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Determination of a site-specific reference dose for methylmercury for fish-eating populations

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Environmental risk-management decisions in the U.S. involving potential exposures to methylmercury currently use a reference dose (RfD) developed by the U.S. Environmental Protection Agency (USEPA). This RfD is based on retrospective studies of an acute poisoning incident in Iraq in which grain contaminated with a methylmercury fungicide was inadvertently used in the baking of bread.[1] The exposures, which were relatively high but lasted only a few months, were associated with neurological effects in both adults (primarily paresthesia) and infants (late walking, late talking, etc.). It is generally believed that the developing fetus represents a particularly sensitive subpopulation for the neurological effects of methylmercury. The USEPA derived an RfD of 0.1 μg/kg/day based on benchmark dose (BMD) modeling of the combined neurological endpoints reported for children exposed *in utero*. This RfD included an uncertainty factor of 10 to consider human pharmacokinetic variability and database limitations (lack of data on multigeneration effects or possible long-term sequelae of perinatal exposure). Alcoa signed an Administrative Order of Consent for the conduct of a remedial investigation/feasibility study (RI/FS) at their Point Comfort Operations and the adjacent Lavaca Bay in Texas to address the effects of historical discharges of mercury-containing wastewater. In cooperation with the Texas Natural Resource Conservation Commission and USEPA Region VI, Alcoa conducted a baseline risk assessment to assess potential risk to human health and the environment. As a part of this assessment, Alcoa pursued the development of a site-specific RfD for methylmercury to specifically address the potential human health effects associated with the ingestion of contaminated finfish and shellfish from Lavaca Bay. Application of the published USEPA RfD to this site is problematic; while the study underlying the RfD represented acute exposure to relatively high concentrations of methylmercury, the exposures of concern for the Point Comfort site are from the chronic consumption of relatively low concentrations of methylmercury in fish. Since the publication of the USEPA RfD, several analyses of chronic exposure to methylmercury in fish-eating populations have been reported. The purpose of the analysis reported here was to evaluate the possibility of deriving an RfD for methylmercury, specifically for the case of fish ingestion, on the basis of these new studies. In order to better support the risk-management decisions associated with developing a remediation approach for the site in question, the analysis was designed to provide information on the distribution of acceptable ingestion rates across a population, which could reasonably be expected to be consistent with the results of the epidemiological studies of other fish-eating populations. Based on a review of the available literature on the effects of methylmercury, a study conducted with a population in the Seychelles Islands was selected as the critical study for this analysis. The exposures to methylmercury in this population result from chronic, multigenerational ingestion of contaminated fish. This prospective study was carefully conducted and analyzed, included a large cohort of mother–infant pairs, and was relatively free of confounding factors. The results of this study are essentially negative, and a no-observed-adverse-effect level (NOAEL) derived from the estimated exposures has recently been used by the Agency for Toxic Substances and Disease Registry (ATSDR) as the basis for a chronic oral minimal risk level (MRL) for methylmercury. In spite of the fact that no statistically significant effects were observed in this study, the data as reported are suitable for dose–response analysis using the BMD method. Evaluation of the BMD method used in this analysis, as well as in the current USEPA RfD, has demonstrated that the resulting 95% lower bound on the 10% benchmark dose (BMDL) represents a conservative estimate of the traditional NOAEL, and that it is superior to the use of "average" or "grouped" exposure estimates when dose–response information is available, as is the case for the Seychelles study. A more recent study in the Faroe Islands, which did report statistically significant associations between methylmercury exposure and neurological effects, could not be used for dose–response modeling due to inadequate reporting of the data and confounding from co-exposure to polychlorinated biphenyls (PCBs). BMD modeling over the wide range of neurological endpoints reported in the Seychelles study yielded a lowest BMDL for methylmercury in maternal hair of 21 ppm. This BMDL was then converted to an expected distribution of daily ingestion rates across a population using Monte Carlo analysis with a physiologically based pharmacokinetic (PBPK) model to evaluate the impact of interindividual variability. The resulting distribution of ingestion rates at the BMDL had a geometric mean of 1.60 μg/kg/day with a geometric standard deviation of 1.33; the 1st, 5th, and 10th percentiles of the distribution were 0.86, 1.04, and 1.15 μg/kg/day. In place of the use of an uncertainty factor of 3 for pharmacokinetic variability, as is done in the current RfD, one of these lower percentiles of the daily ingestion rate distribution provides a scientifically based, conservative basis for taking into consideration the impact of pharmacokinetic variability across the population. On the other hand, it was felt that an uncertainty factor of 3 for database limitations should be used in the current analysis. Although there can be high confidence in the benchmark-estimated NOAEL of 21 ppm in the Seychelles study, some results in the New Zealand and Faroe

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[1] At the time of this analysis, the USEPA RfD for methylmercury was based on data from a poisoning episode in Iraq; however, the RfD for methylmercury is currently based on data from a fish-eating population.

Islands studies could be construed to suggest the possibility of effects at maternal hair concentrations below 10 ppm. In addition, while concerns regarding the possibility of chronic sequelae are not supported by the available data, neither can they be absolutely ruled out. The use of an uncertainty factor of 3 is equivalent to using a NOAEL of 7 ppm in maternal hair, which provides additional protection against the possibility that effects could occur at lower concentrations in some populations. Based on the analysis described above, the distribution of acceptable daily ingestion rates (RfDs) recommended to serve as the basis for site-specific risk-management decisions at Alcoa's Point Comfort Operations ranges from approximately 0.3 to 1.1 μg/kg/day, with a population median (50th percentile) of 0.5 μg/kg/day. By analogy with USEPA guidelines for the use of percentiles in applications of distributions in exposure assessments, the 10th percentile provides a reasonably conservative measure. On this basis, a site-specific RfD of 0.4 μg/kg/day is recommended. *Toxicology and Industrial Health* (2000) **16,** 335–438.

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FOREWORD

Much of the work and progress in the field of risk assessment is found within supporting documents, such as those documents which have been written to assess human exposures for particular situations or to develop risk values for use by government programs. These documents often include a comprehensive evaluation and analysis of the scientific literature and may include new approaches to evaluating data and assessing risk. While government documents may be made available to the public, those developed by the private sector are not usually widely distributed. Because these documents are not generally published, they are not indexed and so are not identified in literature searches.

With this publication, Toxicology Excellence for Risk Assessment (*TERA*) is launching a new effort with *Toxicology and Industrial Health* to publish the supporting documentation from the *TERA* independent peer review program. The following assessment on methylmercury was prepared by The K.S. Crump Group of ICF Consulting for Alcoa, Inc, building on work funded by EPRI. Alcoa pursued the development of a site-specific RfD for methylmercury to specifically address the potential human health effects associated with the ingestion of contaminated finfish and shellfish from Lavaca Bay in Texas. This assessment was prepared and peer reviewed in 1998 based on the data available at that time, including data from the studies in the Seychelles Islands and the Faroe Islands. Because it illustrates several fairly new methods and techniques for risk assessment, it is still timely. The current publication represents a site-specific risk assessment that is media specific, i.e., consumption of fish. The assessment applied the Benchmark dose-reponse model using neurobehavioral data from the non-positive epidemiological study of children in the Seychelles Islands. Estimates of maternal intake were developed using a physiologically based pharmacokinetic model, with the developing fetus as the ultimate target and followed the physiological changes in both the mother and fetus as the pregnancy progressed. Consequently, the extent of exposure to the fetus during different times of the pregnancy could be assessed. Further, a probabilistic Monte Carlo analysis was applied to distributions of physiologically based pharmacokinetic parameters so that the variability in pharmacokinetics expected across a population could be quantified. The result, when coupled with the Benchmark doses, resulted in a distribution of RfD for methylmercury. A risk manager could then select the point on the distribution, i.e., median exposed group (50^{th} percentile) or highly exposed population (90^{th} percentile), to characterize the relevant population. The authors recognize that the science of methylmercury toxicity in humans has continued to move forward with additional studies being published from the Seychelles and Faroe Islands cohorts and that the debate over the choice of the critical study on which to derive the BMDL is still ongoing. USEPA has recently published an update to their 1995 RfD risk assessment on its IRIS database (www.epa.gov.iris). The Agency has utilized a BMDL approach on the Faroe Island data at a 5% response rate, similar to what the NRC/NAS had done in its methylmercury report. Thus the debate over choice of critical study and level of response continue to make this risk assessment one of interest.

Toxicology Excellence for Risk Assessment (*TERA*), a non-profit risk assessment research organization, has developed a two-part program or peer review and distribution of risk values, which seeks to address the need for wider distribution of these efforts. The peer review program provides the opportunity for government, industry, and others to submit their risk assessment documentation for an independent peer review by a panel of risk assessment experts. The results of these peer review discussions are summarized and made available on the Internet (www.tera.org/peer). In addition, the *ITER* database includes risk values approved by the peer review panels alongside the summarized risk values from key government agencies/bodies (www.tera.org/iter). However, the supporting documentation behind the risk values and conclusions of these peer review meetings have not been routinely published or made available.

TERA has developed its peer review program to provide both the public and private sectors with independent peer review of risk assessment values and documentation. Panels

of volunteer experts are convened up to four times a year to review documentation authored by government agencies, consultants, universities, or industry. The process is transparent, with meetings open to the public and summaries of discussions made available on the Internet (www.tera.org/peer). These efforts support *TERA*'s mission to protect public health through development and communication of risk assessment values, improvement of risk methods through research, and education of the public on risk assessment issues.

INTRODUCTION

Alcoa signed an Administrative Order of Consent for the conduct of a remedial investigation/feasibility study (RI/FS) at their Point Comfort Operations and the adjacent Lavaca Bay in Texas to address the effects of historical discharges of mercury-containing wastewater. In cooperation with the Texas Natural Resource Conservation Commission and U.S. Environmental Protection Agency (USEPA) Region VI, Alcoa conducted a baseline risk assessment to assess potential risk to human health and the environment. As a part of this assessment, Alcoa pursued development of a site-specific RfD for methylmercury to specifically address the potential human health effects associated with the ingestion of contaminated fish and shellfish from Lavaca Bay. Application of the published USEPA RfD to this site is problematic; while the study underlying the RfD represented acute exposure to relatively high concentrations of methylmercury, the exposures of concern for the Point Comfort site are from the chronic consumption of relatively low concentrations of methylmercury in fish. Since the publication of the USEPA RfD, several analyses of chronic exposure to methylmercury in fish-eating populations have been reported. The purpose of the analysis reported here was to evaluate the possibility of deriving an RfD for methylmercury, specifically for the case of fish ingestion, on the basis of these new studies. In order to better support the risk-management decisions associated with developing a remediation approach for the site in question, the analysis was designed to provide information on the distribution of acceptable ingestion rates across a population, which could reasonably be expected to be consistent with the results of the epidemiological studies of other fish-eating populations. At the request of Alcoa, ICF Consulting conducted a critical review of the available data for methylmercury, as well as a review of the current RfD recommended by USEPA, for the purposes of development of a site-specific RfD for ingestion of methylmercury in fish.

Inorganic mercury is a naturally occurring element that has been released to the environment from a number of natural sources (volcanic eruptions, erosion of the earth's crust or mercury-bearing rocks, or submersion of vegetation containing mercury[2]) and from human activities (burning of fossil fuels, smelting of metal ores, mining, waste incinerators, or industrial effluents) (Clarkson, 1997). Mercury has been released either directly to surface waters (erosion of mercury-containing soil or rock, submerged vegetation, or industrial effluents) or indirectly by way of initial releases to the atmosphere (volcanic activity, degassing from the earth's crust or industrial emissions) and redeposition onto water bodies. Anthropogenic emissions have contributed to the total global mercury burden, with atmospheric levels increasing since the beginning of the industrialized period (approximately 1850) by a factor of between 2 and 5 (USEPA, 1997).

Anthropogenic emissions of mercury can result in local, regional, and even global deposition of mercury. Mercury can then "cycle" in the environment, with chemical transformations and movement occurring between the air, surface water, and soil compartments of the environment (USEPA, 1997). During cycling, biomethylation of elemental mercury can occur resulting in the formation of organic compounds, such as methylmercury. Recycling of elemental mercury between surface waters and the atmosphere prolongs the impact of anthropogenically derived mercury on aquatic systems and increases the opportunity for methylation. Enhanced human-related mercury deposition, globally, over the past century has probably led to the increased levels of methylmercury currently observed in marine and freshwater fish, rather than current emissions or releases.

In the past, major poisoning incidents occurred in Iraq, where methylmercury was used as a fungicide on grain, and in Minamata, Japan, from industrial releases. Methylmercury poisoning, or Minamata disease, from environmental exposure was first reported in 1956 (Harada, 1995). The poisoning incident was initiated by the release of large amounts of mercury in waste-water discharge from an industrial plant into Minamata Bay. This discharge contaminated marine life in the waters surrounding the facility, resulting in the poisoning of those who ingested fish and seafood from Minamata Bay. The evidence that

[2] Vegetation can remove mercury from the soil and store it in either the root system or above-ground plant foliage. Certain types of vegetation, such as cattails, efficiently remove mercury from soil and have been used in phytoremediation of a test portion of the Florida Everglades. In some areas of the globe, most notably in the Northern Provinces of Canada, construction of hydroelectric plants have resulted in the flooding of large areas resulting in submerged vegetation. Both the release of mercury contained in plant tissue as well as the enhanced environment for microbial conversion of inorganic mercury to organic methylmercury has resulted in increased levels of methylmercury in water bodies distant from industrial sources of inorganic mercury.

Minamata disease was attributable to methylmercury poisoning was:

- patients exhibiting neurological signs and symptoms had eaten large amounts of fish;
- high mercury levels were evident in the hair (or blood or urine);
- neurological signs were present, including sensory disturbances, constriction of visual field, hearing impairment, and cerebellar signs (i.e., impairment of speech and gait, disequilibrium and ataxia); and
- other diseases having similar signs and symptoms were excluded (Harada, 1995).

The disease affected adults, as well as children, with effects observed in the offspring of women who had mild or no symptoms of toxicity. In the Minamata incident, two of the four mothers of severely affected infants recalled no symptoms during pregnancy, confirming the susceptibility of the fetus (Harada, 1995).

Another major outbreak of methylmercury poisoning took place in Iraq in 1971–1972, when seed grain treated with methylmercury fungicide was used to prepare bread throughout rural areas of Iraq (Bakir et al., 1973). This was an acute poisoning episode with exposure lasting for 1–3 months. The consumption of contaminated bread resulted in more than 6000 cases of severe poisoning and approximately 460 deaths. Following exposure at nonfatal levels, methylmercury produced neurological signs and symptoms in adults that ranged from paresthesia, usually the first symptom to appear, to ataxia, dysarthria, and loss of vision. The threshold for paresthesia was estimated to be 100 ppm in hair in adults. Neurodevelopmental effects (i.e., delayed attainment of developmental milestones) were observed in the offspring of mothers exposed to methylmercury who themselves were asymptomatic (Amin-Zaki et al., 1976, 1979; Marsh et al., 1981, 1987).

Results from the Iraqi and Minamata poisoning incidents indicated that symptoms observed in adults could be mild and transient, with paresthesia as the most frequent symptom. However, the severity of effects in the fetus of a mother with mild symptoms or without overt symptoms could be greater. Methylmercury is readily absorbed from the gastrointestinal tract, and once absorbed, readily crosses the blood–brain barrier and the placenta to the fetus (USEPA, 1997). The increased susceptibility of the fetus may be due to the difference in the mechanism of action of neurological damage in the adult compared to that observed in the fetus. In adults, methylmercury ingestion can cause focal atrophy in the developed brain (Hunter and Russell, 1954), while prenatal exposure may cause impaired brain maturation and may inhibit migration of cortical neurones (Choi et al., 1978). Quantitatively, these fetal effects appear at methylmercury levels in the mother during pregnancy that may be as much as an order of magnitude lower than those associated with the earliest effects in nonpregnant adults (Clarkson, 1989). The available data indicate that the fetus may be 5–10 times more sensitive than the adult to brain damage from methylmercury (Clarkson, 1992).

Following the discovery of biomethylation of mercury and bioaccumulation of methylmercury in aquatic food chains and the methylmercury poisoning incidences in Minamata and Iraq, studies were initiated in populations suspected of consuming large amounts of fish. Since the cessation of the use of methylmercury as a fungicide, the most important source of methylmercury exposure in humans is through the consumption of fish and shellfish (Choi, 1989). Field studies indicated that most fish accumulate mercury throughout their lives, often linearly with age (USEPA, 1997). Therefore, the levels of methylmercury currently detected in fish probably resulted from bioaccumulation of mercury over the life of the fish. Accumulation of mercury in fish, shellfish, and cetaceans results in increased human exposures to mercury in populations whose diet includes a high intake of aquatic or marine food (Turner et al., 1980). Average hair mercury concentrations in people who do not consume fish are approximately 2 ppm, while in persons ingesting seven fish meals a week, hair mercury concentrations are on the order of 11.6 ppm (WHO, 1990). Many of the areas studied for the potential toxic effects of methylmercury were selected because the populations of the area ingested aquatic or marine life as a major part of their diet (Grandjean et al., 1992; Kjellstrom et al., 1986, 1989; Marsh et al., 1995a,b; McKeown-Eyssen and Ruedy, 1983a,b; Shamlaye et al., 1995; Wheatley and Paradis, 1995). These evaluations are focused on the potential for neurodevelopmental effects on behavioral, physiological, or cognitive function that may result from prenatal exposure to low levels of methylmercury. One major purpose of these studies is to provide relevant data for regulatory and risk-management decisions regarding allowable levels of methylmercury in environmental media.

Current risk-management decisions regarding methylmercury in the environment have been based, in large measure, on a critical toxicity value, the reference dose (RfD), derived by the USEPA (USEPA, 1997). A chronic RfD is defined as an estimate (with uncertainty spanning perhaps an order of magnitude or greater) of a daily exposure level for the human population, including sensitive subpopulations, that is likely to be without an appreciable risk of deleterious effects during a lifetime (USEPA, 1988). The basis for the USEPA RfD for methylmercury is the retrospective study of latent neurological effects observed in children exposed *in utero*

during the 3-month grain poisoning episode in Iraq that occurred in 1971–1972. The evaluation of this population, published by Marsh et al. (1987), provides the data used by the USEPA for its RfD (USEPA, 1997). When the RfD was developed, uncertainty factors were applied to the estimated apparent threshold value for neurodevelopmental effects, reflecting the state of knowledge regarding, and confidence level in, the toxicity data for methylmercury at that time.

The current RfD has an uncertainty factor of 10 covering two major areas: (1) lack of data, specifically regarding possible multigenerational effects or long-term sequelae of developmental exposures, and (2) variability in the human population, in particular the wide variation in the biological half-life of methylmercury and the variation that occurs in the hair to blood ratio for mercury. However, based on the populations that have been evaluated in the latest available epidemiological studies, the data to evaluate these areas of uncertainty are available.

A major drawback of the Iraqi study as a basis for an RfD for methylmercury at the Point Comfort Facility is that the Iraqi study was an acute, high-level exposure to contaminated grain, whereas the most important source of methylmercury exposure to residents in the Lavaca Bay area is through the chronic, low-level consumption of fish and shellfish. Several studies have been conducted since the Iraqi poisoning incident, which evaluated the potential for developmental neurotoxic effects of methylmercury in populations ingesting aquatic or marine life as a major part of their diet. A no-observed-adverse-effect level (NOAEL) derived from one such study, in the Seychelles Islands, has recently been used by the Agency for Toxic Substances and Disease Registry (ATSDR) as the basis for a chronic oral minimal risk level (MRL) for methylmercury (ATSDR, 1997). Due to the greater relevance of these studies to the question of safe levels of exposure to methylmercury in the Lavaca Bay, it is appropriate to evaluate these studies and their capacity to determine cleanup levels for the Lavaca Bay.

The basic methodology used in determining a site-specific RfD was the same as that used by the USEPA (Barnes et al., 1995; Dourson, 1994; USEPA, 1988, 1994):

- critical review of the available data,
- review of the current RfD,
- selection of the critical study,
- application of benchmark dose (BMD)-response modeling, and
- evaluation of uncertainty.

In this investigation, a critical review of the methylmercury literature was conducted. A review of the basis for the current RfD was also conducted to evaluate its applicability to fish-eating populations. From all the available evidence considered, the critical study and endpoint upon which to base a site-specific RFD for fish-eating populations were determined. The selection of that study was based on a preferred hierarchy, in that human studies, preferably in fish-eating populations, were preferred over animal studies, and chronic exposure was preferred over subchronic or acute.

Based on the calculated BMDL, a quantitative dose–response analysis was then conducted to determine a BMDL for fish-eating populations. The BMDL approach has been proposed as an alternative to the NOAEL approach as the basis for an RfD to be used in setting acceptable exposure limits (Barnes et al., 1995; Crump, 1984; Gaylor, 1989).

Once a BMDL was identified, site-specific daily acceptable intake values were determined. The range of methylmercury intake that could produce an intake equivalent to the BMDL for fish-eating populations were estimated. Application of a physiologically based pharmacokinetic model (PBPK) combined with a Monte Carlo analysis resulted in a distribution of intake values for the critical study. The uncertainty associated with the estimated intake values was then evaluated and uncertainty factors were applied to the distribution estimated intake values to result in a distribution of acceptable daily intake values from which a site-specific RfD for fish-eating populations may be selected.

REVIEW OF RELEVANT EXPERIMENTAL DATA

High doses of methylmercury may result in effects on the neurological, gastrointestinal, cardiovascular, renal, reproductive, immune, and adult nervous systems. In cases of acute poisonings in adults that ingested high doses of methylmercury, such as with the Minimata and Iraqi incidents, the clinical manifestations of methylmercury toxicity included paresthesia, ataxia, and constriction of visual fields (ATSDR, 1997; USEPA, 1997). These effects, as well as alterations in vision, hearing, behavior and motor skills, have also been reported in animals orally exposed to methylmercury (ATSDR, 1997; USEPA, 1997). However, the developing nervous system is particularly susceptible to methylmercury toxicity, and is considered to be the most sensitive endpoint with regard to methylmercury toxicity (ATSDR, 1997; USEPA, 1997).

Review of neurological and neurodevelopmental effects in human populations

Review of the Iraqi Poisoning Incident

A description of the 1971–1972 epidemic of methylmercury poisoning in farmers and their families in Iraq was first

reported by Bakir et al. (1973).[3] Wheat and barley seed grain shipped to Iraq in 1971 had been treated with a methylmercury fungicide, the exact formulation of which is unknown. Some fungicidal formulations were developed to enhance the toxic properties of methylmercury by adding additional components, such as a cyanide anion. Methylmercury-treated wheat and barley seed grain was distributed in Iraq from September 1971 to November 1971. Hospital emissions increased in early January 1972, and from that time until August 1972, 6530 cases of poisoning were admitted to hospitals throughout the country with 459 reported deaths. When other contaminants (e.g., ethylmercury) or other sources of exposure (other foodstuffs, soil, or water) were considered, it was determined that the primary basis of the poisoning was the consumption of homemade bread made with flour from the methylmercury-treated seed grain (Bakir et al., 1973).

The amount of methylmercury exposure could be estimated from bread ingestion for 58 people (Bakir et al., 1973). The mean methylmercury content in wheat flour made from treated grain was 9.1 ppm with a range of 4.8 to 14.6 ppm. The mean concentration in bread was estimated based on an assumed average weight of a loaf of bread to be 1.4 mg/loaf or approximately 700 ppm. From the patients' recollection of the period of consumption of contaminated bread and an estimate of the amount of bread consumed and an average amount of mercury in bread, the body burden of methylmercury estimated for each individual was compared to the blood level for that individual. The estimated body burden was then compared to the frequency of symptoms, at the presumed corresponding blood levels, at the onset of symptoms and at the cessation of exposure. There was a strong correlation between the amount of mercury ingested from bread and the concentration of mercury in blood of these individuals. However, when estimated blood concentrations from bread intake were compared to blood levels from volunteers ingesting a known amount of methylmercury (Bakir et al., 1973), those estimated from recollections of bread ingested tended to underestimate the dose from bread ingestion. According to the patients' reports, the average duration of bread ingestion was 48 days; however, corresponding hair samples in these individuals indicated that exposure lasted 66 days. Total mercury levels in blood were measured and used to estimate the rate of clearance, which varied markedly in different individuals. The mean half-time was 65 days with a range of individual values from 40 to 105 days.

[3] The Bakir et al. (1973) paper provided the initial report of the Iraqi poisoning epidemic. It provides the background on the distribution of poisoning cases by age, sex, and location; the analyses that allowed the determination that the poisoning was caused by methylmercury in homemade bread; the analyses that established the correlation between the amount of mercury ingested in bread and the concentration of mercury in blood; and the determination of the rate of clearance from blood.

The predominant symptoms in adults in this epidemic closely resembled those previously reported in Minamata (Bakir et al., 1973). A mean latency period of 16 to 38 days was reported with the severity of the signs and symptoms dose dependent. In 125 individuals examined (age 9 or greater), as dose increased (increased by either the amount of bread ingested or the duration of ingestion of contaminated bread) the symptoms progressed from paresthesia (loss of sensation in the hands and feet and perioral area), to ataxia (loss of coordination in the gait), followed by visual effects (blurred vision and concentric constriction of the visual field), dysarthia (slurred speech), and loss of hearing.

Effects related to methylmercury intoxication became detectable in this population when the body burden was approximately 25–40 mg of mercury. Blood values were collected from subjects approximately 65 days after cessation of ingestion of contaminated bread. For those collected more than 90 days after exposure, the values were correct to an estimated value at 65 days by assuming a half-life in blood of 70 days duration. Therefore, the estimated dose and corresponding body burden at which symptoms are manifested is likely to be an underestimate. The apparent threshold values for ataxia, dysarthria, deafness, and death were, respectively, at an estimated body burden of 55, 90, 170, and 200 mg of mercury.

Of 21 individuals with blood mercury levels of 101 to 500 ppb, 5% were diagnosed with paresthesia and dysarthria; however, among those with blood mercury levels greater than 4000 ppb (seven individuals), 100% of those examined displayed paresthesia and ataxia, with visual changes, dysarthia, and hearing defects in greater than 66% of the group. Electrophysiological investigations were conducted 7–8 months after cessation of mercury intake. In adults with blood mercury levels greater than 800 ppb, no abnormalities in sensory thresholds and a number of other indications of neurological functions were reported (Bakir et al., 1973).

While paresthesia (at a 10% incidence) was reported at mercury blood concentrations of 0 to 100 ppb, the authors attributed this to factors other than mercury. These calculated body burdens refer to symptoms above the background level of 5–10%. The reported background level may indeed be background in this population or it may be due to, in the case of reported symptoms, inaccurate statements by the patient. According to the authors, many residents of rural districts became aware of the symptoms associated with methylmercury poisoning, and their knowledge may have influenced their answers when describing symptoms to the clinician.

The impact of methylmercury on neurological function of children exposed *in utero* is described in a series of reports (Amin-Zaki et al., 1974, 1976, 1979, 1981; Marsh et al., 1980, 1981, 1987; Seafood Safety, 1991). With time,

the size of the cohort increased as mother/infants pairs were identified and included. The key papers are described in the following paragraphs.

The first report on infants exposed *in utero* consisted of an evaluation of 15 mother–infant pairs examined within 1 to 11 months after the cessation of eating contaminated bread (Amin-Zaki et al., 1974). The majority of infants were 2 months or younger. Clinical manifestations of poisoning were evident in 6 of 15 mothers and included paresthesia, motor weakness, and increased tendon reflexes and to a lesser extent, visual changes and ataxia. All mothers with blood levels in excess of 300 ppb exhibited one or more signs of poisoning. In the infants, those with mercury blood levels at birth in excess of 3000 ppb showed signs and symptoms of severe poisoning, including abnormal reflexes, increased muscle tone, spastic paralysis, decreased hearing, and blindness. It was estimated that exposure started in the third trimester for these four severely impaired infants. One subject with a mercury blood level of 564 ppb showed signs of mild poisoning, while seven other infants with mercury blood levels of 122–636 ppm were asymptomatic.

Two mothers with symptoms had infants that were asymptomatic. The authors concluded that prenatal exposure did not result in a remarkable increase in signs and symptoms for gross measures of neurological function (Amin-Zaki et al., 1974). However, the children were very young, the population was small, and the neurological endpoints evaluated were overt signs and symptoms rather than subtle changes. The study does indicate that at an infant blood level less than 500 ppb, manifestations of overt toxicity are likely to be absent.

As a follow-up, 29 children exposed *in utero* were evaluated up to 4.5 and 5 years of age (Marsh et al., 1980), with earlier interviews for some of these children at 1–1.5 and again at 2–3.5 years of age (Amin-Zaki et al., 1979, 1981). Prenatal exposure was estimated from maternal mercury hair levels; hair sampling took place in rural areas of Iraq between 1972 and 1974. Hair samples from women who were pregnant in late 1971–1972 during the period of potential exposure to methylmercury were included, if their hair was long enough to reflect prior exposure. Several assumptions were made: The mercury concentration in an active hair follicle was assumed to be proportional to the blood mercury concentration; the rate of hair growth was assumed to be 1 cm per month; and mercury, once incorporated into growing hair, was assumed to remain in the hair at deposited levels. Hence, peak mercury concentrations in hair during pregnancy could be reconstructed by evaluation of that section of hair at a distance from the scalp that corresponded to the elapsed time between the time of sampling and the period of pregnancy. Several uncertainties are introduced in this method of exposure: (1) the exact birth dates of the children are unknown and therefore, the time of pregnancy may be off by one or more months; (2) some women preferred to have their hair cut in privacy by their husbands so the cutting technique may have varied, i.e., not at the scalp; and (3) earlier analytical techniques — atomic absorption — may have underestimated the concentration of mercury in hair.

Interviews using standardized questionnaires recorded reported histories and physical examinations (Marsh et al., 1980). One difficulty encountered was in establishing the precise age of the children. No birth dates were recorded and assignment of a birth date for the child was based on the mother's recollection with regard to an important annual event, such as a religious holiday. Some birth dates were assigned based on the time of the mercury poisoning event. Routine neurological examinations were conducted and included consideration of testing for deafness and observations of reflexes, gait, and posture. Mothers, and often grandmothers rather than mothers, were questioned regarding the course of the pregnancy and delivery and when the child attained early milestones of development, i.e., walking and talking. The mother's opinions concerning the child's mental or physical retardation and the presence or seizures were also obtained. Late walking (motor retardation) was defined as the child not standing alone and walking by 18 months, while late talking (speech retardation) was defined as the child's lack of saying two or three meaningful words by 24 months. The presence of mental retardation and seizures was based on the history provided by the mother, while neurological signs were assessed by trained examiners.

Maternal mercury concentrations in hair ranged from 2 to 384 ppm with a median peak mercury level of 25 ppm. Two children whose maternal mercury hair levels were 165 and 209 ppm, were severely affected. Five of the children were apparently normal. The mothers of the five normal children had mercury hair levels of 25 ppm or less. The remaining 22 children had some signs of neurological effects of varying degrees. When divided into three exposure categories of approximately equal numbers of children, there was no significant difference in the frequency of any abnormality in children of mothers with hair mercury levels of 0–11 ppm versus those with hair mercury levels of 12–85 ppm. The difference between 0 and 85 and 99 and 384 ppm was significant for delayed motor and speech development and for seizures. However, statistical analyses were limited by the small number of infant–mother pairs in the intermediate exposure ranges. No infant–mother pairs were available with maternal hair concentrations in the range of 25 to 50 ppm and only four infant–mother pairs were in the 50 to 99 ppm range. The high mercury exposure group also differed in the number of children having multiple symptoms and signs. There was only one infant in the lowest exposure group who had five symptoms and one infant in the midexposure group who had three abnormalities, while 9 of the 10 in the high-exposure group had more

than two abnormalities, and 6 of the 10 reported four or more signs and symptoms.

While covariants were not explicitly considered, a number of potential covariants were not expected to have an impact on these outcomes (Marsh et al., 1980). For example, alcohol consumption was nonexistent and differences in socioeconomic status and nutrition were not apparent across different areas. The mothers were aware that they had ingested contaminated bread and that knowledge may have influenced their replies to questions on developmental milestones and seizures. However, clinical examination alone could not have identified a single case because the signs are not sufficiently specific. The data clearly showed an association between mercury levels in hair and responses, when the range of mercury concentrations was considered, at maternal hair mercury levels above 85 ppm. While clearly establishing levels above which effects should be expected, the sample size may have been too small, in particular in the intermediate dose range, to clearly identify the level at which one should expect no effects.

The cohort evaluated by Marsh et al. (1980) was expanded to 84 mother–infant pairs (Marsh et al., 1981). Peak maternal mercury hair concentrations ranged from 0.4 to 640 ppm in samples of hair collected between 1972 and 1974. The study included children who were not exposed *in utero*. The method of hair collection and mercury analyses in hair were those reported in Marsh et al. (1980). The interviews and examinations conducted were the same as those reported in Marsh et al. (1980). This was actually two subcohorts that represented a clinical series of 29 subjects, augmented by a second series of 55 subjects (WHO, 1990). Although mercury exposure was comparable in both series (average of 78.8 and 80.4 ppm based on atomic absorption), reported symptom frequency and infant sex ratio (M/F) differed significantly in ways that cannot be explained by differences in exposure. Transient paresthesia was reported in 45% of mothers in series 1 and only 7% in mothers in series 2, although the overall exposure, as measured by hair levels, was greater in series 2. Similarly, reported incidences of late walking and late talking were 48% vs. 22%, and 41% vs. 15% in series 1 and series 2, respectively. A significant difference in the sex ratio between the two series was apparently due to an undersampling of female infants in the series 2 cohort.

Children were grouped into exposure categories of 14 children per category (Table 1). Increases in the incidence of late walking were noted at peak hair levels of 18 ppm or greater (the mean in that dose group was 37 ppm). Similarly, late talking, mental retardation and seizures were increased at maternal hair concentrations greater than 18 ppm; however, only late walking and late talking were significantly increased above the lowest exposure group, and no dose–response increase was noted between the 18–67.6 and 68–180 ppm exposure groups. Similarly, with the neurological score (Table 2), an increase in the number of children with moderate scores increased to 3/14 above 18 ppm, while 5/28 children had neurological scores in the severe range at maternal mercury hair concentrations in the two highest exposure groups. Specifically, the maternal hair concentrations were 165, 209, 300, 311, and 320 ppm for these five subjects.

In summary, the authors stated, "Severe neurological deficits were observed in five children whose maternal peak hair mercury concentrations were 165–320 ppm. Minimal symptoms were reported for mothers and children when peak maternal hair levels were below 68 ppm. Minimal neurological signs occurred in children when peak maternal hair mercury concentrations were at an undetermined point between 68 and 180 ppm" (Marsh et al., 1981).

Marsh et al. (1987) re-reported the 81 mother–infant pairs (same cohort as Marsh et al., 1981 minus three pairs). The difference in this study is that a single strand of maternal hair was evaluated using X-ray fluorescence (XRF) spectrometry. Both the peak and mean exposure

Table 1. Symptoms (not signs) in Iraqi children related to maternal hair peak mercury concentration in pregnancy.

Mother				Children				
				Retarded				
Peak hair range	Hg ppm, mean	Neuro symptoms	*N*	Walk	Talk	Mental	Seizures	Total number of symptoms
0.4–6.0	3	1	14	2	1	1	0	4
6.1–10.0	8	2	14	4	1	0	0	5
10.0–18.0	13	1	14	0	0	0	0	0
18.0–67.6	37	5	14	5	7	2	2	16
68.0–180	125	7	14	6	5	3	3	17
204–640	293	6	14	9	6	3	3	21

Source: Marsh et al. (1981).

Table 2. Signs in Iraqi children related to maternal hair peak mercury concentrations in pregnancy.

Maternal hair peak Hg			Signs in children			
Range	Mean	*N*	Normal–mild score 0–3	Moderate 4–6	Severe 7–11	Total number of signs
0.4–6.0	3	14	14	0	0	25
6.1–10.0	8	14	13	1	0	19
10.0–18.0	13	14	14	0	0	19
18.0–67.6	37	14	11	3	0	31
68–180	125	14	10	2	2	44
204–640	293	14	8	3	3	58

Source: Marsh et al. (1981).

during pregnancy were estimated based on the date of hair collection, the assumed average rate of hair growth (1 cm per month), and the month of birth (which was not accurately known). It was stated that the previous method of analyses, atomic absorption, resulted in an underestimate of the true hair concentrations for mercury. Unfortunately, the results reported in Marsh et al. (1987) cannot be compared directly with those in Marsh et al. (1981). These data are reported for the individual mother–infant pairs in Marsh et al. (1987) and as exposure group data in Seafood Safety (1991), which are given in Table 3. Individual scores showed considerable variability. No clear dose–response is noted at maternal hair levels below the 80–139 ppm exposure group (geometric mean 163.4 ppm) as listed in Table 3. Various statistical analyses of these data produce considerably different estimates of an apparent threshold (using a parametric threshold model) or a NOAEL, depending on the definition of background, statistical outliers, and the adverse effect (Cox et al., 1995; Crump et al., 1995). Application of a parametric threshold model resulted in estimates of an apparent threshold for delayed walking that ranged from 10 ppm in maternal hair to >100 ppm, depending on the definition of background (Cox et al., 1989, 1995). In a reanalysis of these data by Crump et al. (1995), there was no convincing statistical evidence of an effect of mercury in children of mothers whose hair concentrations were below 80 ppm. Similar conclusions were reached in this study as those reported in Marsh et al. (1981).

Review of Epidemiological Studies in Fish-Eating Populations

Following the observation of the fetal effects observed in Iraq and the presumption that the fetus was the most sensitive subpopulation, investigations were begun to conduct a more definitive study on the fetal effects of methylmercury. The focus was on women of fertile age in populations that relied mainly on fish as a source of protein. Several populations were identified, including fish-eating communities in the Seychelles (Davidson et al., 1995; Marsh et al., 1995a; Myers et al., 1995a,b), the Faroe Islands (Grandjean et al., 1992, 1994, 1995, 1997), New Zealand (Kjellstrom et al., 1986, 1989), Peru (Marsh et al., 1995b; Turner et al., 1980), Canada (McKeown-Eyssen and Ruedy, 1983a,b; Wheatley and Paradis, 1995, 1996; Wheatley et al., 1979, 1997), and others (Birke et al., 1972; Kyle and Ghani, 1982; Marsh et al., 1974; Skerfving, 1974).

New Zealand

Concern about exposure to high levels of methylmercury in New Zealand from the ingestion of fish was first raised in 1978, when a survey of trace elements in the New Zealand diet (Dick et al., 1978) indicated a much higher than

Table 3. Incidence of neurological effects in children following *in utero* exposure to methylmercury.[a]

			Effects (number of cases)					
Range (ppm)	Geometric mean	Number of subjects	Seizures	Late walking (after 18 months)	Late talking (after 24 months)	"Mental" symptoms	Neurological score over 3	Combined effects[b]
1–3	1.37	27	0	0	2	1	3	5
5–19	10.00	14	0	2	1	0	1	3
20–79	52.53	13	1	2	3	1	4	6
80–319	163.38	12	2	3	4	3	3	8
Over 319	436.60	15	4	12	11	4	9	13

[a]Modified from Seafood Safety (1991).
[b]Summarized from Marsh et al. (1987).

expected mercury content in some commercial fish, in particular shark (Kjellstrom et al., 1989). Shark meat was the most common fish in the fast food "fish-and-chips." In adults eating fish more than three times a week, the hair mercury levels were reported to be as high as 25 ppm (Kjellstrom et al., 1986).

To assess the impact of exposure to mercury in the environment on neurodevelopmental outcomes, a cohort was established in 1978 of the infants born to mothers who had a high fish consumption during pregnancy (Kjellstrom et al., 1986). From the end of 1977 and during most of 1978, all women giving birth at maternity hospitals in the northern part of North Island, New Zealand were asked to participate. The aim of the study was explained to each potential participant.

Maternal hair samples at parturition were collected and questionnaires were obtained from 10,930 new mothers out of 16,293 women who gave birth during the specified time period (Kjellstrom et al., 1986). Umbilical cord blood samples were also collected from as many children as possible as part of routine blood collection (Kjellstrom et al., 1989). The authors noted that the major reason for nonparticipation was that in the largest hospital the number of births was large and the special staff employed to conduct the interviews could not get to all of the patients. Participation from smaller hospitals, where the nursing staff collected the samples was 85%.

The questionnaire given to the women during pregnancy in 1978 focused on the dietary habits, with a special focus on fish and shellfish consumption, frequency that fish was consumed, and size of fish eaten. Fish consumption was divided into fresh fish, canned fish, fish products, shellfish, and cooked fish from take-away shops. Questions were also asked about medications taken during pregnancy, exposure to aerial spraying or handling of pesticides, occupation, and smoking habits.

Based on the results of the questionnaire, 1000 women were selected as high fish consumers, because they had indicated that they consumed fish more than three times per week during pregnancy. Analysis of hair samples for these mothers found that 73 mothers had average hair mercury levels during their pregnancy above 6 ppm, with the highest maternal hair mercury level at 86 ppm. When the types of fish consumed and the association with hair mercury levels was evaluated, the strongest correlation was observed with the ingestion of snapper. In the group with hair mercury levels above 6 ppm, 76% consumed snapper more than once a week and 40% consumed snapper more than three times a week.

An additional questionnaire was also administered after the birth of the children to record sex, weight, age, head circumference, height, any problems during birth, general health, mother's health, and medications taken during pregnancy. Later information on the child's general health, including hearing or sight difficulties and age milestones (i.e., when sitting, walking, and talking were achieved), was also recorded.

As an initial pilot study, the Denver Developmental Screening Test (DDST) was administered to 31 children at age 4 in the home by a nurse. Vision (Sheridan-Gardiner Letter-Matching Test and Stycar Miniature Toy Test) and sensory (Finger Identification, Localization of Tactile Stimuli, and Temperature Recognition Tests) tests were also administered. The DDST is a standardized test of a child's development that is easy to perform in the home. Major function sectors evaluated included gross motor, fine motor, language, and personal–social. The items evaluated in each sector were classified as either passed, failed, or refused, with refused being the classification of choice only if the tester was sure the child could perform the item. A failed score was interpreted as a developmental delay on any one item when at least 90% of the children can pass this item at a younger age. A developmental delay in any of the four major function sectors was scored if there were two or more item delays within a sector or there was one item delay and the age-line in the sector did not go through any item that was passed.

The results of the tests in the high-exposure children (maternal hair level >6 ppm) were compared to the results from a "control" group matched for ethnic group, mother's age, child's birth place, and birth date. However, not all of the identified covariates were considered in the analyses. The results in the high-exposure group for the DDST indicated two abnormal scores and 14 questionable scores, compared to one abnormal score and four questionable scores in the control group. When the number of children with abnormal or questionable scores were combined, the prevalence of these scores was significantly greater in the high-exposure group (16/31), compared to children of mothers with lower hair levels (5/31) ($P<0.005$) (Kjellstrom et al., 1986).

The results of the vision tests indicated a small nonsignificant difference between the two groups. Twenty-six of the children in the exposed group were normal, with 5 children showing a vision deficiency, compared to 28 normal and 2 deficient in the control group. No significant differences were observed between the exposed and control groups in the attainment of developmental milestones.

The main study was conducted when these children were approximately 6–7 years of age to confirm or refute the findings at age 4 using additional confounding variables, additional and more sophisticated tests, and three "control" groups (Kjellstrom et al., 1989). Two control groups had high fish consumption and average mercury hair levels falling into one of two groups (0–3 or 3–6 ppm). The exposed group was classified as having >6 ppm mercury in maternal hair during pregnancy. Another control group of children was identified that had low fish consumption and

whose mothers had maternal hair levels of 0–3 ppm during pregnancy.

It is important to note that the methylmercury measurements used in this study were the average methylmercury concentration over the length of hair assumed to correspond to the time of pregnancy rather than the peak hair level during pregnancy. The latter was the measure used in the Iraqi and Canadian studies. According to Kjellstrom et al. (1986), the average hair levels in the New Zealand mothers were 1.5 times lower than the peak levels.

Umbilical cord blood had been collected from many of the children. For those children selected for the study of 6- and 7-year-olds in 1985, these blood samples were analyzed for lead content. Current lead exposure was assessed by taking samples of garden soil from the homes of some of the participating children. Current blood samples were not taken at the time of study in 1985. The authors note that many children in New Zealand are exposed to high environmental lead levels.

Home interviews, with the child's mother or primary caregiver, were carried out by a number of trained persons at the time the tests were administered to collect additional data on social and environmental factors. As most of the mothers were of Polynesian ethnic origin or born on Pacific Islands for whom English was not their first language (whether these mothers spoke or understood English was not noted), interviewers with a variety of ethnic backgrounds were employed. Questions were asked from standardized questionnaires accompanied by pictures to ascertain information regarding alcohol consumption (different-size glasses or bottles) and other quantitative information, such as income level or educational level. "Social class" was estimated from occupational data. These interviews were supplemented with data from hospital records to include mother's smoking habit, occupation during pregnancy, child's birth weight, gestational age, and Apgar score. It is unclear if maternal alcohol consumption was part of the questionnaire administered in the hospital at the time of birth.

From the original group of 73 children, 60 mother–child pairs in the high-exposure group (maternal hair >6 ppm) were located. A similar number of participants were selected for each of the three control groups. A total of 57 totally matched groups of four children each, along with four incomplete sets (237 children), were evaluated. Matching was on the basis of ethnic group of the mother (European, New Zealand Maori or Pacific Islander), maternal age (±6 years), maternal smoking habits (smoker/nonsmoker), maternal place of residence (urban/rural), and time the mother lived in New Zealand before the child's birth (±10 years).

A battery of 26 psychological and scholastic tests was administered to the children at school (Table 4). The tests administered were those recommended by a WHO Expert Group for indicators of lead neurotoxicity (WHO, 1984). Each child was tested by an educational psychologist and a teacher. A comparison of scores from two psychologists testing the same children was made.

The results of five selected test scales (TOLD, SL, WISC-R performance, WISC-R full, McCarthy perceptual, McCarthy motoric) were compared with mercury exposure and fish consumption for the four groups (Table 5). Multiple regression analyses were conducted of the test results of these five tests with maternal ethnic group, maternal age, maternal smoking habits during pregnancy, mother's time in New Zealand before the birth, and child's sex included as variables. Potential confounding factors included were social class, language spoken at home, older children at home, younger children at home, duration of breast feeding, maternal alcohol intake during pregnancy, child's birth weight, child's maturity at birth, and child's Apgar score. Additional variables not included here, but considered in a reanalysis of all 26 test scores, were age of the child at time of testing and parents' educational level (Crump et al., 1998). Hair mercury levels were expressed as a binary variable indicating a mercury concentration in mothers' hair >6 mg/kg and between 3 and 6 mg/kg. Kjellstrom et al. (1989) performed both standard analyses

Table 4. Psychological tests used in the New Zealand follow-up study.

Test dimension	Specific test used	Reference
I. Academic attainment	a) Clay diagnostic survey	Clay (1972)Clay (1979)
	b) Burt word recognition test	NZCER (1981)
	c) Key math diagnostic arithmetic test	Connolly et al. (1971)
II. Language development	a) Test of language development (TOLD)	Newcomer and Harwitt (1977)
	b) Peabody picture vocabulary test, 1981 version	Dunn and Dunn (1981)
III. Fine and gross motor coordination	McCarthy scales of children's abilities	McCarthy (1972)
IV. Intelligence	a) McCarthy scales	McCarthy (1972)
	b) Weschler Intelligence Scale for Children, Revised (WISC-R)	Wechsler (1974)
V. Social adjustment	a) Everts behavior rating scale	Everts (1983)

Source: Kjellstrom et al. (1989).

Table 5. Comparison of mean psychological test scores for the mercury exposure in New Zealand cohort.

Test variable[a]	Mercury exposure group (ppm hair)				Maximum group difference
Number studied	6–86	3–5.99	0.1–2.99	0.1–2.99	
TOLD-SL	78.8	77.8	74.5	79.6	6.8
WISC-RP	95.9	98.2	96.1	100.0	4.1
WISC-RF	90.6	94.2	91.0	96.0	5.4
MCC-PP	53.8	56.5	56.9	58.3	4.5
MCC-MOT	59.1	62.1	63.0	61.1	3.9

Source: Adapted from Kjellstrom et al. (1989).
[a]TOLD-SL=Test of Language Development; spoken language quotient. WISC-RP=Wechsler Intelligence Scale for Children, Revised; performance scale. WISC-RF=Wechsler Intelligence Scale for Children, revised; full intelligence quotient. MCC-PP=McCarthy Scales of Children's Abilities; perceptual scale. MCC-MOT=McCarthy Scales of Children's Abilities; motoric scale.

and weighted analyses that gave less weight to outliers (Table 6). With the unweighted analysis, only the MCC-PP test was significantly associated with mercury exposure. In the weighted analysis that was conducted, less weight was assigned to outliers (note one mother–infant pair had scores within the normal range, but maternal hair levels were 86 ppm). When weighted scores were assessed, decreased performance on three tests, TOLD-SL, WISC-RF, and MCC-PP, was associated with mercury exposure.

Based on the results of the weighted analysis, the authors concluded that there was an apparent association between prenatal methylmercury exposure in fish and a decreased performance in psychological tests was observed. However, prenatal methylmercury exposure only contributed a small part of the variance of test results compared to ethnic group and social class. The authors also concluded that an average hair mercury level during pregnancy of 13–15 ppm, which is equivalent to a monthly peak of approximately 25 ppm, may be associated with the decreased performance observed in the psychological tests. The authors report that similar, but fewer marked effects, may have occurred in the group with hair mercury levels of 6–10 ppm.

Additional regression analyses of these data in which the scores from all 26 scholastic and psychological tests administered to the 6- to 7-year-old children were conducted (Crump et al., 1998). The actual mother's average mercury hair concentration was used in place of the indicator mercury variables used in the initial analysis. In addition to the nonmercury variables considered in the initial analyses, variables representing the age of the child at the time of testing and the educational level of the parents were included. When reevaluated using either the same nonmercury explanatory variables as were used in the initial analyses (Kjellstrom et al., 1989) or an expanded list of nonmercury variables but employing the actual mercury hair level for each mother, no association between mercury exposure and test outcomes was found. When the scores of one mother–infant pair, a Maori boy whose mother had a hair mercury level of 86 ppm, was removed from the analyses, mercury exposure was significantly associated with decreased scores for the TOLD-SL and MCC-PP tests when only the initial list of nonmercury variables was used. Only the MCC-PP test scores were significantly associated with mercury exposure when the expanded list of nonmercury variables were included. While none of the test scores for this individual were by statistical definition an outlier, his scores were quite influential. These reanalyses confirmed that the most important explanatory variables were ethnicity, education, mother's age, and child's sex rather than mercury exposure.

Seychelles

A study in the Republic of Seychelles was prompted by the results of a study conducted by Matthews (1983) on the determination of hair mercury concentrations in 36 Seychellois. The results of this study indicated that the concentration of mercury in scalp hair ranged from <5 to 45 ppm. These hair concentrations of mercury spanned the lows levels observed in the Iraq poisoning down to average levels in the U.S. (<2 ppm) (Marsh et al., 1995a).

More than 80% of the population of the Seychelles consumes fish meals at least once a day, with fish as the main source of protein, with no contribution from the ingestion of marine mammals. The Republic of Seychelles is 1000 miles away from any continent or large population center, with no local industry as a source of environmental pollution. It is also distant from other sources of pollution (Shamlaye et al., 1995). The population is generally healthy, with low incidences of maternal tobacco and alcohol use or other substance abuse. The health care system is well organized and was capable of and supportive to a child developmental study. Therefore, the Seychelles' population was selected as

Table 6. Analysis of mercury *in utero* exposure on performance tests for the New Zealand cohort.

Test variable	*P* value	
	Unweighted	Weighted
TOLD-SL	0.068	0.0064
WISC-R	0.25	0.072
WISC-RF	0.15	0.019
MCC-PP	0.02	0.0034
MCC-MOT	0.10	0.074

Source: Adapted from Kjellstrom et al. (1989).

a study population to evaluate the effects of methylmercury on the fetus (Marsh et al., 1995a).

With the help of the Seychelles government, a two-phased study was conducted: (1) a cross-sectional pilot study, and (2) a main study. The cross-sectional pilot study was started 2 years before the main study, in 1987, to provide guidance for the main study, which was started in 1989 (Marsh et al., 1995a). In the pilot study, the children were examined at various ages, with a subset of children examined at 66 months of age using the same battery of tests planned for the main study at each age. The pilot study was conducted to identify covariates for the main study and to help determine the number of children that should be evaluated in order to achieve 80% power, with a 5% significance level in the main study. The main study was a double-blind prospective longitudinal study with examinations conducted at 6.5, 19, 29, and 66 months. Narrow time-limited windows were set for the examinations of the children to eliminate age of testing as a confounding variable.

Maternal interviews for the main study confirmed a high consumption of fish during pregnancy (Shamlaye et al., 1995). The median was 12 fish meals per week, with 75% of the women consuming 10 to 14 fish meals per week during pregnancy. Only 8% of the mothers reported eating fewer than five fish meals a week. However, the number of fish meals per week does not take into account the species of fish eaten or the amount of fish eaten.

Maternal scalp hair was used as a biological monitor of the body burden of mercury and, thus, as an indicator of fetal exposure to methylmercury. The results of numerous studies have indicated that at steady state the methylmercury concentration in newly formed hair is directly proportional to the simultaneous concentration in blood (WHO, 1990). Over a period of 4 years (1986–1989), hair samples were obtained for 841 mothers in the pilot study, and 763 mothers in the main study (Cernichiari et al., 1995a). An attempt was made to collect three hair samples from the mothers: on the first visit to the antenatal clinic, at the time of delivery, and 6 months after delivery, with the majority of the mothers providing at least two hair samples. A sample of approximately 50 strands of hair was obtained from the crown of the mothers' heads. Hair samples were analyzed by three different techniques: cold vapor atomic absorption (CVAA), X-ray fluorescent spectrometry (XRF), and gas chromatography/cold vapor atomic fluorescence detection (GC/AFD). The method of GC/AFD could measure methylmercury specifically, while for CVAA, concentrations of organic mercury were calculated as the difference between total and inorganic mercury. Fish samples representing the most often consumed species in the Seychelles were also analyzed by CVAA and GC/AFD.

The pilot study consisted of 789 mother–infant pairs that were evaluated between the infants' ages of 5 and 109 weeks. One or more maternal hair samples were taken over a period of 2 years before the initiation of the pilot study, during their pregnancy and after delivery. The median maternal hair mercury level for the pilot study was 6.74 ppm, with a mean and standard deviation of 7.72±4.77 ppm (Cernichiari et al., 1995a). The children were evaluated using the Revised Denver Developmental Screening Test (DDST-R), as well as given a general medical and neurological examination. The DDST-R provides a measure or motor, perceptual, and cognitive development in children up to 6 years of age (Marsh et al., 1995a). The test measures personal–social, fine motor adaptive, language, and gross motor development. The overall DDST-R is scored as abnormal, questionable, or normal. This test is considered specific, but not very sensitive (Myers et al., 1995a), which means that subtle changes in development may not be readily detected by this test.

The following history was also recorded for each maternal–infant pair: maternal use of tobacco and alcohol during pregnancy, maternal medical history, child's age at testing, child's gender, child's birth weight, 1 or 5 min Apgar score, child's medical history, and the number of persons per room in the child's home. This information was collected in an attempt to adjust for factors that may also have an impact on child development, other than methylmercury exposure.

Only three (<1%) of the children in the pilot study scored abnormal on the DDST-R, with 65 of the children (approximately 8%) obtaining a questionable score. Because of the small number of children who scored abnormal, no analysis was possible. An analysis was conducted with the combination of questionable and abnormal scores. An increasing frequency in the response on the DDST-R was observed with increasing levels of maternal hair mercury levels (P=0.031 one-sided) when the questionable and abnormal scores were combined; however, the standard scoring for the DDST-R is to treat only abnormal scores as failures (Myers et al., 1997a). No association of mercury, with or without adjustment for covariates, was observed with overall neurological examination, limb tone, deep tendon reflexes, or extensor plantar responses.

In preparation for the main study, a subset of 217 children in the pilot study was reexamined at 66 months of age (Myers et al., 1995b). Each child was evaluated within 3 months of his or her 66-month birthday. The test battery was different from the tests used previously in this study, and included the McCarthy Scales of Children's Abilities (with minor modifications), The Preschool Language Scale (with minor modifications), and the Letter–Word Identification and Applied Problems subscales of the Woodcock-Johnson Tests of Achievement. Each of these tests is standardized on Western populations that present a wide range of socioeconomic, ethnic, and cultural variation.

This subset (n=217) was similar to the remaining subset (n=572) for all covariates, with the exception of maternal alcohol consumption. A statistically significantly (P<0.001) smaller number (5%) of the subset reported consuming alcohol during pregnancy, compared to 12% in the remaining cohort mothers. Scores were missing for some children either because they refused certain test items or were fatigued. Missing scores ranged from 6% for the Letter–Word subtest of the Woodcock-Johnson Test to 34% for the General Cognitive Index (GCI) of the McCarthy Scales Test. Significant associations were observed between maternal mercury levels and declines in GCI and Perceptual-Performance subtests of the McCarthy Scales Test. A significant effect of gender was also observed in the CGI, with males scoring lower than females regardless of mercury exposure. There was also a significant association between maternal hair levels of mercury and a decline in scores from the Auditory Comprehension subtest of the Preschool Language Scale, with a similar decrease in the Total Language subtest. This suggested that the effects seen on total language can be attributed to auditory comprehension (Myers et al., 1995b).

There were outlier scores in many of the tests, with scores from one child providing outlying data points in four of the different tests and an influential point in four additional tests. The maternal hair mercury level associated with this child was 23.0 ppm. The maternal mercury hair levels for the other outliers are as low as 5 ppm. When the regression analyses were recomputed after removing outliers, the statistically significant association between maternal mercury concentrations and decreasing neurodevelopmental scores was no longer significant for all endpoints, with the exception of the Auditory Comprehension score, which was only marginally significant (P=0.04). The authors stated that the results of this study should be interpreted with caution until the results of the 66-month evaluations of the main study were completed, because the pilot study was not designed to evaluate all confounding variables.

A cohort of 740 mother–infant pairs was evaluated in the main study. The median maternal hair mercury levels in this study was 5.94 ppm, with a mean and standard deviation of 6.85±4.50 ppm (Cernichiari et al., 1995a). The tests administered at 6.5, 19, 29, and 66 months of age are listed in Table 7. At 6.5 months of age, in addition to the DDST-R test administered in the pilot study, the infants were administered the Fagan's test of visual recognition memory (Infantest). This test, when administered early in infancy, has good predictability of future cognitive outcomes (Marsh et al., 1995a). At both 19 and 29 months, the Bayley Scales of Infant Development (BSID) were administered. These tests have been widely used as a measure of cognitive function (Marsh et al., 1995a). At 66 months, the test battery was similar to that administered to children in the pilot study at 66 months of age. Children were also given a general medical and a neurological examination. Maternal intelligence was also evaluated using the Raven's Pro-

Table 7. Developmental domains evaluated and tests applied in the main study in the Seychelle Islands.

	Age of child (months)			
	6.5	19	29	66
Developmental Domain	DDST-R	BSID MDI	BSID MDI	MSCA GCI
Global–Cognitive		Kohen-Raz	Kohen-Raz	Bender-Gestalt
Visual–Perceptual				MSCA Perceptual
Speech–Language	DDST-R			MSCA Verbal
				PLS Total Language
				Auditory Comprehension
				Verbal Ability
Memory	Fagan Infantest			MSCA Memory
Visual Attention	Fagan Infantest			
Neuromotor Exam	Neurological	BSID PDI	BSID PDI	Bender-Gestalt
	DDST-R			MSCA Motor
Social–Emotional	DDST-R			
Behavioral			BSID IBR	Child Behavior Checklist
Learning-Achievement				Woodcock-Johnson
Audition				Audiometry and Tympanometry

Source: Cernichiari et al. (1995a, 1995b).
BSID=Bayley Scales of Infant Development; DDST-R=Denver Developmental Screening Test-Revised; GCI=General Cognitive Index; IBR=Infant Behavior Record (part of BSID); MDI=Mental Developmental Index (part of BSID); MSCA=McCarthy Scales of Children's Abilities; PDI=Psychomotor Developmental Index (part of BSID); PLS=Preschool Language Scale.

gressive Matrices. This test is a nonverbal test that correlates well with complex measures of cognitive function (Marsh et al., 1995a).

The results of the testing were analyzed for an association between maternal hair mercury level and outcome by multiple regression analysis. The primary analysis for each endpoint consisted of a full model, which included all covariates, and a reduced model, that included a smaller group of covariates. The covariates were selected based on their potential bias to the outcome of the study. The covariates selected for the full model included gender, birth weight, birth order, gestational age, the child's medical history, maternal age, maternal smoking during pregnancy, maternal alcohol consumption during pregnancy, maternal medical history, primary caregiver intelligence, maternal and educational levels, history of breast-feeding, language spoken in the home, and family income. For the reduced model, the covariates gestational age, maternal alcohol and tobacco consumption, family income, and caregiver intelligence were excluded. For continuous endpoints, an examination of the residuals was conducted for each regression analysis. This residual analysis revealed outliers, in some cases, which were removed from further analysis, based on an *a priori* definition of a statistical outlier. A secondary multiple regression analysis was conducted including only children exposed to either ≤ 3 or >12 ppm mercury in maternal hair, using the same covariates as the primary analysis with the exception of caregiver intelligence, which was retained in the 19- and 29-month reduced model.

At the 6.5 month evaluation, the DDST-R was completed on 737 children, with the Infantest completed for 723 children. Three children (0.4%) scored abnormally, with 11 children (1.5%) scoring questionably. Only 3.4% of the children had an overall neurological score other than normal. Because of the small number of abnormal and questionable results, no analysis was possible. The authors concluded that no neurodevelopmental effects related to fetal mercury exposure were observed (Myers et al., 1995a). The only covariate that was significant was breast-feeding, based on the results of the reduced model. Based on the results of this analysis, children who were breast-fed were more likely to have abnormal limb tone ($P=0.02$). The authors cautioned that this was an unexpected outcome, which was not repeated in any other endpoint analysis, and therefore may be spurious.

At the 19- and 29-month examinations, no mercury effect was found for either the full model or the reduced model for the BSID Mental or Physical Development Indices (Davidson et al., 1995). On the more subjective behavior scores of the BSID Infant Behavior Record (IBR) at the 29-month examination, one of the seven measures, activity, did show an association with mercury exposure (Myers et al., 1997a). The mercury×gender interaction for the IBR for activity level was statistically significant for the reduced model ($P=0.0075$) and marginally significant for the full model ($P=0.047$). When activity scores for males and females were analyzed separately, a significant decrease in activity was observed in males ($P=0.0004$), but not females ($P=0.87$). However, these decreases were compared to standard scores for U.S. children. Also, while there was a significant decrease in activity in males, male activity scores were significantly higher than females ($P=0.008$). The authors cautioned that this lowered activity in males may not represent an adverse outcome of increased exposure to mercury (Davidson et al., 1995). The possibility that home environment, or any other social variable, might have interacted with gender, with maternal hair mercury levels, or with both factors was not examined.

During the 19-month examination, nurses obtained information on what age the child walked and talked (Myers et al., 1997b). The mean age that the children walked was 10.7 ± 1.9 months for females and 10.6 ± 2.0 months for males. The mean age that the children talked was 10.5 ± 2.6 months for females and 11.0 ± 2.9 months for males. No association was observed between the age at which the children walked or talked and prenatal exposure to methylmercury. These averages were also somewhat advanced, compared to the mean for these milestones in U.S. children. These milestones were evaluated in order to compare low dose effects in the Seychelles population with those reported for these milestones in the Iraqi study (Myers et al., 1997b). Referring to the Cox et al. (1989) "hockey-stick" analysis in which an apparent threshold for delayed walking was estimated to be 10 ppm mercury in maternal hair, Myers et al. (1997b) stated, "These results [in the Seychelles study] do not support the lowest effect levels in young children following prenatal methylmercury exposure predicted by the dose response analysis of the Iraqi data."

In conjunction with the clinical studies being conducted with the Seychelles Child Development Study, brains from autopsies of infants were examined (Lapham et al., 1995). Sixty-four brain specimens were received for analysis, with 32 judged to be satisfactory for analysis. The cause of death for these 32 infants was a variety of causes not directly involving the nervous system. Most of the deaths were within the first 24 h of life and all were within 24 days postpartum. Where available, six areas of the brain were sampled for histological examination. These areas included the frontal and occipital cortex, temporal cortex with hippocampus, basal ganglia with thalamus, cerebellum, and pons with medulla. Each brain was also analyzed for total mercury and inorganic mercury. Five specimens were removed from analysis due to suspicion of contamination with mercury. Twelve reference infant brains were obtained

from the Autopsy Service at the University of Rochester Medical Center for comparison.

Mercury values in the majority of the specimens from the Seychelles were well above levels observed in most of the reference brains (Lapham et al., 1995). Levels of total mercury were all <300 ppb. No abnormality in cerebral or cerebellar cortical organization was observed; however, the presence of reactive plump astrocytes with pink cytoplasm was observed in the white matter of 22 brains. These observations are indicative of a low-grade destructive process, but did not correlate with elevations in mercury levels. The only developmental abnormality found in the Seychelles infants' brains was rare, tiny islands of heterotopic neurons in the cerebellar white matter in five brains. However, small islands of this sort are an occasional finding in normal brains (Lapham et al., 1995); therefore, their significance in the Seychelles infants' brains is unclear. Overall, this study demonstrated no evidence of toxicity in the brains of infants with total mercury brain levels below 300 ppb.

In summary, the Seychelles study used sophisticated measures of childhood development including a battery of psychological tests appropriate to the child's age when administered. Of the covariates considered, gender, home, breast feeding, and birth weight were found to influence child development. In the main study, the age of the child at examination was an important covariate. Examination through the 29th month has been published, as discussed above. The statistical analyses of the 66th month study have been completed and the a new round of tests in children now 8 years of age is underway (Clarkson, 1997). According to Clarkson (1997), "Despite intensive and repeated testing on both cohorts, we have not found significant adverse developmental effects in these children."

Faroe Islands

Another population with the potential for high mercury exposures from fish ingestion is the Faroe Islands (Grandjean et al., 1992). These islands are located in the North Atlantic between Scotland and Iceland and rely heavily on marine mammals and fish as a source of protein. The results of a questionnaire given to adults in the Faroe Islands indicated a daily consumption of 72 g of fish, 12 g of whale muscle, and 7 g of blubber. Average mercury concentrations in cod fish, which is the most commonly consumed fish in the Faroes, are about 0.07 ppm. Whale muscle in the Faroes area contained an average mercury concentration of 3.3 ppm, about half of which was methylmercury.

Based on this information, a need was perceived to assess the magnitude of fetal mercury exposures in the Faroe Islands. To date, three reports have been published, a pilot study (Grandjean et al., 1992), a birth cohort study (Grandjean et al., 1995), and a study in 7-year-old children (Grandjean et al., 1997). These studies were initiated to determine maternal and fetal exposure levels to mercury, as well as determine any association between mercury exposure *in utero* and any adverse effects.

The pilot study was conducted in the small fishing village or Lorvik (Grandjean et al., 1992). All women between the ages of 20 and 50 years were identified. Sixty-three women were identified that had always resided in Lorvik, and blood samples were obtained from 84% ($n=53$) of these women. A brief questionnaire was given to each woman in the study, to determine demographic information and data on nutritional habits, smoking, and alcohol ingestion.

The women in the pilot study had blood values of mercury that ranged from 2.6 to 50.1 ppb, with only a small proportion of the mercury in the form of inorganic mercury. Lead concentrations were very low. Following the detection of increased mercury concentrations in the blood of the participants in the pilot study, the birth cohort study was initiated.

The birth cohort study was a prospective study of a total of 1386 children born in the Faroese hospitals during a 22-month period during 1986–1987 (Grandjean et al., 1992). Umbilical cord blood, in combination with questionnaire data on maternal habits, was obtained from 1023 of the births (75.1%). Cord blood was also analyzed for mercury, selenium, and lead. Hair samples were also collected from the mothers. In an interview with each mother, a nurse filled in a questionnaire concerning information on the pregnancy and the mother's nutritional habits.

The median umbilical blood mercury concentration in the birth cohort study was 121 nmol/l (approximately 25 ppb) with a 50% range of 65.0 to 201 nmol/l (Grandjean et al., 1992). Mercury concentrations in cord blood and in maternal hair correlated significantly. Consumption of fish was significantly correlated with increased levels of mercury in maternal hair and cord blood, with a more pronounced relationship observed between mercury concentration and the frequency of ingestion of pilot whale meat. Pilot whale intakes were the most important predictors for blood mercury levels, and the variation in intakes between mothers explained 19% of the variation in blood mercury (Grandjean et al., 1992). Based on the results of the questionnaire, approximately 75% of the mothers were abstainers from alcohol ingestion, while all other women reported that they drank alcoholic beverages occasionally. A total of 60% of the mothers were nonsmokers, with approximately 25% smoking fewer than 10 cigarettes/day, approximately 12% smoking more than that, and 3% smoking other types of tobacco.

A study was conducted of a subset of this cohort ($n=583$) that consisted of children visited by a nurse within the first year of life (Grandjean et al., 1994, 1995). The nurses that visited the children filled out a brief question-

naire on milestone development for the children during the first year of life. A hair sample was also collected from these children at about 1 year of age. The children included in the study were mainly characterized by their residence in districts served by nurses and the need of the mother to receive a nurse at the home during the first year of the child's life; therefore, these children may not reflect the average for the Faroe Islands (Grandjean et al., 1994). Of the total cohort (1022 singleton births), questionnaires on milestone development during the first year and maternal hair samples were obtained for 583 children (57.1% of the cohort). No children from Suderoy were included, and coverage in the Torshavn area was incomplete (151 of 413 children) compared to all other districts (432 children of 552). About 15% of the mothers had hair mercury concentrations above 50 nmol/g (approximately 10 ppm), which, according to the authors, is above the level at which fetal toxicity may occur. The geometric mean of maternal mercury hair levels was 17.2 nmol/g (approximately 3.4 ppm) for women in Torshavn and 24.4 nmol/g (approximately 5 ppm) for women from all other districts (other than Suderoy). Cord blood concentrations for this group of children ranged up to 870 nmol/l (174 μg/l). The age at which each child reached three developmental milestones (sits without support, creeps, and gets up into standing position without support), the duration of nursing without supplements, and the age at weaning were obtained (Grandjean et al., 1995).

Breast-feeding was the sole source of nutrition for the infants for a median of 4 months, with weaning occurring at a median age of 7 months (Grandjean et al., 1994, 1995). Concentrations of mercury in the hair of infants increased with increasing duration of breast-feeding ($P<0.0001$). The age at which a child reached the developmental milestones evaluated was not associated with prenatal mercury exposure as measured by concentrations in the umbilical cord blood or in maternal hair at the time of parturition. Rather, achievement of these early milestones was negatively correlated with hair mercury concentrations in the infants at 12 months of age ($P<0.02$), which in turn was dependent on the duration of nursing. This was an unexpected correlation, that is, an advantage associated with increased exposure to mercury, if it is assumed that methylmercury at these levels is a neurotoxicant (Grandjean et al., 1995). The authors concluded that these results indicate that the benefits of breast-feeding outweighed the potential for any methylmercury effect on these milestones of early childhood development.

In 1993, a study was initiated to examine the children of the birth cohort study at school age (approximately 7 years of age) (Grandjean et al., 1997). A total of 917 of the surviving Faroese children of the birth cohort study were examined, 443 in 1993 and 474 in 1994. Past medical history, social factors, and questions to examine the behavior of the children were obtained from the parent accompanying the child for the examination, usually the mother, by a questionnaire. A hair sample was also obtained from the child for analysis. Current information on potential confounders, such as maternal smoking and alcohol consumption, was obtained from a detailed questionnaire filled in by the mother. The battery of tests administered to each of the children included neurophysiological, as well as neuropsychological tests. The neurophysiological tests included tests that measured reversal visual evoked potentials, brain stem auditory-evoked potentials, postural sway, and autonomic nervous system function. A complex battery of neuropsychological tests were administered and are described in Table 8. Because the northeastern Atlantic Ocean is the greatest global environmental reservoir of polychlorinated biphenyls (PCBs) (Larsson, 1985) and because PCBs are known to exist in the blubber of pilot whales (overall average of approximately 30 ppm), as well as the muscle of a pilot whale (averages of approximately 0.6 ppm) (Weihe et al., 1996), stored samples of umbilical cords from the children examined in 1993 were reexamined for PCBs. The sum of the three major PCB congeners (138, 153, and 180) multiplied by 2.0 was used as a measure of total PCB concentration in cord blood.

For analysis of the test outcomes, cord blood concentrations of methylmercury were used as the primary indicator of *in utero* exposure. Additional analyses were also conducted with maternal hair mercury concentrations as the mercury exposure variable. Test scores were analyzed by a series of methods using multiple regression analyses for an association with mercury exposure. Age and sex were obligatory independent variables in all regression analyses. For the neuropsychological test results, maternal cognitive function, based on the caregiver's score on Raven's Progressive Matrices, and the child's acquaintance with computers and computer games was also considered obligatory independent variables. In addition to age, sex, and maternal Raven score, predictors other than methylmercury exposure were identified for each neuropsychological test outcome by backward elimination. Predictors that were important for more than three test outcomes were included in the regression analyses for these tests. An additional analysis of the data (low level analysis) was also conducted that included only those children whose mothers had a hair mercury concentration of <10 ppm. A separate regression analysis was also conducted for adjustment for PCB exposure on just the scores of the children examined in 1993, for which the PCB cord blood concentration was available for inclusion as an independent variable. An additional approach, the Peters-Belson approach, was also used to calculate adjusted outcome variables based on the data from the children with mercury cord blood concentrations below 15 μg/l (control group). Using backward elimination, regression coefficients with a P value below 0.1

Table 8. Neuropsychological tests applied in the Faroese cohort and the developmental function evaluated.

Neuropsychological test administered	Task performed	Function evaluated
Neurobehavioral Evaluation System (NES) Finger-Tapping Test	Child taps a key on the computer for 15 s, first with the preferred hand, then twice with the nonpreferred hand. Two keys are then tapped with both hands twice for the same interval. Score is the maximum number of taps in each condition.	Manual motor ability, focusing specifically on speed
NES Hand–Eye Coordination Test	Child has to follow a sine-wave curve on the computer screen using a joystick. Score is average deviation from the best two trials.	Manual motor coordination
Tactual Performance Test	Blindfolded child place in front of a formboard that contains six cut-out geometric shapes. The six forms are in front of board. Child has to place forms in appropriate place on board, first with preferred, than nonpreferred hand. Score is time to completion for each task.	Tactile processing
NES Continuous Performance Test	Child views a series of animal silhouettes on computer screen. Child required to press a button every time a cat appears over a 4-min interval. Scores are total number of missed responses and average reaction time during the last 3 min.	Vigilance/attention
Wechsler Intelligence Scale for Children-Revised (WISC-R) Digit Spans	Child presented with digit spans of increasing length until the child fails both trials in a series of the same length. Score is total number of correct trials forward only.	Attention and tracking
WISC-R Similarities	Child asked verbally to identify a common category linking two objects or ideas. Scored by WISC-R criteria.	Reasoning and cognitive flexibility
WISC-R Block Designs	Child given red and white blocks to replicate 2×2 and 3×3 designs presented on cards. Scored by WISC-R criteria in combination with a basic score for producing a correct design and bonus points for speed.	Visuospatial organization and reasoning
Bender Gestalt Test	Child asked to draw the nine Bender figures, first to copy and then from immediate recall. Score is errors in the copying condition and the number of recognizable figures summed for the recall score.	Used as a measure of nonspecific brain damage, also function of the right cerebral hemisphere
California Verbal Learning Test (Children)	Child given a list of 12 words over five learning trials, followed by an interference list, followed by spontaneous and cued recall of initial list (short recall). Later, spontaneous and cured recall of the list was requested (long or 20-min delayed recall). A recognition test also administered. Score was total correct in each learning and memory condition.	Several components of short-term memory
Boston Naming Test	Child presented with line drawings of objects and then asked to name them. If no correct response within 20 s, cues were provided. If still no correct response, a phonemic cue. Score was total correct without cues and total correct with semantic and phonemic cues.	Language
Nonverbal Analogus Profile of Mood States	Child was presented with cartoon faces depicting various moods states and a nonverbal response scale consisting of a line between the neutral face and the one depicting a mood state. Score was distance from the neutral face. Score was also a composite of two positive moods and six negative moods.	Mood

Source: Grandjean et al. (1997).

within this control group were used to adjust the outcome data for all children. These adjusted values were then analyzed by regression analysis, with cord blood mercury concentration as the only independent variable.

A significant association between mercury exposure and maternal Raven score ($P<0.001$) was observed and may be partially due to the average score from women residing in Torshavn being 0.7 points higher (Grandjean et al., 1997). Children whose mothers had occasionally ingested alcohol had lower cord blood mercury concentrations, which may have some association with residence in Torshavn, where pilot whale ingestion is decreased and alcohol is more easily available.

For the neurophysiological tests, girls had significantly shorter latencies of evoked potentials than boys, with age being of minimal importance. For the brain stem auditory evoked potential latencies, slight delays were seen for peak I at 40 Hz and significant delays at peaks III and V in those children with higher methylmercury exposure. However, interpeak latencies showed no association with mercury. For the body sway test results, after an adjustment for sex, height, and age, a slight negative association with mercury exposure was reported, suggesting less body sway at higher exposures. The authors concluded that the clinical examinations and the neurophysiological testing did not demonstrate any clear-cut abnormalities related to mercury exposure (Grandjean et al., 1997).

For the neuropsychological tests, a complex series of multiple regression analyses were conducted. The test outcomes were generally associated with age of the child and maternal Raven score, with the significance of other predictors, such as sex, varying. Eighty-five children failed or refused to take the mood test, which seemed to be the most difficult test for children to comprehend. The geometric average cord blood concentration for the children who failed or refused to take the mood test (29.5 ppb) was significantly higher ($P=0.003$) than the average for children who completed the test (22.3 ppb). In most of the models, maternal and paternal vocational or professional training and paternal employment appeared to be important predictors (the authors do not report how); therefore, these covariates were included in the final models for all neuropsychological test result evaluations. For five of the tests, an additional predictor remained significant after backward elimination; however, the authors reported that the addition of these variables to the model for each test changed the mercury effect only slightly. Models between boys and girls were reported to be similar; therefore, no interaction between sex and mercury exposure was identified.

The performance of the children on most of the tests administered on a computer was highly influenced by the child's acquaintance with computer games. Familiarity with computers was categorized as "none," "some," or "much" based on the child's response rather than that of the parent or any more objective measure. For the results of the Finger-Tapping and Hand–Eye Coordination Tests, the computer acquaintance by year interaction term was the only interaction term that remained after backward elimination. However, for the Continuous Performance Test (CPT) reaction time and missed responses, several additional parameters other than computer acquaintance remained in the model after backward elimination. For the CPT the effect of mercury was significant for the first year of test outcomes, but not the second. During the second year of examinations, the team conducting the computer-assisting testing was changed. Because of this change, the authors reported that the supervision specifically on the CPT was less stringent during the second year examinations; therefore, the authors decided to exclude the second year data for the CPT from the analysis. Therefore, for the analysis of the results from the CPT only, approximately one-half of the cohort was excluded.

In the full model, a decrease in performance was reported for the CPT (first year only), the Boston Naming Test, and the short-term reproduction portion of the California Verbal Learning Test with increasing cord blood mercury concentrations. Following the Peters-Belson adjustment, a decrease in the scores for the same tests was reported with increasing cord blood mercury concentrations, with a decrease in performance on the Neurobehavioral Evaluation System (NES2) Finger-Tapping test (preferred hand) and a decrease in long-term reproduction in the California Verbal Learning Test also reported. For the low-level exposure analysis, i.e., children whose mothers' maternal hair level was less than 10 ppm, a decrease in performance was observed for the same tests as with the full model, with a decrease in performance on the reproduction portion of the Bender Visual Motor Test also reported.

Because of feasibility and the psychometric properties of the tests administered, the authors placed emphasis on tests completed by the majority of children and with the widest variability in scores. Further analyses were then conducted on the tests that, according to the authors, most appropriately reflected the following 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 Name Test score after cues), and memory (long-delay recall on the California Verbal Learning Test). The results from these tests were then adjusted by the Peters-Belson adjustment for covariates and children with scores in the lowest quartile were identified. The lowest quartile of scores for each of these tests was then analyzed for any association between decreased performance and mercury exposure. A significant trend was reported for only the attention, language, and memory tests.

PCB concentrations in cord blood were only determined for the subcohort tested in 1993. The geometric mean PCB concentration observed in the 435 umbilical cords analyzed was 1.12 ng/g wet weight (interquartile range 0.57–

1.55 ng/g). In this subset of children, four test outcomes showed an association with the logarithmic transformation of the wet weight PCB concentration before adjustment for mercury (assume CPT average reaction time, Boston Naming Test no cues and with cues, and California Verbal Learning Test long-term reproduction). These four test outcomes in this subset of children were also associated with mercury exposure; therefore, regression analyses were repeated with mercury alone, and with both mercury exposure and PCB exposure included as independent variables. The results of these regression analyses are reported in Table 9.

A statistically significant association between mercury and PCB concentration in cord blood was reported. PCB concentration appeared to be an important predictor for the Boston Naming Test. Three of the test outcomes that were significantly associated with mercury exposure were no longer significant, once adjusted for PCB exposure (Boston Naming Test and California Verbal Learning Test). The only test outcome that retained an association with mercury exposure, following adjustment for PCB exposure, was the CPT average reaction time; however, this is the endpoint for which the second year data, that showed no association with mercury exposure, were not considered.

Cree Indians

Methylmercury contamination of fish in Ontario lakes was originally noted in 1967 (McKeown-Eyssen and Ruedy, 1983a). The Canadian government initiated monitoring of commercial fishing, and in 1969, conducted a survey of mercury levels in fish in Canadian waters (McKeown-Eyssen and Ruedy, 1983a). Measurements of methylmercury in blood and hair were obtained during the early 1970s from members of groups suspected of having been exposed to methylmercury because of subsistence fish eating. In particular, high levels of methylmercury were found among Canadian Indians of northwestern Ontario in 1970 and northern Quebec in 1971.

A few subjects were examined in Winnipeg, northern Ontario, and northern Quebec by physicians who were aware of their exposure (McKeown-Eyssen and Ruedy, 1983a). Mild, diverse neurological abnormalities were reported and it was concluded that six Indians from Quebec definitely had methylmercury poisoning, with an additional ten as probable and nine as possible. These findings prompted an investigation by the Quebec Ministry of Social Affairs, which in 1976, released a report recommending an in-depth study.

As a result, the Cree Indian study of three northwestern communities was formulated in 1977 (McKeown-Eyssen and Ruedy, 1983a,b). The Cree Indians of James Bay in northern Quebec constituted approximately 25% (7000) of Quebec's Indian population. Of the eight Cree Bands, each associated with a permanent community site, three bands, representing 41% of the total James Bay Cree populations were selected for study. These represented both inland (Mistassini and Waswanipi) and coastal (Great Whale) bands and were the groups with the highest reported methylmercury levels. These populations were similar in age and lifestyles — families spent most of the year in the bush, fishing and hunting. Methylmercury exposure occurred due to ingestion of fish or fish-eating animals with methylmercury contamination arising from contamination of lakes and rivers by natural leaching of mercury containing rock, polluted rain and/or industrial effluents.

Hair measurements were available for these communities beginning in 1975 (McKeown-Eyssen and Ruedy, 1983a,b). Peak levels measured approximately 30 ppm for the Waswanipi (early 1995) and approximately 20 ppm for the Mistassini (July to January 75/76) and Great Whale (January 1977). Decreases were noted in 1976–1977 in Mistassini and Waswanipi and in 1977 in Great Whale due to dietary advice.

Table 9. Regression coefficients (βs) for effects of logarithmic transformations of mercury before and after adjustment for PCB concentrations on neuropsychological test results during the first year.

Neuropsychological test	Before adjustment		After adjustment for PCB			
	β	P value	β	P values		
				Mercury	PCB	Both
Continuous Performance Test						
Average reaction time (ms)	39.3	<0.001	37.8	0.002	0.64	0.001
Boston Naming Test						
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)						
Long-term reproduction	−0.99	0.03	−0.78	0.11	0.26	0.05

Source: Grandjean et al. (1997).

The population studied included 175 men and 185 women from Mistassini (86% of the population over 30) and 47 men and 52 women from Great Whale (92.5% of the population over 30). The cohort was examined by one of five neurologists (blind examination) for nystagmus, coordination and gait, tremor, movements and reflexes, sensation, stereognosis, two-point discrimination, constriction of visual field, and audiometrics. Information on age, sex, consumption of alcohol, caffeine, and nicotine, and nutritional status was obtained from interviews. Samples of blood and hair were taken between January 1975 and December 1977 for the analyses of methylmercury and selenium (hair only). Not all subjects were included. A common 6-month sampling period was identified for each community during which the most sampling was taken.

Relatively high participation was achieved in Mistassini (86% of 420 eligible subjects) and Great Whale (92.5% of 107 eligible subjects). Only 67.7% of 195 adults in Waswanipi were able to participate. In all three communities, the nonparticipants were younger and had lower methylmercury exposure than the participating cohort, based on surveys from 1975 to 1977. None of those deemed medically unfit had mercury-related disease.

Results were reported as: (1) prevalence of individual neurological findings and (2) prevalence of "syndrome" of bilateral neurological abnormality usually involving more than one neurological function or tract and which may be considered to be compatible with previously recognized effects of methylmercury toxicity. The neurological abnormalities included: (1) bilateral symmetrical reduction of visual fields not explained by ophthalmological exam, and/or (2) neurological disease. Subjects were considered to display methylmercury syndrome only when neurological findings could not be explained by a definite alternative diagnosis.

In adults, of the six types of neurological abnormalities, no consistent trends in prevalence were seen in relation of increasing age (McKeown-Eyssen and Ruedy, 1983a). For one endpoint, incoordination, the prevalence, unadjusted for mercury exposure, increased in men in the 50–59 and 70+ age groups in both communities and in the 70+ age group in females in Great Whale only. For tremor, increased prevalence was noted in the 50–59 and 70+ age groups in males in Mistassini and in the 70+ age group in males in Great Whale. With coordination, only one individual in each community was scored "severe;" all others were either questionable or mild. Similarly, tremor was classified as severe in only one subject; of five subjects with either moderate or severe tremor, three were under treatment for Parkinsonism. All other endpoints were classified as either questionable or mild, with the exception of abnormality of eye movements, which was classified as severe in six subjects. These observations were conducted without knowledge of methylmercury exposure and the results reported are across all exposure categories.

Of those with reduced visual field on examination (scores $<55^{\circ}$) (20% of both populations), approximately half (35 subjects) were considered by ophthalmological technicians to have constricted visual fields. Only 16 of these were confirmed by an ophthalmologist, and all but three were explained by conditions unrelated to methylmercury exposure. The amount of exposure in these three people was not provided (McKeown-Eyssen and Ruedy, 1983a).

When the syndrome of neurological abnormalities was considered, again no consistent patterns across sex, age, or community were noted. The prevalence by age was higher in men age 50–69 in both communities (although this increase was only slightly higher in Mistassini men). With women, the prevalence was highest in the 50–69 age group for the Mistassini, but in the 70+ age group for Great Whale. When considered by neurological abnormality, incoordination and tremor were the predominant findings, all of which were classified as either questionable or mild. Again, no association with mercury exposure was noted.

The most prevalent findings in these communities were incoordination, tremor, and abnormal reflexes, all of which were physical signs among the Iraqi and Minimata populations. Incoordination was difficult to assess, since many subjects performed poorly on tests of rapid alternating movements but accomplished other tests of coordination without difficulty. Further, several manifestations of mercury toxicity, e.g., constricted visual field, were missing or in very low prevalence in the population (three possible of about 500 examined).

The relationship between neurological findings and methylmercury exposure could not be conducted by comparing the prevalence in these communities with the prevalence in an unexposed community because no comparable population of same age and life style could be identified. Therefore, a case control study was conducted within these communities (McKeown-Eyssen and Ruedy, 1983b). In the adult case study, cases were defined by the results of neurological examinations using *a priori* defined criteria.

After adjusting for confounding variables of age, sex, and alcohol consumption, a positive association between neurological abnormality and methylmercury exposure was significant for the Mistassini participants, but not the Great Whale cohort. In Mistassini, the 1978 hair level provided the best discrimination between cases and controls; however, for two of the five neurologists, the 1975–1976 hair index provided the best discrimination between cases and controls. There was considerable observer variability among the five neurologists. When using their individual findings, three of the five neurologists found no positive

association with mercury exposure after adjustment for age. The differences in ages of the cases and controls made it impossible to match on this variable, and, therefore, the discriminant analyses that was used to adjust for age was subject to modeling assumptions. Adjustments made for alcohol consumption were also uncertain, because of possible inaccuracies in reporting. Alcohol consumption was a more significant contributing variable than mercury exposure. According to the authors, while allowances were made for confounding variables, it remains possible that the effects are not entirely attributable to methylmercury (McKeown-Eyssen and Ruedy, 1983b). Further, the methylmercury levels used in the study did not reflect past exposures, when methylmercury levels were certainly higher. The 1975–1978 levels only reflect part of a lifetime of exposure due to contamination of lakes and rivers from natural leaching of mercury-bearing rock and from polluted rain, and from more recent industrial effluents. Lack of alternative sources of food or employment led to dietary dependence on fish before the mid-1950s. Dietary advice given in 1975–1976 reduced exposures further by 1978. Therefore, any threshold level based on the 1975–1978 time frame would be an underestimate of total exposure according to the authors. The final conclusion for the study conducted by McKeown-Eyssen and Ruedy (1983b) was discussed in Valciukas et al. (1986), which stated, "A recent study of Canadian Cree Indians with such a diet failed to relate methylmercury exposure and neurological findings when, as it was the case in our study, confounding variables were controlled."

Children exposed *in utero* in this St. James Cree population were also evaluated (McKeown-Eyssen et al., 1983). The children were between the ages of 12 and 30 months, with the ages selected to identify children who had been exposed before the time of dietary warnings in this population, after which maternal hair levels fell. The analyses were conducted by first determining which of the neurological responses may be associated with prenatal methylmercury exposure. Then, the children were divided into case and controls, and discriminant analysis was conducted first on the basis of confounding variables (child's age, duration of breast-feeding, mothers age, consumption of beverages containing alcohol or caffeine, smoking) and then on prenatal index of methylmercury exposure. Of all analyses, only abnormality of muscle tone or tendon reflexes in males only was positively associated with methylmercury exposure when adjustment was made for most confounding variables. Other neurological abnormalities were not prevalent and were not associated with exposure. The authors questioned the clinical significance of muscle tone or tendon reflex finding because of the following:

(1) The abnormalities were isolated and of mild severity and doubtful clinical significance; an increased incidence was only achieved when both specific and generalized decreases in tendon reflexes, with or without changes in muscle tone or reflexes, were combined with cases in which tendon or muscle reflexes were increased, with some of these responses noted to be questionable. (Isolated and generalized hyperreflexia was the most prevalent reflex abnormality; however, other reports of methylmercury toxicity indicate that hyperreflexia was prevalent.)

(2) The relationship appeared to be affected by the skewness of the exposure distribution; when expressed as a log normal distribution for maternal hair levels, the effect was no longer significant (P value=0.14).

(3) When arranged by exposure categories, no dose response was found (Table 10). McKeown-Eyssen et al. (1983) stated that according to the calculated odds ratio, a 7-fold increase in incidence would be associated with a 10 ppm increase in maternal hair concentration. However, the actual dose–response support this only if the 2.0–2.9 ppm category is compared with the 13.0–23.9 ppm category.

(4) Lastly, while the analyses conducted attempted to control for confounding variables, they were subject to inaccuracies (in particular with regard to alcohol consumption) and modeling assumptions.

No clear positive association with prenatal methylmercury exposure was found in 234 Cree Indian children ages 12 to 30 months for neurological, physical, mental, and psychosocial development at maternal hair levels ranging from approximately 0.0 to 24 ppm (McKeown-Eyssen et al., 1983). Approximately 15% of the children were born to mothers with maternal hair levels >13 ppm. The apparent NOAEL in this study was >24 ppm. However, tests for subtle neuropsychological effects, such as those conducted in the Seychelles, New Zealand, and Faroe studies were not conducted.

Another group of Cree Indians (autochthons — persons native to Canada was preferred by the study group) was evaluated (Spitzer et al., 1988). The study was conducted in

Table 10. Prevalence of abnormality of muscle tone or reflexes, by prenatal exposure index, for boys.

Prenatal exposure index (μg/g)	Number of boys	Percent abnormal
0.0–1.9	19	15.8
2.0–2.9	18	5.6
3.0–4.9	19	26.3
5.0–6.9	14	0.0
7.0–12.9	14	7.1
13.0–23.9	13	38.5
Total	97	15.1

Source: McKeown-Eyssen and Ruedy (1983b).

July and August, 1977, and consisted of four groups of individuals classified by self-reported symptoms and potential for mercury exposure either due to their location or fish consumption habits. (The Self-Designated Disease Group consisted of those individuals who felt that their health had been adversely affected by mercury exposure from a nearby chlor-alkali plant and were part of a law suit.) Hair mercury levels were assessed for a 20 cm section to reflect 20 months of exposure and a 9 cm section (from the scalp) to assess more recent exposure.

Associations with methylmercury concentrations in whole hair were found for four of the variables including any neurological or ophthalmological abnormalities, coexistent upper and lower extremity tremor, sensory nerve conduction velocity, and abnormalities recorded by data gathering technicians (who examined 179 items) (Table 11). However, in the opinion of the consulting ophthalmologists, all of the abnormalities were unrelated to mercury exposure. Of the subjects reporting neurological signs, only 9 subjects out of 224 had neurological abnormalities that could not be assigned to another definite diagnosis. Tremors of the upper and lower extremities were significantly associated with alcohol consumption. Impaired sensory nerve conduction velocity and increased tremor in extremities were positively associated with levels in the 9 cm length but not with levels in the 20 cm length, that is, there was a positive association at the lower methylmercury level but not at the higher methylmercury levels. When only the 9 cm section was considered, only 23/308 (7%) subjects had methylmercury concentrations in hair at levels >10 ppm, while 86/287 (30%) subjects had concentrations >10 ppm when the 20 cm length was considered. No adjustments in the statistical analyses were made for the distribution of the data, i.e., the data appear to be log normally distributed, or for multiple testing. The authors noted that alcoholism and diabetes in this population had a more marked independent effect on the target variables than exposure to methylmercury.

Spitzer et al. (1988) stated that the data suggested verifiable biological thresholds for mild clinical effects; however, the data were not provided to identify that threshold level. It is important to note that dietary advisories were given to other groups (Waswanipi and Mistassini) in the area in 1976, after which hair levels fell significantly. Therefore, levels in the 20 cm section may be more indicative of past exposure than the 9 cm section. In addition, because of the lack of a dose–response (positive association with lower methylmercury levels but

Table 11. Relationship of target variables with mercury levels in hair.

Target variables	Slope[a] (standard error)		
	Whole hair	20 cm hair	9 cm hair
1. Any neurological or ophthalmological abnormality (prevalence, %)	1.503** (0.582)	0.645* (0.361)	1.462** (0.599)
2. Coexistent upper and lower extremity tremor (prevalence, %)	0.790** (0.279)	0.170 (0.166)	0.940***$^{(>.*)}$ (0.286)
3. Abnormalities recorded by the field neurologist (mean number of findings per subject)[b]	0.0050 (0.0090)	0.0116† (0.0056)	0.0085 (0.0093)
4. Abnormalities recorded by the data gathering technician (mean number of findings per subject)[b]	0.137 **$^{(<.**)}$ (0.056)	0.109 **$^{(<.**)}$ (0.035)	0.114 **$^{(<.**)}$ (0.058)
5. Physical functional disability (mean disability score)	−0.0041 (0.0107)	0.0010 (0.0066)	−0.0030 (0.0111)
6. Stated sickness (prevalence, %)	−0.762 (0.676)	−0.320 (0.418)	−0.212 (0.697)
7. Bilateral neurosensory hearing loss (prevalence, %)	0.447 (0.646)	0.361 (0.401)	0.550 (0.664)
8. Definite EEG abnormalities (prevalence, %)	−0.233 (0.274)	−0.193 (0.170)	−0.286 (0.282)
9. Motor nerve conduction velocities (mean, m/s)	−0.009 (0.082)	0.013 (0.048)	−0.017 (0.089)
10. Sensory nerve conduction velocities (mean, m/s)	−0.216* (0.115)	−0.092† (0.066)	−0.234* (0.115)

Source: Spitzer et al. (1988).

[a]Change in target variable per ppm mercury in hair, adjusted for age, sex, alcoholism, diabetes, and group membership.

[b]Significance assessed by square root transformation of the target variable.

$^{\dagger}P<0.10$, $*P<0.05$, $**P<0.01$, $***P<0.001$ for the significance of the linear association. (Marks in parenthesis indicate the significance of departures from a linear relationship: > indicates a positive quadratic contribution, < a negative quadratic contribution.)

not higher levels), the mild, and in some cases, self-reported nature of the clinical findings, and the presence of significant confounding variables, the finding of this study are suggestive but not conclusive for a mercury-related effect.

However, after consideration of all the findings, Spitzer et al. (1988) stated that no clinical, electrophysiological, or toxicological data revealed neurological or ophthalmological syndrome thought to be related to methylmercury or to any other intoxication other than alcohol. In all patients, neurological or ophthalmological findings could be explained by recognized diseases processes or trauma unrelated to mercury exposure. The frequency and distribution of most general medical problems were consistent with that expected in people of comparable age, socioeconomic status, and living conditions. The exceptions were a high prevalence of diabetes, hearing loss, and visual problems (glaucoma) all unrelated to mercury exposure. No abnormalities associated with methylmercury poisoning (e.g., Minimata disease), such as visual field constriction, were found to be associated with exposure to mercury.

Canadian Aboriginals

All three of these studies (Wheatley and Paradis, 1995, 1996; Wheatley et al., 1997) discuss the 20-year retrospective analysis in Canadian Indian populations in Northern Canada. These studies focus on the communities of Grassy Narrows and Whitedog, but describe the sampling done in other communities.

The potential for significant exposure was first noted in the early 1970s in two northern Ontario communities, Grassy Narrows and Whitedog. Exposure was due to subsistence fishing and employment as fishing guides to sports fishermen. The source of the elevated levels of mercury in fish was attributed to the chlor-alkali plant upstream of these communities. Under the James Bay hydroelectric development project, damming of rivers resulted in significant flooding and immersion of vegetation, which released mercury into the aquatic ecosystem with subsequent methylation. In response to these findings, a program to assess hair and blood samples among potentially exposed individuals was initiated (Wheatley et al., 1979).

Up to December 1992, 71,842 blood or hair tests on 38,571 individuals have been conducted in 514 native communities across Canada. The majority of these samples were hair levels since they provide temporal information about exposure, while the blood sample may reflect episodic resent exposure. Using a hair to blood ratio of 300, based on research with Canadian First Nations (Phelps et al., 1980), hair levels were converted to blood levels. Approximately 77% of the individuals had blood mercury levels of <20 ppb (<6 ppm in hair), with approximately 21.4% of the population with levels of between 20 ppb and 99 ppb (6–29.7 ppm in hair). The remaining 1.6% (608 individuals) had blood mercury of 100 ppb or greater (>30 ppm in hair) including 0.2% (67 individuals) of the population with levels over 200 ppb (>60 ppm in hair). The highest number of individuals with blood levels >200 ppb were from Quebec followed by Ontario, the Northwest Territories and Manitoba. With the exception of the Inuit in the Northwest Territories, who also ate marine mammals, these are fresh water fish eating populations.

All individuals with blood mercury levels over 100 ppb were offered full neurological examinations; however, only 99 accepted and were clinically examined. The examinations included a full clinical neurological assessment including electromyography, audiometry, radiology, and electroencephalography. Heavy emphasis was placed on key methylmercury symptoms, such as paresthesia, and signs including tremor, gait, sensory impairment, and constriction of visual fields. Of those examined, no neurological abnormalities were found in 61 individuals, while 27 were found to have neurological abnormalities due to other causes. Although 11 had neurological findings possibly attributable to mercury, i.e., signs that have been found in persons with mercury poisoning, after controlling for confounders such as age and life style, no definitive diagnosis was made (Wheatley et al., 1979). Other variables, such as age, diabetes, alcohol, were more significant contributors to the outcome than mercury exposure. In none of the papers summarizing these data were the exposure levels of these 11 individuals given (Wheatley and Paradis, 1995, 1996; Wheatley et al., 1979, 1997).

The individuals examined clinically were drawn from among all of the communities evaluated. Although the location of individuals was not specified, given the distribution of individuals with blood levels over 100 ppb, the majority of these individuals lived in Quebec and Ontario as part of either the Cree bands or the communities of Grassy Narrows and Whitedog, respectively. As such, exposure to these individuals has been ongoing since at least the early 1960s for the Ontario communities (i.e., the time the chlor-alkali plant was built) and probably for considerably longer due to natural contamination. Many of these individuals may have been exposed *in utero* and have now been tested as adults without demonstration of late developing effects or effects increasing with age related to mercury exposure.

As part of this assessment and in recognition that fetal exposure may be the more sensitive endpoint, 2405 cord blood samples were taken, with about half of these paired to maternal levels (Wheatley et al., 1997). Of these, 523 children had cord blood samples >20 ppb (21.8%), which was designated as the "acceptable range." Neurological

examinations conducted with these children showed no findings that could be attributed to methylmercury. An in depth neuropsychological study is underway for these children, many of whom are now school age. This evaluation will include tests for memory, attention, executive functions, perceptual functions, and sensory/motor development. In addition, school records and teacher's questionnaires regarding attendance, marks, and social behavior will be compiled. Results are expected in 1998.

Peru

Certain areas of northern Peru eat fish from the Pacific as their main source of dietary protein. Some ocean fish contain as much as 2 ppm mercury, predominantly in the form of methylmercury (Turner et al., 1980). Twenty-three blood samples were obtained from several villages in the coastal region of Peru to analyze for elevated mercury levels. Analysis of these samples indicated a mean methylmercury concentration of 30.5 ng/ml, with values ranging from 6.5 to 120.4 ng/ml. The highest blood mercury concentration was observed in a fisherman from the village of Mancora, indicating a potential area of interest to evaluate for mercury exposure from fish ingestion.

Based on the results of the blood analysis, surveys of the populations and their dietary habits of two small fishing villages on the north coast of Peru, Mancora and Cancas, were conducted. These populations are fairly stable, with most of the inhabitants having been born there or resident for many years. A control population that ate relatively little fish was located approximately 50 km from Mancora, the agricultural village of Morropon. The organic mercury concentrations in the hair of the Morropon inhabitants ranged from 0.1 to 3.5 ppm and was much lower than the levels found in the hair of Mancora and Cancas, confirming Morropon as a good selection for a control population.

A team of nurses and dietitians visited every home in each village and obtained the names, ages, and sex of every member of a household, as well as information on dietary habits. Villagers were invited by the Major of each village to participate in the study. Since all participants were volunteers, all participants could not be matched for age and sex and the study population may not be representative of their respective villages.

Dietary information was obtained from the housewife of each family, especially the intake of fish, with particular attention to swordfish. The participants in the study were given a physical examination and were then examined by a neurologist. A complete neurological examination was conducted, with special attention given to sensation around the mouth and in the limbs. Sensory functions were evaluated with pin prick, vibration, joint-position sensation, two-point discrimination, stereognosis, and touch sensation using von Frey hairs. Coordination was also evaluated by inspection of gait and tandem gait, finger–nose and finger–finger approximation, heel–shin tests, and tests of diadochokinesis (arresting one motor impulse and substituting for it one that is diametrically opposite). Handwriting was evaluated, including the ability to place small pencil dots inside a 2-cm circle and to trace a pencil along a wavy line. Hearing was tested, and speech was observed for dysarthria or dysphasia. Jaeger types were used to test visual acuity, and visual fields were evaluated with a Krimsky eye-cup perimeter. Following the examinations, a venous blood sample was taken from all subjects, with the exception of very small children, and a sample of hair was taken. A separate assessment, *via* a questionnaire administered by the dietitians who were unaware of the interest in paresthesias, was conducted to further evaluate the existence of paresthesias in the extremities separate from complaints by the residents. Samples of all available fish types were also obtained for analysis for the presence of methylmercury.

The average weekly intake of fish per family was 10.1 kg, with the average family size being 6.2 persons. Based on the response of 34 housewives, who purchased food for their families, about the daily fish consumption of their families, daily consumption varied from 0.5 to 3.0 kg. Based on this information, the authors estimated a per capita intake of fish to range from 110 to 660 g/day (Turner et al., 1980). In comparison, the average weekly intake for the control population was 1.9 kg per family, with an average of 6.4 persons per family.

Samples of nine species of fish were obtained from the fish markets in the fishing villages for analysis of methylmercury. Concentrations ranged from 0.04 to 2.29 ppm, with the highest concentrations in a species of shark.

The mercury blood concentrations from the fishing villages ranged from 11 to 275 ng/ml, with a mean concentration of 82 ng/ml. In the control population, the blood concentrations ranged from 3.3 to 25.1 ng/ml, with a mean of 9.9 ng/ml.

Based on the results of the questionnaire, the most common complaints were, in descending order of frequency, distal limb paresthesias, impairment of vision, backache, dizziness, headache, impaired hearing, joint pains, abdominal pain or discomfort, leg cramps, nerve root pain, and weak limbs. Distal-limb paresthesia was also the most common complaint in the control population, with the other complaints differing in frequency. Although paresthesias were observed in the fishing villages, a higher percentage of the control population complained of paresthesias. On direct testing by a physician, none of the paresthesias was accompanied by impaired peripheral sensation. Only one of the 56 persons complaining of

paresthesias showed slight impairment of peripheral touch sensation. This was a 70-year-old man with a blood methylmercury concentration of 95 ng/ml.

Only two other complaints considered to be associated with methylmercury poisoning were reported more frequently in the fishing villages than in the control population: impaired vision (7.9% vs. 2.2%) and impaired hearing (3.7% vs. 1.1%). Of the 15 persons who complained of impaired vision, upon examination by a doctor, it was determined that six had refractive errors, four were illiterate, three had cataracts, and two had no detectable abnormality. Concentrations of methylmercury in the blood of these subjects ranged from 40 to 151 ng/ml. Four of these subjects also complained of paresthesias, two of deafness, and one of dizziness. With the exception of one 82-year old subject who was deaf, no other abnormal neurological signs were detected in the fishing villages. Of the seven subjects who complained of impaired hearing, five showed no detectable hearing loss on audiometry, while the other two had unilateral hearing loss.

The authors' overall conclusions from examining all three populations, both the fishing villages and the agricultural village, were that all three villages consisted of healthy, hardworking individuals without evidence of malnutrition or serious disease. No apparent ill effects from ingestion of considerable quantities of methylmercury from ocean fish were observed (Turner et al., 1980).

A preliminary survey was also conducted of the methylmercury concentrations in women of childbearing age in the region (Marsh et al., 1995b). The authors were interested in identifying a population of women of childbearing age consuming enough marine fish to result in hair mercury concentrations of 20 ppm or higher to investigate whether exposure to methylmercury in marine fish causes the same fetal effects as methylmercury in the seed grain fungicide used in Iraq. During this survey process, it was discovered that almost all babies born in Mancora, an area in which a previous study had been conducted (Turner et al., 1980), had an efficient maternity clinic. The results of the analysis of hair samples from 110 pregnant women indicated hair concentrations ranging from <1 to 38 ppm. Therefore, this area was adopted as a site for further study.

A prospective study was planned to evaluate the relationship between any clinically detectable effects in offspring to maternal methylmercury levels during pregnancy. A maximum of 12 hair samples 1 cm in length or three hair samples 4 cm in length were obtained from 369 pregnant women in Mancora. One hundred ninety-four of their children were examined. Peak and mean maternal hair mercury concentrations were determined. The mothers' mean methylmercury concentration was estimated based on the arithmetic mean for all of the methylmercury concentrations in hair samples collected over the pregnancy. The mothers were asked questions about their labor and delivery and weight of the baby. This information was then compared with medical records. The head circumference, height, weight, and general physical condition of each infant was assessed, with a neurological examination also conducted. During subsequent visits to the clinic, the mothers were also questioned about the achievement of developmental milestones (i.e., age at which the infants progressed to sitting, standing, walking and talking), with the infants reexamined.

The geometric mean of the mean maternal hair mercury levels and the geometric mean of the peak methylmercury levels were similar, because these women were in relatively steady state for methylmercury due to long-term fish consumption (Marsh et al., 1995b). The geometric mean of the mean hair methylmercury levels was 7.05 ppm (range 0.9 to 28.5 ppm), while the geometric mean of the peak hair methylmercury levels was 8.34 ppm (range 1.2 to 30.0 ppm). The authors reported a high correlation between maternal hair levels at the time of birth and mercury levels in newborn hair, indicating the appropriateness of using maternal hair levels as an indicator of *in utero* exposure.

Linear regression analyses indicated no significant correlation between maternal hair levels of methylmercury and birth weight, head circumference, height, measures of infant development (age of sitting, standing, walking, or talking), or neurological signs. The frequency of maternal paresthesia, usually the earliest symptom of adult methylmercury toxicity, or perinatal problems did not increase with increasing maternal hair levels of methylmercury (Marsh et al., 1995b).

Based on these results, the authors concluded that there was no increase in the frequency of neurodevelopmental abnormalities in early childhood of children exposed to methylmercury pre- and postnatally from marine fish in the diet (Marsh et al., 1995b). It is possible that the presence of selenium in fish provides a protective effect from methylmercury, explaining the absence of any observed effects in the Mancora study (Marsh et al., 1995b).

Amazon

The Tapajos River region of the Amazon is currently the most important site of gold mining activities in the Amazon Basin (Lebel et al., 1996). In this area, gold is extracted from the soil or river sediment through amalgamation with mercury. During this process, it has been estimated that over 130 tons/year of mercury is lost to the atmosphere or enters the aquatic environment. Because of the potential exposure to high levels of methylmercury in this area, a study was undertaken to evaluate the origin and fate of mercury contamination. Methylmercury's potential effects on human

health were also studied by: (1) evaluating human exposure to methylmercury to populations in this area, with hair mercury levels as a biomarker; (2) pretesting a neurophysiological test battery in a population in this area; and (3) carrying out preliminary analyses to determine whether any neurophysiological functions are sensitive to changes in mercury hair levels.

An attempt was made to recruit subjects from two villages, Brasilia Legal and Ponta das Pedras, which are downstream from suspected pollution sources. These areas also depend heavily on fish as a main component of their diet. The goal for the study size was to recruit 20 subjects per village (10 male and 10 female) to participate in the study. Subjects were recruited based on a list of adult inhabitants established from a previous study. The first subjects tested were 19 adults in the village of Ponta das Pedras. It was evident following the testing of this group, that the subjects over 35 years of age had more difficulty understanding the tests, compared to younger subjects, and that the older subjects displayed uncorrected near and/or far visual acuity; therefore, in the subjects tested in Brasilia Legal, recruitment was limited to persons 35 years of age or younger. The data presented in the report are the results from 29 persons between the ages of 15 and 35 tested in the two villages.

Two of the subjects had previously worked in the gold mines, one for 8 years and one for 3 months. Four of the subjects reported having suffered from malaria. Fourteen of the subjects were smokers (cigarettes/day ranged from 1 to 20), while 21 of the subjects drank alcohol occasionally or frequently.

Hair samples were taken from all subjects to analyze for total mercury and inorganic mercury levels using cold vapor atomic fluorescence spectrophotometry. Methylmercury was calculated as the difference between total mercury and inorganic mercury.

Tests were administered to the subjects to evaluate visual and motor function. Tests of visual acuity, chromatic discrimination and near visual fields were administered outdoors, due to the absence of electricity. Tests of peripheral visual fields were administered in a darkened room. Subjects were tested for far (6 m) and near (40 cm) visual acuity with optical charts for illiterate individuals. Visual acuity was considered good for resolution at 7.5 m for far acuity and 40 cm for near acuity. Chromatic discrimination was assessed using a color panel upon which participants were required to place 15 caps in order of chromatic similarity. A perfect score for this test was a score of 1, with increasing scores indicating an increase in the deviation of cap order. Near contrast sensitivity was evaluated using three charts that contained 45 circles each, distributed in five rows of increasing sinusoidal grating frequency. The subject was required to indicate whether the gratings were upright, to the left, or to the right, with their hand. Contrast threshold was determined for each eye with three different cards.

Peripheral visual field was measured with a homemade perimeter. This perimeter had two point coordinates, parallel and meridian, that could be cartographically reported as an isopter diagram for each target. A probe light was displaced slowly along a meridian axis from the periphery to the center until the subject could indicate when he/she first detected the light. The procedure was repeated for 12 meridian axis at 30° intervals from 0° (the value of the horizontal plane on the right-hand side of the subject) to 330°. The test was duplicated with two different target equivalents, Target I (used for the detection of threshold values) and Target V (used for the rough estimation of the visual field).

Motor functions tested included maximum grip strength for both hands and manual dexterity. Maximum grip strength was tested using a dynamometer over two trials. Subjects were asked to maintain maximum grip strength for as long as possible, with fatigue estimated as the time, in seconds, for the grip to fall to one-half of the maximum value. To test manual dexterity, subjects were asked to manually turn pegs using the Santa Ana Test. The number of pegs successfully turned 180° in 30 s for two trials was summed for the score for the dominant and nondominant hands.

Statistical analyses were conducted to compare results from the two villages (Mann–Whitney and *t* test). Simple and multiple regression analyses were conducted to evaluate any correlations of hair mercury, age, location, alcohol consumption, and smoking habits with the results of the color vision, grip strength, fatigue, and manual dexterity tests. No statistical analyses were conducted for contrast sensitivity or visual field profiles. The authors only reported the results of visual inspection of these test outcomes with respect to hair mercury levels, location, age, and alcohol consumption.

Hair mercury levels reported for the subjects ranged from 5.6 to 38.4 ppm, with a geometric mean of 14 ppm. The geometric mean hair mercury levels in Brasilia Legal (15.1 ppm) were higher than those in Ponta das Pedras (11.6 ppm); however, they were not statistically significantly different (Mann–Whitney; $P=0.1$). Men (geometric mean 15.9 ppm) also had mercury hair levels higher than women (geometric mean 12.3 ppm), but the difference was not statistically significant (Mann–Whitney; $P=0.23$).

Statistical analyses indicated significant differences between men and women subjects for grip strength ($P<0.05$) and muscular fatigue in the nondominant hand ($P<0.01$). Simple regression analyses indicated that none of the outcomes were related to age. The authors reported that multiple regression analyses of the test outcomes of the color confusion index with respect to hair mercury levels

increased with the log of hair mercury levels; however, the increase, if significant, was only marginal ($P=0.05$). When the village was included in the model, the model became more significant ($P<0.05$), with both elements contributing significantly (village — $P<0.01$; hair — $P<0.01$). Age, alcohol consumption, history of malaria, previous work in the gold mines, or smoking did not influence the model outcome.

No statistically significant relationship was reported for hair mercury level and near visual contrast sensitivity spatial frequencies. The authors reported that visual inspection of the profiles of contrast sensitivity for the individuals with the highest levels of hair mercury (>24 ppm), indicated that these outcomes were reduced. However, the contrast sensitivity scores did not decrease with increasing hair mercury levels.

For the group of subjects as a whole, no association between motor function scores and hair mercury levels were reported; however, when men and women were analyzed separately, women exhibited decreasing scores on the Santa Ana test with increasing total mercury hair levels for the nondominant hands ($P<0.01$) Muscular fatigue did not vary with hair mercury levels. For men, no association between hair mercury levels and manual dexterity, grip strength, or fatigue was reported.

For evaluation of the peripheral visual fields, the authors visually inspected graphs of the results. The subjects were arbitrarily divided into three exposure groups, $\leq$10, >10 to $\leq$20, and >20 ppm mercury levels in hair. The results for each exposure group for each eye and for both Target V and Target I were graphed for visual examination (Figure 1). The two individuals who had worked in the gold mines and those who had malaria were not excluded from the test. The authors reported that based on visual examination of the peripheral visual fields indicated a tendency toward visual field reduction with increasing hair mercury. However, upon statistical evaluation by ICF of each meridian axis point, only one point for Target V and four points for Target I were significantly different between exposure groups (Table 12). Only one point for the left eye was significantly different with increasing mercury hair levels. This was the meridian zero point, which was to the far right side of the individual. The size of the individuals nose could have had an impact on their ability to detect the pin light. Also, with such a small sample size, biological variation could have a significant impact on the results observed. No other points were significantly different for the left eye of the individuals tested. The points that were significantly different for the right eye (Target V — 120°; Target I — 90°, 120°, and 150°) were also in areas where things other than methylmercury exposure may have had an impact on the results.

The authors concluded that overall the results indicated that, with the exception of color vision, test scores fell within the ranges observed in other countries for contrast sensitivity and manual dexterity (Lebel et al., 1996). However, it is questionable as to whether the color vision effects observed are related to methylmercury exposure. The results of studies conducted in nonhuman primates (Rice, 1996) indicate that methylmercury exposure affected low luminance vision (impact on the rods of the eyes) to a greater extent than high luminance vision (impact on the cones of the eyes). Cones form the basis of color perception and are used for day vision, while rods are very sensitive to low levels of illumination and operate well during the night (Rhoades and Pflanzer, 1989). An alteration in color vision would be expected to result from an impact on cones of the eyes, not the rods.

The impact of co-exposure to metallic mercury vapor must be considered in interpreting the color vision results. In the gold mining process, the gold is extracted through amalgamation with mercury. The amalgamate is heated, giving off metallic mercury vapor. It is estimated that the gold mining activity generates the release of over 130 tons/year of mercury into the Amazonian environment, with only 40–45% entering the aquatic environment. The remainder is lost to the atmosphere (Lebel et al., 1996). In workers exposed to metallic mercury vapor *via* inhalation, a grayish-brown or yellow haze on the outer surface of their lenses has been observed (ATSDR, 1997), which could impair color recognition. These effects have not been reported following oral exposure to methylmercury. Therefore, it is questionable as to whether the color vision effects observed in the Amazon cohort are due to ingestion of fish containing methylmercury. While the authors state that there was no exposure to mercury vapor, no sampling data were presented, and given the effects of mercury vapor on the eyes, the potential co-exposure with mercury vapor by the inhalation route cannot be excluded.

The authors suggest that the results of the peripheral visual field tests indicate a tendency toward visual field reduction. However, no statistical analyses of these results were conducted and the conclusion was based only on visual inspection of the data. It is highly unlikely that what is being observed is due to ingestion of methylmercury. Similar tests have been conducted in larger fish-eating populations who had higher methylmercury exposures, with no impact on visual field observed. In the Peruvian population, a total of 190 subjects were examined, with ages ranging from 1.4 to 82, with a mean of 25.4. Hair levels were as high as 52 ppm, with no effects on visual field observed that could be attributed to ingestion of methylmercury (Turner et al., 1980). In the Iraqi population, visual changes were not observed until blood levels reached 7500 ppb (approximately 125 ppm in hair) (Bakir et al., 1973). Therefore, the changes in

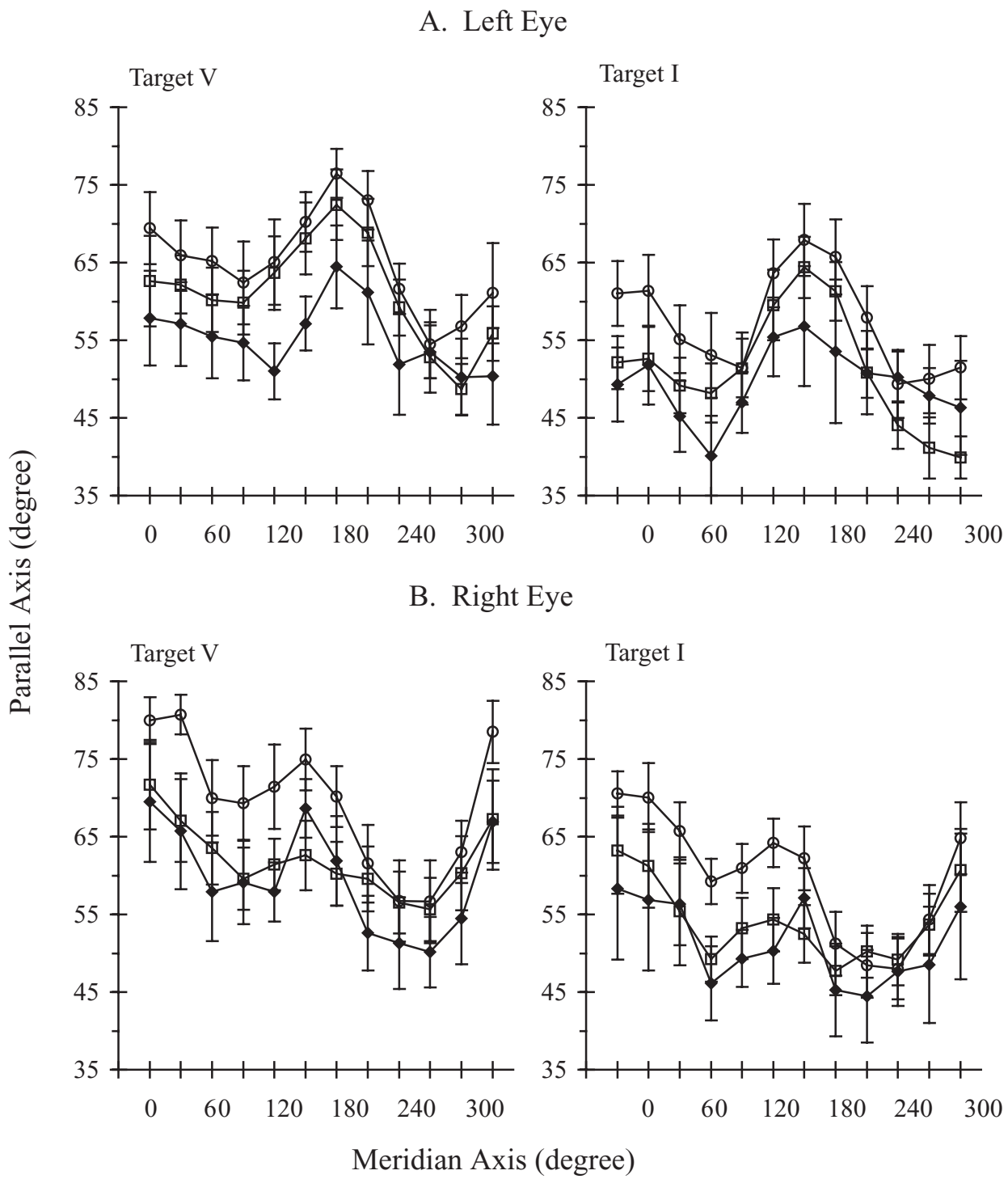

Figure 1. Profiles of visual field for three groups of exposure. -○- ≤10 μg/g ($n=7$); -□- >10≤20 μg/g ($n=15$); -◆- >20 μg/g ($n=6$). 0° of the meridian correspond to the horizontal plane on the right hand side of the subject. The parallel axis value corresponds to the distance from the center of the coupola. Results are plotted for left eye (A) and right eye (B) with Goldman target equivalents I (surface = 0.25 mm^2) and V (surface = 64 mm^2).

visual field reported by the authors is likely due to biological variation or other uncontrolled for confounding variables.

The authors concluded that the results suggest that it is possible to detect early changes in nervous system functions among persons exposed to methylmercury (Lebel et al.,

Table 12. Results of statistical analysis of the peripheral visual fluid outcomes.

Visual field — meridian axis point	*P* value
Left eye V — 0°	0.13
Left eye V — 30°	0.12
Left eye V — 60°	0.10
Left eye V — 90°	0.16
Left eye V — 120°	0.06
Left eye V — 150°	0.06
Left eye V — 180°	0.07
Left eye V— 210°	0.08
Left eye V — 240°	0.09
Left eye V— 270°	0.44
Left eye V — 300°	0.15
Left eye V— 330°	0.09
Left eye I — 0°	0.04
Left eye I — 30°	0.11
Left eye I — 60°	0.07
Left eye I — 90	0.05
Left eye I — 120°	0.29
Left eye I — 150°	0.16
Left eye I — 180°	0.09
Left eye I — 210°	0.08
Left eye I — 240°	0.20
Left eye I — 270°	NC
Left eye I — 300°	0.35
Left eye I — 330°	0.19
Right eye V — 0°	0.15
Right eye V — 30°	0.06
Right eye V — 60°	0.08
Right eye V — 90°	0.09
Right eye V — 120°	0.02
Right eye V — 150°	0.19
Right eye V — 180°	0.13
Right eye V — 210°	0.13
Right eye V — 240°	0.25
Right eye V — 270°	0.21
Right eye V — 300°	0.16
Right eye V — 330°	0.13
Right eye I — 0°	0.12
Right eye I — 30°	0.10
Right eye I — 60°	0.20
Right eye I — 90°	0.01
Right eye I — 120°	0.04
Right eye I — 150°	0.03
Right eye I — 180°	0.21
Right eye I — 210°	0.18
Right eye I — 240°	0.30
Right eye I — 270°	0.48
Right eye I — 300°	0.24
Right eye I — 330°	0.20

Source: Lebel et al. (1996).
NC = not calculated.

1996). However, the potential confounding of exposure to metallic mercury vapor and the small number of subjects evaluated make it impossible to draw conclusions from this study regarding effects following ingestion of fish containing methylmercury.

Review of neurological, developmental, and reproductive effects in experimental animals

Our evaluation of the epidemiological studies focused on human populations that were orally exposed to methylmercury by consuming contaminated fish. Based upon this evaluation, the most relevant endpoints for human health evaluation appear to be neurological, development, and reproductive endpoints. Using these findings, our review of the animal studies focused on investigations where animals were orally exposed to methylmercury. The evaluation further focused on studied that evaluated neurological, developmental or reproductive endpoints. A brief summary of the animal studies is provided in Appendix A. Tables A-1, A-2, and A-3 summarize the neurological, developmental and reproductive studies, respectively, that have been conducted with methylmercury in various animal species.

A review of the animal studies supported the conclusion, based on our evaluation of the epidemiological studies, that neurodevelopmental endpoints are the most sensitive endpoints of methylmercury toxicity. The lowest lowest-observed-adverse-effect level (LOAEL) identified for any neurological, developmental or reproductive endpoint was a developmental LOAEL observed in the offspring of rats administered methylmercury chloride at concentrations of 0.01 mg/kg body weight on days 6–9 of gestation (Bornhausen et al., 1980). A statistically significant reduction in the behavioral performance in the 4-month offspring of dams administered 0.01 mg/kg body weight was observed, based on the results of an operant conditioning program. No effects were observed in the offspring of dams administered 0.005 mg/kg body weight on days 6–9 of gestation (NOAEL). The LOAELs for other types of toxicity, following chronic exposure to methylmercury, were greater than 0.01 mg/kg/day (Burbacher et al., 1988; Chang et al., 1974; Khera, 1973; Mitsumori et al., 1990; Munro et al., 1980; Sato and Ikuta, 1975). Following chronic exposure to methylmercury, the lowest LOAEL was 0.015 mg/kg methylmercury (lowest dose tested), based on the incidence of degeneration of cerebellum and cerebral cortex and necrosis of the dorsal root ganglia in cats administered methylmercury in the diet for 11 months (Chang et al., 1974). For reproductive effects, the lowest LOAEL was 0.05 mg/kg methylmercury, based on decreased sperm speed, decreased sperm forward progression and increased sperm tail defects, in male monkeys that received daily doses of 0.05 mg methylmercury/kg (lowest dose tested), for 20 weeks (Mohamed et al., 1987).

REVIEW OF THE CURRENT RFD FOR METHYLMERCURY[4]

Derivation of the USEPA RfD

The derivation of a reference dose (RfD) for methylmercury has been evolving since the early 1980s as new data have emerged to describe those populations exposed to methylmercury. Initially, the USEPA derived an RfD based on effects noted in adults as a result of the acute poisoning episode that occurred in Iraq during a 1- to 3-month period in 1971 (USEPA, 1980). Based on the data summarized by Clarkson et al. (1976) and Nordberg and Strangert (1976), the USEPA identified multiple central nervous system effects in adults, including ataxia and paresthesia, as the critical effects.

Following the initial publication of reports of neurotoxicity in the Iraqi adult population (Bakir et al., 1973), a series of studies was published that indicated that the developing fetus was more sensitive to methylmercury than the adult (Amin-Zaki et al., 1976, 1981). The possibility that the fetus was the sensitive receptor was first observed with the Minimata outbreak in which high exposures to mothers during pregnancy resulted in offspring with signs of neurotoxicity (Harada, 1978). The sensitivity of the fetus was confirmed in animal experiments (Gunderson et al., 1986; Rice and Gilbert, 1990; Spyker et al., 1972).

The results of several years of investigation of mother–infant pairs were summarized by Marsh et al. (1987) and Seafood Safety (1991) based on the preceding series of studies (Amin-Zaki et al., 1974, 1976, 1979, 1981; Marsh et al., 1980, 1981). The USEPA based the current RfD of 1×10^{-4} mg/kg/day on the incidence of a delayed onset of walking or talking, neurological scores >3, mental symptoms, and seizures in 81 infants whose mothers who had ingested methylmercury contaminated bread at some point either during or just before pregnancy (IRIS, 1994; USEPA, 1997). The RfD was derived as discussed in the following paragraphs.

USEPA Benchmark Estimates of Maternal Methylmercury Hair Concentrations

The peak maternal mercury concentration in hair during the period assumed to correspond to time of pregnancy was used as the surrogate dose metric for doses to the fetus. The individual maternal mercury hair levels and paired infant responses were given in Marsh et al. (1987), and summarization of these individual data into arbitrary "dose groups" was given in Seafood Safety (1991) (Table 3).

[4] At the time of this analysis, the USEPA RfD for methylmercury was based on data from a poisoning episode in Iraq; however, the RfD for methylmercury is currently under review, and it is expected that the new RfD will be based on data from a fish-eating population.

Table 13. Methylmercury benchmark dose estimates (ppm hair) maximum likelihood estimates (BMD) and 95% lower confidence limits (BMDL) from Weibull model for the incidence of effects in children.

Effect	0.10	
	BMD	BMDL
Late walking	34.3	22.4
Late talking	43.8	25.3
Mental symptoms	125.4	67.5
Seizures	124.2	70.5
Neuro score >3	59.1	34.9
Neuro score >4	84.9	48.7
All endpoints	17.1	11.1

Source: USEPA (1997).

A dose–response analysis using the benchmark method (Barnes et al., 1995; Crump, 1984) was applied to each endpoint separately, and to the combination of endpoints using the data in Marsh et al. (1987) and in Seafood Safety (1991). A BMD is a dose (or exposure) that corresponds to a specified level of additional response called the benchmark response (BMR). A BMD is calculated by fitting a mathematical dose–response model to dose–response data. The BMDL, which is a lower statistical confidence bound on the BMD, replaces the NOAEL in the calculation of an RfC or RfD. Each dose group was defined as the geometric mean of the range of peak maternal mercury hair concentrations during pregnancy. The Benchmark models applied by the USEPA (1997) were the Weibull and polynomial models, and the 95% BMDL at the 1%, 5%, and 10% BMRs were calculated. The USEPA (1997) selected the BMDLs based on the Weibull model at the 10% BMR to characterize the dose–response. The use of the Weibull model and a 10% BMR was based on a comparative analysis of the NOAELs identified in approximately 1825 experimental data sets with the BMDL derived from the quantal (i.e., data separated into discrete dose groups) developmental toxicity data for those data sets (Allen et al., 1994a; Allen et al., 1994b; Faustman et al., 1994).[5]

The resulting BMDLs, expressed as maternal mercury hair concentrations, are summarized in Table 13 and, for the individual endpoints, ranged from 22.4 ppm for a delayed onset of walking to 70.5 ppm, based on the reported

[5] The data sets evaluated in these studies were from controlled animal experiments, and were analyzed using additional rather than extra risk. In general, when the background incidence is low or zero, the BMDL estimates based on extra risk and additional risk are almost identical. At a 10% BMR, the BMDLs more closely corresponded to the determined NOAELs for each data set than BMDLs based on either the 1% or 5% BMR levels. While roughly approximating the NOAEL, BMDLs at a 10% BMR were approximately three times less than the corresponding NOAEL and were lower than the NOAEL 95% of the time.

incidence of seizures. Based on the data provided in Marsh et al. (1987), the BMDL for the combination of all neurological endpoints (late walking, late talking, mental symptoms, seizures, or a neurological score >3) was approximately 11 ppm. The USEPA selected 11 ppm as the representative BMDL for these data. The BMDL from the combination of endpoints was selected, "... since the Agency felt that any childhood abnormality is considered an adverse effect and likely to have serious sequelae lasting throughout lifetime" (IRIS, 1994).

Conversion of the Maternal Methylmercury Concentration in Hair to a Daily Dietary Intake

The BMDL of 11 ppm of mercury in maternal hair was converted to an estimated maternal methylmercury intake (in μg/kg/day) using a one-compartment model as described in the following equation:

$$d = (C * b * V)/(A * f * \mathrm{BW}), \quad (1)$$

where d=daily dietary intake (μg methylmercury/kg/day); C=concentration of methylmercury in blood (μg/l); b=elimination constant (days^{-1}); V=volume of blood in the body (l); A=absorption factor (unitless); f=fraction of daily intake taken up by the blood (unitless); BW=body weight (60 kg).

The above equation was used by the USEPA (1997) to estimate that a daily dietary intake of methylmercury of 1 μg methylmercury/kg/day would result in a maternal blood level of 44 μg/l, corresponding to a maternal hair level of 11 ppm. The relationship between C and the maternal hair level was based on a hair to blood ratio of 250:1 for methylmercury in hair (in parts per million) to that in maternal blood (micrograms mercury per milliliter blood). Hair to blood ratios reported in the literature range from 140 to 416 (Birke et al., 1972; Cernichiari et al., 1995a; Den Tonkelaar et al., 1974; Haxton et al., 1979; Kershaw et al., 1980; Phelps et al., 1980; Sherlock et al., 1982; Skerfving, 1974; Soria et al., 1992; Sumari et al., 1969; Tsubaki, 1971a,b; Tyning, 1967). USEPA (1997) selected the Phelps et al. (1980) study as the basis for their hair to blood ratio because of the larger sample size (n=339) and the attention to sampling and analysis. Phelps et al. (1980) report that the actual ratio is probably higher than 200, but less than the observed value of 296; therefore, the USEPA (1997) used a midpoint of 250.

The values for the remaining parameters used in the one-compartment model are listed in Table 14 and were derived as follows. The elimination constant (b) is based on several studies in which clearance half-times for methylmercury from blood or hair were reported to range from 35 to 189 days (Al-Shahristani and Shihab, 1974; Kershaw et al., 1980; Miettinen et al., 1972; Sherlock et al., 1984). The average elimination constant (ln 2/half-life in days) for the four studies was 0.014 day^{-1}. Therefore, the USEPA (1997) selected this average for use in its one-compartment model.

Table 14. Parameter values used by USEPA to estimate daily dietary intake from maternal methylmercury hair concentrations.

Parameter	Value	Reference
Hair to blood ratio (used to derive C)	250	Phelps et al. (1980)
Elimination constant	0.014 day^{-1}	Al-Shahristani and Shihab (1974); Kershaw et al. (1980); Miettinen et al. (1971); Sherlock et al. (1984)
Volume of blood	5 l	Best (1961)
Absorption factor	0.95	Aberg et al. (1969); Miettinen et al. (1971)
Fraction absorbed taken up by blood	0.05	Kershaw et al. (1980); Miettinen et al. (1971); Sherlock et al. (1984)

Based on various experiments, the volume of blood in the body has been determined to be 7% of total body weight (USEPA, 1996). During pregnancy, the blood volume increased approximately 20–30% to about 9% of total body weight (Best, 1961). The USEPA (1997) assumed an average bogy weight of 58 kg and a blood volume of 9% during pregnancy, resulting in a calculated blood volume (V) of 5.22 l; therefore, in the USEPA (1997) model, V was assumed to be 5 l.

The absorption factor (A) was based on the results of studies reported by Aberg et al. (1969) and Miettinen et al. (1972). In the study reported by Aberg et al. (1969), radiolabeled mercuric nitrate was administered in water to three healthy volunteers. Uptake was reported in this study to be >95%. In the study conducted by Miettinen et al. (1972), fish liver homogenate was incubated with radio labeled methylmercury nitrate. This methylmercury homogenate was then fed to fish for a week, after which the fish were killed, cooked, and fed to volunteers. Mean uptake in the volunteers was >94%. Based on these results, the USEPA (1997) selected an absorption factor of 95% (0.95) for derivation of a daily intake of methylmercury.

The fraction of daily intake taken up by the blood (f) was based on the results of three studies (Kershaw et al., 1980; Miettinen et al., 1972; Sherlock et al., 1984). The average fraction of absorbed dose in total blood volume in each of these studies ranged from 5% to 5.9% in volunteers who ingested methylmercury contaminated fish. Five percent (0.05) has been assumed for this parameter by other groups (Berglund et al., 1971; WHO, 1990); therefore, a value of 0.05 for f was used by the USEPA (1997) in its derivation of daily dietary intake of methylmercury.

Application of Uncertainty Factors

According to USEPA guidelines for assessment of non-carcinogenic effects, such as reproductive or developmental toxicity, the dose (e.g., NOAEL or BMDL) for the critical effect from the critical study effects is divided by

uncertainty factors (USEPA, 1988). These uncertainty factors are intended to capture those uncertainties and/or variability inherent in either the extrapolation to the target population (e.g., across species, duration of exposure, sensitive subpopulations, from a LOAEL to a NOAEL) or due to limitations in total data base. While uncertainty factors are applied to a NOAEL or BMDL derived from (usually) a single study, decisions regarding the magnitude of the uncertainty factor(s) are based on a weight of evidence assessment of the total body of literature (Dourson and Stara, 1983; Dourson et al., 1992).

In the derivation of the RfD for methylmercury, USEPA applied a composite uncertainty factor of 10 based on the following rationale. Half of the factor was applied to account for the variability in the human population, specifically the wide variation in a biological half-life of methylmercury and the variations in hair to blood ratios. The other half of the factor was applied to account for lack of data, specifically lack of a two-generation reproductive study and for possible latent chronic effects (USEPA, 1997). Application of an uncertainty factor of 10 to the daily dietary intake of 1 μg/kg/day resulted in a final RfD of 0.1 μg/kg/day or 1×10^{-4} mg/kg/day.

Limitations and uncertainties in the Iraqi data

The series of publications that have chronicled the Iraqi population from the initial poisoning episode through the evaluation of signs and symptoms of neurological effects in children exposed *in utero* have contributed greatly to our understanding of the potential health effects from ingestion of methylmercury. However, there are a number of limitations and uncertainties in these data that clearly indicate that this data set is not suitable as the basis for dose–response analysis and for setting regulatory standards for prolonged human exposure. The major limitations and uncertainties in these data can be summarized as follows:

1. *The exposure data reported do not provide the relevant dose-metric for use in dose–response modeling for populations chronically exposed to low levels of methylmercury.*

As discussed previously, exposure in the Iraqi population was due to an acute poisoning from the consumption of bread made with methylmercury contaminated grain. Exposure continued for 1–3 months rising sharply to a peak level, as measured by analysis of methylmercury in maternal hair, followed by relatively rapid declines in body burden after cessation of exposure (Amin-Zaki et al., 1979; Marsh et al., 1981). Consequently, exposure to pregnant women and to the developing fetus did not occur over the same time frame for all women in the cohort. Some individuals may have already been pregnant for several months before they began consuming contaminated bread, while for others, exposure may have preceded or overlapped the time of conception. While the critical time period of fetal development most susceptible to methylmercury toxicity is not known with certainty, it is likely that a "window of opportunity" exists in which the developing nervous system is more susceptible to this toxicity. Choi and coworkers (Choi, 1989; Choi et al., 1978) hypothesized that there are critical periods for the developing central nervous system with greater susceptibility to methylmercury toxicity. Choi et al. (1978) suggested that the second trimester, in which neuronal migration in the fetal brain occurs, may be the most susceptible time period for the more overt symptoms of poisoning. On the other hand, for effects on the more advanced aspects of the developing nervous system, the third trimester may be the most sensitive period (Amin-Zaki et al., 1979). Given the short duration of the exposure period, which did not encompass the full gestational period, the peak level may not have occurred during the critical period of gestation. In those cases, neurological effects may not have resulted or were less obvious, leading to a conclusion that would result in an underestimate of the dose–response relationship. Conversely, if peak exposures occurred during the critical time period but because of physiological and mechanistic considerations (see discussion in ATSDR, 1997) biological processes for detoxification were overwhelmed, then the dose–response relationship may be overestimated. The timing of the exposure may provide an explanation for the lack of a monotonic dose–response seen with the 81 mother–infant pairs reported in Marsh et al. (1987). For example, there were children with neurological scores of zero whose mothers had methylmercury hair concentrations as high as 294–336 ppm, while some children had neurological scores as high as 5, for maternal methylmercury concentrations as low as 16 ppm.

The effect of the timing of exposure on the fetal dose during different stages of fetal development has been evaluated using a PBPK model (Gentry et al., 2001). When maternal ingestion of methylmercury was simulated to begin 1 month before pregnancy, then a peak hair concentration of 421 ppm corresponded to an average fetal blood concentration in the third trimester of only 0.538 ppm. In contrast, if maternal ingestion of methylmercury began in the sixth month of pregnancy, then for a peak maternal hair concentration of 394 ppm, the average fetal blood concentration in the third trimester was 4.48 ppm. These simulations indicate that the fetal dose for exposure late in gestation is almost ten times higher than when maternal exposure began 1 month before pregnancy for comparable maternal hair concentrations.

Since exact birth dates were unknown in the Iraqi population, any correlation between peak exposure and gestational stage was not possible (Marsh et al., 1980). Therefore, use of a peak hair concentration for all of pregnancy as a dose-metric for a response that may be time

dependent, as in brain development, can lead to misclassification of individual exposures. The misbetween peak maternal hair levels and fetal dose at a critical time period is not a concern for populations chronically exposed at low exposure concentrations. The relevant dose-metric for populations chronically exposed to low levels of methylmercury is expected to be sustained average levels over the duration of pregnancy.

2. *This was a retrospective study in which the cohort was not fully identified at the onset of the study but evolved as the study progressed.*

The results of this retrospective evaluation were published in a series of papers beginning with the report on 15 subjects (Amin-Zaki et al., 1974), which was expanded to 27 mother–infant pairs as reported by Amin-Zaki et al. (1979, 1981) and expanded further to 29 mother–infant pairs as reported by Marsh et al. (1979). The cohort was then expanded to 84 mother–infant pairs (Marsh et al., 1981), with 81 mother–infant pairs forming the final cohort reported in Marsh et al. (1987) and Seafood Safety (1991). The cohort consisting of 84 pairs was actually two subcohorts. These cohorts represented a clinical series of 29 subjects (Marsh et al., 1980), augmented by a second group of 55 subjects (WHO, 1990). Although mercury exposure was comparable in both subcohorts (average of 78.8 and 80.4 ppm hair levels based on atomic absorption), reported symptom frequency and infant sex ratio (M/F) differed significantly in ways that cannot be explained by differences in exposure (Crump et al., 1995). Transient paresthesia was reported in 45% of mothers in the first subcohort and only 7% in mothers in the second subcohort, although the overall exposure, as measured by hair levels, was greater in the second subcohort. Similarly, reported incidences of late walking and late talking were 48% vs. 22%, and 41% vs. 15%, respectively, in the first and second subcohorts. An apparent undersampling of female infants in the second subcohort can be explained by a significant difference in the sex ratio between the two subcohorts (M/F ratios of 1.0 and 2.1). Maternal paresthesia was strongly associated with both late walking and late talking and may be a direct consequence of the subcohort differences noted (Crump et al., 1995). The variation in symptom reporting could have biased dose–response analysis; therefore, these subcohort differences should be controlled for in any dose–response assessment (Crump et al., 1995). However, with the data as published, this is not possible.

3. *The reported results are subject to recall and classification bias.*

The results of the neurological examinations for children consisted of two aspects, both of which had inherent limitations and uncertainties. One phase of the examination, the neurological score, was based on a physical examination of these children for clinical neurological signs. These examinations were conducted in the homes and consisted of such measures as coordination, sensation, reflexes, limb tone, deep tendon reflexes, among others. A scoring system was used with responses graded a score of zero if the result of the neurological examination was deemed "absolutely normal" with scores of 0 to 3 indicative of no definite abnormality (Marsh et al., 1987). Borderline findings and minimal signs were awarded points ≥ 4 with the most severely affected child given a score of 11. No specific criteria for assignment of scores or for a definition of abnormal effects or for any measures taken to achieve consistency in diagnosis among examiners has been discussed in the published literature.

An even greater source of uncertainty, however, was introduced due to the other aspect of the evaluation. The assessment of majority of the measures of neurological effects, i.e., late walking and talking, seizures, mental development, were based on interviews with the mothers, and sometimes other family members, conducted in their homes located in villages in rural areas. Mothers were questioned about their labor and delivery and about the child's early development. Mothers were asked questions, with the aid of an interpreter, regarding the age at which a child attained a developmental milestone (i.e., the age at which the child walked and talked).

Since in most cases, one or more years had elapsed between the actual time when a child first walked or talked and the initial interview with family members, age was determined retrospectively based on maternal recollections. The age of the child at the time of the interview with the primary caregiver averaged 30 months but varied up to 4–5.5 years. It is clear that neither the actual birth date nor the age at which each of these milestones was attained was accurately assessed or based on any recorded data, e.g., postnatal records. Birth dates of children, as well as attainment of developmental milestones, had to be estimated in relationship to some other event, such as a religious holiday or the poisoning episode itself. A disproportionate number of children were reported to have walked at 18 months, whereas only one child was reported to have walked at 17 or 19 months (Crump et al., 1995). In the total cohort, 65% of the months reported at which the child first walked or talked were even multiples of 6 months. Also, among the children with minimal exposure (maternal hair methylmercury levels between 0 and 2 ppm), approximately 65% of those children were reported to have walked exactly at 12 or 18 months. The USEPA (1997), in their quantitative uncertainty analysis (Volume IV, Appendix D), stated that the classification error due to recall bias for late walking and late talking contributed significantly to uncertainty and that the only reliable endpoint upon which to base the dose–response evaluation was the neurotoxicity score.

The impact of this uncertainty surrounding the age of walking and talking was demonstrated by both the (Cox et al. 1989, 1995) and Crump et al. (1995) analyses. As

stated, a disproportionate number of children were listed as starting to walk at precisely 18 months of age, which suggests that age at which walking began was not determined accurately. Crump et al. (1995) showed that if the definition of late walking was defined as 18 months or later rather than later than 18 months, all statistical significance disappeared. Cox et al. (1995) demonstrated that when four individuals with low exposure levels, based on maternal hair, were eliminated from the analyses, the bimodal nature of the distribution was eliminated and the threshold estimated from the late walking data increased from 10 ppm to between 100 and 150 ppm in maternal hair. Reanalyses of these data by Cox et al. (1995) and Crump et al. (1995) indicated that the results of the threshold model were highly influenced by the definition of background. Cox et al. (1995) emphasized that the results of the analysis were highly influenced by four individuals in the low exposure groups and when they were considered "background," the lowest effect level was between 100 and 150 ppm. Crump et al. (1995) using other models indicated that there was no convincing statistical evidence of effects of methylmercury in children of mothers whose mercury hair concentrations were below 80 ppm and the upper limit on the lowest effect level could be as high as 255 ppm. Both analyses demonstrate that these data are not reliable for dose–response analysis for fish-eating populations.

Not only was the age at which a milestone was achieved subject to uncertainty, but also the interpretation by the mother of the definition of such activities introduced uncertainty. The age at which a child walked or talked was defined as the age at which the child could stand and walk unaided or speak two or three meaningful words, respectively. Both definitions leave room for subjective judgement that may vary between women.

The mother's judgement was sought as to the presence of other neurological symptoms, (i.e., impaired vision or hearing, incoordination, or seizures), and of the mother's overall impression as to whether the child's physical and mental development was normal. No neurological tests were administered to identify deficits, such as impaired vision or hearing, and no clear criteria for normality was discussed in the published work. Marsh et al. (1980) noted that while the examination team that conducted the interviews was unaware of the level of mercury exposure, mothers were probably aware that they had eaten contaminated bread and this knowledge may have influenced their answers to questions.

Applicability of the Iraqi data to fish-eating populations

At the time that the current USEPA RfD was derived, the only quantitative data providing a dose–response relationship for methylmercury were the results from the studies of the Iraqi grain poisoning incident (Marsh et al., 1979, 1981, 1987). The RfD is based specifically upon effects evaluated in children exposed perinatally (Marsh et al., 1987). However, in selecting a study for the derivation of an RfD for fish-eating populations, the numerous limitations of this study must be considered, as well as the fact that it does not represent the type of exposure scenario (fish ingestion) of concern. Since the derivation of the current RfD, several studies have been published that evaluate effects in populations that are exposed to methylmercury *via* fish ingestion. These studies are the most representative for the exposures at the Alcoa site, because they represent populations exposed to low levels of methylmercury *via* fish ingestion for chronic periods, not acute, high-level exposures as with the Iraqi poisoning incident.

The USEPA supports its continued reliance on the Iraqi results, rather than those from a fish-eating population, as its critical study, because the USEPA believes that the Iraqi results are consistent with the results from fish-eating populations; however, a detailed analysis suggests that this is not the case. In the Iraqi studies, the effects reported were overt effects (i.e., delayed developmental milestones) readily observable with simple tests. In the fish-eating population studies, large batteries of intense neurological testing were required to detect only subtle changes in neurological function. There is no reason to believe that this population represents a sensitive subpopulation (Cicmanec, 1996; Clarkson, 1997). Rather, the conclusions drawn regarding effects at low doses in the Iraqi population are more likely due to limitations in the data set and the impact of outliers on statistical analyses (Cox et al., 1995; Crump et al., 1995) rather than to low-dose effects in a sensitive population. Indeed, given the differences in exposure (high acute exposure versus chronic low level) and the types of tests (observable signs and symptoms versus tests for subtle behavioral, psychological or cognitive function), there is no reason to expect that the Iraqi study and the fish-eating population studies should give similar quantitative results. Therefore, the use of the Iraqi study for derivation of a site-specific RfD for fish-eating populations is questionable.

DERIVATION OF AN RFD FOR FISH-EATING POPULATIONS

Selection of a critical study

Although the information from poisoning incidents should not be used for the derivation of an RfD for chronically exposed fish-eating populations, both the Iraqi and Japanese poisoning incidents provide information that the fetus does represent a sensitive subpopulation. Data from poisoning events in Japan and Iraq indicate that the fetus may develop neurotoxic effects, even if the mother is

clinically asymptomatic (Amin-Zaki et al., 1979; Harada, 1978). In Minamata, two of the four mothers of severely affected infants recalled no symptoms during pregnancy (Harada, 1978). This demonstrates susceptibility of the fetus in infant–mother pairs with Minamata disease. Results from the Iraqi poisoning incident (Marsh et al., 1987) indicate that even when maternal symptoms during pregnancy are mild and transient, with paresthesia as the most frequent symptom, the possibility of delayed milestones or impacts on neurological scores occurring in the offspring. Therefore, in the determination of an RfD, the critical effect is the impact on neurodevelopment and should be based on data for the developing fetus exposed *in utero*.

All of the studies conducted in fish-eating populations were reviewed to identify quantitative information on the neurodevelopmental effects of methylmercury in the developing fetus. Several populations could not be considered as the critical study for the derivation of an RfD for reasons including: small cohort size, poorly characterized exposure history, lack of data for dose–response assessment, presence of significant potential for confounding (alcohol, preexisting disease), or only gross (less sensitive) neurological endpoints evaluated. For several populations (i.e., in Peru, Canada, and the Amazon), the reported information was restricted to symptoms of methylmercury information in the adult (i.e., paresthesia, visual construction), and therefore, was not considered as a choice for the critical study. Populations in which maternal levels had reached steady state were preferred; therefore, populations such as the Canadian population, whose exposure to methylmercury has significant seasonal variation, were not selected. Dose–response information that could be used in a quantitative analysis, and that was also available in the published reports, was preferred. The review of the available information from fish-eating populations resulted in the identification of only three fish-eating populations that presented quantitative data on neurodevelopmental effects observed in children following *in utero* exposure to methylmercury that did not have the limitations noted above. These were the studies in the Seychelles Islands, the Faroe Islands, and the New Zealand.

The Seychelles study is a large, longitudinal study ($n=740$) conducted in a fairly stable population. The benefits of the Seychelles cohort include that it is a health population with good prenatal and antenatal care with limited confounders, which were identified *a priori* and controlled for in the analyses of the results. The population does not ingest marine mammals (Shamlaye et al., 1995); therefore, co-exposure to PCBs was not a consideration. Also, there was a low percentage of alcohol ingestion in the mothers included in the study (5%), compared to other cohorts (i.e., 25% in the Faroe Islands). The cohort of children has been evaluated at four time points to date (6.5, 19, 29, and 66 months), with a fifth examination (96 months) ongoing using a more extensive battery of tests than those used in previous examinations. Although the Seychelles study published to data is basically a negative study (Clarkson, 1997; Myers et al., 1997b), there is still quantitative data available in the published reports for a dose–response analysis. Therefore, this study would be a good selection as a critical study for the derivation of an RfD.

The New Zealand study is also a well-conducted study in a fish-eating population and provides quantitative information for the derivation of an RfD. Quantitative dose–response analyses have been conducted using the data from the New Zealand cohort, using actual measured hair levels as an indicator of mercury exposure (Crump et al., 1998). Because of the smaller size of the cohort ($n=228$), compared to some of the other fish-eating population studies (i.e., Seychelles and Faroe Islands), the power to detect more subtle neurological effects may be reduced. There are also several issues surrounding the analysis of the New Zealand cohorts, including the question of whether to include data on one individual, that has a significant impact on the results of the study (Crump et al., 1998). However, quantitative information is available from the study, that once adjusted for potential confounders, could be used for the derivation of an RfD.

The cohort of children studied in the Faroe Islands is also a larger cohort ($n=917$), with a very large battery of neurological testing administered, aimed at detecting subtle neurological effects. The population studied is a fairly stable population, whose diet includes a high intake of marine mammals and fish. The children have been evaluated at several times over the first year of life and at approximately 7 years of age. The authors have reported an inverse correlation between methylmercury exposure below 10 ppm, based on maternal hair levels, and tests scores for specific neurological functions. One major limitation of using the latest information published on the Faroe Islands cohort (Grandjean et al., 1997) for the derivation of an RfD is the lack of quantitative data to conduct a dose–response analysis. All that is reported are statistical results of the regression analysis (i.e., P values and regression coefficients), with none of the actual dose–response information provided. This cohort also has several confounders that have the potential to impact the conclusions drawn regarding methylmercury exposure.

The most serious confounder in the Faroe Islands study is co-exposure of the mother to PCBs found in marine mammals, specifically the pilot whale. Effects on cognitive functioning have been reported in children exposed to PCBs *in utero* (Jacobson et al., 1990). Poorer short-term memory has been reported during early childhood of children following *in utero* exposure to PCBs at cord blood levels

of 2.5 (2.0) ng/ml [arithmetic mean (standard deviation)]. Which aspects of short-term memory are specifically affected have not been determined, but a selective attention deficit could be involved (Jacobson et al., 1990). The area of the Atlantic Ocean in which the Faroe Islands are located has been called the greatest global environmental reservoir of PCBs (Weihe et al., 1996). Effects similar to those observed following PCB *in utero* exposure (Jacobson et al., 1990) were observed in the Faroe Islands study (Grandjean et al., 1997). PCB cord blood levels reported for the Faroe Islands (1.12 ng/g wet weight — geometric mean, interquartile range 0.57 to 1.55 ng/g) were similar to those reported for children exhibiting cognitive deficits attributed to PCB exposure in the Jacobson et al. study. Because any effects observed in the Faroe Island cohort are likely to have been a result of co-exposure to PCBs and methylmercury, the only test results that would be relevant for the derivation of an RfD for methylmercury from the Faroe Island data are those that are adjusted for PCB exposure.

The analysis conducted by Grandjean et al. (1997) on effects of methylmercury at maternal hair levels less <10 ppm were not adjusted for PCB exposure; therefore, the outcome of that analysis as published, cannot be considered for the estimation of an RfD for methylmercury. The tests for which the impact of PCB co-exposure on outcome were controlled are shown in Table 9. Only the 1993 subcohort was considered, since PCB data are only available for that subcohort. The only test outcome, after adjustment for PCB exposure, that remains significantly correlated to methylmercury exposure is the CPT score. However, there are limitations with the use of the CPT score as the basis of an RfD. The tests were conducted in two 1-year groups, with different teams supervising the administration of the neurological test each year. The first year of test outcomes (the 1993 subcohort) for the CPT was the only year in which a significant association with methylmercury exposure (without adjustment for PCBs) was reported. No significant correlation was reported for the second year test outcomes (the 1994 subcohort). The authors attributed this discrepancy to the change in teams during the 2 years of testing. When the data for the 2 years were merged, a significant correlation between methylmercury exposure and test scores remained ($P=0.01$); however, due to the lack of PCB information for one-half of the cohort (data only available for children tested during the first year), the impact of PCB exposure cannot be determined for the entire cohort of CPT outcomes. Moreover, the authors also did not retain the second year of CPT outcomes for further analysis, citing less stringent supervision, because the staff administering the computer-assisted tests changed at the end of the first year. However, no test outcomes for any of the other tests were removed from further analysis and an interaction term for year tested was apparently not applied.

Another issue related to all of the tests, including the CPT, conducted on computers is the adjustment for computer familiarity. The children were asked, rather than the caregiver, about familiarity with computer games. The answer from the child was rated as none, some, or much. Because the children were asked instead of an adult, the children may have claimed to be more or less familiar with computers than they actually were. This is a very crude attempt at an adjustment for computer familiarity, so its ability to control for this as a confounder is questionable.

Because of the number of potential confounders and issues not considered in the analyses of the Faroe Island cohort, together with the lack of actual dose–response data in the published report, the Faroes study cannot be used at this time in the derivation of an RfD. The results as presented, especially for the <10 ppm analysis, make it impossible to determine whether the outcomes presented are a result of methylmercury exposure, PCB exposure, a combination of exposure to the two chemicals, or the result of confounding factors. Since there were no quantitative data reported, dose–response evaluation cannot be performed. Additional analyses of the new data would be necessary to resolve many of the issues surrounding the results of this study before it would be useful in the determination of an RfD.

After consideration of the three most relevant studies for the derivation of an RfD, the New Zealand or the Seychelles studies could be used as the critical study for the derivation of an RfD. However, because of the larger cohort involved in the Seychelles study, the Seychelles study was selected as the critical study of choice for derivation of an RfD. However, extensive dose–response analyses have already been conducted for the New Zealand cohort (Crump et al., 1998), and the results of these analyses are compared to those obtained from analyses of the Seychelles cohort.

Dose–Response Analysis

The Benchmark Dose (BMD) Approach

In the past, USEPA has determined an RfC or RfD by applying uncertainty factors to a NOAEL. Whenever a NOAEL was not defined by the available studies, USEPA would use a LOAEL in place of a NOAEL and apply an additional uncertainty factor of 10. This approach was designed for experimental data in which animals were placed in discrete dose groups, and is less appropriate for epidemiological data. When an effect was found in an epidemiological study, a mean (frequently a geometric mean) exposure was often assumed to be a LOAEL. However, all that may have been actually known was that an effect occurred somewhere within the range of exposures in the epidemiological study — a range that might span several orders of magnitude.

More recently USEPA has begun using the benchmark as a replacement for the NOAEL in determining RfCs and RfDs. This approach has now been applied to several chemicals, including mercury, by the Agency (USEPA, 1997). As stated previously, a BMD is a dose (or exposure) that corresponds to a specified level of additional response (the BMR). A BMD is calculated by fitting a mathematical dose–response model to dose–response data. The BMDL, which is a lower statistical confidence bound on the BMD, replaces the NOAEL in the calculation of an RfC or RfD.

A BMD calculation from epidemiological data is illustrated in Figure 2A. The solid line represents the best-fitting regression line to a set of epidemiological data (data not shown). This line is the mean or expected response, $m(e)$, where e represents exposure. Although in this figure the mean response varies linearly with exposure, a nonlinear response could also be considered. In this example, the BMD is defined to correspond to a change in the mean response equal to 0.61 times the standard deviation, σ. (The choice of 0.61 will be explained later.) The BMD calculation may be visualized by drawing a horizontal line from the point on the y axis equal to $m(0)+0.61\sigma$ (the mean response at zero $+0.61\sigma$) to where it intersects the mean response line, and drawing a vertical line from that point down to where it intersects the x axis. The BMD is the value on the x axis where the vertical line intersects the x axis. Note that the BMD reflects the potency of the exposure for causing the health effect, since a stronger

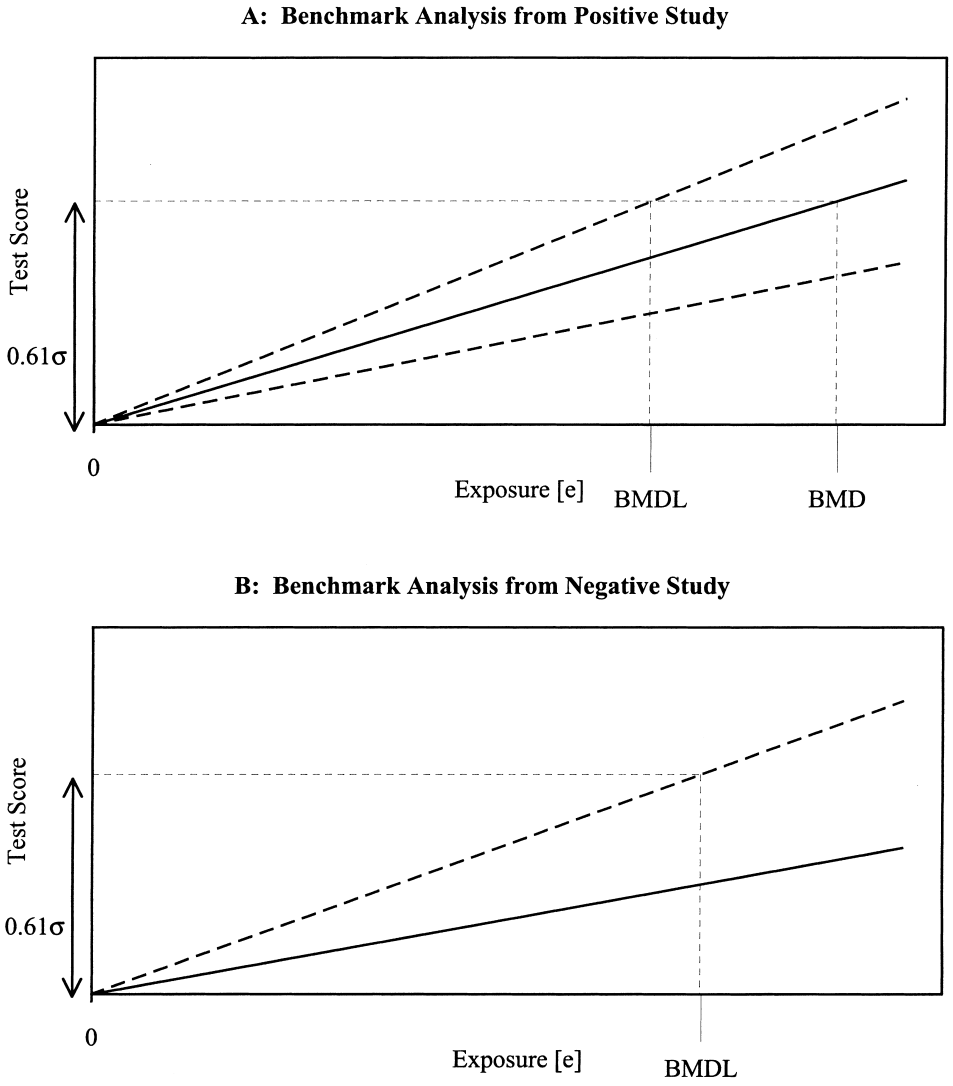

Figure 2. Illustration of a benchmark calculation from a positive study (A) and a negative study (B). Solid line represents best-fitting regression line to test scores (or residuals) from epidemiological study. BMD is defined as exposure associated with increase in mean test score equal to 0.61 times SD of test scores. BMDL is 95% statistical lower bound on BMD. ——— Mean response, m(e); - - - - Lines defining 95% Lower and Upper C.I. on BMD.

(more potent) effect would be represented by a steeper mean response line, which would in turn result in a lower BMD. The BMDL, depicted as a value on the x axis in Figure 2A, represents the lower 95% statistical confidence limit on the BMD. The BMDL reflects both the potency of the exposure and the size of the study (as reflected in the width of the confidence interval). If two studies of unequal size provide the same estimate of effect (i.e., the same mean response line, $m(e)$), the larger study will tend to provide a larger BMDL because it will tend to provide narrower confidence limits.

The dotted lines in Figure 2A provide a visual picture of the 95% lower and upper confidence intervals for the BMD. Specifically, to visually determine the BMDL associated with a given increase, I, in the mean response, one would project a horizontal line from the point $m(0)+I$ on the y axis to where it intersects the dotted line, and drawing a vertical line from that point down to where it intersects the x axis. The BMDL corresponding to an increase in the mean response of I is the value on the x axis where the vertical line intersects the x axis. Note that the dotted line in Figure 2A associated with the BMDL is not an upper 95% confidence limit line for the mean response. That is because the BMDL is a 95% lower bound for the exposure associated with a *change* in response, rather than in the response itself. That also explains why the mean response and the dotted line defining the BMDL converge at an exposure of zero. By definition, the change in response must be zero with zero exposure.

The BMD method has several advantages over the NOAEL, including making better use of dose–response information and reflecting sample size more appropriately (Barnes et al., 1995; Crump, 1984). When evaluating an epidemiological study, use of the BMD method allows one to consider the dose–response over the entire exposure range rather than making the crude assumption often made in connection with the NOAEL that any effect seen in the population would occur at the mean exposure level. In order to apply the BMD method to epidemiological data it is necessary to have access to either the individual exposure levels and responses (preferred) or, if individual data are not available, summary responses grouped by exposure level.

A BMDL can be calculated from a "negative" study as well as from a "positive" one. The calculation of a BMDL from a negative study is illustrated in Figure 2B. In this graph, the slope of the mean response line is smaller than in Figure 2A and consequently the BMD is larger, so large that it cannot be depicted on the scale used. Since the study is negative, the upper bound on the BMD corresponding to any level of increased risk, no matter how small, is undefined; consequently, the dotted line corresponding to the 95% upper bound on the BMD shown in Figure 2A cannot be shown on this graph. Moreover, in a negative study, the slope of the mean response curve could be negative, in which case the estimate of the BMD would also be undefined. Nevertheless, as illustrated in Figure 2B, even in a negative study, the statistical lower bound on the BMD can always be calculated as a finite number (Crump, 1995). It is possible that a BMDL calculated from a negative study reflects only the statistical constraints imposed by the experimental design. Even so, since it is a lower statistical confidence bound, it represents a conservative (in the sense of being health protective) value, since even though a study was negative, exposure could have resulted in a small undetected increase in health effects, and the BMDL represents a statistical lower bound for the potential size of the increase.

Calculation of a BMD involves choosing the dose–response model, the definition of additional response (BMR) used to define the BMD, and the size of the statistical confidence interval represented by the BMDL. Somewhat different approaches must be used to calculate a BMD based on whether the underlying data are quantal or continuous. Quantal data are data that indicate simply whether or not a subject had a particular response. Continuous data, on the other hand, indicate the magnitude of response and theoretically can assume any value in a range. The methylmercury data modeled consist of test scores and consequently are continuous.

The k-power dose–response model (Crump, 1995), which was one of the models used to make BMD calculations reported herein, has the form

$$m(e) = m_0 \pm \beta e^k, \tag{2}$$

where e is the maternal hair mercury level during pregnancy (ppm), $m(e)$ is the expected test score of a child whose mother's mercury hair level was e, and m_0, $\beta \geq 0$, and $k \geq 1$ are parameters estimated from the data. The parameter k was included to allow the dose response for mercury to be nonlinear. It was further assumed that test scores were normally distributed (and consequently represented continuous data) with a variance, σ, that was independent of the hair mercury concentration.

There are two equivalent ways to define the BMD when using this model (Crump, 1995). Both will be described, since each has certain conceptual advantages. One way is to define the BMD as the exposure that corresponds to a given change, BMRC, in the mean response normalized by the standard deviation; i.e., the BMD is defined as the exposure that satisfies $[m(\mathrm{BMD}) - m(0)]/\sigma = \mathrm{BMRC}$. Although this formulation is straightforward, there is no clear-cut, non-arbitrary way for deciding upon an appropriate value for BMRC.

The equivalent alternative approach involves first defining a proportion p_0 of an unexposed population that would be considered to have an abnormal response. The value $p_0 = 0.05$ was used in analyses reported herein, which

is consistent with the convention that the "normal range" of a clinical test is assumed to encompass test results of 95% of a normal population. With p_0 fixed, the probability, $P(e)$, that a subject exposed to e has an abnormal response can be calculated from p_0, σ, and the mean response, $m(e)$.[6] This formulation therefore, provides a link between quantal and continuous data, since quantal data are generally used to model the probability of an adverse response. In benchmark analyses based upon quantal data, the BMD is often defined as the exposure that causes a predetermined increase, BMRQ, in the probability of an adverse response, e.g., as the value that satisfies

$$P(\mathrm{BMD}) - P(0) = \mathrm{BMRQ}. \quad (3)$$

Since we now can calculate $P(e)$ using continuous data, we can use the same expression to define the BMD for continuous data. To complete the definition of the BMD we must choose a value for BMRQ. Allen et al. (1994a,b) determined from analyses of a large number of quantal animal studies that choosing BMRQ=0.1 produced BMDLs that, on average, were similar to, but slightly smaller (more conservative) than, the corresponding NOAELs. The USEPA (1997) likewise selected BMRQ=0.1 in its benchmark calculation for methylmercury based on an analysis of quantal data from the Iraqi study. Therefore, we defined the benchmark using expression (2) with BMRQ=0.1; i.e., we defined the benchmark as the exposure that causes the probability of an abnormal response to increase by 0.1, where an abnormal response is defined so that responses of 5% of unexposed subjects are expected to be abnormal.

Crump (1995) showed that the two approaches described above for defining a BMD from continuous data using the k-power model are completely equivalent, i.e., that given a value for BMRQ, a corresponding value for BMRC can be determined so that BMDs and BMDLs obtained using the two approaches are exactly the same. Crump (1995) also provides a formula for converting from a value for BMRQ to the equivalent BMRC and vice versa. With BMRQ=0.1 and p_0=0.05, the equivalent BMRC is 0.61. Therefore, our definition of the BMD is equivalent to defining the BMD as the exposure for which the mean response above background is equal to 0.61σ. It was noted above that if the underlying data are normally distributed with a common standard deviation, specification of a dose response model, $m(e)$, for the mean response, and specification of a proportion, p_0 of unexposed subjects whose responses are considered abnormal, determines the probability, $P(e)$ of an adverse response as a function of exposure. Alternatively, with normally distributed data and a common standard deviation, one can specify p_0 and a functional form for the probability of an adverse response, and that determines the mean response, $m(e)$. This allows one to define a model for continuous data that corresponds to a predetermined functional form for the probability of an adverse response, and consequently permits the same dose–response model to be applied to quantal and continuous data (Crump, 1995). In the Mercury Study Report to Congress (USEPA, 1997), the USEPA applied the Weibull dose–response model,

$$P(d) = p_0 + (1 - \mathrm{p}_0)\left\{1 - \exp\left[-(Ad)^k\right]\right\}, \quad (4)$$

to quantal data from the Iraqi study. As an alternative to the k-power model, we also applied the Weibull model to the continuous data from the Seychelles study. As with the k-power model, in applying the Weibull model, we selected p_0=0.05, BMR=0.1, and $k \geq 1$. Similar results were obtained.

In the benchmark analysis of the New Zealand data (Crump et al., 1998), potential confounders were accounted for by including other additive terms in the model (1). This approach could not be used with the Seychelles data, since the underlying data for that study were not available. Potential confounders in the Seychelles data were accounted for by applying both the k-power and Weibull models to residual tests scores obtained by the original investigators after adjusting for confounders.

The conventional 95% level of statistical confidence was used in all cases to define the BMDL. A 95% level of confidence was also used in BMD calculations by Allen et al. (1994a,b) and the USEPA benchmark analyses for methylmercury (USEPA, 1997).

Derivation of a BMDL Based on Hair Mercury Levels in the Critical Study

Epidemiological data on neurological measurements in children of mothers exposed to methylmercury are available for an Iraqi population accidentally exposed to high levels of mercury in seed grain (Marsh et al., 1987), and for populations exposed to mercury through consumption of fish in New Zealand (Crump et al., 1998; Kjellstrom et al., 1989), in the Seychelles (Davidson et al., 1995; Myers et al., 1995a) and in the Faroe Islands (Grandjean et al., 1997). As discussed previously, the Iraqi data are less appropriate than the other three studies for derivation of an RfD for the protection of populations exposed to methylmercury primarily through consumption of fish because of the extremely high exposure levels in the Iraqi study, the acute nature of the exposure, and questions regarding the accuracy of the data from this study. Effects upon late walking and late talking found in the Iraqi study were not seen in the Seychelles, where

[6] The probability of an abnormal response is given by $P(e)=1-N\{N^{-1}(1-p_0)-[m(e)-m_0]\sigma\}$ if higher responses are more adverse, and by $P(e)=N\{N^{-1}(p_0)-[m(e)-m_0]\}$ if lower responses are more adverse (Crump, 1995).

mercury exposures were from fish consumption and occurred at lower levels (Myers et al., 1997b). Consequently, the BMD analysis will focus on the studies of fish-eating populations. The Seychelles data appear to be the best currently available for determining an RfD for methylmercury due to its large size, its homogeneous population, its detailed neurological evaluation of children at various ages using an extensive battery of standardized tests that have been widely used to study neurological disorders in children, its careful determination of mothers' mercury hair levels, and the absence of substantial exposures to other known neurotoxins, such as PCBs. Consequently, BMD calculations will be based primarily on the Seychelles data. Other supporting benchmark calculations are provided by the New Zealand study, which was smaller than the Seychelles study and involved a more ethically diverse population. Unfortunately, data have not yet become available from the Faroe Islands study that would permit a BMD analysis.

A joint project has been agreed upon in principle between researchers at ICF Consulting and the University of Rochester to calculate BMDs from the Seychelles data. It is hoped that work will be conducted early in 1998. In the interim, BMDs have been calculated for this report using published scatterplots of residuals of test scores (test scores adjusted for potential confounding variables) versus maternal hair mercury levels. The data from these scatterplots were digitized and analyzed using the benchmark methodology described above. Scatterplots were available for two tests used to evaluate Seychellois children at 6 months of age (Visual Recognition Memory and Visual Attention; Myers et al., 1995a) and three tests applied at 19 and 29 months of age (Mental Development Index at 19 months and at 29 months, and Activity Level at 29 months; Davidson et al., 1995). The scatterplots reproduced from the digitizing process are shown in Figures 3 to 7. Also shown in these figures are the fitted curves from the k-power model associated with the BMD and the BMDL.

Table 15 contains the BMDLs calculated for the five test scores for which scatterplots were available, calculated using both the k-power dose–response model and the Weibull model. Since it was unclear whether reduced or increased activity in children would be considered adverse, BMDLs were calculated using both assumptions. (When lower [higher] were assumed to be adverse, the parameter,

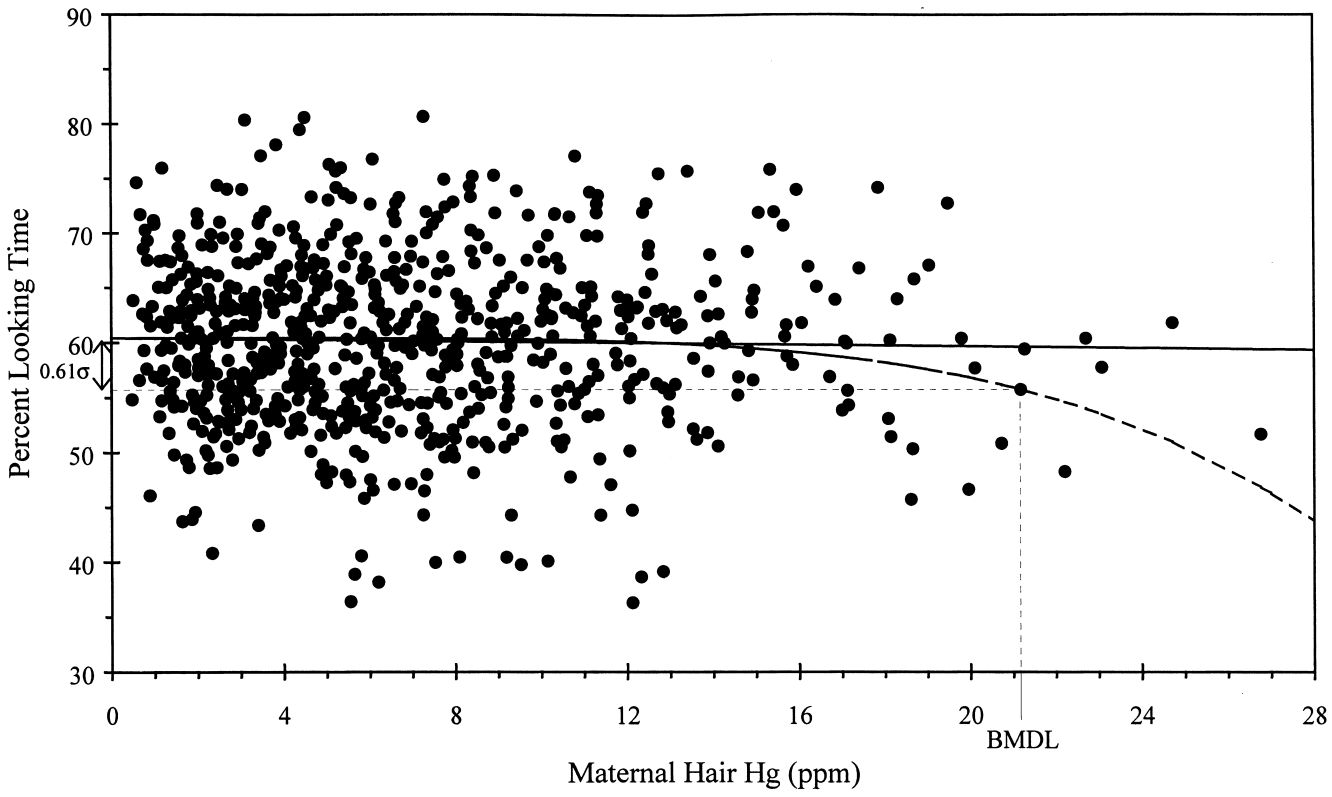

Figure 3. Digitized data for visual recognition at 6 months in Seychellois children (Myers et al., 1995a, Figure 2A). Each data point represents the overall cohort mean + the partial residual. The partial residual is defined as the subject's score adjusted for all variables in the reduced model except mercury (computed by adding the mercury effect to the residual from the reduced model). BMDL is ppm Hg associated with a test score decrement of 0.61σ. ——— Fitted mean response line, m(e), using the k-power model. - - - - Line defined by BMDL, $\sigma = 7.71$.

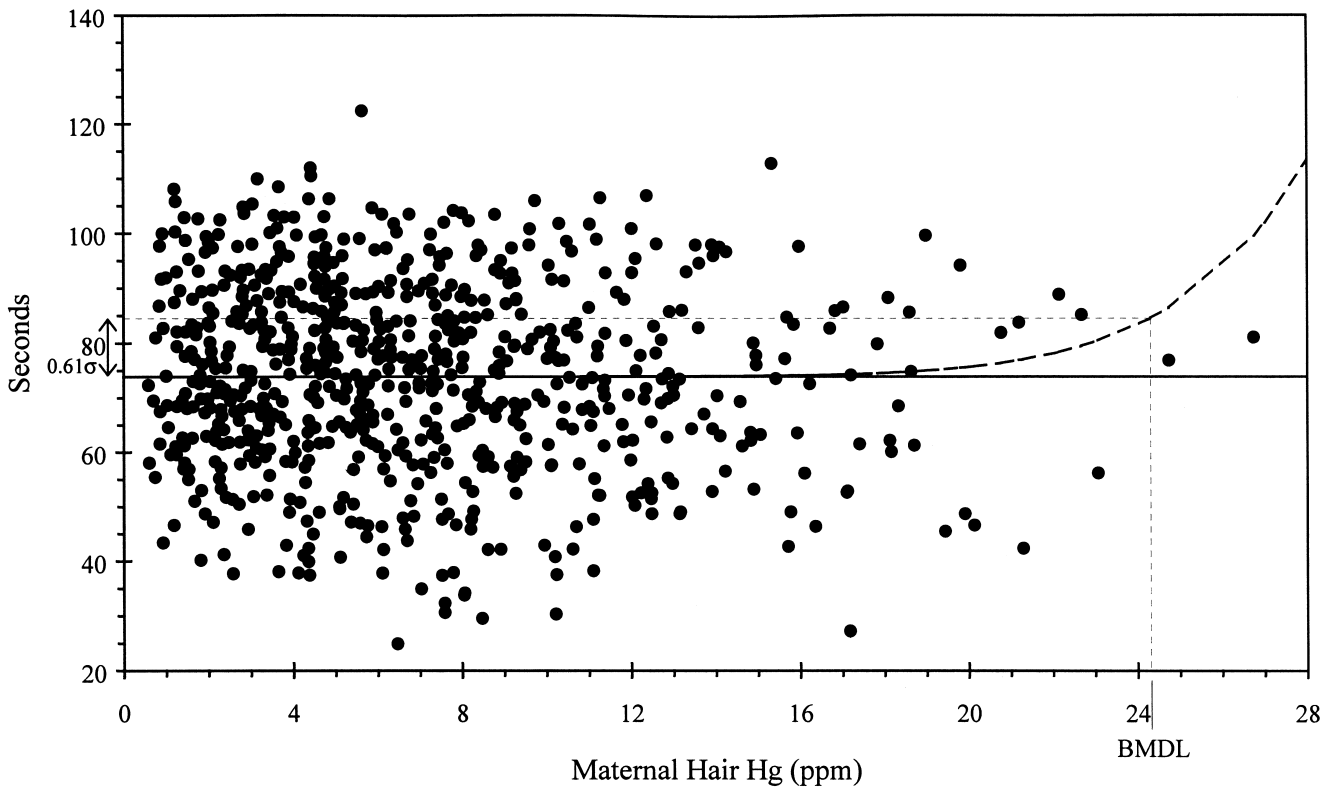

Figure 4. Digitized data for visual attention at 6 months in Seychellois children (Myers et al., 1995a, Figure 2B). Each data point represents the overall cohort mean + the partial residual. The partial residual is defined as the subject's score adjusted for all variables in the reduced model except mercury (computed by adding the mercury effect to the residual from the reduced model). BMDL is ppm Hg associated with a test score decrement of 0.61σ. ——— Fitted mean response line, m(e), using the k-power model. - - - - Line defined by BMDL, $\sigma = 17.4$.

β, in expression (1) was constrained to be nonpositive [nonnegative].)

BMDLs calculated from the Seychelles data (Table 15) ranged from 21 to 26 ppm maternal hair mercury. BMDLs based on the k-power and Weibull models were very similar.

A number of secondary exploratory benchmark calculations (50 in all) were also developed from the Seychelles data by applying the k-power and Weibull models for continuous data to grouped data from results of tests administered to children at 19 and 29 months of age (Davidson et al., 1995; Table 2), by applying the Weibull model for quantal data to grouped data (This model was applied by USEPA (1997) to the Iraqi data.) from neurological tests administered to children at 6 months of age (Myers et al., 1995a, Table 1), and by using the log-transform of maternal hair mercury in these analyses in addition to the untransformed hair mercury value. The 50 BMDLs from this battery of supplemental analyses ranged from 16 to 41 ppm and consequently are quite similar to the results reported in Table 15. Although these analyses are based on grouped data and therefore, are not considered as definitive as the primary analyses presented in Table 15, these exploratory analyses are supportive of the results reported in Table 15.

Comparison to BMDLs Obtained from the New Zealand Data

Table 16 presents BMDLs from Crump et al. (1998) calculated for results of five neurological tests (TOLD — spoken language; WISC-RP — performance IQ; WISC-RF — full scale IQ; MCC-PP — McCarthy scale of perceptual ability; MCC-PP — McCarthy scale of motor coordination) applied to children in the New Zealand cohort at 6 or 7 years of age. These BMDLs were calculated using the k-power model in the same way as it was used in calculating the BMDLs in Table 15 from the Seychelles data. BMDLs obtained from the entire cohort range from 17 to 24 ppm hair mercury and consequently are quite similar to those obtained from the Seychelles data. One New Zealand child, whose mother had a mercury hair level of 86 ppm, was quite influential in this analysis; although there is no apparent statistical or biological reason for omitting this child from the analysis (this child's test scores were not identified as outliers), BMDLs were 2 to 2.5 times smaller when this child was omitted. Because of limitations of the

New Zealand study discussed earlier, it is not believed to provide as reliable a basis for determining an RfD for mercury as the Seychelles study. In particular, since the New Zealand study was considerably smaller than the Seychelles study, BMDLs based on the New Zealand study would be expected *a priori* to be smaller than those based on the Seychelles study. Nevertheless, the BMDLs reported in Table 16 are in the range of those obtained from the Seychelles study.

In summary, the best data currently available for benchmark calculations for methylmercury are from scatterplots of residuals of test scores of children 6, 19 and 27 months of age from the Seychelles study. Interim benchmark calculations obtained by digitizing these data and reanalyzing them resulted in BMDLs that ranged from 21 to 26 ppm hair mercury. These BMDLs are similar to other BMDLs calculated using summarized data from the Seychelles study and to BMDLs calculated from the New Zealand study. The information currently available from the Faroe Islands is not provided in a form that is suitable for performing benchmark calculations. Based on these calculations, we conclude that an appropriate BMDL for calculation of an RfC for methylmercury would be 21 ppm hair mercury.

Estimation of recommended intake for the critical study using a PBPK Model-Based Monte Carlo Analysis

The determination of an acceptable daily intake of methylmercury from fish ingestion requires interrelating several measures of exposure. Specifically, the measure of exposure used in the epidemiological study, mercury in maternal hair, must be related to the measure of exposure desired for the guideline, daily ingested dose. However, the relationship between maternal hair levels and ingested dose is most correctly viewed as a combination of two factors, each of which represents the relationship between one of these external exposure measures and an internal measure of the biologically effective exposure. For the neurotoxic effects of methylmercury on a developing fetus, such a biologically appropriate measure of exposure would be methylmercury concentrations in the fetal brain over the period of greatest susceptibility, which has been hypothesized to be either the second or the third trimester of gestation (Amin-Zaki et al., 1979; Choi et al., 1978). Since

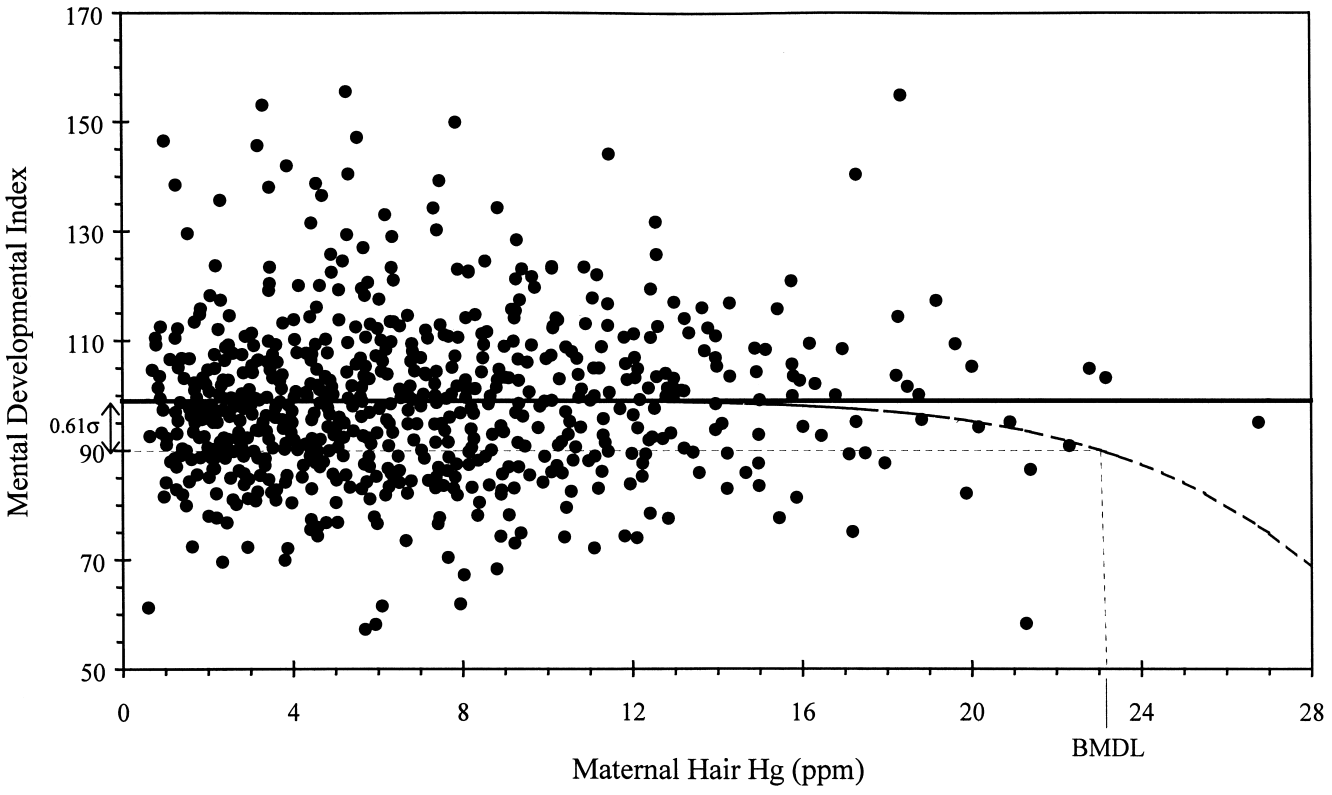

Figure 5. Digitized data for mental development index (MDI) at 19 months in Seychellois children (Davidson et al., 1995, Figure 1A). Each data point represents the overall cohort MDI mean + the partial residual. The partial residual is defined as the subject's MDI score adjusted for all variables in the reduced model except mercury (computed by adding the mercury effect to the residual from the reduced model). BMDL is ppm Hg associated with a test score decrement of 0.61σ. ——— Fitted mean response line, m(e), using the k-power model. - - - - Line defined by BMDL, $\sigma = 14.9$.

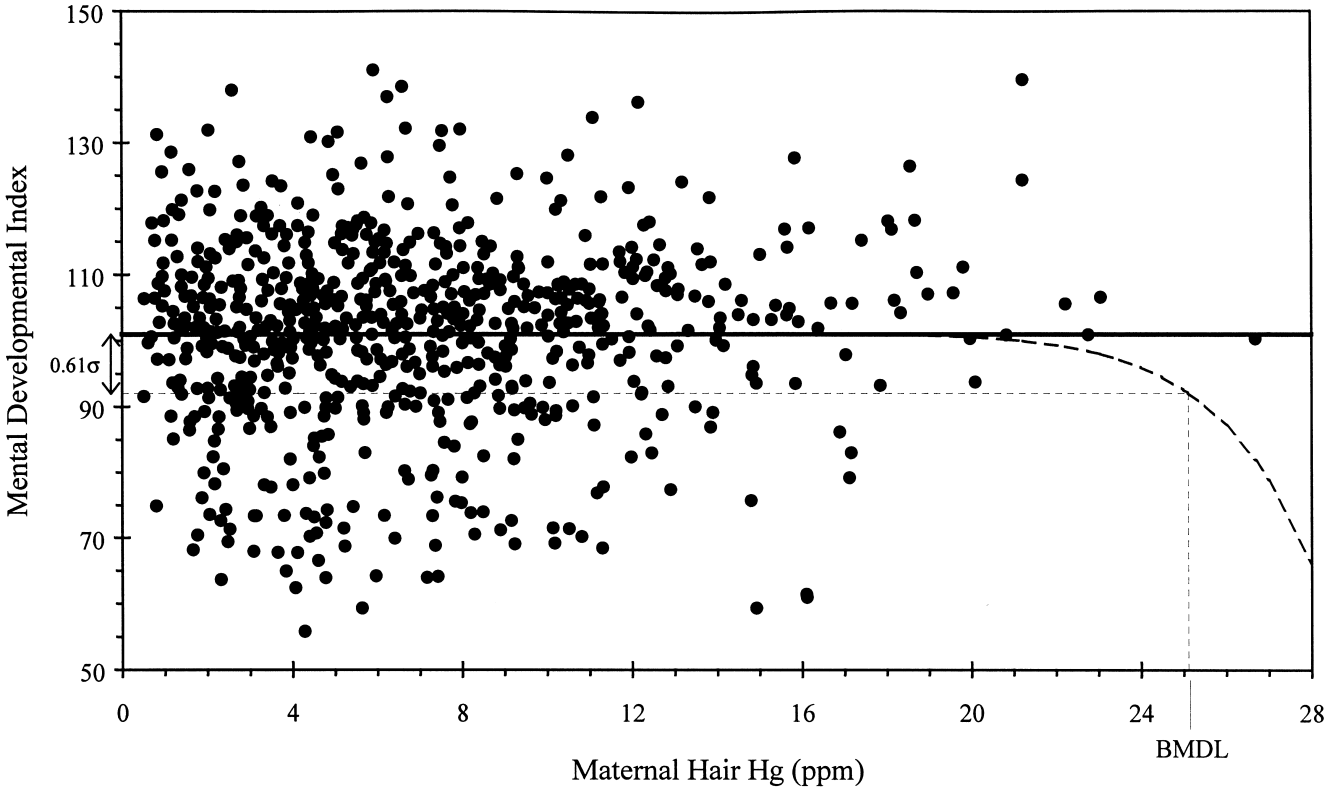

Figure 6. Digitized data for mental development index (MDI) at 29 months in Seychellois children (Davidson et al., 1995, Figure 1B). Each data point represents the overall cohort MDI mean + the partial residual. The partial residual is defined as the subject's MDI score adjusted for all variables in the reduced model except mercury (computed by adding the mercury effect to the residual from the reduced model). BMDL is ppm Hg associated with a test score decrement of 0.61σ. ——— Fitted mean response line, m(e), using the k-power model. - - - - Line defined by BMDL, $\sigma = 14.7$.

it is clearly impossible to obtain such data, a surrogate measure of exposure must be chosen that, preferably, bears a reasonably constant relationship to the desired measure over the exposures of interest, from those observed in the epidemiological studies to those expected in the population being protected. Methylmercury is readily transported across the placenta and into the fetal brain by processes that appear to be both linear and symmetric; therefore, maternal blood levels would appear to be a logical choice. The exposure measure in the Seychelles epidemiology study, average maternal hair mercury concentration during pregnancy, provides a time-weighted-average exposure estimate over a time period surrounding the window of greatest fetal susceptibility. For relatively constant exposures, such as the case of populations regularly ingesting fish containing methylmercury, this average value can reasonably be used as a surrogate for an exposure throughout pregnancy. Therefore, average concentrations of mercury in maternal hair during pregnancy can be used as a surrogate for fetal exposure by using data on the hair:blood partitioning of methylmercury to estimate the concentration in maternal blood corresponding to a given concentration in the hair. However, since the quantity of interest for setting an RfD is the daily ingestion rate, it is also necessary to relate concentrations of methylmercury in hair or blood to the corresponding rates of ingestion. This relationship can be described by making use of the pharmacokinetic information available on methylmercury in humans.

Because of the heterogeneity of the human population, it is generally expected that there will be variability in individual responses to the same exposure to a toxic chemical. Often it is possible to distinguish specific classes of individuals who appear to be more susceptible to a specific effect, such as the fetus in the case of methylmercury neurotoxicity. Noncancer risk assessments typically address this variability by dividing the experimentally determined acceptable exposure level by an uncertainty factor of up to 10 to protect sensitive individuals. PBPK modeling provides the capability to quantitatively describe the potential impact of pharmacokinetic factors on the variability of individual responses. In brief, a PBPK model provides a quantitative structure for determining the effect of pharmacokinetic factors on the relationship between the

external (environmental) exposure and the internal (biologically effective) target tissue exposure, and has been demonstrated to be an important tool for environmental risk assessment (Clewell, 1995; Clewell et al., 1997). When coupled with Monte Carlo analysis, the PBPK model provides a method to assess the quantitative impact of these sources of variability on individual response (as opposed to average population response) by comparing model predictions over the distributions of input parameter values (Clewell and Andersen, 1996).

Studies of human variability (Clewell and Andersen, 1996; Hattis and Silver, 1994; Hattis et al., 1987) have concluded that while pharmacokinetic variation is an important component of the observed interindividual variability in response, pharmacodynamic factors also play a significant role. In particular, the greater susceptibility of the fetus, compared to the adult, to the effects of methylmercury appears to be due to pharmacodynamic differences, specifically, the greater susceptibility of the growing fetal brain to the mitotoxic effects of mercury compared to the adult brain, in which there is essentially no cell division (Choi et al., 1978). However, pharmacokinetic variability can still be expected across a population of mother–fetus pairs. Therefore, this variability should be considered in attempting to relate ingestion rates to hair concentrations.

In their risk assessments for methylmercury, both the USEPA (1997) and ATSDR (1997) employed one-compartment, empirical pharmacokinetic models to describe the relationship between hair concentration and ingestion rate of methylmercury. Parameters in these models were chosen on the basis of data regarding the kinetics and partitioning of methylmercury in human subjects. Based on the selected parameter values, the Agencies then calculated "best estimates" of an average daily ingestion rate that would produce a given hair concentration. The USEPA (1997), which based its RfD on a study of a relatively small Iraqi mother–infant cohort (Marsh et al., 1987), also evaluated the uncertainty in this "dose conversion factor" (DCF) resulting from the potential variability in the pharmacokinetics of methylmercury across the U.S. population, and applied an uncertainty factor of 3 to address this concern in their derivation of the RfD. ATSDR (1997) felt that no uncertainty factor for variability was required in the

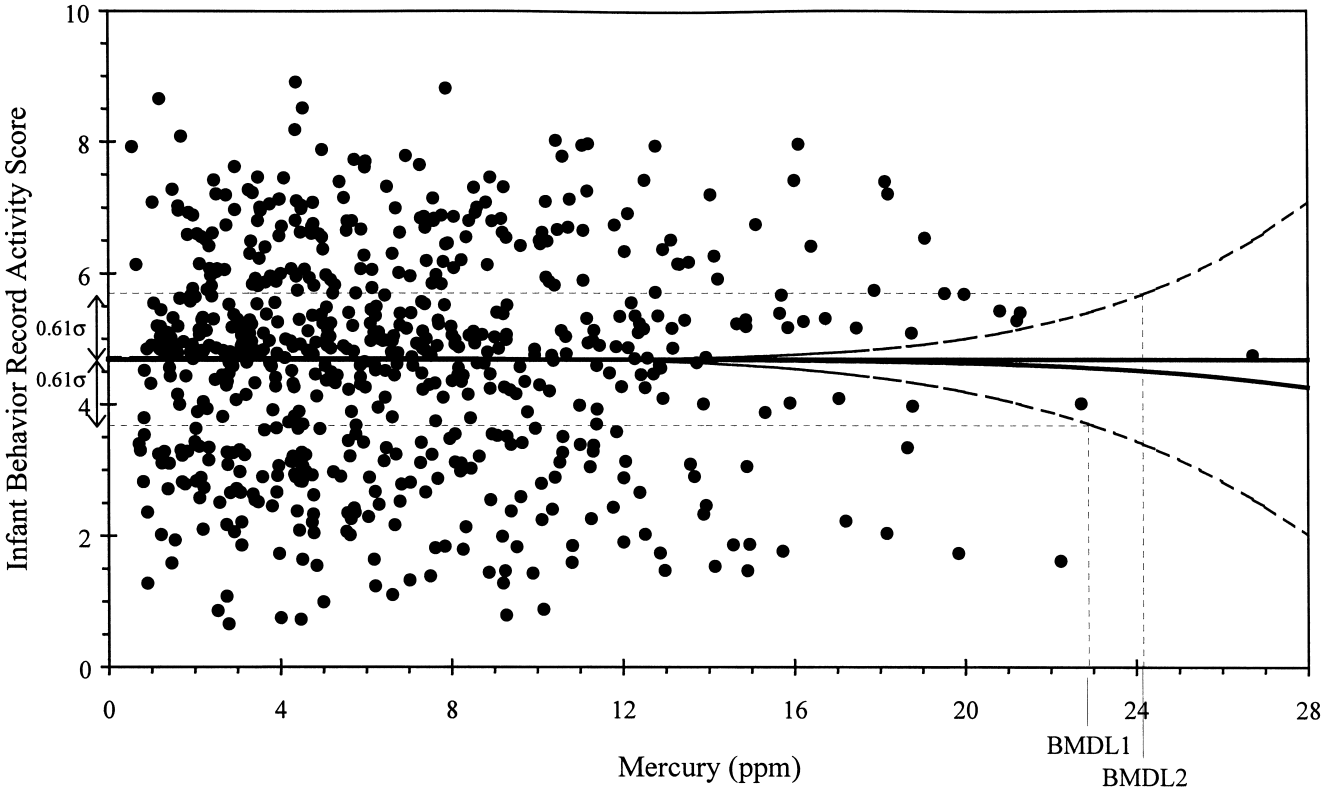

Figure 7. Digitized data for activity level at 29 months in Seychellois children (Davidson et al., 1995, Figure 2). Each data point represents the overall cohort activity level mean + the partial residual. The partial residual is defined as the subject's activity level score adjusted for all variables in the reduced model except mercury (computed by adding the mercury effect to the residual from the reduced model). Activity is scaled from 0 (none) to 9 (much). BMDL is ppm Hg associated with a test score decrement of 0.61σ. ——— Fitted mean response line, m(e), using the k-power model. - - - - Line defined by BMDL, $\sigma = 1.66$. BMDL1 = reduced activity adverse; BMDL2 = increased activity adverse.

case of its minimal risk level (MRL), which was based on the much larger Seychelles cohort.

In this section, a similar procedure to that performed by the USEPA (1997) will be described. However, the procedure differs from that used by the Agency in two respects: (1) instead of an empirical pharmacokinetic model, a PBPK model will be used, and (2) instead of predicting a "best estimate" and uncertainty region, an analysis of the variability in the relationship between ingestion rate and hair concentration (i.e., the DCF) will be used to define the distribution of ingestion rates over the population of women of childbearing age in the U.S. that would be associated with the hair concentration at the BMDL found in the Seychelles. This approach assumes, consistent with the evaluation by ATSDR (1997), that the impact of interindividual variability, both pharmacokinetic and pharmacodynamic, in the Seychelles population is adequately addressed by the use of a large cohort in the study defining the BMDL. Therefore, only the impact of variability in the U.S. population is addressed in this analysis.

The use of a PBPK model in place of an empirical compartmental description in an analysis of variability provides several benefits. The principal benefit is the structural framework the model provides, which defines the functional relationship between the physiological, chemical, and pharmacokinetic factors determining the uptake, disposition, and clearance of methylmercury in an individual. In the empirical approach it is necessary to combine parameters that are primary determinants of kinetic behavior, such as body weight and hair/blood partition, with parameters that are empirical measures of the kinetics resulting, in part, from these primary determinants, such as the fraction of methylmercury body burden in the blood and the half-life for its excretion. In this case, the latter two parameters reflect the results of complex underlying processes, and are functionally dependent on the former two parameters. However, in the compartmental description any functional relationship between the parameters must be determined empirically, an approach that is often hindered by the lack of adequate data (USEPA, 1997). Another advantage of the PBPK model is its ability to integrate data obtained for different exposure scenarios, routes of exposure, and species in order to test the ability of the model's functional structure to properly simulate the determinants of methylmercury pharmacokinetics.

Table 15. Benchmark (BMDL)[a] maternal hair concentrations (ppm) based on the Seychelles study.

Test	*k*-Power model	Weibull model	Reference
Results at age 6 months			
Visual Recognition Memory	21	21	Myers et al. (1995a), Figure 2A
Visual Attention	24	24	Myers et al. (1995a), Figure 2B
Results at age 19 months			
Mental Development Index	23	22	Davidson et al. (1995), Figure 1A
Results at age 29 months			
Mental Development Index	25	26	Davidson et al. (1995), Figure 1B
Activity Level			Davidson et al. (1995), Figure 2 (males and females combined)
Increased activity	23	22	
Decreased activity	24	24	

[a]95% statistical lower bound on maternal mercury hair concentration associated with an increase of 0.1 in the probability of an adverse effect (or, equivalently with this model, an adverse change in the mean response equal to 1.1 times the standard deviation of the response).

Table 16. Benchmark (BMDL)[a] maternal hair concentrations (ppm) based on the New Zealand study (Crump et al., 1998).

Test	*k*-Power model	
	All New Zealand children	Omitting child with high mercury level
TOLD-SL	20	9.5
WISC-RP	24	10
WISC-RF	21	10
MCC-PP	17	7.4
MCC-MOT[b]	21	9.8

[a]95% statistical lower bound on maternal mercury hair concentration associated with an increase of 0.1 in the probability of an adverse effect (or, equivalently with this model, an adverse change in the mean response equal to 1.1 times the standard deviation of the response). Significant covariates other than mercury were also included in the models.
[b]In order to achieve normally distributed residuals, $-\ln\{101-[\text{test score}]\}$ was used as the independent variable.

Methodology

The PBPK model of methylmercury in the human used in this analysis has been described previously (Gearhart et al., 1995). The structure of the maternal model (Figure 8A) is based to a large extent on the PBPK model of methylmercury in the rat developed by Farris et al. (1993), and includes compartments for the plasma, red blood cells, brain, kidney, liver, gut tissue, other richly perfused organs, slowly perfused tissues (representing primarily muscle and skin), placenta, urine, intestinal contents, and feces. Compartments for hair and remaining nonperfused tissues (bone, cartilage) are also included in the model. Enterohepatic recirculation of methylmercury is described by the excretion of methylmercury in the bile (kb) and its subsequent reabsorption into the gut tissue (kr). Oral absorption is modeled as zero-order stomach emptying (k0) followed by intestinal absorption (kr). The transport of methylmercury and its conversion to inorganic mercury (ki)

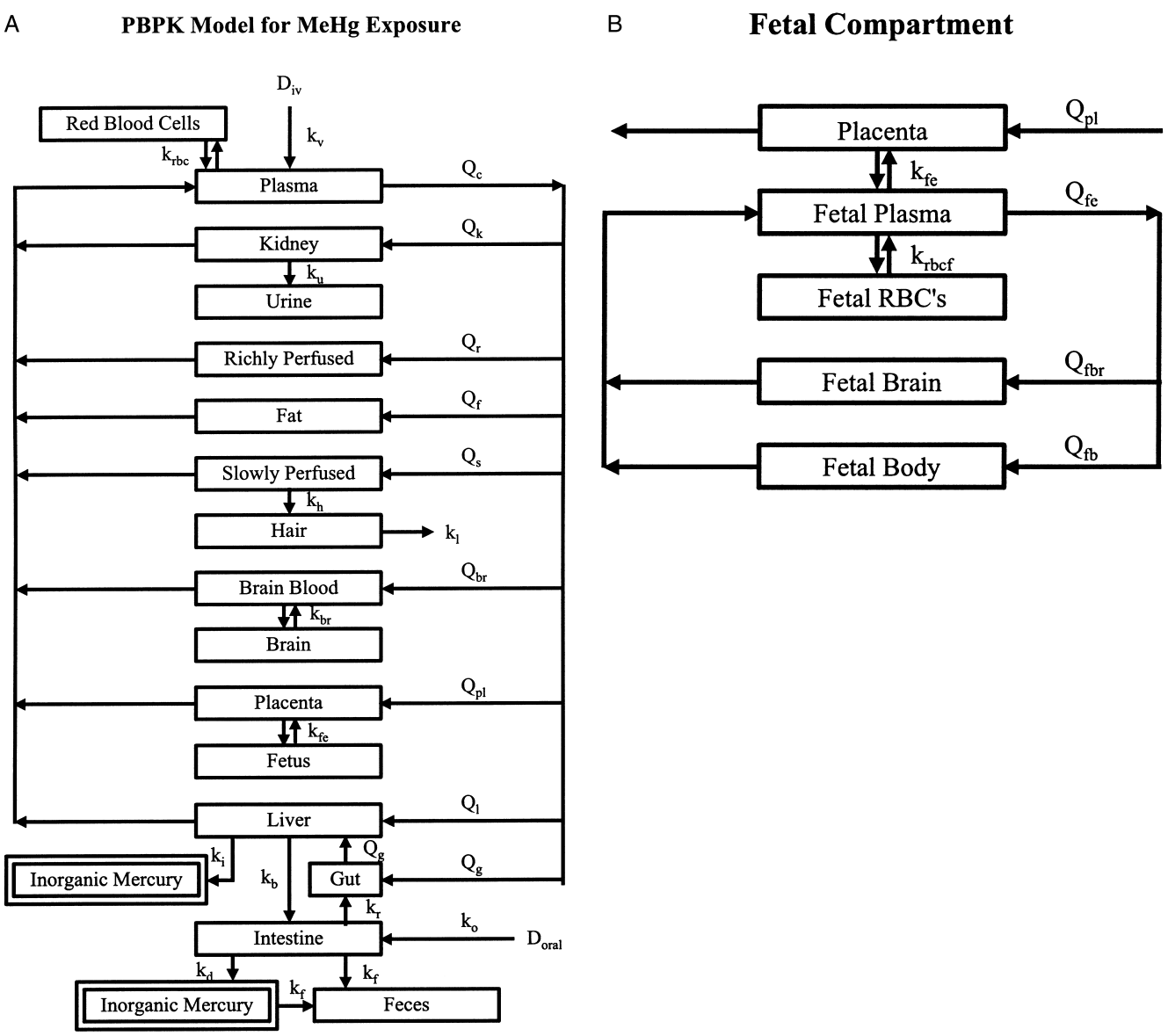

Figure 8. (A) PBPK model for MeHg exposure. (B) Fetal compartment.

in the model is described by linear processes. Distribution in the blood is assumed to be plasma-flow limited, with the exception of transport across the placenta (kfe), blood–brain barrier (kbr), and red cell membranes (krbc and krbcf), which are considered to be diffusion limited. The most important excretion mechanisms for mercury are excretion in hair (kh) and conversion of methylmercury to inorganic mercury by the gut flora (kd), with subsequent excretion of inorganic mercury in the feces (kf). Urinary excretion (ku) only becomes important at the higher experimental doses used in animals, in which cases renal damage often occurs. Following the approach of Farris et al. (1993), loss of hair (kl) and (in the case of rodents) reingestion of hair by preening (klr) are also described.

The fetal portion of the model (Figure 8B) consists of four compartments, which grow during the time of gestation: plasma, RBCs, brain and the remaining fetal tissue. Increases in maternal tissue during pregnancy are also described in the case of plasma, RBCs, richly perfused tissues (representing changes in the uterus and mammary glands), fat and fluid. The time-course for these physiological changes during pregnancy and gestation (see Appendix B) was taken from Hytten and Leitch (1971). Maternal dietary intake also increases over the course of pregnancy, based on data for U.S. women (Hytten and Leitch, 1971). The prepregnancy tissue volumes (*V*s) and blood flows (*Q*s) in the model are standard values taken from Brown et al. (1997) and ICRP (1975), while the tissue/blood partition coefficients (*P*s)

were based on tissue mercury data from Berlin et al. (1975), Kitamura et al. (1976), Kawasaki et al. (1986), and Vahter et al. (1994). Tissue volumes are scaled in the model in proportion to body weight, while blood flows and kinetic parameters (in the form of clearances) are scaled in proportion to body weight raised to the three-quarters power (USEPA, 1992). The kinetic parameters in the model were estimated either from the physiological literature or by simultaneously fitting data from a number of methylmercury pharmacokinetic studies for a variety of dosing scenarios in both monkeys (Charleston et al., 1994; Gunderson et al., 1986; Kawasaki et al., 1986; Lind et al., 1988; Mottet et al., 1987; Rice et al., 1989) and humans (Birke et al., 1972; Hislop et al., 1983; Miettinen et al., 1972; Sherlock et al., 1984; Smith et al., 1994).

The resulting model is able to accurately describe both the uptake and clearance of methylmercury in hair and blood for human volunteers ingesting various diets of methylmercury in fish (Gearhart et al., 1995). Plots showing the ability of the model to simulate the timecourse of methylmercury in hair and blood for two of the key fish-consumption studies (Hislop et al., 1983; Sherlock et al., 1984) are presented in Appendix C (Figures C-6 and C-7). For the application described in this report, the model was run at a constant daily dietary intake of methylmercury (1 μg/kg/day) until steady state was achieved in all maternal tissues. At this point (600 days into the exposure) the pregnancy was initiated and the dosing was continued until conception, at which time the average maternal hair concentration during pregnancy was calculated. Plots of the maternal hair and blood concentrations predicted by the model for this scenario are shown in Figure 9. The concentrations at the left end of the plots represent the steady-state conditions. The slight ($\sim$5%) increase in maternal hair concentration during pregnancy results from the increased dietary intake over this period. The maternal blood concentration, on the other hand, decreases steadily during pregnancy due to the dilution effect of the increase in maternal tissues (primarily fat) and fluids and of the growing fetus. This difference in behavior between the hair and blood results in a change in hair/blood partition over pregnancy, as discussed in Appendix C. Figure 10 shows the agreement of the model predictions for methylmercury in maternal and fetal blood with experimental data from a monkey exposed to a constant diet of 50 μg/kg/day (Gunderson et al., 1986). To obtain this simulation, physiological parameters for the monkey were used in the PBPK model in place of those for the human and the changes during pregnancy in the PBPK model were scaled from the human to the monkey on the basis of body weight (see Appendix B). The model reproduces the decrease in maternal blood concentration over pregnancy observed in this study, and is in reasonable agreement with both maternal and fetal blood concentrations. Appendix C also includes a plot (Figure C-8) that demonstrates the ability of the human version of the PBPK model to simulate maternal hair and blood concentrations during pregnancy, as well as fetal cord blood concentrations, for a mother–infant pair from the Iraqi poisoning incident (Amin-Zaki et al., 1976).

The PBPK model just described could simply be used directly with "best estimate" parameter values for humans to perform the conversion from hair concentration to daily ingestion rate. However, in order to provide a more complete description of the distribution of ingestion rates in a population that could result in a given hair level it was necessary to determine the variation in the model parameters that could be expected from one individual to another. Therefore, probability distributions for each of the model parameters were determined from the literature (see Appendix C) and used in a Monte Carlo analysis to determine the impact of pharmacokinetic parameter variability on the relationship between methylmercury ingestion rate and hair concentration. In the Monte Carlo simulation method, a probability distribution for each of the PBPK model parameters is randomly sampled, and the model is run using the chosen set of parameter values. This process is repeated a large number of times until the probability distribution for the desired model output has been created. In this study it was found that 1000 iterations were adequate to ensure the reproducibility of the mean and standard deviation of the output distributions as well as the 1st through 99th percentiles. To the extent that the input parameter distributions adequately characterize the variability in the inputs, and assuming that the parameters are reasonably independent, the resulting output distribution will provide a useful estimate of the variability associated with the model outputs.

Random sampling for the Monte Carlo analysis reported here was performed with the Latin hypercube method. Latin hypercube sampling provides a thorough coverage of the distributions using fewer iterations than the standard Monte Carlo method.

In performing a Monte Carlo analysis it is important to distinguish uncertainty from variability. As it relates to the impact of pharmacokinetics in risk assessment, uncertainty can be defined as the possible error in estimating the "true" value of a parameter for a representative ("average") person. Variability, on the other hand, should only be considered to represent true interindividual differences. Understood in these terms, uncertainty is a defect (lack of certainty) that can typically be reduced by experimentation, and variability is a fact of life that must be considered regardless of the risk assessment methodology used. Unfortunately, in practice it is often difficult to differentiate the contribution of variability and uncertainty to the observed variation in the reported measurements of a particular parameter (Allen et al., 1996).

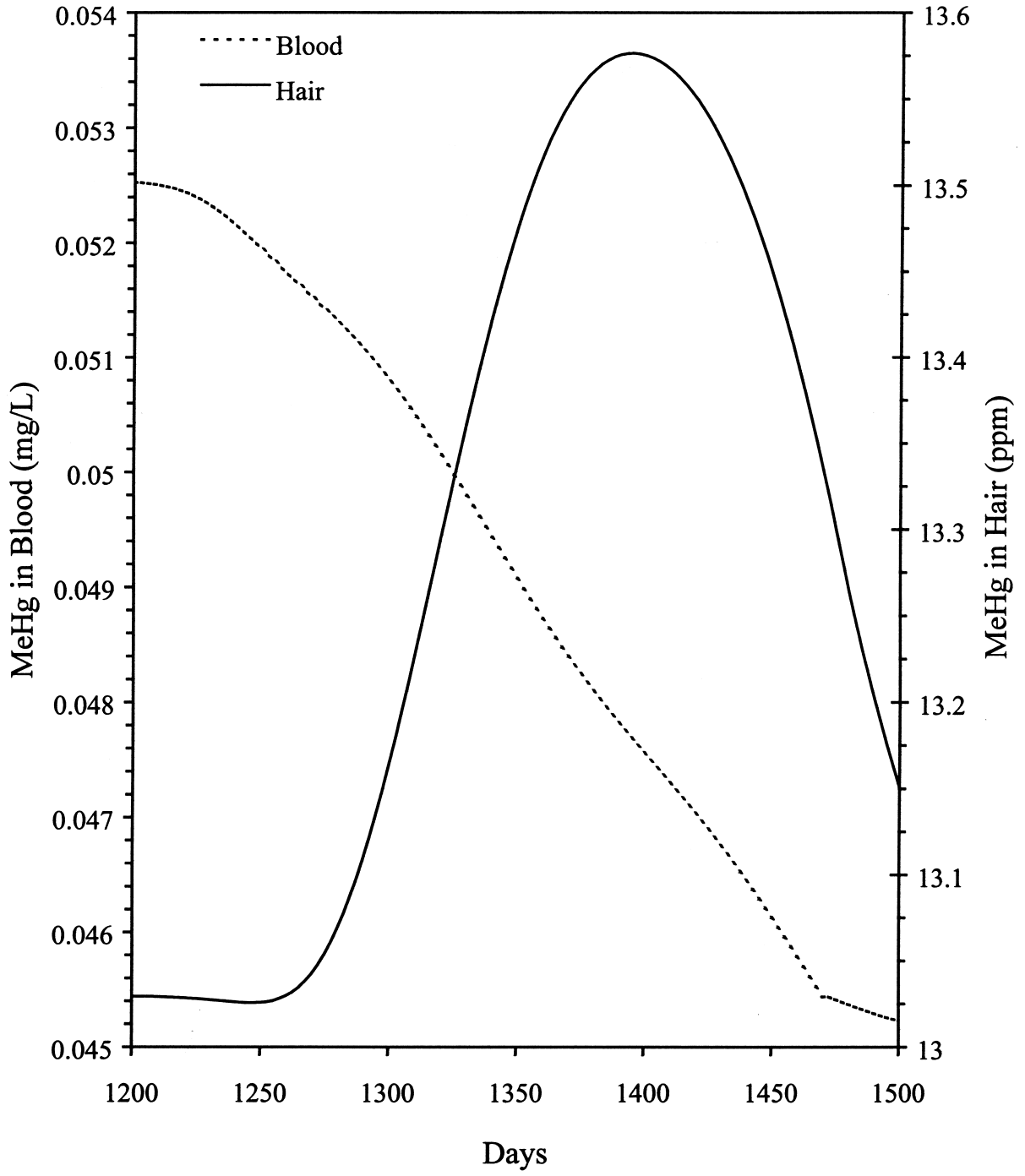

Figure 9. Maternal hair and blood concentrations during pregnancy predicted by the PBPK model for a daily MeHg ingestion rate of 1 μg/kg/day.

The parameter distributions used in the Monte Carlo analysis described here were chosen to represent interindividual variability; however, where there was doubt regarding whether differences between studies represented experimental uncertainty or population variability the conservative position was taken that the differences should be assumed to reflect interindividual variability. For example, it is likely that the study-to-study differences in the means of reported evaluations of the hair:blood partition coefficient for methylmercury reflect experimental bias due to differences in the analytical methodologies used rather than to real differences in the populations. If the differences

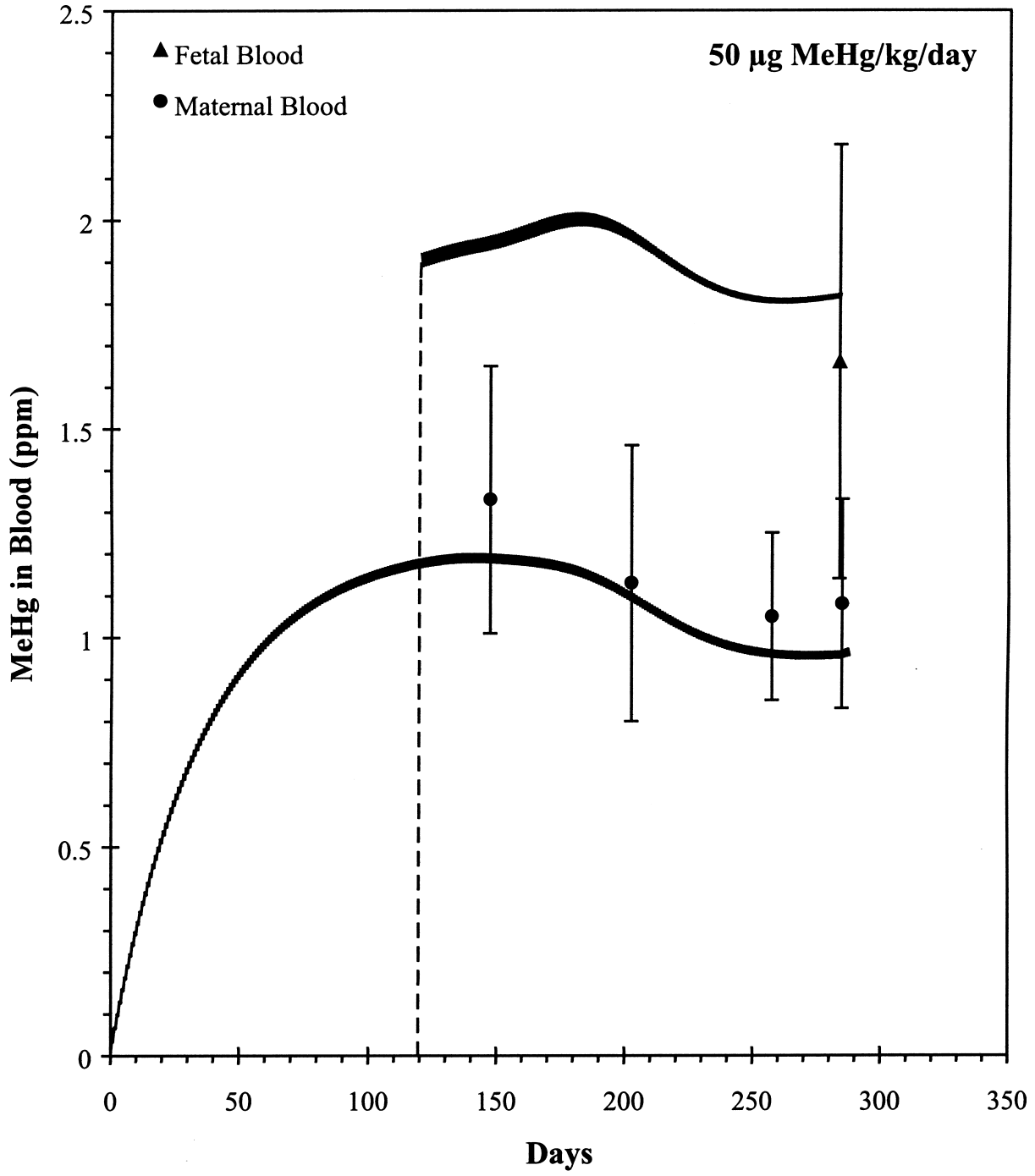

Figure 10. Maternal and fetal blood concentrations of MeHg before and during pregnancy in 10 monkeys (*Macaca fascicularis*) administered an average of 54 μg MeHg/kg/day. The data points represent the mean ± one SD for the 10 adult females and their 10 infants (data from Gunderson et al., 1986). The PBPK model was scaled from humans to monkeys as described in the text.

in reported means were indeed due to experimental bias, the study means could have been combined in an unweighted fashion, and a coefficient of variation could have been separately determined from one or more of the larger studies. A narrower distribution would have been obtained if the studies were combined in this way. However, the assumption of experimental bias cannot be supported by comparison data, so the distribution calculated in Appendix C assumes all of the reported measurements represent estimates of a single distribution. An elegant approach for

separately documenting the impact of uncertainty and variability is "two-dimensional" Monte Carlo, in which distributions for both uncertainty and variability are developed and multiple Monte Carlo runs are used to convolute the two aspects of overall uncertainty; unfortunately this approach still requires the ability to distinguish variability from uncertainty, a highly problematic decision in the case of most PBPK parameters (Allen et al., 1996).

To the extent that a particular PBPK model correctly reflects the pharmacokinetic and metabolic processes underlying the delivery of the active form of a chemical to the target tissue, PBPK modeling also provides a means for identifying the important physiological and biochemical parameters affecting individual risk. The technique for obtaining this information is known as sensitivity analysis and can be performed by two different methods (Clewell et al., 1994). Analytical sensitivity coefficients are defined as the ratio of the change in a model output to the change in a model parameter that produced it. To obtain a sensitivity coefficient by this method, the model was run for the exposure scenario of interest using the preferred values of the input parameters, and the resulting output (e.g., hair concentration) was recorded. The model was then run again with the value of one of the input parameters varied slightly. In this analysis a 1% change was used. The ratio of the resulting incremental change in the output to the change in the input represents the sensitivity coefficient. For example, if a 1% increase in an input parameter resulted in a 0.5% decrease in the output, the sensitivity coefficient would be -0.5. Sensitivity coefficients >1.0 in absolute value represent amplification of input error and would be a cause for concern. An alternative approach is to perform a simple correlation analysis of the model outputs and the input parameters. Both methods have specific advantages. The analytical sensitivity coefficient most accurately represents the functional relationship of the output to the specific input under the conditions being modeled. The advantage of the correlation coefficients is that they also reflect the impact of interactions between the parameters during the Monte Carlo analysis.

The parameter distributions used in the Monte Carlo analysis, expressed as means and coefficients of variation (CVs, where CV=standard deviation/mean), are defined in Table 17. The justification for the distributions of the key parameters used in the PBPK model during the Monte Carlo analysis are provided in Appendix C. In most cases, the means of the distributions are essentially identical to the parameter values identified during the development and validation of the model described above. The exceptions are those for which specific data more relevant to the specific population of interest (U.S. women of childbearing age) were available. For example, the distribution of body weights was obtained from the NHANES III database, and includes only women of childbearing age (14–45 years, inclusive) in the U.S. Distributions for the remaining kinetic and partitioning parameters not described in Appendix C were estimated in a similar fashion from available data. In some cases, where no data on the distribution of a parameter could be found, the parameter was assumed to have a distribution consistent (in a conservative direction) with those of similar parameters for which data were available (e.g., see discussion of PG in Appendix C). To avoid physiologically implausible values, all distributions were generally truncated at three standard deviations above and below the mean, and normal distributions were also truncated at 1% of the mean (to avoid negative or zero values). In the case of body weights, the extreme values were obtained directly from the NHANES III database.

Due to its physiological structure, many of the parameters in the PBPK model are interdependent. For example, the blood flows must add up to the total cardiac output and the tissue volumes must add up to the body weight. Failure to account for the impact of Monte Carlo sampling on these mass balances can produce erroneous results (Clewell et al., 1994). In addition, some physiological parameters are naturally correlated, such as cardiac output and respiratory ventilation rate, and these correlations should be taken into account during the Monte Carlo analysis (Allen et al., 1996). In its Monte Carlo analysis for methylmercury, the USEPA (1997) considered three correlations between parameters: blood volume with body weight, fraction of methylmercury in blood with body weight, and hair:blood partition with elimination half-life. The first and last of these correlations result naturally from the model structure, but the second does not. We reviewed the study (Sherlock et al., 1984) that served as the basis for the correlation between the fraction of methylmercury in blood and body weight in the USEPA (1997) analysis. It appears that the observed correlation actually reflects a higher ratio of men to women in the groups with the larger average body weights. Men have relatively less fat, per kilogram body weight, than women, and fat has a much lower partition for methylmercury than the other tissues. Thus, the negative correlation between fraction of methylmercury in the blood and body weight observed by the USEPA (1997) can be understood physiologically as a positive correlation between fraction of methylmercury in blood and fraction of fat in the body. To consider the impact of this correlation on the Monte Carlo analysis, an approach for explicitly treating correlations between fractional tissue volumes and body weight was developed (Appendix C) and was used in the primary Monte Carlo analysis. None of the other key input parameters in the PBPK model are expected to be significantly correlated.

As described above, the Monte Carlo analysis was performed in several steps. First, the parameter distributions were sampled to obtain the required number of parameter sets. Then the PBPK model was run with each of the

Table 17. Parameter distributions for Monte Carlo analysis.

Parameters		Means	CV	Upper bound	Lower bound	Distribution
Plasma flows (fraction of cardiac output)						
QCC	Cardiac output (l/h for 1 kg animal)	20.0	0.22	33.2	6.8	normal
QBrBC	Brain	0.114	0.30	0.217	0.011	normal
QFC	Fat	0.052	0.30	0.099	0.0052	normal
QGC	Gut	0.181	0.33	0.360	0.0018	normal
QKC	Kidney	0.175	0.30	0.333	0.0175	normal
QLC	Liver	0.046	0.32	0.0902	0.01	normal
QRC	Richly perfused tissues	0.183	0.30	0.348	0.0183	normal
QSC	Slowly perfused tissues	0.249	0.30	0.473	0.0249	normal
Qplm	Placenta (l/h per kg)	58.5	0.35	119.9	10.0	normal
QFeC	Fetal (l/h per kg)	54.0	0.30	102.6	10.0	normal
Tissue volumes (fraction of body weight)						
BW	Body weight (kg)	67.77	0.26	139.87	30.81	normal[a]
VBrC	Brain	0.02	0.30	0.038	0.002	normal
VBrBC	Brain plasma	0.007	0.30	0.013	0.0007	normal
VFC	Fat	0.273	0.24	0.47	0.076	normal
VGC	Gut	0.017	0.15	0.025	0.0094	normal
VHC	Hair	0.002	0.50	0.005	0.0001	normal
VIC	Intestine	0.014	0.30	0.027	0.0014	normal
VKC	Kidney	0.004	0.30	0.0076	0.0004	normal
VLC	Liver	0.026	0.25	0.046	0.0065	normal
VPC	Plasma	0.041	0.14	0.058	0.024	normal
VRBCC	Red blood cells	0.024	0.12	0.033	0.015	normal
VRC	Richly perfused tissues	0.10	0.30	0.19	0.01	normal
VSC	Slowly perfused tissues	0.35	0.16	0.52	0.18	normal
VRem	Remainder of body (nonperfused)	0.122	0.30	0.23	0.012	normal
Partition coefficients for MeHg						
PBr	Brain/blood	3.0	0.30	6.93	1.19	lognormal
PBrB	Brain blood/plasma	1.0	0.30	2.31	0.397	lognormal
PF	Fat/Blood	0.15	0.30	0.347	0.060	lognormal
PFe	Fetal plasma/placenta	2.0	0.30	4.62	0.794	lognormal
PG	Gut/blood	1.0	0.70	5.45	0.123	lognormal
PHB	Hair/blood partition coefficient	248.66	0.70	1361.68	30.4	lognormal
PK	Kidney/blood	4.0	0.30	9.24	1.59	lognormal
PLiv	Liver/blood	5.0	0.30	11.6	1.99	lognormal
PPl	Placenta/blood	2.0	0.30	4.62	0.794	lognormal
PRBC	Red blood cell/plasma	12.0	0.30	27.7	4.76	lognormal
PRBCFe	Red blood cell/plasma for fetus	14.0	0.30	32.4	5.56	lognormal
PR	Richly perfused tissues/blood	1.0	0.30	2.31	0.397	lognormal
PS	Slowly perfused tissues/blood	2.0	0.30	4.62	0.794	lognormal
Kinetic parameters (l/h for 1-kg animal)						
kbrici	Incorporation of brain to inorganic Hg	5.0e−5	0.0			lognormal
kbrili	Loss of inorganic Hg from brain	0.001	0.0			lognormal
kbrini	Brain MeHg to inorganic Hg (/h for 1 kg animal)	1.2e−5	0.30	2.77e−5	4.76e−6	lognormal
kbi	Biliary clearance of MeHg	0.0001	0.30	2.31e−4	3.97e−5	lognormal
kbri	Brain/brain plasma diffusion of MeHg	0.01	0.30	0.0231	3.97e−3	lognormal
kdi	MeHg to inorganic Hg in intestine	0.0001	0.30	2.31e−4	3.97e−5	lognormal
kfi	Fecal excretion of MeHg	0.0002	0.36	5.36e−4	6.60e−5	lognormal

Table 17. (continued)

Parameters		Means	CV	Upper bound	Lower bound	Distribution
khi	Excretion of MeHg into hair	7.0e−6	0.25	1.42e−5	3.25e−6	lognormal
kii	Loss of MeHg to inorganic Hg in liver	1.0e−5	0.30	2.31e−5	3.97e−6	lognormal
klri	Reingestion of hair	0.0	0.0			lognormal
krbci	RBC to plasma diffusion of MeHg	1.5	0.30	3.47	0.596	lognormal
kri	Intestinal reabsorption of MeHg	0.005	0.30	0.0116	1.99e−3	lognormal
Fetal kinetic parameters (l/h)						
kfe	Placenta/embryo diffusion of MeHg	1.0	0.50	3.69	0.217	lognormal
krbcfe	RBC to plasma diffusion of MeHg in fetus	100.0	0.50	369.0	21.7	lognormal

[a]Normal distribution used to permit correlation of BW and VFC during Monte Carlo analysis (lognormal distribution used in Monte Carlo analysis without correlation of BW and VFC).

parameter sets. The output of the Monte Carlo simulation was a distribution of hair concentrations (peak and average during pregnancy) and excretion half-lives associated with an ingestion rate of 1 μg/kg/day. To obtain the ingestion rate distributions, the hair distributions were inverted (to obtain a distribution of DCFs in μg/kg/day/ppm) and multiplied by the BMDL.

Results

The results of the sensitivity analysis are shown in Table C-1 of Appendix C. The parameters identified as most significant for relating dietary ingestion to hair concentration by the analytical sensitivity analysis were the hair excretion rate constant (khi), hair:blood partition coefficient (PHB), the body weight (BW), gut tissue:blood partition coefficient (PG), fecal excretion rate constant (kfi), fractional fat volume (VFC) and fractional slowly perfused tissue volume (VSC). These results were the basis for the selection of the parameter distributions to be more fully documented in Appendix C. The same parameters were also identified as significant by correlation analysis, with the exception of VSC. In the Monte Carlo analysis in which the relationship between percent fat and body weight was treated explicitly, limited correlations of several of the other tissues with hair concentration were induced; however, these correlations were not observed when the Monte Carlo analysis was repeated without the explicit fat:body weight adjustment. The most sensitive parameters for predictions of half-life were similar to those for hair concentration, with the addition of the partition coefficient for slowly perfused tissue:blood.

As a test of the predicted variability in global pharmacokinetic behavior of methylmercury for a population, the distribution of model-predicted half-lives for the clearance of methylmercury resulting from the Monte Carlo analysis was compared with empirical data from human studies. The performance of the Monte Carlo with respect to half-life provides a useful surrogate for the prediction of hair concentrations associated with a given dietary intake, because the sensitivities of these two outputs to the various input parameters are very similar (see Table C-1 in Appendix C). The mean and standard deviation for the half-life distribution predicted by the Monte Carlo analysis were 61±35 days. For comparison, a pooled distribution calculated from four published studies (Kershaw et al., 1980; Miettinen et al., 1972; Sherlock et al., 1984; Smith et al., 1994) had a mean and standard deviation of 49±7.5 days. Thus, the central tendency of the distribution for this model output is conservative but consistent with observations, while the Monte Carlo analysis tends to overestimate the observed variability. The large standard deviation produced by the Monte Carlo analysis clearly reflects the fact that the distributions calculated for the input parameters reflect not only interindividual variability, but also uncertainty regarding the true value of the parameters. As discussed above, including some level of uncertainty in the variability distributions is unavoidable. Fortunately, the impact of this overestimate of variability for the current risk assessment is conservative; that is, overestimating the variability in the outputs produces a lower RfD estimate in the portion of the exposure distribution that will be used in decision making. Specifically, in the case of calculating the distribution of dietary intakes associated with the BMDL, the dietary intakes associated with percentiles below the mean will tend to be underestimated (the estimates will be conservative).

The distribution of dietary methylmercury ingestion rates corresponding to the hair mercury concentration identified as the BMDL for the Seychelles study, 21 ppm, is shown in Figure 11. The geometric mean for this distribution is 1.60 μg/kg/day, with a geometric standard deviation of 1.33, and the percentiles are shown in Table 18. Several additional Monte Carlo analyses were performed to investigate the sensitivity of the resulting distribution to the approach used for the analysis. In the first alternative case, the explicit treatment of the correlation between the fractional fat volume and body weight was removed. In the second alternative case, only the seven key parameters

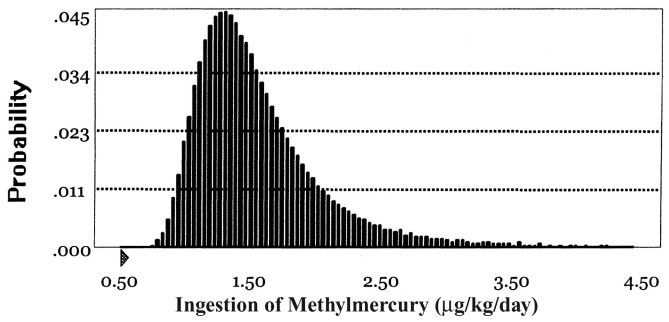

Figure 11. Methylmercury ingestion associated with BMDL in Seychelles population.

described in Appendix C were varied, and all the rest of the parameters in the model were fixed at their preferred (mean) values. In the third alternative case, all of the parameter distributions were changed to lognormal instead of the mix of normal and lognormal shown in Table 17. In all three cases, the resulting mean, standard distribution, and 5th through 50th percentiles in the distribution of ingestion rates were within 1% of the values obtained in the primary analysis. Somewhat greater differences were observed in the 1st percentile as well as in the 95th and 99th percentiles of the distribution.

The variability in ingestion rates predicted by this PBPK analysis is compared with two previous compartmental analyses (Stern, 1997; USEPA, 1997) in Table 19. In the PBPK analysis, the ratio of the ingestion rate at the 5th percentile in the distribution (1.04 μg/kg/day) to the median ingestion rate (1.58 μg/kg/day) is 0.66. The same ratio in the compartmental analyses is approximately 0.5. The somewhat greater variability predicted by the empirical one-compartment analyses probably results primarily from the inability of the empirical approach to represent the functional relationships between parameters, as already discussed. As mentioned earlier, in the analysis performed by Stern (1997) only one relationship was described explicitly: blood volume with body weight, while in the USEPA (1997) analysis three correlations between parameters were considered: blood volume with body weight, fraction of methylmercury in blood with body weight, and hair:blood partition with elimination half-life. In the PBPK model each of these relationships, as well as all the others, is defined functionally within the structure of the model and interacts with the parameter selections during the Monte Carlo analysis. For example, the USEPA (1997) estimated a correlation of -0.5 for hair:blood partition with half-life; the correlation observed in the PBPK Monte Carlo analysis was -0.66 (Table C-1). In the case of the PBPK analysis, however, the variability in the half-life was not used as one of the inputs to determine the variability of DCFs for hair, rather it was an output predicted in parallel with the DCFs.

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

Table 18. Percentiles in the distribution of daily methylmercury ingestion rates for women of childbearing age in the U.S. corresponding to the NOAEL (BMDL) hair concentration observed in the Seychelles fish-eating population study.

Percentile	Daily ingestion rate (μg/kg/day)
1	0.86
5	1.04
10	1.15
25	1.31
50	1.58
75	1.90
90	2.30
95	2.59
99	3.31

Table 19. Comparison of predicted variability of ingestion rate from Monte Carlo analyses.

Ratio of 5th percentile to median of distribution		
USEPA	Stern (1997)	PBPK
0.5	0.5	0.66

is an artifact of the simplified model structure. The basic description of the one-compartment model is:

$$\frac{VdC}{dt} = d^*\mathrm{BW}^*A^*f - b^*V^*C \quad (5)$$

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

Comparison of PBPK Approach with Empirical One-Compartment Models

A separate analysis was conducted to compare the estimated daily intake values using an empirical, one-compartment model, with those determined using the Monte Carlo approach described above. This comparison was conducted to determine how point estimates or preferred values (means of distributions from Monte Carlo analyses) would compare to the distribution derived using the BMDL of 21 ppm from the Seychelles study. As described earlier in this report, the form of the one-compartment model is

$$d = (C^*b^*V)/(A^*f^*\mathrm{BW}), \quad (6)$$

where d=daily dietary intake (μg methylmercury/kg/day); C=concentration of methylmercury in blood (μg/l); or C=concentration in hair/hair:blood partition (PHB); b=elimination constant (days^{-1}); V=volume of blood in the body (l); A=absorption factor (unitless); f=fraction of daily intake taken up by the blood (unitless); BW=body weight (kg).

Daily ingestion rates (d) were estimated using the preferred parameters for the one-compartment model reported by ATSDR (1997), Stern (1997) and USEPA (1997). In the case where parameter distributions were defined, the nominal values (Stern, 1997, Table 5; USEPA, 1997, Table D-2) reported for those parameters were used. These preferred parameters are shown in Table 20. The right most column of Table 20 shows the corresponding means for the same parameters in the PBPK Monte Carlo analysis described here. It should be noted that in three cases (PHB, V, and BW) these represent the means of the input parameter distributions, but in the other three cases (b, f, and A) they represent predictions (outputs) of the PBPK model rather than inputs.

The main differences in the preferred values between ATSDR (1997), Stern (1997), and USEPA (1997) were due to differences in the studies selected to define the parameters. Stern (1997) defined more than one nominal value for several of the parameters due to uncertainty regarding the priority to be given to different studies. There were only minor differences between the ATSDR (1997) parameters and the two sets of parameters used by the USEPA (1997), resulting in relatively little difference in the estimated ingestion rates. Several of the parameter estimates used by Stern (1997), on the other hand, were significantly different from those selected by the two Agencies.

In order to have a consistent basis for comparison, the same initial hair concentration was assumed. The concentration in the blood "C" for input into the model was determined based on the BMDL of 21 ppm and the hair to blood ratio. To estimate C, the following equation was used:

$$\mathrm{C} = \mathrm{BMDL}/\mathrm{P_{HB}}. \quad (7)$$

The hair to blood partition coefficient (PHB) used by the USEPA and the ATSDR is based on several studies, with the most weight on information reported by Phelps et al. (1980). The value used by Stern (1997), on the other hand, is based on two other studies, Birke et al. (1972) and Kershaw et al. (1980). The mean used in the PBPK analysis

Table 20. Parameters for use in a deterministic one-compartment model and comparison with PBPK model inputs/outputs.

	USEPA RfD	USEPA Monte Carlo	ATSDR	Stern (1997)	PBPK
P_{HB}	250	250	250	292	249[a]
b ($t_{1/2}$)	0.014 day^{-1} (50 day)	0.013 day^{-1} (53 day)	0.014 day^{-1} (50 day)	0.011/0.014 day^{-1} (63/50 day)	0.011 day^{-1} (61 day)[b]
V (l)	5	5	4.2	3.6/3.8	4.4[a]
f	0.05	0.059	0.05	0.067/0.077	0.059[c]
A	0.95	0.95	0.95	0.94	0.98[c]
BW (kg)	60	55	60	63	68[a]

[a]Mean of parameter distribution used in Monte Carlo analysis.
[b]Mean of predictions from Monte Carlo analysis with PBPK model (not an input parameter).
[c]Prediction of PBPK model using mean parameter values (not an input parameter).

was calculated from these three studies plus seven others in the literature, as discussed in Appendix D.

As discussed by Stern (1997) and in Appendix C, an analysis of the published data on the excretion half-life for methylmercury is consistent with a value of approximately 50 days (b=0.014 day^{-1}), and this value was used in most analyses. Stern (1997) also calculated a value of 63 days (b=0.011 day^{-1}) from a study of the population of concern (Iraqi) for his analysis. As discussed in Appendix C, the mean half-life predicted by the PBPK Monte Carlo analysis was 61 days.

For its assumption of volume of blood in the body, the USEPA (1997) assumed that blood volume is 7% of body weight, and that an increase of 20–30% occurs during pregnancy, resulting in a blood volume of approximately 8.5–9% body weight. Assuming a body weight of 58 kg and a blood volume of 9%, this resulted in an estimated blood volume of 5.22 l, which was rounded to 5 l for the one-compartment model. ATSDR (1997) assumed a female body weight of 60 kg and a blood volume of 7%, resulting in a value of 4.2 l for *V*. Stern (1997) estimated a blood volume from three reported studies of blood volume in women. The mean value used in the PBPK analysis (the sum of the means for plasma and RBC volumes) represents an average of a large number of reported estimates for women, as summarized in Altman and Dittmer (1961) and Diem and Lentner (1971).

Estimates of the fraction of methylmercury in the blood range from 0.05 to 0.077 depending on the data analyzed (ATSDR, 1997; Stern, 1997; USEPA, 1997). Using the mean input parameter values, the PBPK model predicts a fraction of methylmercury in the blood of 0.059.

The fraction of methylmercury absorbed is generally considered to be well over 90% (ATSDR, 1997; USEPA, 1997). Stern used a value of 94% based on Miettinen et al. (1972). When the PBPK model was run under the conditions of the experimental protocol described in Miettinen et al. (1972), it predicted an absorption of 98%.

The USEPA (1997) used a default body weight of 60 kg, rounded from a value of 58 kg, for an adult female. ATSDR (1997) also used a default value of 60 kg for an adult female. Body weights used in the Stern (1997) and PBPK analyses were based on the NHANES III database.

The ingestion rates estimated using each set of preferred parameters are reported in Table 21. In comparing these estimates to percentiles in the distribution of daily methylmercury ingestion rates from the PBPK Monte Carlo Analysis, the preferred parameters of the USEPA RfD, USEPA Monte Carlo, and ATSDR analyses result in daily ingestion rates that correspond to approximately the 75th, 50th, and 50th percentiles, respectively. The range of ingestion rates calculated from the various parameter estimates in Stern (1997), on the other hand, are much lower, around the 1st percentile of the distribution. It should be noted that the predictions of the PBPK model using the mean parameter values have been validated against human data (e.g., Figures C-6, C-7, and C-8). It seemed unlikely that the much different parameterizations selected by Stern (1997) could provide an acceptable fit to these same data. Therefore, a one-compartment model was exercised for the purpose of comparing the ability of the various parameterizations in Table 21 to reproduce actual human data. As shown in Figure 12A, it is possible to reproduce the data from Sherlock et al. (1984) reasonably well with the one-compartment description. The lines in this figure reflect, from top to bottom for each dose, the parameterizations of the EPA Monte Carlo analysis, the ATSDR analysis, and the EPA RfD analysis, as listed in Table 21. In contrast, the parameterizations used in the analysis by Stern (1997), as shown in Figure 12B, greatly overestimate the blood concentrations associated with the measured ingestion rate. Coupled with the fact that Stern (1997) also used a higher hair:blood partition coefficient than the other analyses, the parameterization selected by Stern significantly overestimates the ratio of hair concentration to ingestion rate. Thus, the ingestion rate estimates calculated by Stern (1997) represent very conservative estimates.

Table 21. Comparison of ingestion rates (μg/kg/day) estimated using a deterministic approach to rates estimated using a Monte Carlo approach.

Percentile	Monte Carlo	Deterministic
1	0.86	0.6–0.9 (Stern, 1997)
5	1.04	
10	1.15	
25	1.31	
50	1.58	1.7 (ATSDR, USEPA MC)
75	1.90	2.0 (USEPA)
90	2.30	
95	2.59	
99	3.31	

Application of uncertainty factors

The Seychelles study was selected as the critical study upon which to base both dose–response analyses to derive the BMDL and pharmacokinetically derived estimates of intake. Based on the analyses described in the preceding sections, the BMDL selected from the Benchmark analyses was 21 ppm maternal mercury hair concentrations, which when used with the PBPK-derived DCFs resulted in a distribution of daily dietary intake values represented in Table 17. The last step in the derivation of an RfD is to evaluate the overall sources of uncertainty and variability to determine the need for uncertainty factors and the magnitude of such factors. These factors are intended to

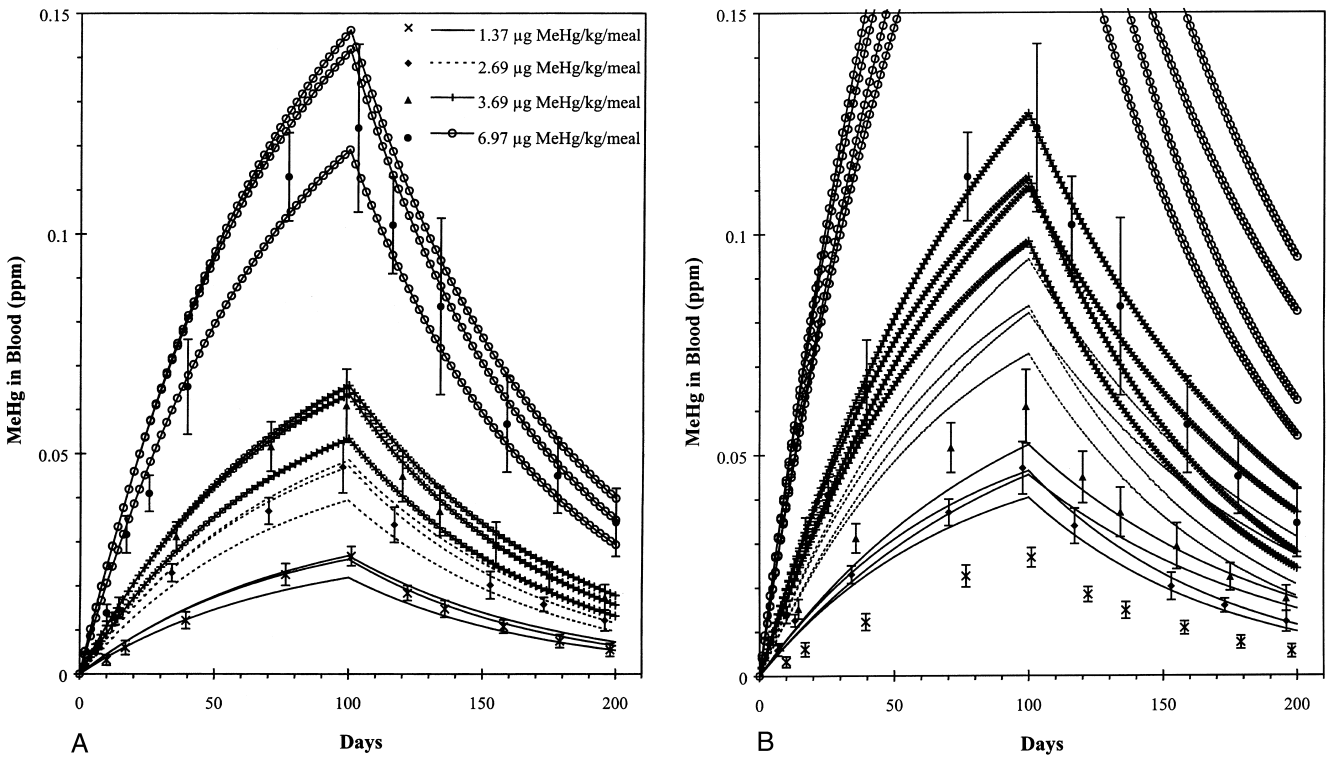

Figure 12. (A)Time course of MeHg in whole blood from human subjects consuming MeHg-contaminated halibut at 1.37 ($n=5$), 2.69 ($n=4$), 3.69 ($n=6$), or 6.97 ($n=5$) μg MeHg/kg/meal for three meals/week over approximately 96 days and followed after elevated ingestion levels ceased for approximately 100 days. The predictions of the PBPK model for this data set are shown in Figure C-7. The data points with bars represent the mean ± one SD for each group (data from Sherlock et al., 1984), and the lines represent the one compartment model simulation using parameters from USEPA (1997) RfD (lowest line at each dose), ATSDR (1997) (middle line), and USEPA (1997) Monte Carlo (highest). (B) Time course of MeHg in whole blood from human subjects consuming MeHg-contaminated halibut at 1.37 ($n=5$), 2.69 ($n=4$), 3.69 ($n=6$), or 6.97 ($n=5$) μg MeHg/kg/meal for three meals/week over approximately 96 days and followed after elevated ingestion levels ceased for approximately 100 days. The predictions of the PBPK model for this data set are shown in Figure C-7. The data points with bars represent the mean ± one SD for each group (data from Sherlock et al., 1984), and the lines represent the one compartment model simulation using parameters from Stern (1997) analysis.

capture those uncertainties (and in some cases, variability) inherent in either the extrapolation to the target population (e.g., across species or duration of exposure or for variability in sensitivity in the target populations) or due to limitations in total data base. While uncertainty factors are applied to a NOAEL or BMDL derived from (usually) a single study, decisions regarding the magnitude of the uncertainty factor(s) are based on a weight of evidence assessment of the total body of literature. The rationale for the continued use of the uncertainty factors applied by the USEPA for an RfD based on the Iraqi study was first considered. The rationale for application of uncertainty factors and the magnitude of those factors based on the use of the Seychelles data was then assessed.

Uncertainty Factors Applied by the USEPA in the Derivation of the RfD Based on the Iraqi Population

In the derivation of the RfD for methylmercury, USEPA applied a composite uncertainty factor of 10 based on the following rationale for two main areas of uncertainty: (1) to account for the variability in human pharmacokinetics (i.e., the parameters used in the one compartment model), and (2) database inadequacy, specifically, due to a lack of a two-generation reproductive assay in animals and concerns regarding the potential for long-term sequelae from developmental effects. The necessity (or lack thereof) for the application of an uncertainty factor for these reasons is discussed in the following sections.

Uncertainty for Human Variability

The USEPA considered the application of an uncertainty factor to the daily dietary intake estimated using the one-compartment model necessary to account for variability in those kinetic parameters that determined the relationship between mercury hair levels and estimated intake. The USEPA applied a deterministic approach, in that each parameter in the algorithm for estimating intake was a single point value. These values were derived from the literature and the most representative value, as identified by the USEPA, was used in the calculation. The USEPA conducted an uncertainty analysis in which each parameter

in this algorithm was defined by the distribution of values found in the literature. For example, hair to blood ratios ranged from approximately 140 to 416. For each parameter, the shape of the distribution was determined, e.g., lognormal, triangular, etc., and Monte Carlo simulation was used to create a distribution of intake values that could achieve a maternal hair level of 11 ppm. The ratio of the 95th percentile to the median of the resulting distribution was a factor of 2. However, the USEPA did not use any of the values from that distribution, i.e., the 50th or 5th percentile, as the basis for the RfD. Instead, the deterministic value derived from the preferred value was simply divided by factor of 3.

In the current analysis, an additional uncertainty factor for variability in kinetic parameters is no longer necessary because, through the use of PBPK modeling with distributions for each parameter in the PBPK model, a distribution of intake values that captures and quantifies the variability in each parameter was generated. Thus, the full range of biologically plausible values for the parameters have been incorporated into the PBPK/Monte Carlo analyses used in the estimation of intakes corresponding to the BMDL based on the Seychelles data. The estimated values for intake at the 50th and 5th percentile generated using this PBPK model differed by only a factor of 1.5, with a difference of a factor of 1.4 between the 50th and the 10th percentile. Rather than use a fixed uncertainty factor, such as a factor of 1.5, the selection of the intake value to use is left to the risk manager.

Uncertainty Due to Lack of Data on Chronic Sequelae

The USEPA incorporated as part of its composite uncertainty factor consideration of possible "sequelae of developmental neurotoxicity effects and adult paresthesia" (USEPA, 1997). Chronic sequelae can be defined as the progression of existing symptoms or the delayed or latent appearance of previously undetected symptoms either with continued exposure to or following cessation of exposure to methylmercury. Delayed and/or progressive neurological effects have been described in the poisoning episodes in Iraq (Clarkson, 1992), in Minimata and Niigata (Harada, 1982, 1995), and in animal studies (Evans et al., 1977; Rice et al., 1989; Spyker, 1975). These and other data were reviewed to assess the likelihood that either overt symptoms of neurotoxicity (e.g., paresthesia, visual disturbances) or more subtle effects (e.g., cognitive dysfunction) would develop or increase in severity later in life in children exposed *in utero* to methylmercury as a result of maternal ingestion of fish containing low levels of methylmercury. Upon review of these studies and the basis of concern for chronic sequelae following *in utero* exposure to methylmercury, the data strongly indicate that the likelihood of delayed appearance and/or progression of neurological effects is highly dose and dose-rate dependent. Based on the weight of evidence, discussed below, it is highly unlikely that chronic sequelae will result in populations exposed *in utero* to methylmercury as a result of maternal consumption of fish when methylmercury intake levels through fish consumption are at or below the levels recommended for this site-specific RfD. This conclusion is based on the following main issues.

- Latency (chronic sequelae) between exposure and onset of signs and symptoms of neurotoxicity has been seen only in populations exposed to high concentrations of methylmercury (Clarkson, 1992; Harada, 1982, 1995).
- Longitudinal studies in animals have reported delayed onset of neurological endpoints comparable to those seen in human populations (Evans et al., 1977; Rice et al., 1989; Spyker, 1975); however, the appearance, latency and the severity of those effects are highly dose and dose-rate dependent (Evans et al., 1977; Spyker, 1975).
- No association between lifetime, low-level methylmercury exposure (probably including *in utero* exposure) and selected neurophysiological and neurobehavioral tests was found in adult fish-eating populations (McKeown-Eyssen and Ruedy, 1983a,b; Turner et al., 1980).
- No long-term effects on cognitive function were detected in juvenile or adult monkeys exposed *in utero* to methylmercury (Gilbert et al., 1993; Rice, 1992, 1996) at levels that exceeded those expected for children exposed *in utero* due to maternal consumption of fish containing low levels of methylmercury.
- While the mechanism of action for chronic sequelae is not known with certainty, the evidence suggests mechanism of action for adult exposure that is different from those for *in utero* exposures and that the evidence suggests that there is a threshold for such effects (USEPA, 1997).

Each of these points is discussed in subsequent paragraphs.

Development of Delayed Effects in Poisoning Episodes.

Signs and symptoms of neurotoxicity in some of the exposed Iraqi patients were not evident until weeks or months after cessation of exposure (Clarkson, 1992). In Minimata, initial diagnoses of the disease in adults and in children exposed *in utero* (congenital) were based on the presence or absence of signs and symptoms characteristic of acute methylmercury poisoning. Thirty-three adults were initially characterized as having acute or subacute poisoning and 40 children were classified as congenital cases (Harada, 1982). These patients were followed for years by the staff of Kumamoto University with the results of these follow-up examinations reported periodically.

In 1969, 8 to 14 years after the initial examination of 50 individuals (33 adults and 17 children), all surviving patients were examined again by the staff of Kumamoto

University (Tokuomi et al., 1975). While no individual fully recovered, of the 22 adults examined in 1969, no change in the severity of symptoms was noted in approximately 30% to 35% of the individuals, and improvement in symptoms was noted in 50% to 60% of the individuals examined. The parameters investigated were mental impairment, constriction of visual field, hearing impairment, cerebellar function (dysarthria, incoordination), pyramidal dysfunction (reflexes), peripheral nerve dysfunction (paresthesia). Deterioration was found in two individuals in each of the symptoms of constriction of visual field, hearing, coordination, and sensory disturbance. The authors did not state whether it was the same two individuals for each parameter. The mercury content in hair in these individuals, which according to Tokuomi et al. (1975) was measured 10 months after the onset of symptoms, ranged from 96 to 705 ppm.

The progress of Minimata children exposed *in utero* and born between 1955 and 1959 has been followed for more than 20 years (Harada, 1978, 1995; Tsubaki and Irukayama, 1977). Harada (1978) reported that after 15 years of follow-up of 37 cases of congenital Minimata disease, improvement in motor function was more noticeable in mild cases than in those classified as moderate, and in some milder cases, only "clumsiness of movement" remained. "Intelligence disturbance," which was not further defined, did not improve. Further improvement was noted in these patients when examined in an additional follow-up examination of 33 cases in 1981 (Harada, 1995). At this time, some improvement, although not total recovery, was noted in all the measured parameters except for signs of mental retardation and dysarthria. Analyses of hair samples for mercury content did not begin until 1960 at which time people had already ceased ingesting local seafood (Harada, 1995). When mercury hair levels were measured 5 to 8 years after giving birth, maternal mercury hair levels ranged from 1.82 to 191 ppm, while that of congenital patients measured 5.25 ppm to 110 ppm (Harada, 1995). Mercury levels in preserved umbilical cords of these congenital patients was 1 ppm or higher reaching greater than 4 to 5 ppm in some patients (Harada, 1995).

In the follow-up examinations symptoms of some of these initial patients became "atypical." Neurological examinations were then extended in 1964 and 1972 to 21 of the mothers of patients with congenital Minimata disease. A high incidence of signs and symptoms of Minimata disease were noted in these women, and it was concluded that mild, incomplete, or atypical cases of Minimata disease may have existed from the outset (Harada, 1995). The investigation was expanded in 1973 to inhabitants (exposed as adults) of the three most highly polluted areas in Minimata. About 30% of the approximately 1000 inhabitants examined exhibited one or more neurological signs of mercury poisoning compared to 15% of the those examined in a slightly polluted area (Goshonoura) and a control area (Ariake). In addition, approximately 50% of the inhabitants in the Minimata area complained of various symptoms, such as weakness, numbness or forgetfulness, that were attributed to methylmercury, which resulted in a revision and expansion of the criteria for designation as a Minimata disease patient, thereby greatly increasing the number of persons who were then eligible for compensation (Harada, 1982).

The cases in adults diagnosed after the initial outbreak were designated "chronic" Minimata disease patients (Harada, 1982, 1995). According to Harada (1982, 1995), some of the patients that initially had atypical, incomplete, or slight symptoms, among both the mothers of congenital patients and inhabitants of the highly contaminated areas, had symptoms that were gradually progressing or were delayed and gradually appeared after cessation of exposure.[7,8] Harada (1995) stated that progressing cases were found more frequently among elderly persons in whom typical symptoms such as sensory disturbances of limbs, constriction of visual field, and ataxia appeared to be exacerbated.

Whether these individuals had mild, atypical cases that existed from the outset and were not diagnosed because of the initial criteria for Minimata disease, or whether the symptoms were delayed in appearing and progressive, this population was exposed to sustained high levels of contamination for a number of years, based on the levels of methylmercury contamination in Minimata Bay from the early 1950s until the early 1960s. Mercury hair levels in the mothers of congenital patients, measured approximately 5 or more years after giving birth, ranged from about 2 to 191 ppm. The mercury content in hair samples for the other inhabitants in the Minimata area

[7] The number of individuals with delayed or progressive effects among those examined was not given. One symptom identified as exhibiting either a delayed onset or progressive change was constriction of visual field (Harada, 1995). However, the reference cited pertains to subjects in Niigata, not Minimata, and refers to 13 patients with mercury hair levels between 52 and 528 ppm who were tested yearly for 7 years (Iwata et al., 1975). In several of these patients, constriction of visual field, which was present during the initial examination, progressed further but fixed and improved somewhat after 5 years. Two subjects with mercury hair levels of 310 and 425 ppm had normal visual fields.

[8] Harada (1995) cited the outbreaks in Niigata and China as other examples of delayed onset saying that symptoms appeared up to 5 years after cessation of exposure. Clinical follow-up studies from Niigata suggested that there was a latency period of several years between peak exposure, as measured by hair levels, and the onset of symptoms (Tsubaki et al., 1978). However, this delay in the onset of symptoms was only reported in four patients and the length of the latency period was unrelated to mercury concentration in hair (maximum hair concentrations in the range of 50 to 300 ppm) (WHO, 1990). Further, according to WHO (1990), "The delayed cases were mild, showing nonspecific symptoms, so that cases of methylmercury poisoning could not be diagnosed with complete certainty."

were not reported. However, given the typically high fish and shellfish ingestion rate in this area (an average of 316 to 459 g/day for men and 69 to 251 g/day for women) (Harada, 1982) and the very high methylmercury content in fish and shellfish measured in Minimata Bay in the late 1950s (e.g., 11–39 ppm in *Hormonya mutabilis*, 24 ppm in sea bream, 11 ppm in gray mullet, 36 ppm in crab and 20 ppm in short neck clams) (Harada, 1982, 1995), methylmercury intake would have been significantly higher in Minimata than for the fish-eating populations studied in other parts of the world. Harada (1995) calculated that a fisherman ingesting an average of 334 g/day of fish would ingest 0.048 mg/day of total mercury a day based on mercury levels in fish in 1984. At that ingestion rate, the average mercury content in fish in 1984 had to have been about 0.15 ppm. As noted, mercury levels in fish and shellfish in the late 1950s, when the highest exposure took place, were considerably higher. If the average contamination if marine food was 10 to 20 ppm during the time of the Minimata poisoning, mercury intake for a fishermen ingesting 334 g/day would be approximately 7 mg/day or approximately 50 to 100 μg/kg/day. In the Seychelles population, the average intake was estimated, based on the median maternal mercury hair levels, to be about 0.5 μg/kg/day (ATSDR, 1997).

Development of chronic sequelae in this population is likely to be a high-dose phenomenon and not directly applicable to other fish-eating populations exposed to chronically to very low levels of methylmercury. Harada (1995) stated, "Admittedly, Minimata is not an example of a simple long-term trace contamination given that excessive contamination existed until 1960, followed by more than 20 years of relatively low contamination levels."

Evidence for Dose and Dose-Rate Effects in the Development of Chronic Sequelae in Animals. Longitudinal studies in animals have evaluated the potential for delayed neurotoxicity (Evans et al., 1977; Rice, 1989; Spyker, 1975). The studies by Evans et al. (1977) in macaque and squirrel monkeys and Spyker (1975) in mice demonstrated that the progressive development of neurotoxic effects was dose dependent.

Spyker (1975) administered 0, 0.5, 1, 2, 4, or 8 mg/kg methylmercury dicyandiamide intraperitoneally to pregnant mice on days 7, 9, 12, or 13 of gestation. Offspring of treated and control mice all appeared unaffected at weaning; however, in offspring from dams treated with 8 mg/kg performance was significantly different from controls in two behavioral tests: the open-field test and the swimming evaluation. By adulthood (6 months to 1 year), offspring in the highest dose group began showing signs of overt neurological effects (e.g., tremor, incoordination), while animals from all other dose groups showed no overt signs. In motor ability tests conducted at 12 to 15 months, only animals from the 4-mg/kg-dose group performed poorly compared to controls (apparently the 8-mg/kg-dose group was not tested). By middle age (1 to 1.5 years) neurological differences between controls and the high-dose groups became obvious. According to the authors, both the severity of the deficit and the length of time before detection was dose dependent.

Evans et al. (1977) reported that the latency decreased and the severity of neurotoxicity increased in macaque and squirrel monkeys as the mean mercury blood concentration increased. The earliest noted critical effects were reduced sensitivity to visual stimuli of low luminance and an intention tremor upon reaching for a small object at a distance. As exposure continued, additional signs appeared and included constricted visual fields, somesthetic impairment, and ataxia. All monkeys with mean mercury blood concentrations >2 ppm developed symptoms within approximately 100 days, while 5/6 with mean blood concentrations between 1.0 and 2.0 ppm demonstrated toxic effects within 135 to 450 days. Only 1/4 monkeys with mean blood levels between 0.5 and 1.0 ppm showed any signs of toxicity even when exposure continued for 1000 to 1400 days.

Rice (1989) reported signs of delayed neurotoxicity in macaque monkeys given 0.05 mg/kg/day methylmercury first in infant formula and then in gelatin capsules with corn oil until 7 years of age. At 13 years old, during routine animal care, it was noted that some of the individuals (three controls and five treated) appeared "clumsy and hesitant" when in the large exercise area. Cage observations were admittedly subjective with one treated monkey appearing normal, two assessed as marginally clumsy and one treated animal described as extremely clumsy. In a more objective assessment, overall, treated monkeys performed more poorly in the "raisin pick-up test" (P=0.047), i.e., took longer and had greater difficulty retrieving the raisins, than controls. These observations have been reported for the group of monkeys exposed postnatally, but similar observations have not been reported in the group of Macaque monkeys exposed *in utero* and postnatally up to 4.5 years old.[9]

[9] Somatosensory function was assessed in both groups at age 15 for one group and age 18 for the other (Rice and Gilbert, 1995), since it was speculated that the "clumsiness" observed in the postnatal exposure only cohort may have been in part due to somatosensory damage (Rice, 1996). Treated monkeys had elevated vibration detection thresholds at the three highest frequencies of the five tested compared to controls, although there was no dose–response for the *in utero* exposed group. In the *in utero* exposed group, the two remaining monkeys in the high-dose group were only "slightly impaired," while the monkey in the lowest dose group showed more impairment than the animals in the two higher treatment groups. This test was not administered to these animals at any earlier ages; therefore, classification as a delayed effect can not be made.

In these studies, the doses to animals were considerably higher than that expected from the ingestion of fish. However, in order to conduct a quantitative comparison across species, the dose should be defined in terms of the target organ dose, in this case, the brain (Burbacher et al., 1990a). Burbacher et al. (1990a) conducted a qualitative and quantitative comparison of neurological endpoints across species. When expressed as the dose to the brain, Burbacher et al. (1990a) noted there was a quantitative similarity in neurobehavioral effects at high doses across species, i.e., similar effects were noted at the same dose level to the brain. At lower levels of exposure, when similar categories of neurobehavioral functions were compared, effects across species were also quantitatively similar, especially for the macaque monkey and humans.

The dose levels at which delayed neurotoxicity was observed in the animal studies can be compared to the potential for these effects in humans exposed at a comparable level. This can be approximated according to Burbacher et al. (1990a) by comparing the blood levels and brain to blood ratios. The peak and steady state blood levels in monkeys in the Rice (1989) study were 1.4 and 1.1 ppm, respectively, which would correspond to a brain level of approximately 3.3 and 2.9 ppm, respectively (brain:blood ratio of 2.6, from Burbacher et al. (1990a). To reach corresponding brain levels in humans (brain:blood ratio ranging from 3 to 6, from Kitamura et al., 1976 and Burbacher et al., 1990a, respectively), the corresponding maternal blood level at steady state would have to be approximately 0.25 ppm to 0.5 ppm with a corresponding maternal mercury hair level about 65 to 125 ppm (assuming a hair to blood ratio of 250), which is approximately the level at which ataxia was observed in the Iraqi poisoning episode and within the range of hair levels in mothers of congenital patients who were diagnosed with delayed chronic Minimata disease. Given the brain levels in these monkeys from birth to 7 years of age, it is not surprising that mild signs were noted in aged animals. No data have been published thus far reporting signs of delayed effects in animals exposed *in utero*. However, as discussed in one of the following sections, the mechanism of neurotoxicity for adult exposure and subsequent demonstration of effects is likely very different from the neurotoxic mechanism with *in utero* exposure. These mechanistic differences provide a plausible explanation for the difference in cage observation in the two groups of monkeys.

Lack of Evidence for Delayed Neurological Effects in Adults in Fish-Eating Populations. Studies have been reported on stable fish-eating populations exposed chronically to methylmercury at concentrations lower than the Iraqi and Minimata cohorts, but above what would be expected for U.S. populations exposed to methylmercury in fish. These more recent studies provide a better indicator of the potential for latent chronic effects and were reviewed, along with the animal data, to assess the need for continued consideration of the potential for sequelae as a contributor to uncertainty requiring quantification.

Observations of the adult Peruvian population (Turner et al., 1980), which were, according to the study authors, "probably exposed *in utero* to blood methylmercury concentrations higher than those usually observed in the US, but showed no clinical ill effects," support the suggestion that chronic sequelae are not of concern in populations exposed to low levels of exposure from fish ingestion. The dietary histories of the Peruvian cohort indicated a steady intake of fish. Analysis of long segments of hair indicated that most of the subjects were close to steady state. Therefore, the exposure was a steady, low-level exposure over an extended period of time, presumably starting *in utero*. The mean age of the Peruvian population was 25.4 years with a range of 1.4 to 82 years of age.

The adult population ($n=190$) was examined by a neurologist, who conducted a complete neurological examination, including sensory and visual tests (Turner et al., 1980). The sensory modalities examined were pin prick, vibration, joint-position sensation, two-point discrimination, stereognosis, and touch sensation employing von Frey hairs. Coordination was assessed by inspection of gait, finger–nose and finger–finger approximation, heel–shin tests, and tests of diadochokinesis. Fine motor skills were assessed by examining handwriting and the ability to place small pencil dots inside a 2-cm circle and to trace a pencil along a given wavy line. Speech was assessed for dysarthria or dysphasia. Hearing was tested with a pure-tone audiometer. Visual acuity and visual fields were examined. The authors of the Peru study of adults (Turner et al., 1980) state that there was nothing to suggest psychological impairment in any subjects, which suggests the lack of subclinical effects on behavioral or cognitive function; however, no formal psychological testing was performed.

Evaluation of complaints in the Peru study initially indicated a significant increase in the incidence of the complaint of impaired vision in the exposed population. However, upon examination, four cases of impaired vision were due to illiteracy and three cases were due to cataracts. No cases of visual field constriction, a characteristic of methylmercury poisoning, were observed in the exposed or the control populations, with intakes as high as 20 μg/kg/day possible. No other mercury-related effects were noted in this population. In particular, no effects on pin prick response, measures of coordination and vibration sensitivity, or fine motor skills (which were noted in aged monkeys), were present in this population. None of the classical symptoms associated with methylmercury poison-

ing were reported to be elevated in the exposed population in Peru. Also, the incidence of paresthesia was increased in the control population, compared to those exposed to high levels of methylmercury. Based on these results, no indication that an increase in the incidence of effects related to methylmercury exposure was observed in the aged of this population. Based on the estimated per capita intake of fish (110–660 g/day), the average concentration of mercury in all fish tissues analyzed (0.58 ppm), and a standard body weight (65 kg), estimated mercury intake for the population rages from approximately 1 to 6 μg/kg/day for fish-eating populations. However, as estimated above, the mercury levels to monkeys when compared on a brain level were much larger than expected for this population.

Native Indians in either the Cree bands in Quebec or the communities of Grassy Narrows and Whitedog in Ontario also represent stable populations who have been exposed to methylmercury since at least the early 1960s (i.e., the time the chlor-alkali plant was built) and probably for considerably longer, due to natural contamination (McKeown-Eyssen and Ruedy, 1983a,b). Many of these individuals have been exposed chronically for most of their lives, beginning with *in utero* exposures in some cases. They have been tested as adults without demonstration of late developing effects or effects increasing with age related to mercury exposure in Peru.

Lack of Delayed or Persistent Effects on Cognitive Function in Adulthood in Nonhuman Primates. In a longitudinal study in children in the Seychelles Islands, children were administered age-adjusted tests for neurobehavioral and cognitive function repeatedly from age 6 months to 66 months with no treatment-related effects found for any of the tests conducted (Clarkson et al., 1998; Davidson et al., 1995). Since many of these tests are designed to be predictive of behavioral and cognitive function later in life, these negative results support the hypothesis that effects will not be manifested when this population reaches adulthood. However, this hypothesis could only be tested with a lifetime longitudinal study of this population or a similar population in which repeated testing as adults is conducted and all intervening confounding influences over their lifetime are controlled. Such a study would be very difficult, if not impossible to conduct. However, neurobehavioral effects in nonhuman primates exposed *in utero* have been evaluated in longitudinal studies using a number of tests indicative of cognitive function, and have been reviewed to assess the evidence for the presence of delayed or progressive effects in monkeys.

These studies have been conducted largely at two research facilities by Burbacher and colleagues at the University of Washington and Rice and colleagues in the Health Protection Branch (HPB), Health Canada. The effects of prenatal exposure on measures of cognitive function was assessed at the University of Washington in macaque monkeys exposed *in utero* at maternal exposure levels of 0, 0.05, or 0.07 mg/kg/day. At birth, methylmercury blood levels in treated offspring ranged from 1.04 to 2.46 ppm, which would correspond to a brain level of 2.7 to 6.4 ppm (using the brain:blood ratio of 2.6 reported in Burbacher et al., 1990a). Offspring tested at 2 weeks of age took longer to learn in an object permanence task, which tests the ability of the infant to realize an object placed out of sight is still present (Burbacher et al., 1986). When tested at 1 month of age in visual recognition tests, the control animals showed differential visual attention to novel stimuli compared to the treated monkeys, indicating impaired visual recognition memory performance (Gunderson et al., 1988). Visual recognition tests are sensitive indicators of impairment in both macaque and human infants (Gunderson et al., 1988), and human performance on visual recognition tests, such as the Fagan test, is correlated with childhood intellectual status (Gunderson et al., 1988; Rice, 1996). Social behavior, as measured by a decrease in social play and an increase in nonsocial passive behavior, was observed to be different in treated monkeys compared to controls when observed from 2 weeks of age up to 8 months (Burbacher et al., 1990b). These monkeys were reevaluated at 7 to 9 years of age (Gilbert et al., 1993). The animals were evaluated for spatial delayed alternation, a procedure for testing spacial memory in young and adult monkeys, which corresponds in functional testing to the object permanence task, a spacial memory task for infants, administered to these monkeys at two weeks. In contrast to the results obtained when these monkeys were tested at 2 weeks (Burbacher et al., 1986), no differences between treated and control animals were noted at any dose group. No differences were found in initial button training or number of sessions to reach criterion on 0.1-s delay procedure. There were no differences between control and treated monkeys on performance on the variable delay schedule, and treated monkeys performed significantly better than controls on the fixed delay sessions. The authors concluded that, "*in utero* methylmercury exposure did not adversely affect the spatial memory of adult monkeys when tested on a delayed alternation task and may have facilitated performance on this task" (Gilbert et al., 1993). The authors further stated that, "The results of the present study do not support the hypothesis that *in utero* exposure to methylmercury produces long-term deficits in spatial memory as measured by performance on a spatial delayed alternation task." According to the authors, this test was sensitive enough to detect severe impairment following *in utero* exposures to lead (Brozoski et al., 1979) and PCBs (Levin et al., 1988). These monkeys were also tested on a delayed nonmatching to sample task designed to reassess visual recognition memory, which was found to be impaired when

these animals were tested at 1 month of age (Rice, 1996). No difference in controls and treated groups were found, which indicates that *in utero* exposure had no long-term effects on visual recognition memory (Rice, 1996).

In the research program at HPB, macaques exposed *in utero* were evaluated for effects on cognitive function as infants and then as juveniles (Rice, 1992, 1996). Mothers were dosed at 0, 0.01, 0.025, and 0.05 mg/kg/day before and during pregnancy and offspring were continued on treatment postnatally at the same dose levels administered to the mothers until 4 to 4.5 years of age. Infant blood levels were 0.46, 0.93, and 2.66 ppm at birth and decreased slowly to 0.2, 0.25, or 0.6 ppm at steady state (Rice, 1992). Infants were tested using a series of complicated nonspatial discrimination reversal tasks and a test for fixed-interval (FI) performance. The discrimination reversal paradigm used has been shown to be sensitive to impairment produced by lead (Rice and Gilbert, 1990) and PCBs (Schantz et al., 1989) in monkeys. The FI performance has been demonstrated to be sensitive to lead exposure in infant monkeys (Rice, 1988). During infancy monkeys were tested 7 days per week for 16 to 21 h per day. Infants were trained on a visual discrimination task, after which a series of discrimination reversals was instituted, in which the previously correct stimulus became the incorrect one. No impairment in cognitive function as measured in this task was found, and treated infants learned the task quicker than controls. When tested using an intermittent schedule of reinforcement, the FI schedule, controls exhibited better temporal discrimination in FI performance according to the median pause times. However, the mean pause times did not differ between treated and controls, and there was no difference in either the overall rate of response or the run rate. Additionally, there was no significant difference between groups over the 30 sessions in the response pattern. These monkeys were retested as juveniles using the same battery of tests while still being exposed to methylmercury (Rice, 1992, 1996). No differences were found between groups, and indeed, treated monkeys acquired the task more quickly than controls, and were less distracted by irrelevant cues early in the experiment. In addition to these tests for cognitive function, both research groups found that none of the groups of treated monkeys were impaired in their ability to learn the complex tasks required for the various assessments of sensory system function (Rice, 1996). Several different types of tests were conducted at several stages of the lifetime of monkeys exposed either *in utero* and up to 4.5 years of age or in monkeys exposed from birth to 7 years of age. The last series of tests were administered when these animals were 18 years of age (Rice, 1996).

While not every domain of cognitive function has been explored in these monkey studies, an impressive array of studies has been administered. The types of tests administered are some of those recommended in the USEPA testing battery and are designed to test major aspects of cognitive function, such as memory and learning (Weiss and Cory-Slechta, 1994). These studies provide convincing evidence that persistent alterations in cognitive function are highly unlikely in populations exposed *in utero* to low levels of methylmercury by way of maternal fish ingestion.

These studies provide evidence that developmental exposure to methylmercury did not result in persistent or delayed effects on cognitive function. However, alterations in sensory system function were found in these same monkeys (see Appendix A). Additionally, Rice (1996) stated in a review of the rodent data that impairment in motor function was clearly the "most salient effect," while tests of cognitive function were largely negative or showed a very weak high-dose effect. It is possible that either sensory system functioning is more sensitive to the effects of methylmercury than those of the cognitive domain or cognitive domains have a greater reserve or compensatory capacity than sensory system function. If that is the case, then effects on sensory system function should be detected earlier and at lower doses than an impact on cognitive function. A lack of effects on sensory system function could indicate that effects on cognitive function should not be expected either.

Possible Mechanism of Action for Chronic Sequelae. In the Minimata (Harada, 1995) and Iraqi (Bakir et al., 1973) adult populations, acute high-level exposure to methylmercury resulted in latent periods of a few days to several years reported before clinical manifestations of signs and symptoms of neurotoxicity in some individuals. For Minimata patients, the signs were mild and atypical, and not thought originally to be associated with methylmercury. The mechanism(s) by which methylmercury may produce latent neurotoxicity is unknown. The biological rationale for a latency period between exposure and the onset of symptoms may be related to methylmercury metabolism (USEPA, 1997). Metabolic demethylation of methylmercury may set up a cascade of biochemical events. Homolytic cleavage of the carbon–mercury bond may release methyl free radicals (Ganther, 1978). These free radicals are expected to initiate a chain of events involving peroxidation of lipid constituents of neuronal cells (USEPA, 1997). The onset is delayed for the period of time that cellular systems are able to prevent or repair the effects of lipid peroxidation. When cellular defense mechanisms are overwhelmed, rapid and progressive degeneration of the tissue results (USEPA, 1997). Following exposure to low levels of methylmercury, the normal defense and cellular repair mechanisms keep the lipid peroxidation in check such that there is no or minimal

tissue damage and no overt evidence of toxicity. However, as exposure increases, cellular repair mechanisms may eventually be overwhelmed or depleted (i.e., the threshold is exceeded), and tissue damage with overt signs of toxicity may result.

Following intraperitoneal injections of methylmercury chloride in animals, the formation of reactive oxygen species have been observed in the brains of rodents (Ali et al., 1992; LeBel et al., 1990, 1992), indicating the occurrence of lipid peroxidation. The results of an *in vitro* study by Sarafian and Verity (1991) in rat cerebellar granule cells demonstrated that methylmercury produced time- and concentration-dependent increases in lipid peroxidation and decreases in reduced intracellular glutathione, an endogenous component that provides protection against the effects of free radicals, and therefore against the neurological effects of methylmercury.

The mechanism of neurotoxicity, and hence the potential for delayed effects, may be different in persons exposed *in utero* rather than as adults. A comparison of autopsy brain sections of adults and *in utero* exposed children in the poisoning episodes in Iraq and Minimata revealed significant differences in brain architecture and histopathology (Harada, 1978; Clarkson, 1992). Similar effects have also been observed in animals exposed to high doses of methylmercury *in utero* (Choi, 1989; Geelan et al., 1990; Inouye and Kajiwara, 1988a; Reuhl et al., 1981; Rodier et al., 1984).

The potential effects that methylmercury may have on the developing human nervous system were evaluated by (Lapham et al., 1995). The histological features of 32 brains obtained from Seychelles infants were compared to 12 reference brains from infants who died at the Rochester (New York) Medical Center. In each infant, the cause of death was unrelated to the nervous system. The areas of the brain examined were the frontal and occipital cortex, temporal cortex with hippocampus, basal ganglia with thalamus, cerebellum, and the pons with the medulla. There were no consistent histological changes identified in the brains from the Seychelles infants when compared to the reference brains, and no correlations could be made with mercury brain levels and a particular histologic change. In contrast, the brains from two infants from the Iraqi population that were exposed to high doses of methylmercury *in utero* exhibited histologic changes that were indicative of neurodevelopmental abnormalities, such as alteration in the laminar patterns and normal layers of the cerebral cortex and changes in neuron location in the cerebrum and cerebellum. These changes were suggestive of alterations in neuronal migration and formation of tissue architecture.

The results reported by Lapham et al. (1995) indicate that exposures to high doses of methylmercury *in utero*, as in the case of the Iraqi poisoning incident, may result in disruptions in neural development and alterations in tissue architecture. A likely sequel to these histological changes would be overt evidence of toxicity. However, there was no evidence of methylmercury associated changes in infant brains from the Seychelles population following chronic low-level *in utero* exposures. In comparison to adult exposure, *in utero* exposure results in more diffuse changes and reduction in cellularity in selected brain areas (Clarkson, 1992). It has been suggested that the mechanism for neurotoxicity from *in utero* exposure may be disruption of microtubule formation, and consequential interruption of mitosis and neuronal migration (Abe et al., 1975; Choi et al., 1978; Rodier et al., 1984; Sager, 1988; Sager et al., 1982, 1983). The initiating event may be the binding of methylmercury to sulfhydryl groups on tubulin (Sager, 1988), a component of microtubules. The relative sensitivity and/or resistance of cell types and brain areas suggest that these neurotoxic mechanisms are processes that would be expected to have a threshold either because of the reserve capacity of cellular components or because of repair processes.

The potential for delayed effects expressed following *in utero* exposure may not be due to actual progressive cellular damage, as may be the case for adult exposure effects as described above, but rather delayed expression of *in utero* damage as different domains are called into play to allow expression of different functions. For example, in the Iraqi population, diagnoses of some cases designated as "cerebral palsy" were delayed. However, in general, symptoms of cerebral palsy due to causes other than methylmercury are also delayed in appearing (Harada, 1995).

Such an underlying mechanism would strongly suggest that if a decrement in performance is not noted in a child at the age at which that child should be able to perform that function, then it is likely that the "domain" responsible for that ability has not been impaired, either because cellular repair processes have prevented any damage or the brain area has more than enough capacity to compensate. In either case, the threshold or virtual threshold has not been exceeded. A lack of decrement in performance, as in the Seychelles study, in age-specific behavioral tests designed to assess subtle measures of behavioral and cognitive performance should be viewed with confidence as predictive of future functioning into adulthood. The test battery administered in the Seychelles are among those recommended by USEPA, and have been widely used and accepted as predictive of future functioning (Weiss and Cory-Slechta, 1994; White and Proctor, 1995).

What complicates the issue with methylmercury and other potential neurotoxicants is the seemingly unanswerable question with regard to aging when reserve capacity may be called upon to compensate for the aging process. While this is a difficult, if not impossible, hypothesis to test for any chemical, for methylmercury this possibility can be assessed

by studies in adults, such as in Peru, where 20% to 25% of the population evaluated was 50 years old or older (up to 82 years of age) and in nonhuman primates exposed *in utero*, as discussed in the following sections. No decrement in a variety of neurological parameters was found in the Peru population chronically exposed to low levels of methylmercury in fish (Turner et al., 1980). No lasting decrement was found in cognitive function in adult nonhuman primates exposed *in utero* (Gilbert et al., 1993; Rice, 1992) at doses that were greater than those to which either the Peru population or the Seychelles population were exposed.

The critical concepts are dose and threshold. A number of factors indicate that this threshold may indeed be higher when low levels of methylmercury are ingested in fish. Protective mechanisms may be operative in conjunction with ingestion of fish. For example, studies have provided evidence that the antioxidants selenium and vitamin E are protective against the effects of methylmercury (Chang et al., 1977, 1978; Imura and Naganuma, 1991). Selenium may offer some protection by forming selenium–mercury complexes, thereby interfering with mercury transport into the brain (USEPA, 1997). Fish and shellfish are nutritional sources of selenium and vitamin E (Egeland and Middaugh, 1997). Therefore, it seems likely that the protection from the neurotoxic effects of methylmercury provided by selenium and vitamin E would be operative in fish-eating populations chronically exposed to low levels of methylmercury.

Another protective effect that would be active in fish-eating populations was proposed by Clarkson (1995). According to Clarkson (1995), large neutral amino acids, such as leucine, methionine, and phenylalanine, would inhibit the transport processes by which mercury enters the brain, thus decreasing the amount of mercury that reached the target tissues. Large quantities of these amino acids are found in fish. Therefore, brain uptake of mercury following ingestion of fish would be decreased due to the inhibitory effects on mercury transport by these amino acids in comparison to methylmercury ingested in grain, such as in the Iraqi population, or as the methylmercury chloride in apple juice in some of the monkey studies. As a result, the amount of mercury that would reach the target tissue, the brain, would be decreased. It has been suggested that the differences in the length of the delay period between the Iraqi and Minimata poisoning episodes may be due in part to the selenium in the fish ingested by the Japanese (USEPA, 1997).

Based on the proposed mechanisms for neurotoxicity in adult and *in utero* exposure, a threshold exists, and that following exposures to high levels of methylmercury, such as in the Iraqi and Minimata poisoning incidents, the defense mechanisms may be overwhelmed and latent neurotoxic effects may result. However, with chronic low-level exposures as would be expected in a fish-eating population, the threshold for the development of latent methylmercury induced neurotoxic effects, i.e., chronic sequelae, would not be exceeded, due to the presence of several protective elements, i.e., selenium, vitamin E, large neutral amino acids, as well as the body's natural defense and repair mechanisms.

Weight of Evidence for the Potential for Chronic Sequelae in Fish-Eating Populations. The weight of evidence indicates that the development of chronic sequelae is dose dependent, and such effects are highly unlikely in populations exposed *in utero* to low methylmercury levels (i.e., at the levels proposed for the site-specific RfD) from maternal consumption of fish containing methylmercury.

- Delayed neurotoxicity has only been seen in humans in poisoning episodes in Iraq and Minimata and in animals exposed to high doses that equal or exceed those seen in the Iraq and Minimata cases.
- The postulated mechanism of action for neurotoxicity strongly indicates the presence of a threshold for these delayed effects.
- Studies in human fish-eating populations exposed to low levels of methylmercury (Turner et al., 1980) and studies in nonhuman primates (Rice, 1992; Gilbert et al., 1993) provide no evidence of delayed effects.

Based on a weight of evidence, it is highly unlikely that chronic sequelae will result in populations exposed by way of fish ingestion to very low levels of methylmercury. Clarkson (1997), one of the investigators in both the Iraqi studies and the Seychelles studies, recently stated that

> ... our experience to date in fish consumers is that extended exposure does not increase the risks of poisoning of the kind seen in Iraq. The specter of a long delay in onset of symptoms from chronic repeated yearly exposures does not seem to be real.

The available data indicate that chronic sequelae may occur only following ingestion of concentrations of methylmercury much higher than those to which fish-eating populations are exposed.

Uncertainty Due to Lack of Data from a Two-Generation Reproductive Study

The USEPA (1997) also adjusts its NOAEL for uncertainty due to the lack of a two-generation reproductive study in animals. USEPA (1996) indicates that a two-generation reproductive study is conducted to detect effects caused by prenatal and postnatal exposure, as well as effects on germ cells, that could be transmitted to and

expressed in the next generation. However, based on the reasons described in the USEPA (1996) Guidelines for Reproductive Toxicity Risk Assessment for conducting a multigeneration reproductive study, uncertainty due to lack of a two-generation study for methylmercury is unwarranted for two reasons:

1. the proposed mechanisms for developmental effects are not heritable, and
2. the lack of effects in germ cells following oral exposures to high doses of methylmercury.

The site-specific RfD for fish-eating populations is based on the most sensitive endpoint, developmental effects on the nervous system, that has been observed following *in utero* exposures as evidenced by both human and animal information. The mechanisms for these neurodevelopmental effects are believed to be the result of methylmercury interfering with microtubule formation, thus disrupting mitosis, which results in decreased cellularity in certain areas of the brain. It has been demonstrated *in vitro* that methylmercury binds to sulfhydryl groups on tubulin, a component of microtubules (Sager et al., 1983). This leads to damage to the microtubules, and subsequently, mitotic arrest (Sager, 1988), with the external granular layer of the cerebellum appearing to be the most sensitive area of the brain (Sager et al., 1982). Based on the mechanism of action proposed for neurodevelopmental effects, it is not an endpoint that would be expected to be transmitted to and expressed in the next generation.

A review of the available literature revealed one study, a modified dominant lethal study conducted in male mice, where the potential effects of methylmercury on spermatogenesis were evaluated following oral exposures. Dominant lethal studies are typically conducted in males because male germ cells are generally considered to be more sensitive than females. This modified two-generation dominant lethal study was designed to evaluate the potential heritability of any effects in male mice exposed to methylmercury *in utero* (Inouye and Kajiwara, 1988b). In this study, groups of 12 pregnant female mice (P_0) were administered 15 mg methylmercury chloride/kg body weight, on days 14 (Group 1) or 17 (Group 2) of gestation. An additional group of 11 pregnant mice served as untreated controls (Group 3). The F_1 males were mated to unexposed females to produce the F_2 generation.

The study authors reported that all of the F_1 males were fertile. There were no statistically significant differences in litter size, fetal mortality or the incidence of gross fetal anomalies in the Group 1 or Group 2 F_2 fetuses, when compared with the controls. The only statistically significant changes reported were decreases in mean fetal body weight and decreases in the number of ossified vertebrae in Group 2 F_2 fetuses, when compared with the controls. However, the observed changes were not typical of effects on germ cells. The magnitude of these changes was small, approximately 1–3%, with mean fetal body weights of 1.06 ± 0.085 and 1.02 ± 0.109 g reported in the control and Group 2 fetuses, respectively. The values for the number of ossified vertebrae were also very close, with 35.3 ± 1.11 reported in the Group 2 F_2 fetuses, compared with 35.7 ± 0.96 reported in the controls. Also, based on the data reported in Inouye and Kajiwara (1988b), the average litter size in the Group 2 F_2 litters were greater than the average litter size in the controls by approximately one pup/litter. It is possible that a larger litter size could have resulted in decreased fetal weights. Therefore, the effects on mean fetal body weights and the number of ossified vertebra were likely biological variation, rather than related to treatment with methylmercury. Moreover, it seems unlikely that *in utero* exposures would result in an effect on the germ cells of the F_1 males that could be transmitted to the F_2 generation resulting in slight growth retardation of the F_2 fetuses. The results of this study do suggest, however, that *in utero* exposures to high doses of methylmercury do not result in male-mediated heritable effects in the subsequent generation, and provide no evidence that methylmercury may cause effects on germ cells.

The available literature also provided no evidence that affects on germ cells may be a more sensitive endpoint than neurodevelopmental effects. In the study by Inouye and Kajiwara (1988b), the P_0 females received 15 mg methylmercury chloride/kg body weight *via* oral gavage. However, the lowest neurodevelopmental LOAEL identified in animal studies was 0.01 mg methylmercury chloride (Bornhausen et al., 1980). This LOAEL was based on effects observed in the offspring of female rats that had received methylmercury chloride on gestation days 6 through 9. The lowest reproductive LOAEL was 0.05 mg methylmercury/kg body weight and was based on decreases in sperm speed and forward progression and increased sperm tail defects in male monkeys that had been exposed for 20 weeks (Mohamed et al., 1987). Therefore, the adjustment of the NOAEL due to the lack of a two-generation study is not warranted based on the negative results of Inouye and Kajiwara (1988b), and the lack of evidence that affects on germ cells or reproduction represent a more sensitive endpoint than neurodevelopmental effects.

Information from epidemiological studies in which the population has been exposed chronically to methylmercury from ingestion of fish provides further evidence that adjustment of the NOAEL for uncertainty due to the lack of a two-generation study is unwarranted. Current human exposure to methylmercury in fish is not only related to current emissions, but past emissions, especially airborne transport and global deposition of elemental mercury. The fish-eating populations that have been studied, other than

poisoning incidents, are fairly stable populations, with most of the families inhabiting the area for generations. For example, the Peruvian populations studied by Turner et al. (1980) and Marsh et al. (1995a,b) are fairly stable, with about 90% of the population having either been born there or a resident there for many years. Seventy percent of protein intake for this population is from ingestion of ocean fish, with fish ingestion initiated at the time of weaning, approximately 9 months of age (Turner et al., 1980). This population (Turner et al., 1980) could be characterized as chronically exposed, probably for several generations, to methylmercury from consumption of fish.

The Peruvian population represents probable multigenerational exposures. Although reproductive effects were not specifically evaluated in this population, the information provided in the reported studies suggests the absence of the reproductive problems that have been observed in animal studies exposed at high doses [i.e., decreased reproductive successes (Burbacher et al., 1988)]. In the Peruvian population, the number of persons per household (6.2 persons) in the population eating large amounts of fish was similar to the number of persons per household in the population who consumed relatively little fish (6.4 persons) (Turner et al., 1980). Thus, it would appear that the reproductive capability of the subjects in the village exposed to high concentrations of methylmercury *via* ingestion of fish is similar to that observed in persons in the control village.

In summary, the proposed mechanism of neurodevelopmental effects would not be expected to be passed to following generations. Also, there is no evidence that methylmercury exposures result in heritable effects on germ cells, or that effects on germ cells or reproduction are the most sensitive endpoint. Further, information from epidemiological studies suggests that chronic low-level exposures to methylmercury *via* fish ingestion over multiple generations have no effect on reproduction. Collectively, these results indicate that an uncertainty factor for the lack of a two-generation reproductive study is not necessary for an RfD based on a fish-eating population.

Uncertainty Factors for Fish-Eating Populations

The need for an uncertainty factor due to the use of the Seychelles study for derivation of a site-specific RfD was assessed. As discussed in early sections of this report, the Seychelles study is a large, well-conducted longitudinal study in a well-characterized and well-monitored population. Sophisticated measures of child development including a battery of psychological tests appropriate to the child's age were administered. Ten covariates were considered, four of which, gender, home, breast feeding, and birth weight, were found to influence child development. In the main study, 740 children were examined at the same age ±2 weeks. Confounding variables were anticipated, and protocols to evaluate the impact of confounding variables on outcome were considered. As now published, there is no positive association between methylmercury exposure and any neurodevelopmental functional endpoint evaluated (Clarkson, 1997; Marsh et al., 1987). Unpublished results of the 66-month study also show no association with methylmercury exposure; a new round of tests in children now 8 years of age is underway (Clarkson, 1997). According to Clarkson (1997), "Despite intensive and repeated testing on both cohorts, we have not found significant adverse developmental effects in these children. In fact, the children are developing very well. For example, their developmental milestones were earlier than western norms."

Even when well-designed and conducted, epidemiological data will have some degree of uncertainty, usually because of the sample size and the inability to control for all confounders. Statistical analyses of epidemiological populations can be highly influenced by the presence of statistical outliers. This was evident in the reanalysis of Iraqi study by Cox et al. (1995), when removal of four individuals who could have been classified as "background" resulted in a change of the estimated "threshold" dose from 10 to >100 ppm.

In the Seychelles pilot study, four individuals were also identified in the Seychelles as statistical outliers when assessed by an *a priori* statistical analysis. However, no statistical outliers were identified in the main study and, therefore, no subjects were excluded from the analysis. An influential point did have an impact on the Benchmark analysis of the New Zealand data. While not a true statistical outlier by the *a priori* statistical criteria, when this individual was removed from the Benchmark analyses, the lowest estimated BMD for this cohort was lowered from 17 to 7.4 ppm maternal mercury hair concentration. While there is no compelling statistical or biological reason to exclude this individual from the analyses, it is an uncertainty to be considered.

Uncertainty is also introduced by the recently published results in the Faroe Islands (Grandjean et al., 1997). The authors stated that subtle effects on neurological development are evident at maternal hair levels <10 ppm. No dose–response data are provided to determine the NOAEL in this case. Moreover, the validity of this conclusion remains to be assessed since some of the important confounding factors, in particular co-exposure to PCBs, have not been controlled for in the evaluation of neurodevelopmental outcomes for the members of the cohort in the 10 ppm and less range. While it is highly likely that any observed development deficits in that dose range are due, at least in part, to PCB exposure, it is not possible to attest to this point with certainty, given the data as published. Further, while the weight of evidence provides a convincing argument that chronic sequelae are highly unlikely to occur in populations

Table 22. Distribution of RfD values.

Percentile	RfD (μg/kg/day)
1	0.29
5	0.35
10	0.38
25	0.44
50	0.53
75	0.63
90	0.77
95	0.86
99	1.10

exposed at low levels of methylmercury through ingestion of fish, it cannot be proven with absolute certainty.

It is therefore, recommended that an uncertainty factor of 3 be applied to the distribution of intake values listed in Table 18 to account for the abovementioned concerns in deriving a corresponding distribution of RfDs (Table 22). Since the distribution of intakes is a linear function of the maternal hair level, application of an uncertainty factor of 3 is essentially the same as using an uncertainty-adjusted BMD of 7 ppm rather than the 21 ppm derived from the Seychelles study. This hair level is lower than the 7.8 ppm, which is the lowest BMDL derived from the New Zealand study, when the influential individual is excluded from the analysis. It also provides a measure of conservatism to address the concerns raised by the Faroe Islands report.

RECOMMENDED SITE-SPECIFIC RFD FOR FISH-EATING POPULATIONS

We have derived a distribution of acceptable intakes from which to select a site-specific RfD for fish-eating populations (Table 22). This assessment has used the methodology for RfD determination outlined in USEPA guidelines for the assessment of reproductive and developmental effects (USEPA, 1996). This assessment has also incorporated suggestions made by the USEPA Science Advisory Board in their review of the USEPA report to Congress.[10]

Based on a review of the available literature on the effects of methylmercury, a study conducted with a population in the Seychelles Islands was selected as the critical study for this analysis. The exposures to methylmercury in this population result from chronic, multigenerational ingestion of contaminated fish. This prospective study was carefully conducted and analyzed, included a large cohort of mother–infant pairs, and was relatively free of confounding factors. The results of this study are essentially negative, and a NOAEL derived from the estimated exposures has recently been used by the ATSDR as the basis for a chronic oral MRL for methylmercury. In spite of the fact that no statistically significant effects were observed in this study, the data as reported are suitable for dose–response analysis using the BMD method. Evaluation of the BMD method used in this analysis, as well as in the current USEPA RfD, has demonstrated that the resulting 95% BMDL represents a conservative estimate of the traditional NOAEL, and that it is superior to the use of "average" or "grouped" exposure estimates when dose–response information is available, as is the case for the Seychelles study.

BMD modeling over the wide range of neurological endpoints reported in the Seychelles study yielded a lowest BMDL for methylmercury in maternal hair of 21 ppm (range of 21 to 26). This BMDL was then converted to an expected distribution of daily ingestion rates across a population using Monte Carlo analysis with a PBPK model to evaluate the impact of interindividual variability. The resulting ingestion rate distribution had a geometric mean of 1.60 μg/kg/day with a geometric standard deviation of 1.33; the 1st, 5th, and 10th percentiles were 0.86, 1.04, and 1.15 μg/kg/day. In place of the use of an uncertainty factor of 3 for pharmacokinetic variability, as is done in the current RfD, one of these lower percentiles of the daily ingestion rate distribution provides a scientifically based, conservative basis for taking into consideration the impact of pharmacokinetic variability across the population. On the other hand, it was felt that an uncertainty factor of 3 for database limitations should be used in the current analysis. Although there can be high confidence in the Benchmark-estimated NOAEL of 21 ppm in the Seychelles study, some results in the New Zealand and Faroe Islands studies could be construed to suggest the possibility of effects at maternal hair concentrations below 10 ppm. In addition, concerns regarding the possibility of sequelae, while not supported by the available data, can neither be completely ruled out. The use of an uncertainty factor of three yields a NOAEL of 6 ppm in maternal hair, which provides additional protection against the possibility that affects could occur at lower concentrations in some populations.

Based on the analysis described above, the distribution of acceptable daily ingestion rates (RfDs) recommended to serve as the basis for site-specific risk-management decisions at the Alcoa's Point Comfort Operations ranges from approximately 0.3 to 1.1 μg/kg/day, with a population median (50th percentile) of 0.53 μg/kg/day. Additional percentiles of this distribution are shown in Table 22.

The selection of which percentile of the distribution to apply at a particular site is a risk-management decision; however, such a decision should consider the underlying data used to develop these numbers. In particular, the

[10] The SAB recommended (1) the greater use of data from fish-eating populations, (2) the incorporation of PBPK modeling to characterize the dose to the developing fetus, and quantify variability, and (3) making use of all information, including animal data, to better characterize uncertainty.

following information should be considered. These RfDs were based on evaluation of very subtle effects using highly sensitive tests in the target population already identified as the most sensitive subgroup. No endpoint other than neurodevelopmental effects have been shown, either in studies of human populations or in experiments with animals, to exhibit greater sensitivity. The BMDL used as starting point for the distribution of intake values is the 95% lower bound on the maximum likelihood estimate of dose. In a study of reproductive and developmental endpoints in laboratory animals, estimation of the BMDL at a 10% BMR was shown to approximate the NOAEL but was estimated to be lower than the NOAEL 95% of the time (Allen et al., 1994a,b). Hence, the BMDL calculated from the Seychelles population provides a conservative estimate of the NOAEL.

Given these considerations, a value based on the 50th percentile, 0.5 μg/kg/day, would appear to be adequately protective. This value is identical to the MRL for methylmercury recently derived by ATSDR from the same study. However, to more fully capture the range of possible intakes in a population, an RfD could be selected in a manner analogous to the use of Monte Carlo analyses when applied in stochastic estimates of exposure to persons from chemicals contained in environmental media. According to USEPA (1992) guidelines, a reasonable definition of a most highly exposed individual is that individual exposed at the 90th percentile of the distribution of estimates of exposure. Analogously, the RfD would then correspond to the value at the 10th percentile of the pharmacokinetically derived distribution of intakes or 0.4 μg/kg/day (rounded from 0.38 μg/kg/day). On this basis, we recommend an RfD for fish-eating populations of 0.4 μg/kg/day. The range of acceptable values would include 0.53 μg/kg/day at the 50th percentile to 0.29 μg/kg/day at the 1st percentile, with values of 0.38 at the 10th percentile and 0.35 at the 5th percentile.

APPENDIX A

Table A-1. Summary table for neurological toxicity from oral exposure to methylmercury

Reference	Species no./ sex group	Doses (units)	Exposure frequency/ duration	NOAEL	LOAEL	Effect
Sato and Ikuta (1975)	Cynomolgus monkeys, M/F, 1/group (*exception*: 0.02 mg/kg/day group contained two monkeys)	0.01, 0.02, 0.03, 0.04, 0.21 mg Hg/kg/day MMC	Orally administered *via* a pellet for 62–327 days.	0.02 mg Hg/kg/ day	0.03 mg Hg/kg/day	Neurotoxic effects
Comment: This experiment omitted the use of a control group. The high-dose-treated monkey was the first to manifest symptoms of mercury toxicity after 57 days of administration. Signs of toxicity included seizures, loss of activity, tremor of the trunk, general clumsiness, weakness in grasping, ataxic gait, and anorexia. Histopathological examination of the high dose monkey revealed changes with astroglial proliferation in the occipital, parietal, and temporal lobes, which were similar to histopathological changes observed in people suffering from Minimata disease. Primarily, the most severe damage was observed in the calcarine and insular cortices. The 0.03 mg/kg/day treatment group did not show any detectable clinical signs of toxicity after 327 days of mercury administration. However, ultrastructural study did reveal changes in the nerve cells in the calcarine cortex in this group at that 327-day treatment period. It should be noted that although the LOAEL was identified as 0.03 mg/kg/day, the monkey administered 0.04 mg/kg/day did not develop any clinical or histopathological abnormality. Due to the small number of animals given each dose of methylmercury chloride, the power of this study to identify a NOAEL or LOAEL is limited.						
Evans et al. (1977)	Macaque monkeys, 6 M/6 F	Macaque monkeys: 0.4, 0.5, 0.6, 1.0 mg Hg/kg	Macaques were administered MMC *via* food biscuits.	Macaques: 0.4 mg Hg/kg	Macaques: 0.5 mg Hg/kg	Neural signs consisting of reduced sensitivity to visual stimuli and constricted visual fields were observed in both species of monkeys.
	Squirrel monkeys, 10 M	Squirrel monkeys: 0.1, 0.2 mg Hg/kg	Squirrel monkeys were administered MMC *via* gastric tube.	Squirrel: 0.1 mg Hg/kg	Squirrel: 0.2 mg Hg/kg	
Comment: The aim of this study was to identify indices of minimal intoxication resulting from chronic exposure. In order to achieve steady whole blood concentration of mercury in the range of 1 to 4 ppm, the range associated with approximately a 50% incidence of neurological effects in human exposed to methylmercury in Iraq, macaques were "primed" with doses of four or five treatments of 1 mg mercury/kg at 5-day intervals. The macaques were then administered maintenance doses of 0.4, 0.5, or 0.6 mg/kg as "maintenance" doses. Squirrel monkeys received a single priming dose of 1 or 2 mg mercury/kg, with maintenance dosing initiated 77 days after priming. Mercury concentrations in the brain were also determined. The highest Hg concentrations were observed in three areas of the brain: the calcarine region of the occipital cortex, the lateral geniculate body (not examined in squirrel monkeys), and corpus striatum. The description of the effects observed in this study was limited.						

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Table A-1. (continued)

Rice and Gilbert (1982)[a]	Macaque monkey, 5 treated, 2 controls, sex not specified	0 or 0.05 mg/kg/day	Methylmercury was administered in infant formula until weaning, then in gelatin capsules. Dosing continued until offspring were 6 1/2–7 years old (Rice, 1989)	None	0.05 mg/kg/day (ODT)	Impairment of spatial visual function

Comment: Testing occurred between 3 and 4 years of age. Test was to determine the animal's ability to see various frequency components of objects at high or low luminance. Blood levels in treated animals peaked at 1.2–1.4 ppm and declined after weaning from infant formula to steady-state level of 0.6–0.9 ppm. At high luminance, two of the treated animals had contrast sensitivity functions indistinguishable from controls, while two were impaired at high frequencies, and one was severely impaired at low frequencies. At low luminance, all treated monkeys (5/5) were impaired, although the patterns of deficit (frequencies at which impairment was observed) varied among monkeys. Two monkeys were impaired at frequencies greater than one cycle per degree, one was impaired at lower frequencies, while one was impaired at middle frequencies. Deficits under low luminance did not correlate with deficits in high luminance. Temporal vision effects on these animals are reported in Rice and Gilbert (1990).[a]

Rice and Gilbert (1990)	Macaque monkeys, 3 controls, 5 treated	0 or 0.05 mg/kg/day	Methylmercury was administered in infant formula until weaning, then in gelatin capsules. Dosing continued until offspring were 6 1/2–7 years old	See comment	See comment	None

Comment: This study reports results of two separate tests. This is the first experiment that was conducted on the same group of animals as those reported in Rice and Gilbert (1982), with a visual test conducted when the animals were 5 to 5 1/2 years that had not been conducted previously, temporal visual function. For the monkeys exposed as described above, temporal visual function in exposed individuals was superior to controls in both high/low luminance tests and narrow field tests.

Rice and Gilbert (1992)	Macaque monkeys, 3 control, 5 dosed	0 (vehicle control) or 0.05 mg/kg/day	Dosing from birth to 7 years of age initially in infant formula and then in gelatin capsules with corn oil	none	0.05 mg/kg/day (ODT)	Impaired high-frequency hearing (increased hearing thresholds)

Comment: NOTE: These test and control animals are the same as those in Rice and Gilbert (1982), reported above. These tests were performed when the monkeys were 14 years old–7 years after exposure had ceased. Tests were for "pure tone detection thresholds" at various frequencies. Frequencies between 125 and 31,500 Hz were used in the tests. One treated monkey (#34) exhibited normal detection thresholds at all frequencies (note: this same monkey had highly impaired spatial vision when tested at age 3–4). Conversely, #46 was the most impaired in the present study, but exhibited normal spatial visual function in the previous test. The other four treated monkeys (#35, 36, 39, and 46) showed impaired high-frequency hearing with differences in degree of impairment. Three of the four affected showed effects only at high frequency (25,000 Hz). It should be noted that no tests of hearing were done during the period of dosing.

Rice (1989)	Macaque monkeys, 3 or 4 controls, depending on test, and 5 treated	0 (vehicle control) or 0.05 mg/kg/day	Dosing from birth to 6.5 to 7 years of age initially in infant formula and then in gelatin capsules with corn oil	none	0.05 mg/kg/day (ODT)	Impaired fine motor performance, neurological assessment for touch sensitivity, motor assessment (degree of mobility and resistance to movement of joints)

Comment: NOTE: These test animals and two controls are the same as those in Rice and Gilbert (1992, 1982), reported above. These tests were undertaken when some treated monkeys, at age 13–14, began to show "signs of gross toxicity in the form of clumsiness in the large exercise cages." Fine motor performance was tested by "raisin pick-up test," in which time to retrieve raisins from recessed squares of a Plexiglas container were measured. Two control monkeys could not be tested on one hand (one preferred and one nonpreferred hand) because one finger could not be bent due to a previous break. Treated monkeys took longer to retrieve the raisins ($P=0.047$; Mann–Whitney U test). For the neurological exams, control male #06 was not used since he would not tolerate handling by strangers. He did, however, participate in the raisin retrieval test. Another control male from a different study was used for the neurological tests. These tests included a battery of sensory assessments (e.g., touch, pin prick). These tests showed that there were significantly more "no responses" in treated than in control animals. There were no differences between the two groups on motor assessment. Cage observations were admittedly subjective in nature, but are as follows: treated male #36 appeared normal; treated monkeys 46 and 39 were marginally clumsy and treated monkey 35 was extremely clumsy. The authors state these tests are "admittedly crude," but state that this group of monkeys is "displaying overt signs at 13 years of age that are presumably attributable to past exposure to MeHg."

Table A-1. (continued)

Rice and Gilbert (1995)	Macaque monkeys, 3 controls, 5 treated	0 or 0.05 mg/kg/day from birth	Dosing from birth to 7 years of age initially in infant formula and then in gelatin capsules with corn oil	none	0.05 mg/kg/day (see comments)	Threshold of detection of vibration by amplitude over five frequencies.

Comment: These test animals are the same as those in Rice and Gilbert (1982, 1990, 1992), and Rice (1989). The results of Rice (1989) on neurological effects must be carefully looked at in terms of the present study. These tests *began* when test animals were 18 years old (max. age in captivity is 36 years). Blood levels peaked at 1.2–1.4 ppm and declined after weaning from infant formula to steady-state levels of 0.8–1.1 ppm. Vibration thresholds were determined over frequencies of 25–250 Hz. The underside of the tip of the monkey's middle finger was positioned in contact with a blunt probe attached to a vibrator. Monkey #34 tested normal, three others (#s 36, 39, 46) had "substantially elevated vibration thresholds" at the three highest frequencies. Although based on the results of this study, 0.05 appears to be the LOAEL, in comparing these results to previous results for this monkeys, these effects raise questions. Monkey #35 had severely impaired vibration sensitivity in fingers of both hands at all but the lowest frequency tested. In comparing these results to Rice (1989), monkeys 34 and 35 (normal sensory function and severely impaired, respectively, in present study) were the most impaired in terms of clumsiness or reluctance to move; #34 scored high on "no response" to pin prick in Rice (1989). Monkey 39 was moderately impaired in the present study, but was the most impaired in terms of failure to respond to pin prick in Rice (1989). The authors stated that the present study results "... represent a permanent effect of MeHg exposure ..." and "... may represent permanent peripheral neuropathy." However, based on the results as of this study as reported, the determination of a NOAEL or LOAEL is questionable.

Munro et al. (1980)	Wistar rats, M/F, 50/group	0, 0.002, 0.01, 0.05, 0.25 mg Hg/kg/day MMC	MMC was fed *via* diet daily for 26 months.	0.05 mg Hg/kg/ day	0.25 mg Hg/kg/day	Neurotoxicity

Comment: The MMC was dissolved in corn oil before used in the diet. The dietary concentration of MMC was corrected on a weekly basis in order to maintain a constant dosing level. At 0, 6, 12, 15, and 17 months of the study, 10 rats from each group of 50 were anesthetized lightly and blood was collected for the determination of hematocrit, hemoglobin, and WBC (total and differential). This study reflected the sensitivity of male rats to MeHg toxicity compared to female rats when examining mortality, hematology, and renal pathology. No statistical tests were carried out between tissue Hg levels of males and females in the 0.25 mg Hg/kg/day dose group. Both male and female rats dosed at high level had decreased body weights. However, male rats also had decreased hematocrit and hemoglobin values, overt signs of neurotoxicity, and increased mortality rates, whereas females had minimal clinical signs of neurotoxicity when compared to controls. Histologically, demyelination of the dorsal root and peripheral nerves were reported in the 0.25 mg Hg/kg/day males.

Leyshon and Morgan (1991)	*Exp. 1*: Wistar rats, M, 5, 6, 5/respective treatment group. *Exp. 2*: Wistar rats, M, 5/group	*Exp. 1*: 0, 50 mg Hg/kg. *Exp. 2*: 0, 20 mg Hg/l H_2O.	*Exp. 1*: Dosed by gastric gavage for 15 days. *Exp. 2*: Daily administration for 14 or 42 days *via* drinking water.	*Exp. 1*: None. *Exp. 2*: None	*Exp. 1*: 50 mg Hg/kg (ODT). *Exp. 2*: 20 mg Hg/l H_2O (ODT)	Neurologic effects

Comment: Two experiments were carried out in order to utilize two different analytical techniques. In Exp. 1, rats were dosed for 15 days and killed 1, 14, and 28 days after the final dose. Exp. 2 consisted of exposing rats *via* drinking water with sacrifices at 14 and 42 days of exposure. In Exp. 1, the hind-leg crossing reflex was observed in the treated rats and was characterized by cerebellar cortical atrophy of the granule cell layer. Hg concentrations were reported to be greatest in the kidney, followed by the liver and cerebellum. In Exp. 2, the hindlimb crossing reflex was also observed in the treated rats. As with Exp. 1, Hg concentrations were greatest in the kidney, with smaller concentrations observed in the liver and cerebellum.

Miyakaw et al. (1976)	Wistar rats, M, 5/group	0, 1 mg	1 mg given orally for 10 days	None	1 mg (ODT)	Developmental defects

Comment: Male Wistar rats (five/group) were administered 1 mg of organic mercury compound (CH_3HgSCH_3) orally for 10 days. Upon examination of the sural nerve, myelinated nerve fibers were reduced and Schwann cells enveloping small myelinated nerve fibers were increased.

Postl et al. (1973)	Sprague–Dawley rats, M, 15/group	0, 2.0, or 2.5 mg/100 g body weight	Single dose	2 mg/100 g body weight	2.5 mg/100 g body weight	Statistically significant differences were observed in T-maze testing, open-field tests and open-field retests, when compared to controls.

Comment: Male Sprague–Dawley rats were force-fed single doses of MMC *via* cocoa butter or 1-2-propanediol in three experiments. In Exp. 1 and 2, a concentration of 2.0 mg/100 g body weight was fed to 15- and 21-day-old rats (15/group), respectively, and 2.5 mg/100 g body weight was fed to 60-day-old rats (15/group) in Exp. 3. Each experiment contained an additional group of 15 rats, which served as controls. Rats treated with 2.0 mg/100 g body weight showed no alterations in T-maze and open-field performances, when compared to controls. Rats treated with 2.5 mg/100 g body weight MMC took significantly longer time to reach the criterion of 9 out of 10 responses during the T-maze testing,

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Table A-1. (continued)

responded less than control rats for latency and areas of traversed in open-field tests, showed a greater response than control rats for inactivity and circling, showed less response than control rats for areas traversed and standing upright during open-field retesting, and showed a greater response than control rats for inactivity. No lesions of the brain that were attributable to treatment were observed during histological examination.						
Dellinger et al. (1995)	Hooded Long–Evans rats, F, 12/group	Treatment: 0, 0.2, 0.4, 0.8 ppm total Hg. Challenge: 2.5 mg/kg, s.c. D-amphetamine	Treatment: 90 days. Challenge: single dose	0.8 ppm total Hg	None	No statistically significant behavioral alterations were observed during the experimental or challenge phase, when compared to controls, except for the latency period between the N1 and N3 peaks in visual (flash) evoked responses in Hg-exposed rats.
Comment: Hooded Long–Evans female rats (12/group) were fed fish samples containing 0, 0.2, 0.4, or 0.8 ppm total Hg for 90 days. Standard behavioral assessments were conducted before dosing, and on days 1, 7, 14, 55, and 85 of dosing. Motor activity was evaluated before dosing and on days 30, 60, and 90 of dosing. Following the 90-day dosing regime, each rat was administered a challenge dose of 2.5 mg/kg D-amphetamine, subcutaneously. At 6 months post feeding, three rats from each group were surgically implanted with epidural electrodes, with a 2-week recovery period, to test auditory and visual activity. A statistically significant increase in body weight gain was observed in the 0.8-ppm group, when compared to controls. A statistically significant increase in latency period between the N1 and N3 peaks in visual (flash) evoked responses was observed in Hg-exposed rats, when compared to controls.						
Elsner (1991)	Wistar rats, F, 16/group. Offspring: 4/sex/group	0, 1.5 or 5 mg/l MMC	Females were offered MMC in the drinking water *ad libitum* 2 weeks before pairing until the end of lactation	None	1.5 mg/l MMC	Behavioral performance deficit
Comment: Animals were subjected to force-sensitive lever press tests to determine potential behavioral alterations. Results indicated slightly increased locomotor activity in the high-dose group, when compared to controls. A greater frequency of path iteration was observed in a dose-dependent manner. No treatment-related effects were observed at any of the time variables. Statistically significantly reduced performance was reported in the treated groups and a dose-dependent increase in the percentage of upper failures was observed in the final two training phases, with statistically significant increases in upper failures and forces reported in the high-dose group.						
Beattie et al. (1996)	Hooded Sprague–Dawley M/F, 7–8/group	Hg contaminated: walleye (0.57 μg Hg/ml), walleye spiked with MMC (Hg not pecified), whitefish (0.055 μg Hg/ml), carp (0.08 μg Hg/ml), trout (0.19 μg Hg/ml) or salmon (0.04 μg Hg/ml). Challenge: Hg contaminated: 2.5 mg/kg D-amphetamine	Rats were fed diets containing 30% fish to 70% chow ratio for 45 or 90 days. Challenge: Rats were given a single subcutaneous injection following the 90-day feeding exposure.	None	See comments	
Comment: Minimal effects were reported in the 45–day study. A statistically significant increase in brain weight was observed in animals fed walleye (1.855 g), when compared to animals fed whitefish (1.794 g). Significant increases in brain Hg levels were reported in rats that were fed walleye or spiked walleye. Statistically significant behavioral responses were observed in animals fed the Hg-contaminated diets and during the challenge phase, when compared to animals fed the control diet. A LOAEL could not be calculated using the units as reported in the article.						
Hughes and Annau (1976)	CFW F mice; offspring 4/litter	0, 1, 2, 3, 5, or 10 mg/Hg /kg BW MMOH	Pregnant dams were administered MMOH on day 8 of gestation by peroral injection.	2 mg Hg/kg BW	3 mg Hg/kg BW	Alterations in behavioral responses

Table A-1. (continued)

Comment: Behavioral tests indicated statistically significant increases in the number of trials to criterion and in the number of escapes in session one and significant decreases in the number of avoidances in session one in two-way active avoidance experiments. In pups from the 3 or 5 mg Hg/kg biological- or foster-mother treated groups, when compared to controls. Also, statistically significant increases in the number of trials to criterion in the shuttle box test in pups from the 3 or 5 mg Hg/kg biological- or foster-mother treated groups were observed, when compared to controls.

Mitsumori et al. (1990)	B6C3F1 mice, 60/sex/group	0, 0.4, 2, 10 ppm MMC	MMC mixed with chow and fed to mice for 104 weeks.	*Males*: 0.4 ppm MMC; *Females*: 2 ppm MMC	*Males*: 2 ppm MMC; *Females*: 10 ppm MMC	Chronic nephropathy

Comment: Neurologic effects were reported in the high-dose groups and included posterior paralysis, encephalopathy, and peripheral sensory neuropathy. An increase of chronic nephropathy was observed in both males and females, and in males, testicular atrophy and glandular stomach ulcer were observed. There were significant increases in the incidence of renal adenoma and/or carcinoma (16/60) and tubular cell hyperplasia (14/60) inmales of the high-dose group. Fisher's exact test was used to determine the statistical significance of the incidence of histopathological lesions between the control group and each of the treated groups.

Sager et al. (1982)	BALB/c mouse pups, M/F, 10 control, 14 treated	0, 8 mg Hg/kg body weight	Single oral dose administered postnatally day 2.	None	8 mg Hg/kg body weight (ODT)	Alteration in cerebellar tissue architecture

Comment: Single-dose administration of MeHg to 2-day-old pups resulted in histologic changes in the external granular layer of the cerebellar cortex 24 h posttreatment. These changes included statistically significant decreases in cell numbers, decreases in the size of nuclei and decreases in the numbers of late mitotic figures, when compared to the controls. However, mitotic activity was not altered.

Chang et al. (1974)	Juvenile cats, M and F, 15 controls, 16 treated	0, 0.5 ppm Hg	Animals fed Hg-contaminated tuna for 11 months	None	0.5 ppm Hg (ODT)	Neurological disturbances such as ataxia, weakness, and incoordination and histological changes in the brains of exposed animals.

Comment: Results indicated that 3 of the 16 Hg-treated animals developed observable neurological disturbances such as mild to severe ataxia, weakness, and incoordination of movements. Histopathological examination revealed lesions in the nervous system, with the most prominent lesions seen in the cerebellum. Study did not give histopathological results for control animals.

Charbonneau et al. (1976)	Cats, 8–10/4–5 sex/group	0, 3, 8.4, 20, 46, 74 or 176 μg Hg/kg/day	Cats were fed MMC or MeHg-contaminated fish 7 days/week for up to 2 years.	20 μg Hg/kg/day	46 μg Hg/kg/day	Neurological impairment

Comment: Clinical signs of toxicity were observed in animals receiving 176 and 74 μg Hg/kg/day at 14 or 40 weeks, respectively. Pathological examination revealed nervous system effects consisting of loss of nerve cells with replacement by reactive and fibrillary gliosis. Neurological impairment was observed in animals receiving 46 μg Hg/kg/day after 60 weeks. However, neurological impairment did not intensify for the rest of the exposure period. Clinical signs of toxicity included ataxia, loss of balance and motor incoordination. Following 2 years of treatment, no treatment-related effects were observed in animals in the 20, 8.4, or 3 μg Hg/kg/day groups. There were no MeHg-related effects noted in hematology, urine analysis, BUN levels and terminal bone marrow tests in any of the treated groups.

NOTE: MeHg=methylmercury; MMC=methylmercuric chloride; MMD=methylmercury dicyandiamide; MMOH=methylmercury hydroxide; ODT=only dose tested.

[a]It should be noted that in the various Rice, and Rice and Gilbert publications, only two cohorts of animals are used in experimentation over 18 years. The first cohort is a group of two controls and five dosed animals, with the treated animals receiving 50 μg/kg/day from the time of birth until approximately 7 years of age. The second group is composed of offspring from females receiving 0, 10, 25, or 50 μg/kg/day before and during pregnancy. The females were treated for two different lengths of time before mating — for either 4 months or up to 2 years. The offspring from these mothers (numbering 5, 1, 2, and 5, respectively, per dose group) received doses, following birth equivalent to those given their mothers. As these two cohorts aged, numbers on test sometimes changed due to individuals dropping out from illness or other reasons. In the group tested *in utero* and postnatally, test results were often combined for the two highest dose groups.

Table A-2. Summary table for developmental toxicity from oral exposure to methylmercury

Reference	Species no./sex group	Doses (units)	Exposure frequency/duration	NOAEL	LOAEL	Effect
Newland et al. (1994)	Squirrel monkey, 3 M	0, 0.7 to 0.9 ppm MeHg in maternal blood	MeHg was administered by gavage to pregnant monkeys between weeks 11 and 14.5 of gestation through approximately week 22 of gestation.	Indeterminate	Indeterminate	

Comment: Monkeys were trained to lever press at 5–6 years of age. Concurrent reinforcement schedules were employed on two levers. During training, monkeys received reinforcements equally from each lever. After training, the reinforcements changed where one lever resulted in more reinforcements.

(continued on next page)

Table A-2. (continued)

The monkeys were tested to see how quickly they could transition. In the monkeys exposed to methylmercury *in utero*, transitions to changes in reinforcement were much slower. This study is limited due to the small number of animals tested.

Rice and Gilbert (1990)[a]	Macaque monkeys, 5 live births from controls, 1 from low-dose, 2 from intermediate, and 5 from high-dose	*In utero* to maternal levels of 0, 0.01, 0.025, or 0.05 mg/kg/day; postnatally to equivalent doses	Dams dosed 3× per week for several months until steady-state blood levels were achieved; offspring dosed 5× per week at same dose as mothers through duration of test; until 4 to 4 1/2 years old.	See comment	See comment

Comment: This is the second of two experiments reported in Rice and Gilbert (1990). In this study, monkeys were exposed *in utero* (mothers given 0, 10, 25, or 50 μg/kg/day) to maternal blood levels of mercury that averaged 0.37, 0.75, and 1.42 ppm for the three dose groups, respectively. Infants were born with blood levels averaging 1.7 times those of their mothers. Although the high-exposure group initially consisted of five animals, one of the offspring had myopia in both eyes severe enough to interfere with spatial visual function at high frequencies, and two of the offspring were born with clear signs of methylmercury intoxication; however, only the two animals with clear intoxication were eliminated from the visual testing, reducing the high-exposure group to three animals. Over the course of additional dosing, offspring had average levels of 0.21, 0.35, and 0.65 for the three dose groups. These offspring were tested for both spatial and temporal visual function (age for spatial tests 3 months after dosing ceased (4.3–4.6 years), tests for temporal visual function were done at ages 6 to 6 1/2 years The authors reported that spatial vision was impaired in treated animals under both high- and low-luminance conditions, but that dose–response could not be determined (2 high-dose monkeys were normal, one mid- and low-dose monkey were impaired; two more high-dose monkeys could not be tested due to illness). When all examined animals are pooled, three of six exhibited impaired spatial vision. For these same animals, temporal visual function was impaired at middle to low frequencies at high luminance, but all treated monkeys were superior to controls under low-luminance conditions. The meaning of these tests is unclear. However, the authors report that "... low-level developmental MeHg exposure in the monkey is unlike adult MeHg exposure in its effects on visual system function." They suggest that diffuse cortical damage from developmental exposure may result in degeneration of some visual functions with "concomitant over representation of others," also stated as "... the developing visual system may be able to remodel as a result of early insult by a neurotoxic agent."

Rice (1992)	Macaque monkeys; 5 live births from controls, 1 from low-dose, 2 from intermediate, and 5 from high-dose (see comments)	*In utero* to maternal levels of 0, 0.01, 0.025, or 0.05 mg/kg/day. Equivalent doses to offspring following birth	Details of exposure to females as reported in Rice et al. (1989): females were given MeHg three times a week until all reached at least 99% of their estimated equilibrium values (up to 2 years) using a one-compartment model, after which they were put on a breeding program. Females were dosed throughout pregnancy.	0.05 mg/kg/day

Comment: These test animals are the same as those on test in Group 2 of Rice and Gilbert (1990), since reported blood levels are almost identical (maternal blood Hg levels averaged 0.33, 0.78, and 1.41 ppm in the three treated groups, respectively. Infants averaged 0.20, 0.25, or 0.60 ppm at steady state). Number of animals actually tested is difficult to track. Infants were started on operant training as soon as self feeding (within 1 week of birth). It is unclear when infant testing began; juvenile testing took place between 2 and 3 years of age. One animal in the high-dose group was unsuitable for test, leaving four high-dose animals. Results from the high-exposure animals were combined with those from the two mid-dose animals. The one low-dose animal was not included in test "due to lack of reliable data" (Rice and Gilbert, 1995). Animals were tested for both discrimination reversal (DR) and fixed-interval performance (FI) as infants and for DR as juveniles. These studies failed to show deficits in nonspatial discrimination reversal performance. "In fact, treated monkeys performed marginally better than controls during initial task acquisition during infancy and as juveniles." The authors also stated that the treated monkeys performed "differently" from controls on fixed-interval tests "... as evidenced by shorter pause times in the treated group." The authors inferred that this endpoint reflects "... poorer temporal discrimination" due to MeHg exposure. However, there is no information to substantiate this statement. Therefore, this study, overall, is a negative study.

Rice and Gilbert (1995)	Macaque monkeys, 5 live births from controls, 1 from low-dose, 2 from intermediate, and 5 from high-dose	*In utero* to maternal levels of 0, 0.01, 0.025, or 0.05 mg/kg/day. Equivalent doses to offspring following birth	Females dosed before and during pregnancy. Newborns dosed with equivalent doses as mothers to approx. age 4.	Indeterminate	Indeterminate

Table A-2. (continued)

Comment: These test animals are the same as those in Rice (1989) and Rice and Gilbert (1982, 1990, 1992). The results of Rice (1989) on neurological effects must be carefully looked at in terms of the present study. These tests *began* when test animals were 18 years old (max. age in captivity is 36 years). Mothers of exposed offspring had blood Hg levels at parturition of 0.37, 0.75, and 1.42 ppm for the three dose groups, respectively. Blood Hg of infants decreased following birth, to average levels of 0.21, 0.35, or 0.70 for the three dose groups. After dosing ceased, blood levels were 0.01–0.02 ppm. Control animals were also at these blood Hg levels (0.01–0.02 ppm). Vibration thresholds were determined over frequencies of 25 to 250 Hz. The underside of the tip of the monkey's middle finger was positioned in contact with a blunt probe attached to a vibrator. In monkeys exposed *in utero* and postnatally, both high-exposure monkeys exhibited only slight impairment, while both monkeys from the lower dose group showed marked impairment at all but the lowest frequency [no dose response]. This group of monkeys has exhibited no overt signs of toxicity, in contrast to the group exposed postnatally. At this age of testing, these monkeys are past the age when gross signs began to manifest themselves in the monkeys exposed postnatally only. Because of the lack of dose response for effect in this cohort, a NOAEL or LOAEL could not be determined.

Gunderson et al. (1986)	Macaque monkeys, infants, 10 from control females, 8 from low-dose, 2 from high-dose	*In utero* to maternal levels of 0, 0.05 or 0.07 mg/kg/day	Daily doses in apple juice; onset of dosing time is not precise, but stated as "... before being mated." Females were dosed throughout pregnancy.	Indeterminate	Indeterminate

Comment: Mean blood Hg level of offspring at birth was 1.69 ppm, at onset of testing (190 days postconception, ~31 days postnatal), 1.04 ppm. At birth, the two offspring of 0.07 mg/kg/day females had blood levels of 1.94 and 1.57, within the same range as the 0.05 mg/kg/day group (0.88–2.46 ppm); however, higher blood levels were observed in the 0.05 mg/kg/day group. None of the exposed mothers showed signs of toxicity. This study is based on the premise that both human and rhesus macaque subjects show longer response latencies to new items than to familiar ones (termed the "novelty paradigm"), with "... the inference being that the entire memory store must be searched to rule out the absence of these stimuli from the original list." The animals were tested using an adaptation of Fagan's Test of Infant Intelligence, using abstract black and white patterns. Infants were tested at 31, 41, and 51 days of age. There were no differences in mean duration of looks to novel or familiar patterns between groups. However, control animals spent significantly longer looking at novel stimuli, as expected. In contrast, treated animals did not look statistically significantly longer at the novel stimuli. The authors conclude that the treated infants "... perform at a significantly lower level than do controls on a test of visual recognition memory." However, because statistical analyses were conducted on the pooled data from two different dose groups, and individual data were not reported, a NOAEL or LOAEL could not be determined.

Gunderson et al. (1988)	Macaque monkeys, 8 from control mothers, 7 from low-dose, 2 from high-dose	*in utero* to maternal levels of 0, 0.05 or 0.07 mg/kg/day	Additional information on dosing before pregnancy (see Gunderson et al., 1986). Three females had long exposures (mean 747 days) before conception, 6 had short exposure (mean 168 days).	Indeterminate	Indeterminate

Comment: This is a follow-up on the animals reported in Gunderson et al. (1986) to "... determine whether they would continue to show a novelty response when given more challenging problems." Two control animals were eliminated because of fussiness during the test sessions and an additional methylmercury animals was eliminated because of severe birth trauma. The test administered was similar to that reported in the Gunderson et al. (1986) report; however, instead of abstract shapes for recognition stimuli, the animals were shown a series of five pair of slides of pigtailed macaque faces. In addition to the blood Hg levels reported previously, blood levels just before onset of testing had a mean of 0.54 ppm, significantly lower than those at birth. Tests were performed when animals were between 50 and 60 days old. Controls directed a mean of 60% of looking time to the novel stimuli, which is statistically significantly different ($P<0.01$) from chance (i.e., 50%). Treated animals directed a mean of 53%, which was not statistically different from chance and was, therefore, termed "random." Five of the nine exposed infants looked longest at the novel stimuli, a value not differing from chance. The authors state that the current test using photographs is a more complicated "social recognition problem" than that described above using abstract shapes. The authors conclude that these results "... provide further evidence that [exposed] infants ... perform differently than nonexposed controls on visual recognition tests." However, they also concluded that the control and exposed groups did not differ significantly from each other in the proportion of looking time directed to novel stimuli. There was also no relationship between MeHg blood levels and dose or between blood levels and magnitude of novelty response. Because of the grouping of the exposed animals for analysis, no NOAEL or LOAEL could be determined.

Burbacher et al. (1986)[b]	Macaque monkeys, 12 from control mothers, 12 from dosed mothers (9 from short-term; 3 from long-term)	*In utero* to maternal levels of 0 or 0.05 mg/kg/day	Treatment before conception of mothers was termed short-term (4 months) or long-term (2 years). All dosed females received mercury throughout pregnancy.	None	*In utero* to maternal levels of 0.05 mg/kg/day (ODT)	Performance on object permanence tests — retrieval of object in plain site, object partially hidden, and object fully hidden

Comment: The assessment of object permanence is considered an established test of early cognitive development in humans and in nonhuman primates. This study is part of the same test series as Gunderson et al. (1986) and Gunderson et al., (1988). In fact, Gunderson et al. (1986) used a subgroup of

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Table A-2. (continued)

infants on test in the current study. Mean maternal blood Hg level at delivery was 0.96 ppm for treated mothers and that of their infants at birth was 1.565 ppm. Mean Hg blood level of infants at beginning of test was 1.1 ppm. The plain reach test began at 14 days of age. Criterion was achieved when the infant successfully picked up the object on 8 of 10 consecutive trials. After criterion was reached on plain reach task, testing on three hiding tasks began. The object to be retrieved was placed in front of an opaque horizontal screen (no hiding), halfway behind the screen (partial hiding), or completely behind the screen (full hiding). Performance of the offspring of short-term exposed mothers and that of offspring of long-term exposed mothers and their controls did not differ significantly for maternal blood Hg, rate of infant blood Hg clearance, plain reach, no hiding, and full-hiding tasks. Therefore, data from both exposed groups were combined. However, offspring from long-term treated mothers performed significantly differently for partial-hiding task compared to the other groups and these data were not combined with the other treated group (offspring from short-term treated mothers). Two comparisons were made for each test: mean number of sessions required to achieve criterion and mean age at which criterion was met. There was no significant difference in age to criterion for the plain reach test, but number of sessions was significantly higher (2-way ANOVA) in treated infants for this test. For the no-hiding test, there was no difference in either number of sessions or age to achieve criterion. For the partial-hiding task, performance of infants from long-term treated mothers was significantly different (requiring more sessions) from both infants of short-term treated mothers and controls ($P<0.05$). There were no significant differences in age to criterion. Overall performance of infants from short-term treated mothers did not differ from controls. The performance of MeHg-exposed Infants was significantly retarded compared to the control infants for the full-hiding task. Treated animals required significantly more sessions and were significantly older than controls at the time criterion was met. Note: The text is not in agreement with footnotes on significance in Table 4; therefore, results of the text are reported when there is disagreement. It is noted in the text that the results on delayed reaching were significantly affected by three treated infants who repeatedly refused to orient to the object during the first several days of plain reach testing, but after successful orientation, these individuals performed well. The authors stated that "Because the attentional problems exhibited by these infants cannot be separated from possible motor deficits, the differences in the reaching responses of MeHg-exposed and control infants cannot be clearly interpreted at this time." The performance of the full-hiding task requires object permanence and treated infants performed significantly poorer than controls. On average, exposed infants required nearly twice as many sessions and were over 1 month older than control infants when they could retrieve the fully hidden object. Because the attentional problems had been overcome well before the full-hiding task was initiated, this factor is thought to have no effect on the full-hiding results.

Burbacher et al. (1990b)	Macaque monkeys, 13 from control mothers, 12 from exposed mothers (9 from short-term; 3 from long-term)	*In utero* to maternal levels of 0 or 0.05 mg/kg/day	Females were dosed for either 4 months (short-term) or 2 years (long-term) before mating, and throughout pregnancy.	None	*In utero* to maternal levels of 0.05 mg/kg/day	Effects on social behavior gauged by six categories of social behavior and seven of nonsocial behavior.

Comment: These are the same animals reported in Burbacher et al. (1986) (see maternal and infant-at-birth-blood Hg levels reported above). Testing began when infants were 2 weeks old, continuing until 18 months of age. Testing was conducted in a playroom containing shelves, ladders, toys, and a two-way observation window. Effects of MeHg exposure were tested using ANOVA. The interaction of MeHg exposure and age was not significant, but the difference between MeHg and control offspring tended to increase with age for nonsocial passive behavior and decrease with age for social play. The amount of social play, and social behavior in general, exhibited by the MeHg-exposed offspring (almost exclusively "rough and tumble") was consistently lower than the control infants. This decrease in social play was accompanied by an increase in the amount of nonsocial passive behavior. This leads the authors to conclude that maternal intake of MeHg before and during pregnancy affects social behavior of exposed offspring.[b]

Gilbert et al. (1993)	Macaque monkeys, 11, 9, 2, 2 offspring per dose group, respectively	*In utero* to maternal levels of 0, 50, 70, or 90 μg/kg/day	Treatment of females daily before and during pregnancy (treatment before pregnancy for either 4 months or up to 2 years).	90 μg/kg/day — highest dose tested (HDT)		Performance on fixed- or variable-delay tasks.

Comment: Female adult monkeys were exposed to 0, 50, 70, or 90 μg MeHg/kg/day in apple juice before and throughout pregnancy. At approximately 7–9 years of age the offspring were trained to respond on a lit button for drink reward, with variations on response, such as fixed and variable delay (spatial delayed alternation task). Data from all treated animals were combined into one treated group due to small number of test animals and overlapping blood Hg levels. There were no differences between treated and control monkeys in initial training. On fixed delay tests, treated monkeys performed better than controls. Authors report that results indicate that *in utero* MeHg exposure did not adversely affect spatial memory of adult monkeys when tested on a delayed alternation task and may even have facilitated performance. Note: Many of these test animals were also used in a previous study reported positive for effects on long-term deficits in spatial memory (Burbacher et al. 1986).

Chen et al. (1983)	Macaque monkeys, M, 6 groups, 1/group.	80–125 μg MeHg/kg/day in apple juice. A control subject was not utilized in this study.	*Monkey #1*: 125 μg MeHg/kg/day for 3.5 months *Monkey #2*: 80 μg/kg/day for 7 months *Monkey #3*: 80 μg/kg/day for 12 months	None	None	None

Table A-2. (continued)

			Monkey #4: 100 μg/kg/day for 10 months *Monkey #5*: 80 μg/kg/day for 15 months *Monkey #6*: 90 μg/kg/day for 10 months			

Comment: Sacrifice of monkeys 4, 5, and 6 was delayed for 5, 2.5, and 4.5 months, respectively, to allow for body clearance of MeHg, one of the parameters evaluated in this study. These three monkeys showed a sharp decrease in blood Hg concentrations after ceasing the MeHg treatment. Changes in the ultrastructure of the kidney, liver, and intestine were observed in monkeys 1–3. In monkeys 3–5, ultrastructural changes in the liver and intestine were less severe than the changes observed in monkeys 1–3. Clinical laboratory tests, including hematocrit, plasma glucose, liver function tests, electrolytes and kidney function tests were conducted on blood samples collected weekly from each monkey. No treatment-related effects on any of these parameters were reported. A NOAEL and a LOAEL could not be determined due to the use of only one monkey/group and the failure to employ a control group.

Khera and Tabacova (1973)	Wistar rats, F, 5 groups, 35/group.	0, 0.002, 0.01, 0.05, or 0.25 mg Hg/kg/day	MMC was administered in the diet for 122 days.	Indeterminate	Indeterminate	

Comment: No treatment-related effects on body weight gain, behavior, pregnancy, or histological kidney changes were reported in the Hg-exposed dams, when compared with the controls. There were no treatment-related macroscopic or microscopic changes in the brain, spinal cord, or viscera of fetuses from treated dams, when compared to the control fetuses. However, there was a statistically significant dose-related positive trend in the incidence of eyelid defects. The author stated that the aberrations observed in the eyelid development were not directly related to methylmercury treatment, but mercury was a contributory factor, "in some unexplained way."

Bornhausen et al. (1980)	Wistar rats, 10/sex/group	0, 0.005, 0.01, and 0.05 mg/kg body weight	Pregnant rats were administered MMC dissolved in distilled water by stomach tube on gestational days 6, 7, 8, and 9.	0.005 mg/kg body weight	0.01 mg/kg body weight	Reduction in behavioral performance

Comment: The offspring (10/group) were randomly assigned to one of three lever boxes to determine behavioral performance tested by the "differential reinforcement of high rates" (DRH) program. In the first step of the test session (DRH 2/1), animals were required to press the lever twice within 1 s to obtain a food pellet. The second and third steps required the lever to be depressed four times in 2 s (DRH 4/2) and eight times in 4 s (DRH 8/4), respectively, to obtain food pellets. During the 1-h DRH session for each step, 5-min periods of light and 5-min periods of dark were alternated, with no reinforcement for the dark period. Results of the behavioral performance testing resulted in more distinct dose-dependent differences in test performances in the second (4/2) and third (8/4) test sessions. Animals in which the mothers were treated with 0.05 MMC showed the highest deviation from control values in the two-way analysis of variance. No behavioral alterations were observed in offspring of mothers treated with 0.005 MMC. No significant sex-dependent effects on DRH performance were observed in a three-way analysis of variance. All animals, with the exception of males treated prenatally with the highest MMC dose, showed a decrease in lever presses during 5-min periods of no light, when compared to 5-min periods of light.

Musch et al. (1978)	Wistar rats, F, (9/group)	0, 0.05, 2.0 mg/kg	Pregnant dams were administered MMC in distilled water by stomach tube on days 6, 7, 8 and 9 of gestation.	None	0.05 mg/kg	Statistically significant differences in behavioral performance tests, when compared to controls.

Comment: The female offspring (nine/group) were randomly assigned to one of three lever boxes to determine learning ability using the differential reinforcement of high rates (DRH) operant conditioning schedule. In the first step of the test session (DRH 2/1), animals were required to press the lever twice within 1 s to obtain a food pellet. The second and third step required the lever to be depressed four times in 2 s (DRH 4/2) and eight times in 4 s (DRH 8/4), respectively, to obtain food pellets. Statistically significant differences were observed between the three test sessions. Animals showed more distinct dose-dependent deficiencies in test performances in the second (4/2) and third (8/4) test sessions. Bar press activity was increased more prominently between the first (2/1) and second (4/2) test sessions in all groups.

Zenick (1976)	Holtzman albino rats, F, 5/group. Offspring: 8/litter	0, 2.5 mg Hg/kg/day as MMC	Pregnant dams were administered MMC *via* drinking water from onset of pregnancy to 21 days after birth; Offspring were administered MMC *via* drinking water from 21 days of age to 30 days of age.	None	2.5 mg Hg/kg/day MMC (ODT)	Alterations in visual evoked potentials

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Table A-2. (continued)

Comment: A statistically significant reduction in visual cortex (VEP) latencies for peaks N1, P1, and P2 was observed in 30-day-old offspring from dams treated during gestation or nursing, when compared to controls. Offspring directly exposed to MMC for 9 days after weaning also showed a statistically significant reduction in VEP latencies for peaks N1, P1, and P2, when compared to controls.

Olson and Boush (1975)	Female rats (strain unspecified) Offspring: 20 (Group 1, marlin), 19 (Group 2, tuna), and 20 (Group 3, controls)	0 or 2 ppm Hg/kg diet	Pregnant dams were divided into three groups and fed diets containing 0 or 2 ppm Hg/kg diet, in the form of Pacific Blue marlin or tuna, from day 1 of gestation through the duration of the experiment.	None	2 ppm Hg (ODT)	Learning deficit

Comment: A statistically significant decrease in the size of the Group 1 pups was reported, when compared to control pups. Statistically significantly slower development was noted with regard to swimming behavior and righting reflexes in Group 1 pups over days 7 through 15, when compared to controls. Group 1 pups were shown to be significantly inferior to control animals in the righting reflex test on days 14 through 17 and Group 2 pups on days 15 and 16. Motivational tests showed a statistically significant increase in speed by the high- vs. the low-hunger rats. A statistically significant learning deficit was observed in Group 1 pups, when compared to control rats and to Group 2 pups. Hg levels measured in the pups from the three groups were ranked in increasing order as follows: controls, Group 1, and Group 2.

Fredriksson et al. (1996)	Female Sprague–Dawley rats, 12/group; Offspring: 4 M/group	0, 2 mg MeHg/kg/day, 0.1 mg HgE/kg/day, or MeHg+HgE	Pregnant dams were exposed to (1) MeHg n days 6–9 of gestation and vehicle control on days 14–19 of gestation; (2) HgE on days 14–19 of gestation and vehicle control on days 6–9 of gestation; (3) exposed to MeHg on days 6–9 of gestation and HgE on days 14–19 of gestation	2 mg MeHg/kg/day (ODT)	0.1 mg HgE/kg/day (ODT)	Alterations in behavioral responses

Comment: Pregnant dams received oral doses of MeHg or were exposed to HgE vapor at a concentration of 1.8 mg/m^3 air for 1.5 h/day. Offspring were subjected to spontaneous motor activity and swim maze learning tests at 4 months of age and radial arm maze activity and learning at 5 months of age. A statistically significant increase in locomotion, rearing, and total activity was observed in offspring from groups receiving HgE and MeHg+HgE, when compared to controls. Statistically significantly longer latency times in the swim maze learning test and statistically significant increases in the number of ambulations and rearings, latency, and number of errors in the radial arm maze test were observed in offspring from HgE and MeHg+HgE groups, when compared to controls. The total Hg concentration in the tissue of the brain of offspring from all treated groups was statistically significantly greater than concentrations observed in the brain tissue of control animals.

Schreiner et al. (1986)	Wistar rats, 6/sex/group	0, 1.5, and 5.0 mg MMC/l; Challenge: 0.5 mg/kg amphetamine	Rats had been exposed prenatally to MMC *via* drinking water.	1.5 mg MMC/l	5 mg MMC/l	Impairment of visual discrimination

Comment: A statistically significant reduction of the intertrial interval (ITI) response rate in the visual discrimination phase was observed in high-dose males, when compared to controls. Results of the visual discrimination reversal test showed statistically significant increases in passiveness and latency in the high-dose males and latency in the high-dose females, when compared to controls. Amphetamine (0.5 mg/kg) was given to animals on the fourth day of reversal testing as a challenge dose. No significant effects were observed following the challenge phase.

Stoltenburg-Didinger and Markwort (1990)	Wistar F rats, offspring M and F (no. unspecified)	0, 0.025, 0.05, 0.5, 5.0 mg MMC/kg	Pregnant females were administered MMC by gavage on days 6 through 9 of gestation.	0.05 mg MMC/kg	0.5 mg MMC/kg	Behavioral alterations

Comment: Significantly impaired swimming was reported in offspring from the highest dose group, when compared to controls. In the visual discrimination reversal task, males in the highest dose group showed less nosepoking during the intertrial interval, when compared to controls. Increased passiveness during locomotion testing was observed in males in the highest dose group, when compared to controls. On days 180 and 210, increased auditory startle amplitude was observed in the two higher dose groups, when compared to controls. Abnormalities, such as stubby, mushroom-shaped and long, thin and tortuous-shaped spines, were observed in animals in the highest dose group, when compared to controls. Does not indicate any statistical analysis was performed in the study.

Table A-2. (continued)

Vorhees (1985)	Sprague–Dawley rats, F, 16, 15, 17, 19/respective treatment group	0, 2, 6 mg MeHg/kg/day	2 control+2 treatment groups dosed on gestational days 6–9.	None	2 mg MeHg/kg/day	Neurobehavioral deficits

Comment: The high dose produced significant increases in gestation length and decreases in maternal weights and postnatal survival from days 1–21. Specific developmental abnormalities noticed in the high-dose pups were early incisor eruption rates, reduced body weight at day 60, and delayed vaginal patency. Neurobehavior disturbances in the pups included significantly slower surface righting (high-dose), accelerated geotaxis (low-dose), and delayed swimming development (high-dose). Increased water maze times were reported in pups exposed to either 2 or 6 mg Hg/kg.

Buelke-Sam et al. (1985)	**Rats, 16 litters/group	Vehicle controls, 0, 2, 6 mg/kg MMCl	Administered by gavage on gestational days 6–9.	None	2 mg/kg MMCl	Neurotoxicity

Comment: This was a collaborative study grouping the data collected from six different laboratories. Neurological discrepancies were assessed by examining the following parameters: negative geotaxis, olfactory discrimination, auditory startle, 1-h activity, 23-h activity, activity following pharmacological challenge to D-amphetamine, and visual discrimination task. No treatment related effects were observed in animals that underwent the negative geotaxis and olfactory discrimination performance tests. Significant dose effects were noticed in 1-h activity levels, and 1-h activity levels challenged with D-amphetamine. The visual discrimination test recorded fewer total correct responses from the high-dose group when compared to controls. The high-dose group also displayed increased responses in the startle task. The purpose of this study was not to provide more prenatal exposure information for MMCl, but to determine the consistency of data within each of the six laboratories involved. **Animal strain could not be determined from this article.

Cuomo et al. (1984)	Sprague–Dawley female rats, male pups (8/group)	0, 8 mg MMC/kg	Pregnant dams were administered 8 mg MMC/kg on day 8 of gestation.	None	8 mg MMC/kg (ODT)	Behavioral alterations including stereotyped sniffing, locomotor activity and avoidance latency.

Comment: Fifteen- and 22-day-old pups prenatally exposed to MMC were challenged with apomorphine, a dopaminergic agent, s.c.; 15-, 22-, 40-, and 60-day-old pups prenatally treated with MMC were challenged with clonidine hydrochloride, a noradrenergic agent, s.c. MMC pretreated rats showed a statistically significant increase in ^{3}Hspiroperidol binding sites in striatal membranes at 22 days of age, when compared to controls; however, at 40 and 60 days of age, the affinity (Kp) and number of ^{3}Hspiroperidol binding sites were not affected in rats treated prenatally with MMC, compared to the affinity and number of binding sites in the controls. *Stereotyped behavior*: Rats treated prenatally with MMC and challenged with 1 mg apomorphine/kg at 15 days of age or with 0.5 or 1.0 mg apomorphine/kg at 22 days of age showed a statistically significant increase of stereotyped sniffing, when compared to controls. Modifications in stereotyped behavior were not observed in rats at 40 or 60 days of age, which had been treated prenatally with MMC and challenged with apomorphine. *Locomotor activity*: There were no significant differences in locomotor activity in rats of 15, 22, 40, or 60 days of age that had been treated with MMCA. However, statistically significant decreases in locomotor activity were observed in both control and MMC treated rats challenged with clonidine at 15, 22, 40, and 60 days of age. *Passive avoidance*: Rats (60 days old) pretreated with MMC, showed statistically significantly shorter avoidance latency, when compared with controls. Body weights were not affected by MMC treatment at any postnatal age, when compared to controls.

Dyer et al. (1978)	Long–Evans hooded F rats, 4 treated, 6 control; Offspring: F 18 controls, 13 treated	0, 5 mg/kg MMC	Pregnant dams were administered a single dose of MMC *via* corn oil by gastric intubation on day 7 of gestation.	None	5 mg MMC/kg (ODT)	Increased P1–N1 amplitudes and decreased P2 and N2 latencies.

Comment: Female offspring from treated and control dams were implanted with recording electrodes at 60 days of age. The visual evoked potentials of the female offspring were monitored at four flash intensities. Results of the study showed statistically significantly increased P1–N1 and N1–P2 amplitudes and shorter P2 and N2 latencies in Hg-treated animals, when compared to control animals.

Fowler and Woods (1977)	Charles River female rats (20/group), fetuses (no. unspecified)	0, 3, 5, or 10 ppm in drinking water of dams	Females exposed daily *via* drinking water 4 weeks before mating and through gestation day 19.	Dams: 10 ppm; Fetuses: 3 ppm	Dams: None; Fetus: 5 ppm	Dams: None; Fetuses: ultrastructural morphometric changes in liver cells; decreased volume density of mitochondria, increased volume density of nuclei

Comment: Dams sacrificed at gestation day 19 and fetuses removed and examined. In order to obtain sufficient sample size, livers from all fetuses at each dose level were pooled for each replicate experiment. No signs of neurotoxicity were observed in dams and gross inspection of fetuses from treated dams showed no evidence of fetal mortality or teratogenicity. Morphometric analyses were conducted on three randomly selected samples from five fetal livers at each dose level. Authors report study indicates that continuous maternal exposure to MMOH during pregnancy produces dose-related pronounced ultrastructural and functional alterations of fetal rat liver mitochondria. Specifically, increased mitochondrial volume density, decreased monoamine oxidase activity was reported at 5 and 10 ppm. Significant decreases in the activity of cytochrome oxidase and ALA synthetase were reported in the 10 ppm group.

Stoltenburg-Didinger and Markwort (1990)	Female Wistar rats, offspring M and F (no. unspecified)	0, 0.025, 0.05, 0.5, 5.0 mg MMC/kg	Pregnant females were administered MMC by gavage on days 6 through 9 of gestation.	0.05 mg MMC/kg	0.05 mg MMC/kg	Developmental defects of the spine

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Table A-2. (continued)

Comment: Significantly impaired swimming was reported in offspring from the highest dose group, when compared to controls. In the visual discrimination reversal task, males in the highest dose group showed less nosepoking during the intertrial interval, when compared to controls. Increased passiveness during locomotion testing was observed in males in the highest dose group, when compared to controls. On days 180 and 210, increased auditory startle amplitude was observed in the two higher dose groups, when compared to controls. Abnormalities, such as stubby and mushroom-shaped and long, thin and tortuous-shaped spines, were observed in animals in the highest dose group, when compared to controls. Does not indicate any statistical analysis was performed in the study.

Suter and Schon (1986)	HAN-Wistar F rats, 20/group; pups 4/sex/group	0, 1.5, 5.0, or 15 mg MMC/l *via* drinking water	Females were administered MMC *via* drinking water 13 days before pairing and through weaning, on day 21 postpartum.	None	1.5 mg MMC/l	Developmental alterations

Comment: Blood samples taken from dams 12 days following the start of treatment, at birth, and at weaning showed a dose-dependent increase in mean blood levels of MMC. Newborn pups showed similar blood levels to the dams in the lower dose group and approximately 1/3 lower levels as the dams in the mid-dose group. Pups from the mid-dose group showed a statistically significant increase in the delay in testes descent, when compared to controls. A statistically significant increase in the occurrence of vaginal openings was observed in low- and mid-dose pups, when compared to control; however, no delay was observed. A statistically significant increase in impairment of righting reflex and swimming behavior was observed in the mid- and low-dose pups, when compared to controls. A statistically significant increase was observed in the overall affected pups in the mid-dose group, when compared to controls.

Yip and Chang (1981)	Charles River rats	0, 2.0 mg MMC/kg BW	Daily for 8 weeks; oral administration	None	2 mg MMC/kg BW (ODT)	Reduction of dorsal root fibers

Comment: Examination with light and electron microscopy revealed extensive Wallerian-like degeneration in the dorsal root fibers of MMC-treated animals. A significant reduction in dorsal root fibers was also noted in treated animals, when compared to controls. The toxic effects of MMC to the dorsal root ganglion was determined to be dorsal root fibers > ganglion neurons > ventral root fibers.

Geyer et al. (1985)	Sprague–Dawley rats, female, 5/group; Offspring: 4/sex/group	0, 0.25, 1.25, 2.5, or 5.0 mg MMC/kg	Pregnant dams were administered doses of MMC on days 6–15 of gestation by gavage.	0.25 mg MMC/kg	1.25 mg MMC/kg	Neurobehavioral deficits

Comment: Females treated with 5.0 mg MMC/kg produced no live offspring. A statistically significant decrease in total litter weight was observed in offspring from dams treated with 2.50 mg MMC/kg. Statistically significant early incisor eruption and eye opening and delayed vaginal patency and testes descent was reported in the offspring from dams treated with 2.5 mg MMC/kg, when compared to controls. A statistically significant delay in surface righting and increased number of falls in the negative geotaxis test was observed in offspring from the 2.5 mg MMC/kg group, when compared to controls. A statistically significant decrease in group effect of the number of exits from circle was observed in offspring from the 2.5 mg MMC/kg group in the pivoting tests, when compared to controls. Offspring from the 1.25- and 2.5-mg/kg treatment groups showed statistically significant lower scores for direction and angle in the swimming tests, when compared to controls. Open-field testing results showed that group effects of offspring from the 2.5-mg/kg treatment group showed statistically significant increases in the central beam breaks, when compared to controls. Offspring from the 2.5-mg/kg treatment group showed a statistically significant increase in auditory stimulus and tactile stimulus on day 72, when compared to controls. Offspring from the 0.25-mg/kg treatment group also showed a statistically significant increase in auditory stimulus on day 72, when compared to controls.

Fuyuta et al. (1978)	Wistar rats, F, 20/group.	0. 2.5, 5 or 7.5 mg MMC/kg in H_2O.	Daily dosing on days 7–14 of gestation.	2.5 mg MMC/kg	5 mg MMC/kg	Hydrocephaly and wavy ribs.

Comment: In the dams, statistically significant decreases in body weight gains were observed in the high-dose group throughout gestation, when compared with controls. Decreases in body weight gain was observed in the 2.5- and 5-mg/kg dams at some time points. Water and food consumption was significantly decreased in the 5- and 7.5-mg/kg dams, when compared with controls. Clinical signs of neurotoxicity were reported in 9/20 of the high-dose dams. Total malformations were significantly increased in the mid- and high-dose groups. The incidence of wavy ribs and hydrocephaly was increased in the mid- and high-dose groups. In addition, the incidence of cleft palate, generalized edema, brain lesions, absence of vertebral centra and defects of the sternum were increased in the high-dose group, when compared with controls.

Nolen et al. (1972)	Charles River rats, F, 20/group.	0, 0.02, 0.2, 4 mg MMC/kg	Pregnant rats were administered MMC *via* drinking water on gestational days 6–14.	None	0.02 mg MMC/kg	Bladder defects, undescended testes, skeletal defects.

Comment: The high-dose dams experienced a significant reduction in weight gain during the treatment period, which coincided with reductions in food and water intake. A statistically significant increase in the number of dead fetuses was reported in the high-dose group, when compared to the controls. Significant increases in fetal bladder defects and missing fifth sternebrae were reported in all treated groups, when compared with the controls. Significant increases in the incidence of undescended testes were reported in the low-dose group only. Extra ribs were reported in the low- and high-dose groups and incomplete calcification of the skeleton was reported in the low-dose group when compared with the controls. Based on these results, our derived LOAEL differs from the LOAEL reported in the Report to Congress.

Table A-2. (continued)

Fuyuta et al. (1979)	ICR mice (20 F/group), fetuses	0, 10, 15, 20, or 25 mg MMC/kg	Females given single oral dose *via* distilled water on day 10 of gestation.	None	10 mg MMC/kg	Fetal malformations such as cleft palate and hydronephrosis and skeletal variations.

Comment: A significant decrease in body weight gain was observed on gestation days 11–14 in dams treated with 25 mg MMC/kg; however, body weight gains were comparable to body weight gains reported in the controls during late gestation. Mean fetal body weights (male and female) were statistically significantly decreased in fetuses from dams that had received 15, 20, or 25 mg MMC/kg, when compared to mean fetal body weights from fetuses from control dams. Fetuses from dams treated with 15, 20, or 25 mg MMC/kg had a 31.8, 63.7, and 100%, respectively, incidence of malformations. The incidence of cleft palate was statistically significantly increased in fetuses from dams that had received 15, 20, or 25 mg MMC/kg, while the incidence of hydronephrosis was statistically significantly increased in fetuses from dams that had received 20 or 25 mg MMC/kg, when compared with the controls. The incidence of incomplete fusion of the sternebrae was statistically significantly increased in all dose groups, when compared with the controls.

Rodier et al. (1984)	DUB/ICR female mice, 7 treated, 8 control; fetuses (no. unspecified)	0, 8 mg Hg/kg	Dams were given a single *per os* dose of MeHg or vehicle (5 mM Na_2CO_3) on day 12 of gestation.	None	8 mg Hg/kg (ODT)	Developmental alterations of the brain (alterations in mitosis).

Comment: A statistically significant decrease in the mean weights of treated fetuses was observed 48 h following treatment, when compared to control fetuses. A statistically significant increase in the mean thickness and the mean cell numbers of the midbrain was observed in treated fetuses at 48 h postexposure, when compared to controls. The mean total mitotic figures and the mean mitotic index in the cortex were statistically significantly increased at 48 h postexposure in fetuses from treated dams, when compared to controls. Statistically significant increases in the mean number of early mitotic figures in the cortex and the rhombic lip was observed 48 and 24 h postexposure, respectively, when compared to controls. Statistically significant decreases in the mean number of late mitotic figures in the hippocampus, midbrain, and rhombic lip were observed at 24, 48, and 48 h postexposure, respectively, when compared to controls. Statistically significant decreases in the mean percent of mitotic figures that are late were observed in the hippocampus and cortex, 24 h following treatment, when compared to controls.

Inouye and Kajiwara (1988a)	C3H/HeN female mice, total of 35 dams divided into three groups; F1 pups: 6/untreated dam F2 fetuses: no. unspecified	Dams: 0, 15 mg/kg (12 mg Hg/kg)	Dams were administered MMC *via* distilled water orally on day 14 or 17 of pregnancy.	None	15 mg MMC/kg (ODT)	Developmental alterations (decreased fetal body weights and decreased ossified vertebrae).

Comment: A total of 35 pregnant mice were divided into three groups in which 15 mg/kg MMC was administered orally in distilled water on gestation day 14 (Group A) or 17 (Group B) of pregnancy or were untreated (Group C). The mice were allowed to give birth and the newborn pups were placed with untreated dams (6 pups/dam) acting as foster mothers. Hg analysis was conducted on 25 female offspring on 1, 17, 14, 21, and 28 days of age. Neurochemical analysis of the brain was conducted on half of the male offspring at 9 weeks of age. The remaining F1 generation was placed with untreated mates of the same strain at 12 weeks of age and allowed to mate. On day 18 of pregnancy, females were put to death by cervical dislocation and examined to determine the number of resorption sites and the number of fetuses. The fetuses (F2) were examined for external malformations. *P* values less than 0.01 were considered to be significant. A statistically significant reduction in fetal body weight and the number of ossified vertebrae were observed in the F2 fetuses from the dams that had been treated on gestation day 17, when compared to controls.

Khera and Tabacova (1973)	Virgin Swiss-Webster mice, F. Experimental design does not state number of mice/group.	0, 0.001, 0.01, 0.1, 1, 5,10 mg MMC/kg/day in corn oil	Daily oral doses from days 6–17 of gestation	None	None	Statistical comparisons were not conducted.

Comment: Two separate experiments were conducted: body weight, viability, appearance, and behavior of pups were observed in one experiment, and in the second, pups were sacrificed in order to examine the cerebelli histologically. For all histological evaluations, the anterior region and posterior region of the cerebelli from Hg-treated mice were compared with the corresponding anterior and posterior regions from treated control animals. Doses of 1 mg MMC/kg/day caused histological alterations of the cerebellar region. These changes were not associated with any obvious adverse clinical effects and were not observed at doses below 1 mg MMC/kg/day. However, there is no evidence that statistical analyses were conducted.

Colomina et al. (1997)	Swiss mice, F. Experimental design does not state number of mice/treatment group.	0, 2 mg MMC/kg/day.	MMC was administered *via* gavage on gestational days 15–18.	None	2 mg MMC/kg/day	Decreased percentage of live pups at birth, decreased viability index, developmental defects.

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Table A-2. (continued)

Comment: One of the treatment groups consisted of pregnant animals placed in cylindrical holders for 2 h/day on gestational days 15–18. This was considered to be the "restraint" group, and produced stress in the animals. The MMC-treated group had a statistically significant delay in pinna development and a delay in eye opening. The MMC+restraint group also showed a significant reduction in the percentage of live births and reductions in the viability and lactation indexes. There were no significant differences following a battery of neurobehavioral tests in offspring from MMC treated dams or offspring from the MMC+restraint dams, when compared to offspring from control dams.

Thuvander et al. (1996)	BALB/c mice, F, 72, 27, 37, respectively, for the listed treatment groups.	0, 0.5, 5 mg Hg/kg diet	The dams received the test diets for 10 weeks before mating and during gestation and lactation.	None	0.5 mg Hg/kg	Increase in total numbers of CD4+ lymphocytes in the thymus

Comment: The mice were exposed for a 10-week period, before mating, during gestation and lactation. The offspring were exposed until day 15 of lactation. The dams in the high-dose group had statistically significantly increased body weights at weeks 4, 5, 8, and 9 before mating. A significant decrease in body weights was reported in pups (10-day-old) from the high-dose dams, when compared with the controls. Statistically significant increases in spleen weight (10 days), number of splenocytes (10 and 22 days), and the number of thymocytes (22 days) were observed in pups from dams that had received 0.5 mg Hg/kg diet, when compared with the controls. Following analysis of the lymphocyte types in the thymus, at 10 days, statistically significant decreases in the percent CD4+ lymphocytes and significant increases in the percent CD4+CD8+ lymphocytes were reported in pups from the low-dose dams. In pups from the high-dose dams, at 10 days, the percent CD4+ lymphocytes was decreased, when compared to the controls. At 22 days, the total number of CD4+ lymphocytes and the total number of CD4+CD8+ lymphocytes was significantly increased in pups from the low-dose dams. In pups from the high-dose dams, statistically significant increases in the total number of CD4+ lymphocytes, percent of CD8+ lymphocytes and the total number of CD8+ lymphocytes were observed. At 50 days the only significant change reported was an increase in the percent of CD8+ lymphocytes in the high-dose group. A statistically significant increase in the proliferation response was reported in splenocytes from pups in dams that had received the 5-mg MMC/kg diets. Natural killer cell activity was significantly increased in pups from the high-dose dams at day 22, when compared to the controls. However, this effect was not reported at 50 days.

Yasuda et al. (1985)	JCL:ICR mice, F, 50/group	0, 25 mg MeHg/kg	Single dose administered on gestational day 12.	None	25 mg MeHg/kg	Cleft palate

Comment: The purpose of this experiment was to determine the critical time in gestation that in which cleft palate is produced in order to better understand the mechanism of cleft palate formation. There were no maternal deaths reported. Cleft palate was induced in 99% of the fetuses for groups treated on day 12, 8th hour, and on day 12, 16th hour of gestation. On day 12, hour 20, cleft palate formation was induced in 89.6% of the fetuses. There were no cleft palates reported in the control fetuses; however, statistical comparisons with the treated groups and the controls were not conducted.

Yasuda et al. (1985)	JCL:ICR mice, F, 10/group	0, 12.5, 15, 20 or 25 mg MMC/kg	A single oral dose was administered on gestational day 10 or 12.	15 mg/kg	20 mg/kg	Decreased fetal body weights, increased incidence of cleft palate and dilatation of the renal pelvis.

Comment: Statistically significant decreases in mean fetal body weights (male and female) were reported in pups from dams that had received 20 or 25 mg/kg on gestational day 10 or 12, when compared with the controls. The number of fetuses with cleft palate was statistically significantly increased in pups from dams that had received 20 or 25 mg/kg on gestational day 10 or 12, while the number of fetuses with dilatation of the renal pelvis was statistically significantly increased in fetuses from dams that had received 20 or 25 mg/kg on gestation day 10 and in fetuses from the dams that had received 25 mg/kg on gestational day 12, when compared with controls.

Inouye et al. (1985)	C3H/HeN mice, female, 10/group	0, 20 mg MMC/kg	Pregnant dams were administered a single oral dose of MMC on gestation day 13, 14, 15, 16 or 17.	None	20 mg/kg MMC (ODT)	Behavioral impairment

Comment: Long-term behavioral impairment was observed in offspring from all treated groups. Righting movement, tail position during walking, flexion of the hind limbs, and spontaneous locomotor activity were altered. However, the results of the behavioral tests were not analyzed statistically. Histopathological examination showed reduced brain weight), reduced size of the nucleus caudatus putamen, and simplified cerebellar folial patterns. Whole brain weights were statistically significantly reduced in female offspring from groups treated on day 13 and day 14 of gestation, when compared to controls.

Fuyuta et al. (1978)	C57BL mice, F, 10/group.	0, 2.5, 5, 6, or 7.5 mg MMC/kg in H_2O.	Daily dosing on gestational days 6–13.	None	2.5 mg MMC/kg	Decreased ossification of the occipital bone and absence of ossification of the sternebrae.

Comment: Dams in the high-dose group gained significantly less weight, when compared with the controls. The total number of malformations was increased 2.5, 5, and 6 mg/kg/day groups. Significant increases in the incidence of cleft palate and fused thoracic vertebrae were reported in the 5- and 6-mg/kg groups. Significant increases in the incidence of decreased ossification of the occipital bone and absence of ossification of the sternebrae, were reported in the 2.5-, 5-, and 6-mg/kg groups, when compared with the controls.

Reuhl et al. (1981)	Golden hamsters, F, 10/group.	0, 2, 10 mg MMC/kg	*Group A*: single oral dose 10 mg MMC/kg on gestational day 10.	None	2 mg MMC/kg	Pathological alterations in the cerebellum of pups.

Table A-2. (continued)

			Group B: daily oral dose of 2 mg/kg on gestational days 10–15.			

Comment: CNS deterioration in the cerebellum, marked by lysosome and lipid droplet accumulation, was reported. Primarily, degenerative changes of neurons and neuronal processes were observed. These degenerative changes involved both the nerve cell body and the dendrites. The data were not analyzed statistically.

Inouye and Kajiwara (1988b)	Hartley guinea pigs, female, no. unspecified;	Dams: 0, 7.5 mg MMC	Pregnant dams were orally administered a single dose of 7.5 mg MMC on one of days 21, 28, 35, 42, or 49 of pregnancy.	None	7.5 mg MMC (ODT)	Degenerative cerebral changes

Comment: A statistically significant decrease in the body weight of treated dams was observed at 5, 6, and 7 weeks of treatment, when compared to controls. A statistically significant decrease in the brain weight of treated dams was observed at 3, 4, 6, and 7 weeks of treatment, when compared to controls. The highest concentration of Hg in the dams was in the kidney. However, fetal hair was observed to have the highest concentration in the fetus. Blood levels analyzed at 9 weeks of pregnancy showed higher levels of Hg in the fetus than in the dam. Gross necropsy revealed that fetal brains from dams treated at 3, 4, and 5 weeks of pregnancy were smaller, when compared to controls. Degenerative cerebral changes were observed in fetuses from dams treated at 6 and 7 weeks of pregnancy, when compared to controls.

NOTE: MeHg=methylmercury; MMC=methylmercuric chloride; MMD=methylmercury dicyandiamide; MMOH=methylmercury hydroxide; ODT=only dose tested.

[a]It should be noted that in the various Rice, and Rice and Gilbert publications, only two cohorts of animals are used in experimentation over 18 years. The first cohort is a group of two controls and five dosed animals, with the treated animals receiving 50 μg/kg/day from the time of birth until approximately 7 years of age. The second group is composed of offspring from females receiving 0, 10, 25, or 50 μg/kg/day before and during pregnancy. The females were treated for two different lengths of time before mating — for either 4 months or up to 2 years. The offspring from these mothers (numbering 5, 1, 2, and 5, respectively, per dose group) received doses, following birth equivalent to those given their mothers. As these two cohorts aged, numbers on test sometimes changed due to individuals dropping out from illness or other reasons. In the group tested *in utero* and postnatally, test results were often combined for the two highest dose groups. Although these studies provide valuable information, they are limited by the use of such a small number of animals. The cohort of animals exposed postnatally is also limited because only one dose level was tested.

[b]Burbacher et al. (1990b) presented analysis of their study and of three other studies (Burbacher et al., 1986; Gunderson et al., 1986, 1988) performed at the same laboratory and having similar treatment protocols. They state that these results indicate that maternal intake of MeHg before and during pregnancy affects both social behavior and cognitive functioning of exposed offspring. However, comparison of the behavior scores with those on recognition memory and object permanence tasks did not reveal a consistent pattern of responses across assessments (tested by Pearson r). Correlation coefficients were low and not statistically significant. An additional comparison of MeHg effects across the three "assessment procedures" using Student's t tests indicated higher t values for the two cognitive assessments, object permanence being the highest ($P<0.001$) compared to the social behavior effects.

Table A-3. Summary table for reproductive toxicity from oral exposure to methylmercury

Reference	Species no./ sex group	Doses (units)	Exposure frequency/ duration	NOAEL	LOAEL	Effect
Mohamed et al. (1987)	Macaque monkeys, M, 3/group	0, 50, or 70 μg MeHg/kg/day	Given orally on a daily basis *via* apple juice for 20 weeks.	None	50 μg MeHg/kg/day	Decreased sperm speed, sperm forward progression and increased sperm tail defects.

Comment: An increase in blood Hg levels was observed in treated animals when compared to control animals. A statistically significantly lower mean sperm concentration and mean total sperm count was observed in one animal from the low-dose group and one animal from the high-dose group, when compared to baseline values. However, the average sperm concentration and average total sperm count for each treated group were not statistically significantly lower, when compared to baseline values. The mean scores for sperm speed and forward progression were statistically significantly lower during the treatment period, when compared to baseline means. A statistically significant increase in mean total abnormal sperm tail defects was observed in the high- and low-dose groups during the treatment period, when compared to control means. However, the power of the statistical tests are greatly reduced by the low number of animals/dose group.

Burbacher et al. (1984)	Macaque monkeys, females; 8 controls, 7 in each dosed group	0, 50, or 90 μg/kg/day	Dosing occurred through four menstrual cycles (approx. 124 days) after which females were mated. Dosing continued through pregnancy.	50 μg/kg/day (see comments)	90 μg/kg/day	Reproductive dysfunction; conception and delivery of viable offspring.

Comment: Mean blood level ("during breeding and/or pregnancy") of females receiving 50 μg/kg/day was 1.03 ppm; for those receiving 90 μg/kg/day the level was 2.03 ppm. Controls had blood levels of 0. Menstrual cycles were unaffected in females exposed to MeHg. Number of animals conceiving were 8/8 controls, 5/7 in 50 μg/kg/day group, and 4/7 in 90 μg/kg/day group. However, these differences were not statistically significant. No control or low-dose animals aborted; two of the four pregnant high-dose animals aborted, but this was not statistically significant. Reproductive failure was associated with significantly higher mean blood Hg (1.99 ppm) than reproductive success (1.06 ppm) ($P<0.02$). When

(continued on next page)

Table A-3. (continued)

treated females were divided into groups according to blood Hg levels the results were as follows: Those with levels under 1.0 ppm delivered viable offspring; of the four treated females with blood levels between 1.0 and 1.5 ppm, two delivered viable offspring; only one of the six females with blood level over 1.5 ppm delivered a viable offspring ($P=0.02$). Four females receiving 90 μg/kg/day MeHg began showing signs of MeHg toxicity after their pregnancy had ended.

Burbacher et al. (1988)	Macaque monkeys, females, 15 controls, 16 low-dose, 7 mid-dose, and 7 high-dose	0, 50, 70, or 90 μg/kg/day in apple juice	Dosing occurred through four menstrual cycles after which females were mated. Dosing continued throughout pregnancy and afterwards or until signs of MeHg toxicity were observed.	50 μg/kg/day	70 μg/kg/day	Decreased reproductive success rate.

Comment: Mean blood Hg levels after 10 weeks of dosing (steady state) were 1.28 (at 50 μg/kg/day), 1.62 (at 70 μg/kg/day) and 2.03 (at 90 μg/kg/day). No changes in menstrual cyclicity were related to MeHg exposure. The reproductive success rate (no. viable/no. females) decreased significantly at 70 and 90 g/kg/day dose levels (Fisher's exact, $P<0.05$); conception rate was unaffected. Females with mean blood Hg level of 1.5 ppm or greater had a significantly lower rate of viable deliveries (40%) than those with mean blood level of 0.5 to 1.0 ppm (100%) or of controls (90%). No exposure related effects on offspring were identified. Overt signs of toxicity were observed in six females following pregnancy. These effects were associated with blood Hg levels above 2.0 ppm. Burbacher et al. (1984) is identified as a preliminary report for the current study.

Khera (1973)	*Exp. 1*: Wistar rats, 15–20 M; *Exp. 2*: Wistar rats, 15 M; *Exp. 3*: Wistar rats, 14–10 M	*Exp. 1*: 0, 1, 2.5, 5 mg/kg/day; *Exp. 2*: 0, 1, 2.5, 5 mg/kg/day; *Exp. 3*: 0, 0.1, 0.5, 1 mg/kg/day	*Exp. 1*: 7 days; *Exp. 2*: 7 days; *Exp. 3*: 125 days for first three doses, and 95 days at 1 mg/kg/day.	0.1 mg/kg/day	0.5 mg/kg/day	Reduction in mean litter size/pregnancy attributatable to preimplantation losses.

Comment: The long-term experiment, which consisted of 21 mating trials, each of 5 days duration, was conducted. In this long-term experiment, 25–30 days of dosing at 1 mg/kg MMC and 85–90 days of dosing at 0.5 mg/kg MMC, produced a decrease in the average number of viable implants. The decrease became more pronounced in successive trials. However, this effect was not produced with administration of the low dose. Short-term exposures (7 days) produced a dose-related reduction in incidence of fertile matings. This effect was not observed in the long-term exposures. Arithmetic means of reproductive statistics were calculated over all mating trials during or after treatment, and these means were analyzed as functions of dose.

Geyer et al. (1985)	Sprague–Dawley rats, female, 5/group; Offspring: 4/sex/group	0, 0.25, 1.25, 2.5, or 5 mg MMC/kg	Pregnant dams were administered doses of MMC on days 6–15 of gestation by gavage.	None	2.5 mg MMC/kg	Decreased mean litter weight.

Comment: Females treated with 5.0 mg/kg MMC produced no live offspring. A statistically significant decrease in total litter weight was observed in offspring from dams treated with 2.50 mg/kg MMC.

Suter and Schon (1986)	HAN-Wistar Female rats, 20/group; pups 4/sex/group	0, 1.5, 5.0, or 15 mg MMC/l *via* drinking water	Females were administered MMC *via* drinking water 13 days before pairing and through weaning, on day 21 postpartum.	5 mg MMC/l	15 mg MMC/l	Decreased litter size and increased perinatal and prenatal mortality.

Comment: All dams survived treatment; however, MMC was toxic to dams in the high-dose group as demonstrated by reduced weight gain during pregnancy, ataxia, and slight paresis of the hind legs before term and significantly decreased litter sizes and perinatal and postnatal mortality.

Fuyuta et al. (1978)	Wistar rats, F, 20/group.	0. 2.5, 5 or 7.5 mg MMC/kg in H_2O.	Daily dosing on days 7–14 of gestation.	5 mg MMC/kg	7.5 mg MMC/kg	Increased resorptions and deaths and decreased number of live fetuses and average litter size.

Comment: In the dams, statistically significant decreases in body weight gains were observed in the high-dose group throughout gestation, when compared with controls. Decreases in body weight gain was observed in the 2.5- and 5-mg/kg dams at some time points. Water and food consumption was significantly decreased in the 5- and 7.5-mg/kg dams, when compared with controls. Clinical signs of neurotoxicity were reported in 9/20 of the high-dose dams. Statistically significant decreases in the number of live fetuses and average number of fetuses/litter were reported in the high-dose group, when compared with controls.

Nolen et al. (1972)	Charles River rats, F, 20/group	0, 0.02, 0.2, 4 mg MMC/kg	Pregnant rats were administered MMC *via* drinking water on gestational days 6–14.	4 mg MMC/kg	None	None

Comment: The high-dose dams experienced a significant reduction in weight gain during the treatment period, which coincided with reductions in food and water intake. There were no statistically significant differences in the number of litters, average number of corpora lutea, average number of implantations, average number of resorptions, average number of live fetuses or fetal weights in any Hg-treated group, when compared with the controls. A statistically significant decrease in the number of dead fetuses was reported in the low- and mid-dose groups, when compared to the controls.

Table A-3. (continued)

Khera (1973)	*Exp. (4)*: Swiss-Webster mice, 10–12 M; *Exp. 5*: Swiss-Webster mice, 12–13 M	*Exp. 4*: 0, 1, 2.5, 5 mg/kg/day; *Exp. 5*: 0, 1, 2.5, 5 mg/kg/day	*Exp. 4*: 7 days; *Exp. 5*: 5 days.	5 mg/kg/day MMC	None	High dose produced no postimplantation losses or reduction of fertility.
Comment: Arithmetic means of reproductive statistics were calculated over all mating trials during or after treatment, and these means were analyzed as functions of dose.						
Yasuda et al. (1985)	JCL:ICR mice, F. 10/group	0, 12.5, 15, 20 or 25 mg MMC/kg	A single oral dose was administered on gestational day 12 or 18.	25 mg/kg	None	None
Comment: No statistically significant differences in the number of implantation sites, percent dead and resorbed fetuses, number live fetuses or the total number of male fetuses were reported in any treated group, when compared with the controls.						
Fuyuta et al. (1979)	ICR mice (20 F/group), fetuses	0, 10, 15, 20, or 25 mg MMC/kg	Females given single oral dose *via* distilled water on day 10 of gestation.	None	10 mg MMC/kg	Decreases in the number of live fetuses and average fetuses/litter
Comment: A significant decrease in body weight gain was observed on gestation days 11–14 in dams treated with 25 mg MMC/kg; however, body weight gains were comparable to body weight gains reported in the controls during late gestation. Statistically significant decreases in the number of live fetuses and the average number of fetuses/litter were reported for dams that had received 10 or 25 mg MMC/kg. Mean fetal body weights (male and female) were statistically significantly decreased in fetuses from dams that had received 15, 20, or 25 mg MMC/kg, when compared to mean fetal body weights from fetuses from control dams. No other significant differences were reported.						
Khera and Tabacova (1973)	Virgin Swiss-Webster mice, F. Experimental design does not state number of mice/group.	0, 0.001, 0.01, 0.1, 1, 5, 10 mg MMC/kg/day in corn oil	Daily oral doses from days 6–17 of gestation.	None	None	None
Comment: All dams that were administered the high dose failed to survive. Six of the nine dams that were administered 5 mg/kg/day failed to litter. No effects on mean number of pups/litter, mean number of live pups and birth, mean number of stillborns and deaths on day 1, mean pup weights and postnatal survival were reported by the authors. However, the data were not analyzed statistically.						
Colomina et al. (1997)	Swiss mice, F. Experimental design does not state number of mice/treatment group.	0, 2 mg MMC/kg/day	MMC was administered *via* gavage on gestational days 15–18.	None	2 mg MMC/kg/day	Decreased percentage of live pups at birth, decreased viability index.
Comment: One of the treatment groups consisted of pregnant animals placed in cylindrical holders for 2 h/day on gestational days 15–18. This was considered to be the "restraint" group, and produced stress in the animals. The MMC-treated group had a statistically significantly lower percentage of live pups at birth and a decreased viability index. The MMC+restraint group also showed a significant reduction in the percentage of live births and reductions in the viability and lactation indexes.						
Hughes and Annau (1976)	CFW female mice; Offspring 4/litter	0, 1, 2, 3, 5, and 10 mg Hg/kg BW (MMOH)	Pregnant dams were administered MMOH on day 8 of gestation by peroral injection.	2 mg Hg/kg body weight MMOH	3 mg Hg/kg body weight MMOH	Decreased litter size
Comment: A statistically significant decrease in the number of pups/litter was observed in the litters from animals treated with 3, 5, and 10 mg Hg/kg as MMOH, when compared to controls. A statistically significant decrease in body weight was observed in pups from groups treated with 3, 5, or 10 mg Hg/kg as MMOH at weeks 1, 2, and 3, when compared to control animals. However, body weights of animals from these groups were similar to controls by week 9 (56 days).						
Hughes and Annau (1976)	CFW female mice; offspring 4/litter	0, 1, 2, 3, 5, or 10 mg/Hg/kg BW MMOH	Pregnant dams were administered MMOH on Day 8 of gestation by peroral injection.	2 mg Hg/kg BW	3 mg Hg/kg BW	Alterations in behavioral responses
Comment: A statistically significant decrease in the number of pups/litter was observed in the litters from animals treated with 3, 5, or 10 mg Hg/kg as MMOH, when compared to controls. A statistically significant decrease in body weight was observed in pups from groups treated with 3, 5, or 10 mg Hg/kg as MMOH at weeks 1, 2, and 3, when compared to control animals. However, body weights of animals from these groups were similar to controls by week 9 (56 days).						
Fuyuta et al. (1978)	C57BL mice, F, 10/group	0, 2.5, 5, 6, or 7.5 mg MMC/kg in H_2O	Daily dosing on gestational days 6–13.	2.5 mg MMC/kg	5 mg MMC/kg	Decreased fetal body weights.
Comment: Dams in the high-dose group gained significantly less weight, when compared with the controls. The high dose was embryocidal. Statistically significant increases in the number of resorption and deaths were reported in the 6- and 7.5 mg/kg groups, when compared with the controls. The number of live fetuses was significantly decreased in the 6-mg/kg group and the body weights of male fetuses were significantly decreased in the 5 and 6 mg/kg groups, while the body weight of female fetuses was decreased in the 5-mg/kg group, when compared with the controls.						

NOTE: MeHg=methylmercury; MMC=methylmercuric chloride; MMD=methylmercury dicyandiamide; MMOH=methylmercury hydroxide.

APPENDIX B

Documentation of Fetal Model

The following equations document the portion of the model related to changes during pregnancy and gestation, as depicted in Figures 9 and 10, and Table B-1 lists the values for the coefficients used in these equations. Table B-2 lists the monkey parameter values that differ from the human values. These were used to generate Figure 10.

$$\text{Time} = \text{time since conception} \tag{8}$$

$$\text{TScale} = \frac{6480.0}{\text{LPreg}} \tag{9}$$

6480.0 is the length of human pregnancy (hours).

$$\text{FracInc} = 1.0 + \begin{cases} (0.130 \times \exp(15.44 \times \exp(-0.00183 \times \text{TScale} \times \text{Time}))), \\ \quad \text{where}(\text{TScale} \times \text{Time}) < (24.0 \times 7.0 \times 24.0) \\ (0.129 \times (\sin(0.000374 \times \text{TScale} \times \text{Time}))), \\ \quad \text{where} \quad (\text{TScale} \times \text{Time}) \geq (24.0 \times 7.0 \times 24.0) \end{cases} \tag{10}$$

FracInc is the fractional increase in dietary intake over pregnancy.

$$\text{VP} = \text{BW} \times (\text{VPC} + (0.03 \times \exp(-6.881 \times \exp(-0.000705 \times \text{TScale} \times \text{Time})))) \tag{11}$$

Table B-1. Values for coefficients used in equations.

Parameter	Human value	Monkey value
Fetal brain volume		
VBrFeA	0.50	0.06
VBrFeB	−20.93	−71.826
VBrFeC	−6.19e−4	−1.77e−3
Fetal weight		
VFeA	3.50	0.65
VFeB	−16.08	−20.897
VFeC	−5.67e−4	−1.13e−3
VFeD	3.50	0.0
VFeE	−140.2	0.0
VFeF	−7.01e−4	0.0
Placenta volume		
VPlA	0.85	0.25
VPlB	−9.434	−6.570
VPlC	−5.23e−4	−7.96e−4
Fetal plasma volume		
VPFeC	0.0425	0.0425
Fetal red blood cell volume		
VRBCFC	0.0425	0.0425

Table B-2. Monkey parameter values that differ from human values.

Parameters		Value
Plasma flows (fraction of cardiac output)		
QCC	Cardiac output (1/h for 1 kg animal)	23.0
QBrBC	Brain	0.06
QFC	Fat	0.025
QGC	Gut	0.148
QKC	Kidney	0.123
QLC	Liver	0.046
QRC	Richly perfused tissues	0.343
QSC	Slowly perfused tissues	0.255
QPlM	Placenta (1/h per kg)	15.6
Tissue volumes (fraction of body weight)		
BW	Body weight (kg)	3.0
VFC	Fat	0.15
VGC	Gut	0.023
VIC	Intestine	0.023
VKC	Kidney	0.006
VPC	Plasma	0.045
VRBCC	Red blood cells	0.029
VRC	Richly perfused tissues	0.05
VSC	Slowly perfused tissues	0.55
VRem	Remainder of body (nonperfused)	0.081
Partition coefficients for MeHg		
PHB	Hair/blood partition coefficient	125.0
PRBC	Red blood cell/plasma	22.0
Kinetic parameters (1/h for 1-kg animal)		
kbi	Biliary clearance of MeHg	3.0e−5
khi	Excretion of MeHg into hair	1.5e−5

$$\text{VRBC} = \text{BW} \times (\text{VRBCC} + (0.0075 \times \exp(-6.530 \times \exp(-0.00059 \times \text{TScale} \times \text{Time})))) \tag{12}$$

$$\text{VR} = \text{BW} \times (\text{VRC} + (0.025 \times \exp(-5.354 \times \exp(-0.000467 \times \text{TScale} \times \text{Time})))) \tag{13}$$

$$\text{VF} = \text{BW} \times (\text{VFC} + (0.09 \times \exp(-12.91 \times \exp(-0.000797 \times \text{TScale} \times \text{Time})))) \tag{14}$$

$$\text{VFe} = \text{VFeA} \times \exp(\text{VFeB} \times \exp(\text{VFeC} \times \text{Time})) + \text{VFeD} \times \exp(\text{VFeE} \times \exp(\text{VFeF} \times \text{Time})) \tag{15}$$

$$\text{VPI} = \text{VPIA} \times \exp(\text{VPIB} \times \exp(\text{VPIC} \times \text{Time})) \tag{16}$$

$$\text{VBrFe} = \text{VBrFeA} \times \exp(\text{VBrFeB} \times \exp(\text{VBrFeC} \times \text{Time})) \tag{17}$$

$$\text{VPFe} = \text{VPFeC} \times \text{VFe} \tag{18}$$

$$\text{VRBCFe} = \text{VRBCFC} \times \text{VFe} \tag{19}$$

APPENDIX C

Estimation of Key Parameter Distributions for Monte Carlo Analysis

The development of a Monte Carlo analysis with a PBPK model is a complex process that requires the critical review of large amounts of literature, not only literature that is chemical-specific, but also literature that describes the various values that have been obtained for biological parameters. In order to focus on the parameters in the PBPK model that had the greatest impact on the development of the site-specific RfD, a sensitivity analysis was conducted. The results of this analysis indicated that the most sensitive parameters in the PBPK model were the hair excretion rate constant (khi), the fecal excretion rate constant (kfi), the hair to blood partition coefficient for methylmercury (PHB), the gut to blood partition coefficient (PG), the body weight (BW), the volume of the fat compartment (VFC) and the volume of slowly perfused tissue (VSC) (Table C-1). The basis for the distributions for each of these parameters is discussed below.

Hair Excretion Rate Constant (khi)

No information on the ratio of hair excretion (khi) for the total body was directly available from the literature. However, a distribution for khi could be developed based on the available information on scalp hair excretion together with an analysis of data on mercury levels monitored in hair

Table C-1. Parameter sensitivity.

Parameter	Analytical sensitivity coefficient			Pearson correlation coefficient[a]		
	AvgCH	PeakHair	HLife	AvgCH	PeakHair	HLife
Tissue volumes						
BW	0.24	0.24	0.22	0.19/0.10[b]	0.18/0.10[b]	0.07/0.16[b]
VFC	0.08	0.08	−0.17	0.15/0.02[b]	0.15/0.02[b]	−0.03/−0.07[b]
VBrC	−0.01	−0.02	0.02	−0.09/0/03[b]	−0.09/0.03[b]	−[c]/0.04[b]
VKC	−[c]	−[c]	0.01	−0.10/0.04[b]	−0.10/0.04[b]	0.07/−[b,c]
VLC	−[c]	−[c]	0.09	−0.06/−0.02[b]	−0.06/−0.02[b]	0.04/0.04[b]
VPC	0.02	0.01	−[c]	−0.13/−0.04[b]	−0.13/−0.04[b]	−[c]/−0.03[b]
VRBCC	0.02	0.02	−[c]	−0.13/−0.06[b]	−0.13/−0.06[b]	−[c]/0.02[b]
VRC	0.03	0.03	0.01	−0.05/−[b,c]	−0.05/−[b,c]	−0.02/0.03[b]
VSC	0.09	0.09	0.35	−[c]/0.01[b]	−[c]/0.01[b]	0.08/0.05[b]
VRemain	0.03	0.04	−0.09	−0.13/0.03[b]	−0.13/0.03[b]	−0.05/−0.07[b]
Partition coefficients for MeHg						
PBr	−0.02	−0.02	0.03	−0.04	−0.04	−[c]
PBrB	−0.02	−0.02	0.03	0.02	0.02	−[c]
PF	−[c]	−[c]	0.04	0.02	0.02	0.06
PFe	−[c]	−0.02	−[c]	−0.02	−0.02	−0.04
PG	−0.13	−0.13	−0.11	−0.32	−0.32	−0.14
PHB	0.22	0.23	−0.80	0.421	0.45	−0.66
PK	−[c]	−[c]	0.01	−[c]	−[c]	−0.02
PLiv	−0.04	−0.04	0.08	−0.04	−0.04	0.05
PR	−[c]	−[c]	0.09	−0.03	−0.04	0.05
PS	−[c]	−0.02	0.61	−[c]	−[c]	0.30
Kinetic parameters (l/h)						
kbrini (/h)	−0.02	−0.02	−0.02	−0.04	−0.04	−[c]
kbi	−0.01	−0.01	−0.01	−0.04	−0.03	−0.06
kdi	−0.06	−0.06	−0.05	−[c]	−[c]	−0.01
kfi	−0.13	−0.13	−0.09	−0.23	−0.23	−0.07
khi	−0.77	−0.75	−0.80	−0.66	−0.65	−0.34
kii	−0.02	−0.02	−0.02	−0.07	−0.07	−0.04
kri	0.06	0.06	−[c]	−0.08	−0.08	0.01

[a]Kendall's and Spearman's correlation coefficients were consistent with Pearson's.
[b]Coefficients for simulation with/without correlation of VFC with BW.
[c]Less than 0.01 in absolute value.

and blood from human volunteers following ingestion of fish meals containing methylmercury (Hislop et al., 1983; Sherlock et al., 1984). An excretion rate for hair can be calculated using the following equation:

hair excretion rate

$$= \mathrm{DS} \times \frac{\pi}{4} \times \mathrm{DI} \times \mathrm{GR} \times \mathrm{FD} \times \mathrm{SA} \times \mathrm{CF1} \times \mathrm{CF2} \times \mathrm{CF3} \quad (20)$$

where DS=hair density (g/ml); DI=hair diameter (mm^2); GR=growth rate (mm/day); FD=follicle density ($hairs/cm^2$); SA=skin area (cm^2); CF1=conversion factor 1 (ml/mm^3); CF2=conversion factor 2 (kg/g); CF3=conversion factor 3 (d/h).

A review of the literature was conducted to locate information on each of the parameters necessary for estimating the scalp hair excretion rate. Information on the range of values or the mean and standard deviation were collected for each component of the equation in an attempt to estimate a distribution for scalp hair excretion. A particular attempt was also made to locate information for each parameter for women of childbearing age (approximately 14 to 45). A description of the distribution for each parameter in the above equation is discussed below, followed by a description of the distribution of scalp hair excretion, the estimation of the preferred value of khi, and how the variability of scalp hair excretion was used to describe the variability of khi for the Monte Carlo analysis.

Hair Density (DS)

A description of the variation in hair density was presented by Robbins (1994). The density of human hair was determined by the method of Abbott and Goodings (1949). Robbins reported that the density of chemically unaltered hair, at a 60% relative humidity, ranged from 1.320 to 1.327 based on the results of analysis of four lots of hair (dark-brown European hair and three samples taken from heads of volunteers). For the development of a distribution for scalp hair excretion rate, it was assumed that hair density would range from 1.320 to 1.327, with the form of the distribution being uniform, because of the lack of a mean or information about the shape of the distribution (Figure C-1).

Hair Diameter (DI)

Rushton et al. (1983, 1990) conducted two studies to characterize alopecia in both women in men. This assessment included conducting a unit area trichogram in control women and men who had experienced no episodes of hair thinning, excessive hair shedding, or general health disturbances. In Rushton et al. (1983), unit area trichograms were conducted for the occupital region of the scalp in 10 control females. Hair diameter ranged from 55 to 77 μm, with a mean of 67 μm (SD 2). For the assumption of the shape of the distribution for hair diameter, information contained in Rushton et al. (1990) was used. In this study, hair diameter values for 11 control females were plotted. The shape of the distribution of these values was close to normal, with slight skewing to the right. Therefore, the distribution of DI was assumed to be normal (Figure C-2).

Growth Rate (GR)

Numerous articles were located in the literature in which the growth rate of human hair was reported (Flesch, 1954; Barman et al., 1965; Pecoraro et al., 1969; Pelfini et al., 1969; Saitoh et al., 1969; Shahristani and Shihab, 1974; Kershaw et al., 1980; Phelps et al., 1980; Hislop et al., 1983; Cox et al., 1989; Friedel et al., 1989; Hayashi et al., 1991). The values

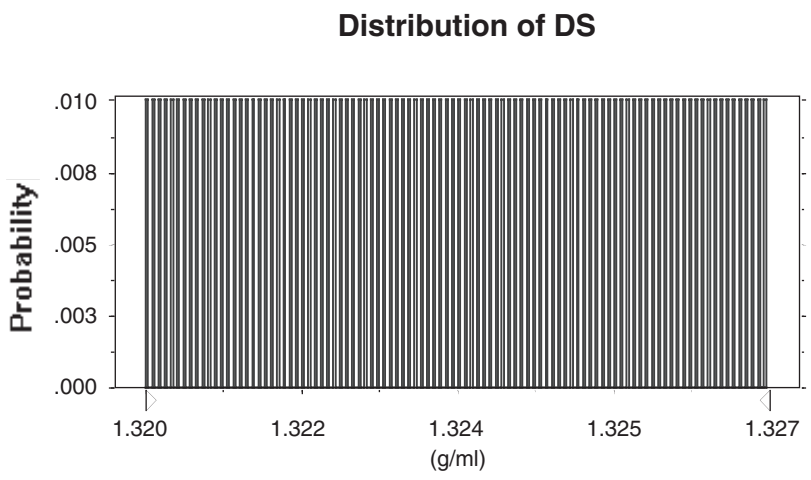

Figure C-1. Distribution of DS.

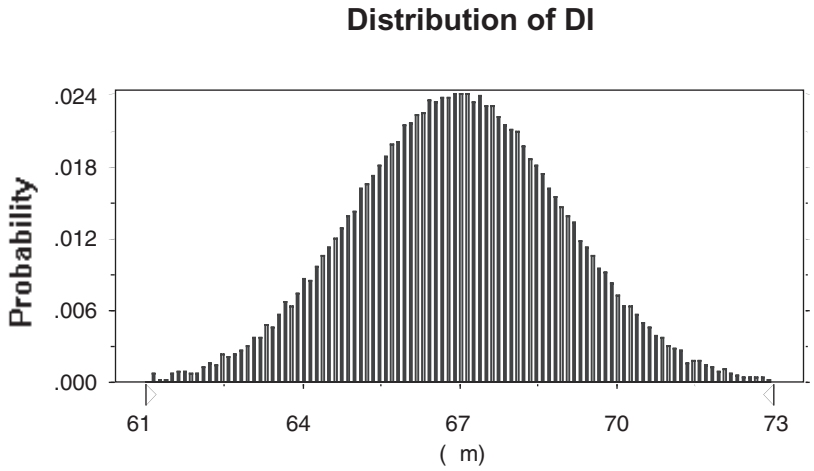

Figure C-2. Distribution of DI.

reported ranged from 0.313 to 0.451 mm/day. These growth rates were evaluated in both males and females from populations including Arabs, Caucasians, Japanese, and North American Indians. The range of values reported is not large, indicating that sex or race has little influence in hair growth rate. Because the population for which the site-specific RfD is being developed is a heterogenous population, there was no reason to prefer a single hair growth rate reported in the literature. Therefore, the distribution of hair growth rate was assumed to be a uniform distribution ranging from 0.313 to 0.451 mm/day (Figure C-3).

Follicle Density (FD) and Scalp Area (SA)

The product of these two parameters provides an estimate of the number of hairs on the scalp. Although information was available on follicle density (Barman et al., 1965; Pecoraro et al., 1969; Rushton et al., 1990), the available literature provided no information on the variation in the area of the scalp. However, a range of estimates of the visible hairs on the scalp was provided in ICRP (1992). Number of visible hairs reported on the scalp were 90,000, 110,000, 140,000, and 140,000 for red, black, brown, and blond hair, respectively. For the distribution of FD×SA (number of visible hairs), it was assumed that the number of scalp hairs would range from 90,000 to 140,000, with the form of the distribution being uniform (Figure C-4).

Distribution of Scalp Hair Excretion

A Monte Carlo analysis was conducted to develop a distribution for scalp hair excretion rate. Ten thousand iterations were conducted to ensure stabilization of the distribution. The resulting mean and standard deviation were $(8.61 \pm 2.15) \times 10^{-6}$. Values ranged from 2.94×10^{-6} to 1.95×10^{-5}. The Kolmogorov D statistic (Stephens,

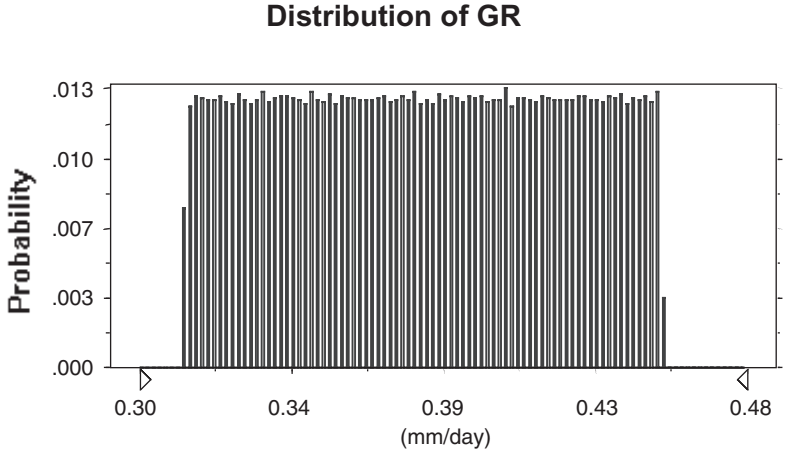

Figure C-3. Distribution of GR.

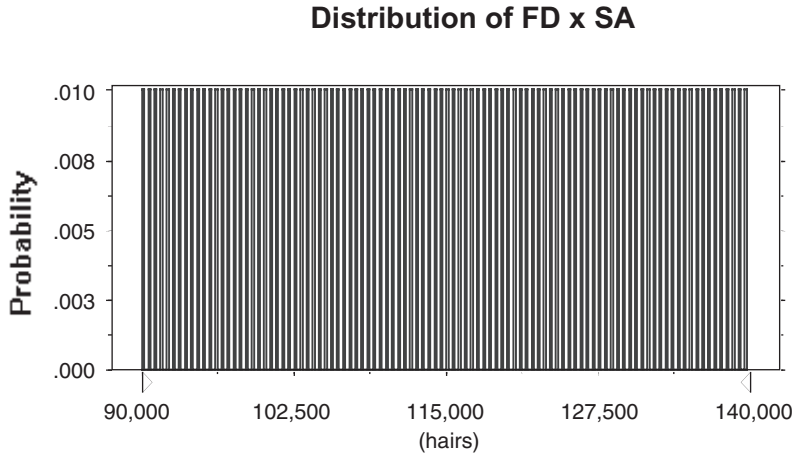

Figure C-4. Distribution of FD×SA.

1974) was calculated on the distribution of values generated for scalp hair excretion rate to determine whether the distribution was consistent with a normal distribution. The statistical analysis indicated that the distribution was neither normal or lognormal; however, upon viewing the normal probability residual plots, the distribution of scalp khi values appeared to be consistent with either a lognormal or a normal distribution, except at the extreme ends. Overall, a lognormal distribution appeared to provide superior fit. Visual inspection of the histogram of the scalp hair excretion rate values indicated a distribution that was skewed to the left; therefore, the form of the distribution was assumed to be lognormal (Figure C-5).

The distribution for scalp hair excretion was used as the starting point for the development of the distribution for khi. The shape of the distribution and the variability in khi was assumed to be the same as that estimated for the scalp hair excretion rate. To obtain the mean value of khi, the PBPK model was fit to empirical data on blood and hair levels of methylmercury. Because khi was the most sensitive parameter in the model (Table C-1), estimation of this parameter was easily accomplished. The fitting of the model to the data from Amin-Zaki et al. (1976), Hislop et al. (1983), and Sherlock et al. (1984) is presented in Figures C-6 through C-8. Using the initial khi of 8.6×10^{-6} resulted in an overprediction of blood and hair levels of mercury (Figures C-6 and C-7). Decreasing the value of khi to approximately 7.7×10^{-6} resulted in a better fit to the data from Hislop et al. (1983) and Sherlock et al. (1984). However, the majority of this data were from men. Based on the data of Sherlock et al. (1984), women have biological half-life that is approximately 9% longer than men, which is

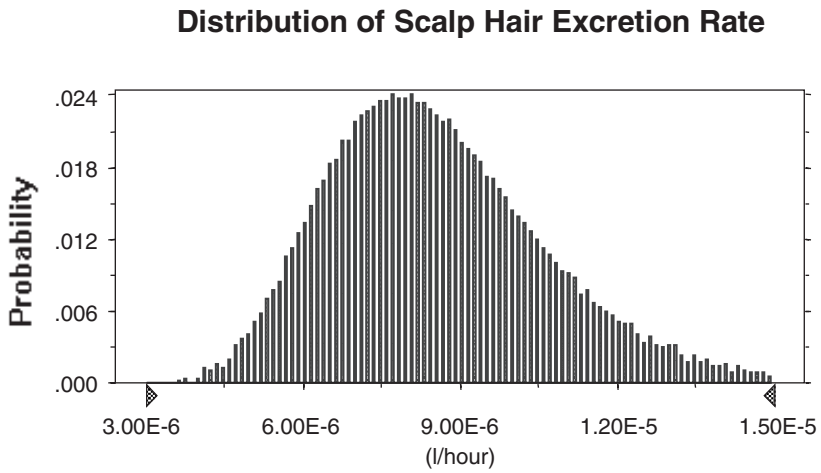

Figure C-5. Distribution of scalp hair excretion rate.

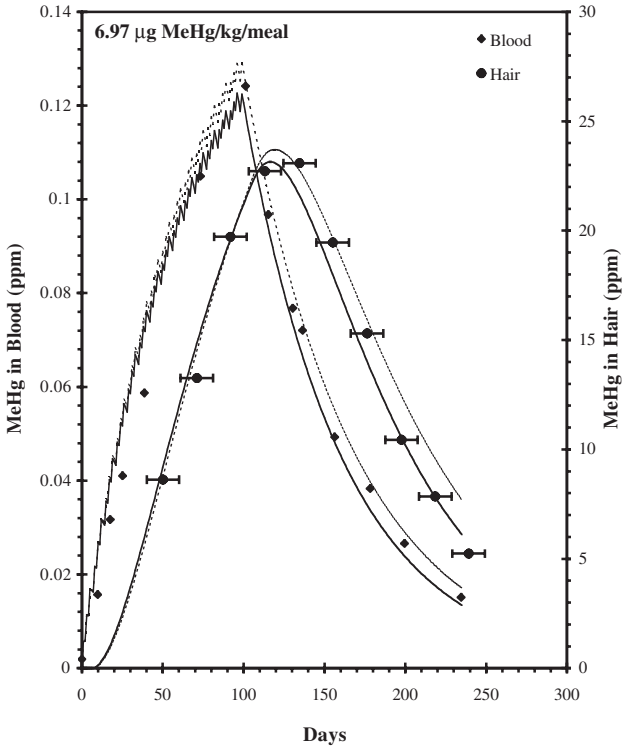

Figure C-6. Time course of MeHg in whole blood and hair from a human subject consuming naturally contaminated halibut at 6.97 μg HeHg/kg/meal for three meals/week for approximately 96 days and followed after elevated ingestion levels ceased for approximately 100 days. The lines represent the PBPK model simulations for blood and hair, while the data points with bars represent the mean ± 10 days representing the effect of hair growth on MeHg determination in hair (data from Hislop et al., 1983). The solid line represents the model prediction with a hair excretion rats constant (khi) of 8.6×10^{-6}, while the dotted line represents the model prediction for khi = 7.7×10^{-6}.

probably attributable to differences in hair excretion rates. The khi was adjusted down further to 7×10^{-6} and attempt was made to fit data from Amin-Zaki et al. (1976) (Figure C-8). In this study, time course data on blood and hair levels were available from a 29-year-old Iraqi woman who was exposed during pregnancy to methylmercury during the contaminated grain incident. Information on the baby's blood levels at term were also available. This fitting process indicated that the most appropriate value for khi in women was 7.0×10^{-6}. Once the mean value of khi was approximated, the distribution for khi was developed using the variability in scalp hair excretion (coefficient of variation of 0.24). The shape of the distribution of khi was also assumed to be lognormal.

Hair:Blood Partition Coefficient (PHB)

Numerous studies have reported hair:blood partition coefficients for total mercury (Tejning, 1967; Sumari et al., 1969; Tsubaki, 1971a,b; Birke et al., 1972; Den Tonkelaar et al., 1974; Skerfving, 1974; Haxton et al., 1979; Kershaw et al., 1980; Phelps et al., 1980; Turner et al., 1980; Sherlock et al., 1982; Soria et al., 1992; Cernichiari et al., 1995). A table summarizing the reported results of these studies is provided (Table C-2). These studies were reviewed to identify information for use in the development of a PHB distribution for the Monte Carlo analysis. Initially, the data reported for a small subcohort of Seychellois women (Cernichiari et al., 1995) was to be used as the basis for the distribution of PHB values for the derivation of the site-specific RfD for methylmercury; however, a recent publication (Stern, 1997) has questioned whether this information should be used in the derivation of an RfD for methylmercury because the PHB was determined at the time of delivery in pregnant women. Therefore, instead of using a PHB based on a single study, a critical review of all of the available information on hair to blood ratios in the literature was conducted. The results of the literature review indicated that there was no relationship between the hair to blood ratios and ethnicity, or no biological reason that one hair to blood

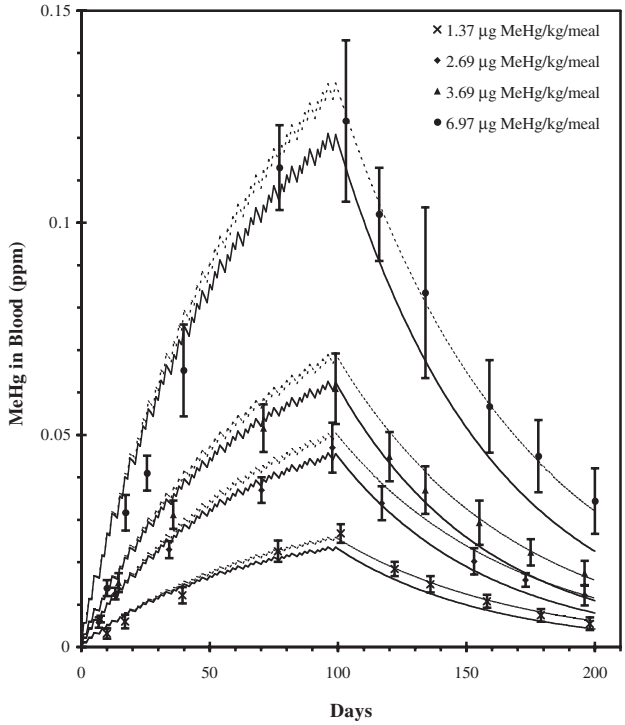

Figure C-7. Time course of MeHg in whole blood from human subjects consuming MeHg-contaminated halibut at 1.37 ($n=5$), 2.69 ($n=4$), 3.69 ($n=6$), or 6.97, ($n=5$) μg MeHg/kg/meal for three meals/week over approximately 96 days and followed after elevated ingestion levels ceased for approximately 100 days. The lines represent the PBPK model simulations for each group (solid line: khi = 8.6×10^{-6}; dotted line: khi = 7.0×10^{-6}), and the data points with bars represent the mean ± one standard deviation (data from Sherlock et al., 1984).

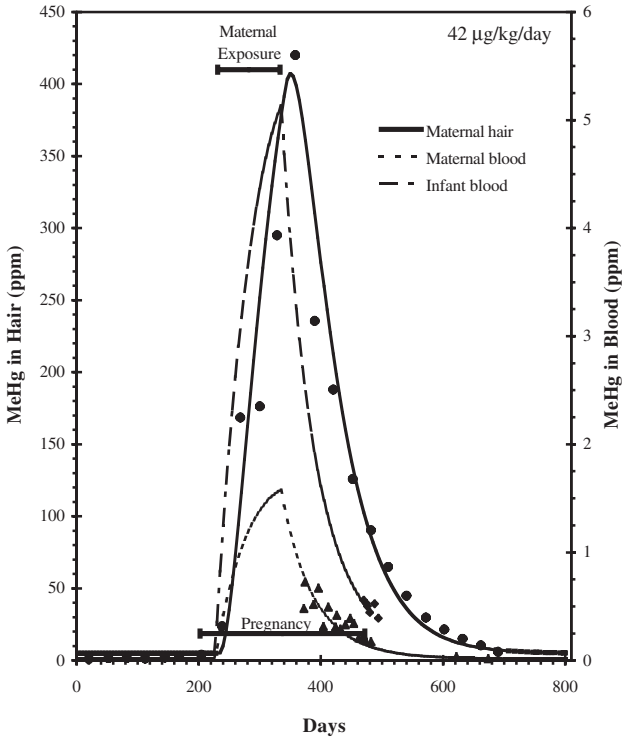

Figure C-8. Simulation (lines) of data on MeHg concentrations in maternal hair, maternal blood, and infant blood from Amin-Zaki et al. (1976). Maternal blood and hair samples were taken from a pregnant woman who had consumed MeHg-contaminated bread for approximately 90 days before being admitted to the hospital. The infant's blood was sampled from birth to about 30 days. The dose input to the model was adjusted to reproduce the peak concentration of MeHg in maternal hair. The parameter values used in the model are the same as the mean values used in the Monte Carlo analysis.

ratio would be preferred over another. Because slight changes in methodology for determining mercury levels in biological media can have a great impact on the blood:hair ratio observed (Sherlock et al. (1984) reported that a slight change in the methodology for determining blood levels of mercury resulted in an underestimation of blood levels of approximately 30%), the variation observed between the reported hair to blood ratios is likely experimental error or bias. Therefore, an attempt was made to combine all information available from the literature into a "global" distribution of PHBs to capture the variability in experimental. The methodology for the development of the global distribution is described in Appendix F. Information that could be used for the development of the global distribution was obtained from ten of the available studies (Sumari et al., 1969; Birke et al., 1972; Skerfving, 1974; Haxton et al., 1979; Kershaw et al., 1980; Phelps et al., 1980; Turner et al., 1980; Sherlock et al., 1982; Soria et al., 1992; Cernichiari et al., 1995).

For two of these studies (Soria et al., 1992; Cernichiari et al., 1995), hair and blood levels were determined in pregnant women at the time of delivery. Because blood volume and possibly hair growth rate may change significantly during pregnancy (Stern, 1997), the PBPK model was used to adjust the levels observed at delivery to steady-state levels before pregnancy for incorporation into the distribution. This resulted in a mean of 360 for Cernichiari et al. (1995) rather than the reported value of 416, and a mean for Soria et al. (1992) of 189 rather than 218. For two additional studies (Haxton et al., 1979; Sherlock et al., 1982), some of the authors had reported a problem with the methodology used for determining mercury levels in blood samples (Sherlock et al., 1984). The method used, a modification of Magos (1971), resulted in determination of blood mercury levels that were approximately 30% too low; therefore, the PHB for these two studies was adjusted by a factor of 1/1.3 to reflect this information for incorporation into the global distribution. This resulted in the use of means of 282 and 192 for Haxton et al. (1979) and Sherlock et al. (1982), respectively, rather than the reported values of 367 and 250.

Table C-3 contains a summary of the information used in developing the global distribution for PHB. The mean was approximately 245 (SD 173), with the shape of the distribution lognormal (Figure C-9), consistent with previous analyses (Stern, 1997; USEPA, 1997).

Fecal Excretion Rate (kfi)

An average fecal excretion rate for an adult female of 110 g/day was obtained from ICRP (1992). A similar value (115.3 g/day) for adult males was reported in Geigy Scientific Tables (Diem and Lentner, 1970), for which a standard deviation was available (41.1 g/day). Therefore, the variability of fecal excretion in males was used to assess the variability of kfi in females. For use of this value in the PBPK model, this value was adjusted to liters per hour (l/h; assuming a density of 1) and adjusted by $BW^{3/4}$ to obtain the scalable input parameter. The resulting distribution used for kfi is a mean of 0.0002 l/h, with a coefficient of variation of 36%. The shape of the distribution was assumed to be lognormal (see Figure C-10), although use of a normal distribution had little impact on the results of the Monte Carlo simulations.

Gut to Blood Partition Coefficient (PG)

The gut to blood partition coefficient was determined based on the results of a studies in which heavy metals were evaluated in the tissues of 30 cadavers (Sumino et al., 1975). PG was estimated to be 1, based on the levels of methylmercury observed in the small intestine and the large intestine compared to levels in the blood. The variation in PG based on the cadaver data could not be determined because the individual data were not provided. For another partition coefficient, PHB, for which a large amount of data was available to develop a distribution, the

Table C-2. Hair to blood ratio (total Hg).

Reference	Hair to blood ratio	Number of subjects	Hg range in whole blood (μg/l)	Hair samples		Distance to scalp
				Hg range in hair (ppm)	Length (mm)	
Sumari et al., 1969	157	30	5–268	1–56	–	–
Soria et al., 1992	218	16	2.4–9.1	0.3–2.16	–	at scalp
Tejning, 1967[a]	230	51	4–110	1–30	–	axillary
Skerfving, 1974	230	60	44–550	1–142	5	at scalp
Haxton et al., 1979	250	173	0.4–26	0.1–11.3	20	–
Tsubaki, 1971b[a]	260	45	2–800	20–325	–	–
Birke et al., 1972	286[b]	8	7–650	2.2–185	5	at scalp
Den Tonkelaar et al., 1974[c]	280	47	1–40.5	<0.5–13.2	–	–
Kershaw et al., 1980	291[c]	5	–	–	5	at scalp
Phelps et al., 1980	296	339	1–60	1–150	10	at scalp
Sherlock et al., 1982	367	96	1.1–42.3	0.2–21	24	–
Cernichiari et al., 1995	416	27	0.5–26.7		10	at scalp
Tsubaki, 1971a	370	~25	–	–	"longer tuft"[a]	–
Turner et al., 1980	190	140	11–275	1–48	–	at scalp

[a]Information obtained from USEPA (1997).
[b]Ratio of methylmercury in hair to methylmercury in blood.
[c]Based on repeated measurements at different time points (3–8 ratios per individual) of the ratio of 5-mm hair segments to corresponding 2-week average blood levels (assuming hair growth of 1.1 cm/month).
– Not reported.

coefficient of variation was approximately 70%. While different color and racial characteristics contribute to the variation in hair partitioning, no similar effects are likely in the case of intestinal tissue. Thus, since the interindividual variability of PG would be expected to be less than that for PHB, a conservative estimate of variation for PG was assumed to be 70%. The shape of the distribution was assumed to be lognormal (Figure C-11) to be consistent with PHB.

Body Weight (BW)

The distribution for BW was based on body weight data for 2681 women, 14 to 45 years of age, from the Third National Health and Nutrition Survey (NHANES III) (National Center for Health Statistics, 1995). The mean BW value was 67.77 kg (SD, 17.36 kg). The range of ages for determination of the distribution of BW was selected based on the range of maternal ages (Shamlaye et al., 1995) in the Seychelles population that is the basis for the derivation of the site-specific RfD. The Kolmogorov *D* statistic (Stephens, 1974) was calculated for the distribution of values generated for BW to determine whether the distribution was consistent with a normal distribution. The statistical analysis indicated that the distribution differed from normal; however, the distribution was also significantly different from a

Table C-3. Information used for the development of the global distribution of the hair to blood ratio (PHB) for methylmercury.

Reference	*R*	Regression slope	Regression standard error	*N*	Mean PHB	SD
Cernichiari et al., 1995a	–	360.0	35.0	27	360.0	181.9
Soria et al., 1992	–	–	–	16	188.7	52.8
Kershaw et al., 1980	–	–	–	5	290.6	41.2
Birke et al., 1972	–	286.4	11.5	8	286.4	32.6
Sumari et al., 1969	–	156.7	11.9	30	156.7	65.2
Skerfving, 1974	0.860	230.0	17.9	60	230.0	138.8
Sherlock et al., 1982	0.837	282.3	19.0	96	282.3	186.5
Haxton et al., 1979	0.600	192.3	19.6	173	192.3	257.9
Phelps et al., 1980	0.935	296.0	6.1	339	296.0	112.6
Turner et al., 1980	0.770	190.0	13.4	140	190.0	158.6

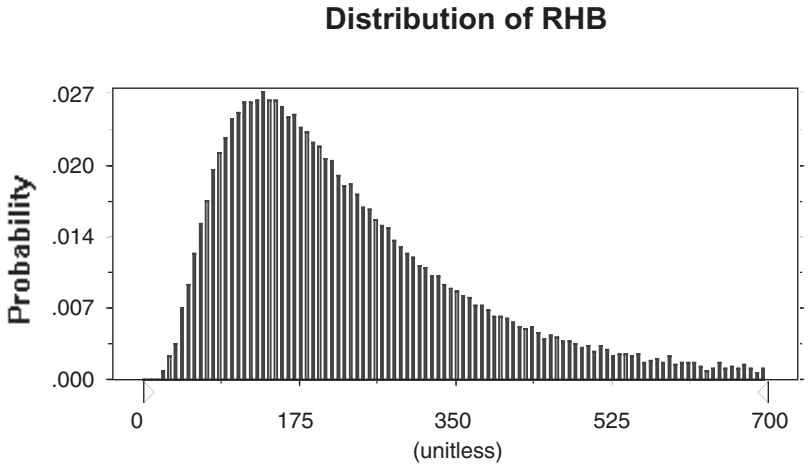

Figure C-9. Distribution of PHB.

lognormal distribution. In the case where the volume of the fat compartment was correlated with body weight, it was assumed that BW is normally distributed (see Figure C-12). This assumption facilitated the performance of a Monte Carlo simulation with this correlation incorporated. In the case where the Monte Carlo was performed without the fat/body weight correlation, a lognormal distribution was used.

Volume of the Fat Compartment (VFC)

The volume of the fat compartment (VFC) was estimated based on information provided by Pollock et al. (1975). In this study, body density and percent body fat was determined in 83 females aged 18–22, and 60 females aged 33–50. The mean percent body fat for the 18–22 year old females was 24.8 (SD 6.4), with the mean percent body fat in the 33–50 year old women being 29.8 (SD 6.7). This information was combined to result in a mean value of 27.3%, with a coefficient of variation of 24%. The shape of the distribution was assumed to be normal (Figure C-13), although the use of a lognormal distribution did not significantly change the results of the Monte Carlo analysis. In the primary analysis, VFC was assumed to be correlated with BW.

Volume of Slowly Perfused Tissue (VSC)

The slowly perfused tissue compartment consists of muscle and skin. For purposes of the PBPK model all tissue volumes are expressed as a fraction of BW. Therefore, information on the fraction of BW that consists of muscle mass and skin was combined to estimate VSC. Limited information was available on the mass of muscle

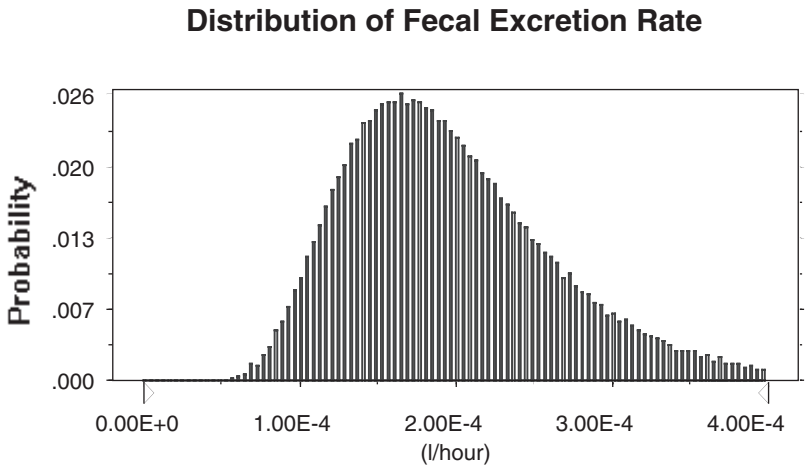

Figure C-10. Distribution of fecal excretion rate.

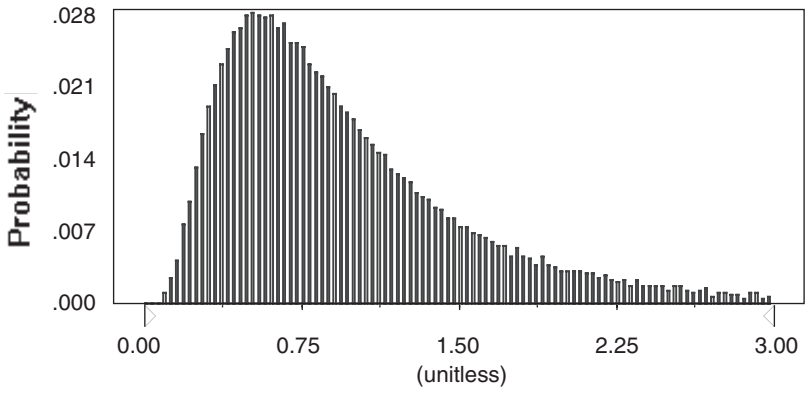

Figure C-11. Distribution of PG.

tissue in the body. A variety of indirect methods are available for estimating muscle mass; however, very few of these indirect methods have been validated against direct cadaver evidence. One study was available in which 13 female cadavers were dissected and gross tissue weights determined (Clarys et al., 1984). Body weights before dissection were also available so muscle mass could be expressed as a fraction of BW. The weights of the cadavers ranged from 44.3 to 74.2 kg, with gross muscle weights ranging from 13.3 to 23.4 kg. The mean muscle mass as a fraction of BW was estimated to be 0.296 (SD 0.047).

For estimation of the weight of skin, information from ICRP (1992) was used. The skin of an adult female is approximately 5.4% of BW (3000 g in a 55.3-kg adult female).

For VSC, the fraction of BW contributed by skin and muscle mass were combined to result in a fraction of BW contributed by slowly perfused tissue of 35%. Because muscle mass constitutes the majority of this compartment, the variability from the cadaver information was used to assess the variability of VSC. This resulted in a coefficient of variation of VSC of 16%. In the primary analysis, the shape of the distribution for VSC was assumed to be normal (Figure C-14); however, use of a lognormal distribution did not significantly change the results of the Monte Carlo analysis.

Correlation between BW and VFC

In addition to developing distributions for individual parameters, it was assumed that BW and VFC were correlated. Using underwater weighing, Hansen et al. (1993) estimated the correlation between body weight and % fat in premenopausal females to be 0.73. Accordingly, BW and VFC were assumed to be bivariately normally distributed with a correlation of $\rho=0.73$ (Hansen et al., 1993). Once these parameters were generated, the fractions $f_1, \ldots, f_k$ of body weight in the remaining k compartments

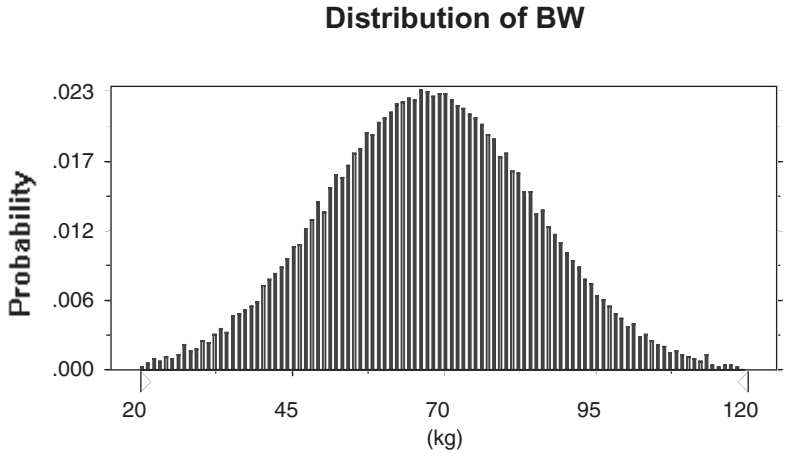

Figure C-12. Distribution of BW.

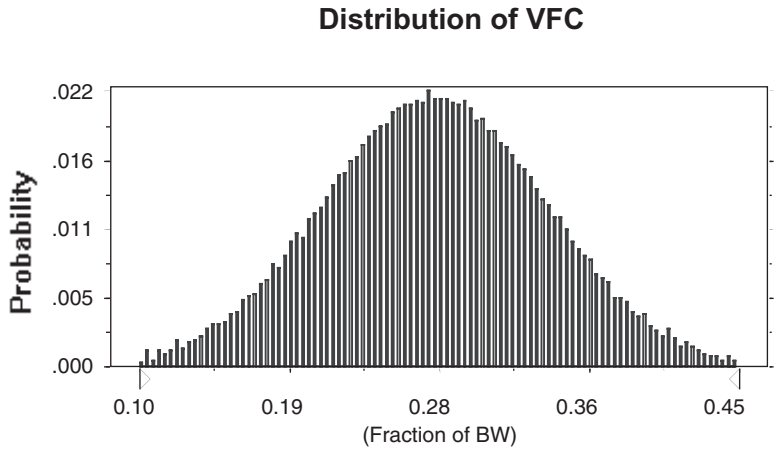

Figure C-13. Distribution of VFC.

were developed by generating k independent normal "pseudo fractions," f_1', ..., f_k', and the actual fraction of body weight in the ith compartment was computed as $f_i = f_i'(1-\text{VFC})/(f_1'+f_2'+\ldots+f_k')$. All of the distributions (for BW, VFC, f_1', ..., f_k') were censored above at the input mean plus four times the input standard deviation and below and the larger of the mean minus four times the standard deviation, and 1% of the mean. This approach insured that organs weights were all positive and summed to the total body weight. The input means and standard deviations for the pseudo fractions f_1', ..., f_k' were selected by trial and error so that the actual fractions, f_1, ..., f_k, had the desired means and standard deviations. It was also verified that the censoring had no noticeable effect on the means and standard deviations of the variables, or upon the correlation between BW and VFC.

Validation of Parameter Distribution Selection

To validate the selection of parameter distributions for the Monte Carlo analysis, the distribution of biological half-lives of methylmercury predicted by the model was compared with half-lives published in the literature. The half-lives reported from the most applicable data to fish-eating populations (Miettinen et al., 1971; Kershaw et al., 1980; Sherlock et al., 1984; Smith et al., 1994) ranged from 32 to 70 days, with a mean of approximately 49 days (SD 7.45 days). In one of the studies, information on half-life was available for both men and women. In the study conducted by Sherlock et al. (1984), the mean half-life in males was 49.7 days (SD 7.47; $n=14$), while the mean half-life for women was 54.2 days (SD 3.62; $n=6$), indicating that excretion of methylmercury in women is slower than in men, possibly due to a lower

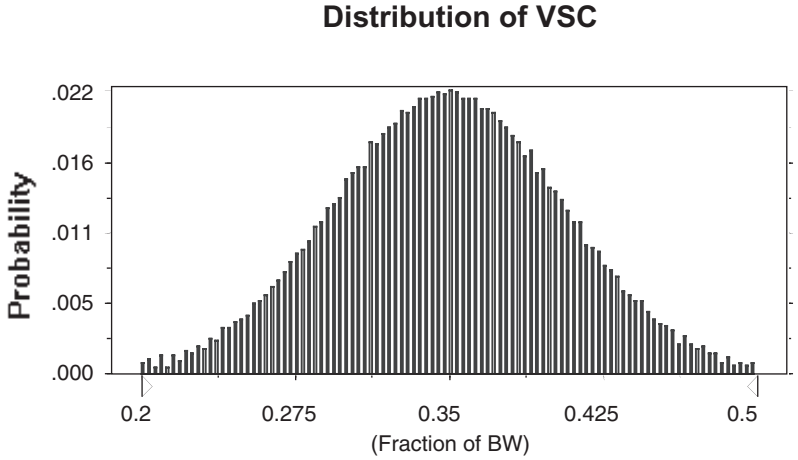

Figure C-14. Distribution of VSC.

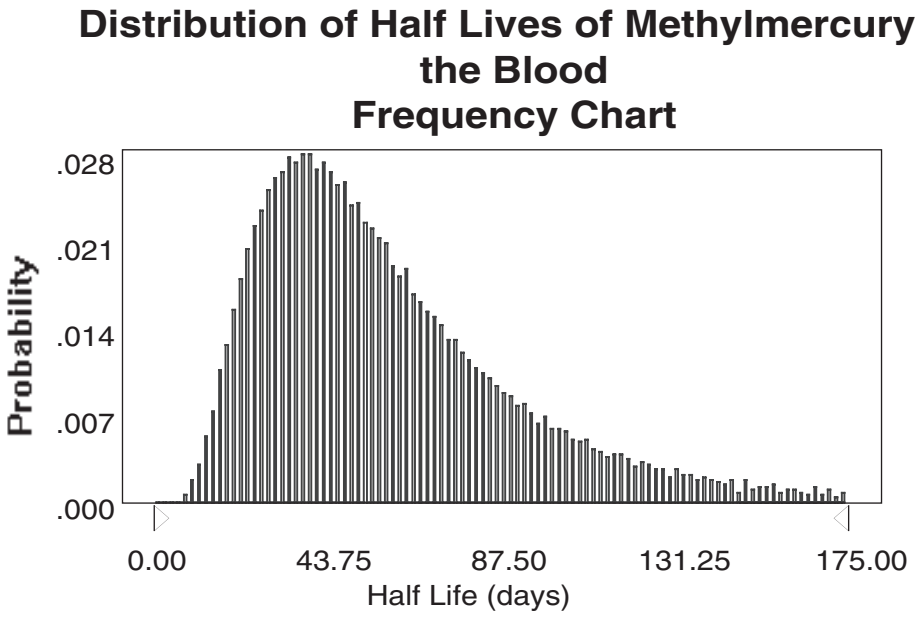

Figure C-15. Distribution of half lives of methylmercury in the blood frequency chart.

hair excretion rate. The distribution of half-lives output by the PBPK Monte Carlo analysis indicated a mean half-life of 61 days, with a standard deviation of 35 days (Figure C-15). The mean half-life output by the model is slightly larger than the reported mean value for women reported in the literature, with the variability being larger. However, this would result in more conservative estimates of the ingestion rates for the basis of the site-specific RfD.

APPENDIX D

Statistical Methods Used for Development of Distributions

Global Mean for Hair to Blood Ratio Distribution

A global mean and standard deviation were computed from nine independent studies (Sumari et al., 1969; Birke et al., 1972; Skerfving, 1974; Haxton et al., 1979; Kershaw et al., 1980; Phelps et al., 1980; Turner et al., 1980; Sherlock et al., 1982; Soria et al., 1992). In one case (Soria et al., 1982), the hair to blood ratio of methylmercury was defined by the sample size n, the estimated mean , and the estimated standard deviation, s. However, in most of the studies only the linear coefficient, sample size, and the standard error from a regression analysis of the blood concentrations of methylmercury to the hair concentrations of methylmercury were available. In a few cases, the correlation coefficient, R, or its square was given instead of the standard error. In the cases where the linear regression coefficient, sample size, and either the standard error or the correlation coefficient were given, the mean and standard deviation of the hair to blood ratio were determined based on the formulas given below.

Given the following definition of the regression equation:

$$\text{HairConc} = h$$
$$\text{Bloodconc} = b$$
$$h = axb + \text{error} \tag{21}$$

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

$$\text{se}(\hat{a}) = \frac{\hat{a}x\left(\frac{1}{R^2} - 1\right)}{(n-2)} \tag{22}$$

From this, we determined the following.

$$\hat{a} = \frac{\sum b_i \, x \, h_i}{\sum b_i^2} \approx \text{mean of } \frac{h}{b} = \bar{x} \tag{23}$$

$$Var\, h = \sigma^2 = Var_{\text{error}^2} \tag{24}$$

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

and

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

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

$$\begin{aligned} Var\left(\tfrac{h}{b}\right) &= E\big(Var\big(\tfrac{h}{b}|b\big)\big) + Var\left(E\big(\tfrac{h}{b}|b\big)\right) \\ &= \sigma^2 \times E\big(\tfrac{1}{b^2}\big) + 0 \\ &= \sigma^2 \times E\big(\tfrac{1}{b^2}\big) \end{aligned} \tag{26}$$

If we use the approximation

$$E\left(\frac{1}{b^2}\right) \approx \frac{n}{\sum (b_i)^2} \tag{27}$$

then

$$Var\left(\frac{h}{b}\right) = n \times Var\ \hat{a} \tag{28}$$

and

$$s \text{ of } \left(\frac{h}{b}\right) = \sqrt{n} \times se(\hat{a}) \tag{29}$$

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

Given n_i, $\bar{x}_i$, and s_i^2 for each study i, where

$$s_i^2 = \frac{1}{n_{i-1}} \sum_j \left(x_{ij} \bar{x}_i\right)^2 \tag{30}$$

and

$$N = \sum_i n_i \tag{31}$$

then

$$\text{Global mean} = \bar{x} = \frac{1}{N} \sum_i \sum_j x_{ij} = \frac{1}{N} \sum_i (n_i \times \bar{x}_i) \tag{32}$$

$$\begin{aligned} \text{Global } s^2 &= \tfrac{1}{N-1} \sum_i \sum_j \left(x_{ij} - \bar{x}_i + \bar{x}_i - \bar{x}\right)^2 \\ &= \tfrac{1}{N-1} \sum_i \left(\sum_j \left[\left(x_{ij} - \bar{x}_i\right)^2 + n_i (\bar{x}_i - \bar{x})^2 \right] \right). \\ &= \tfrac{1}{N-1} \left(\sum_i (n_i - 1) s_i^2 + \sum_i n_i (\bar{x}_i - \bar{x})^2 \right) \end{aligned} \tag{33}$$

The global mean and standard deviation for the hair to blood ratio were used in the pharmacokinetic model to determine a distribution for the global DCF. The units for the DCF calculated by the pharmacokinetic model are milligrams per kilogram per day ingestion of methylmercury per part million of methylmercury in the hair.

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