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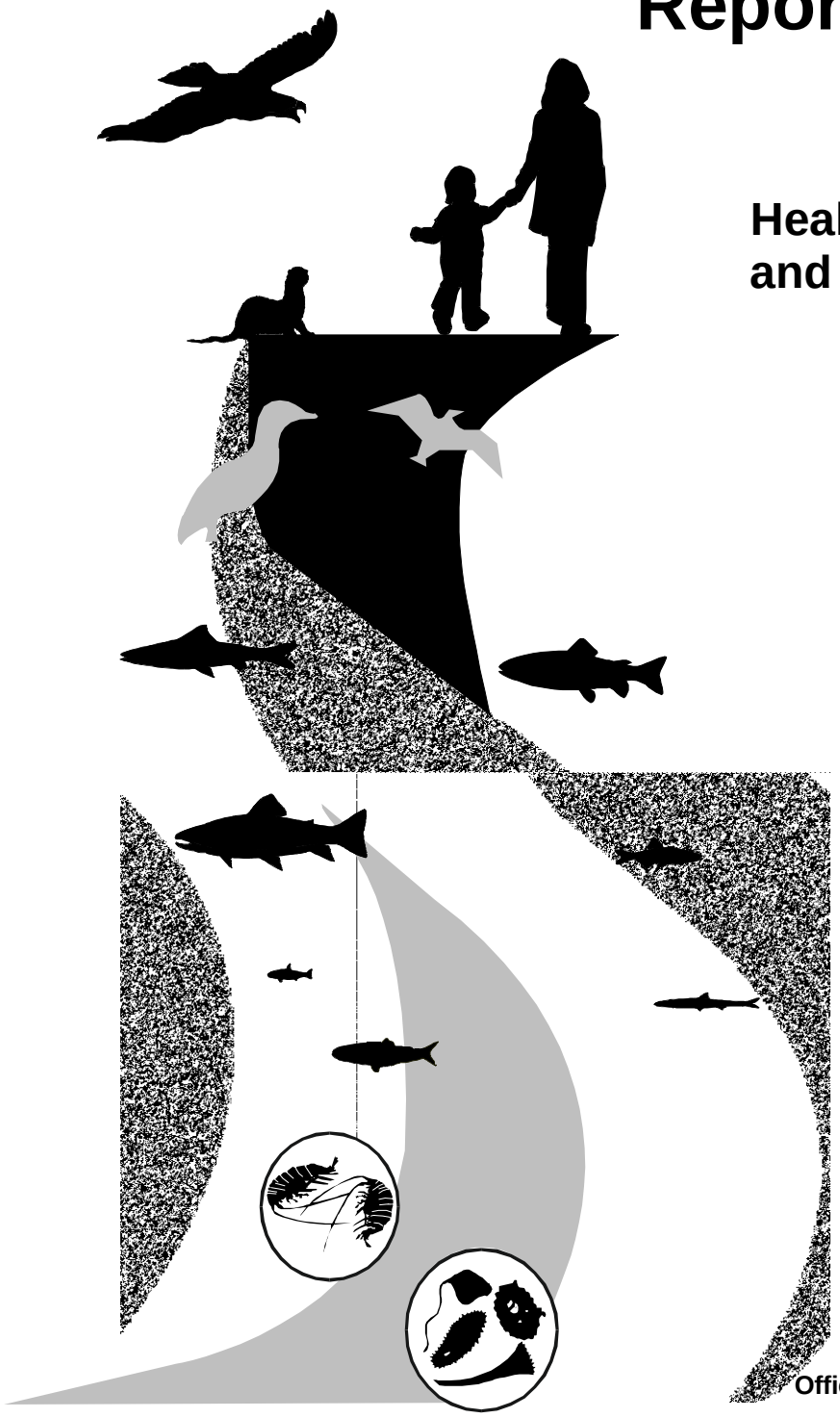

Volume V: Health Effects of Mercury and Mercury Compounds

Office of Air Quality Planning & Standards
and
Office of Research and Development

MERCURY STUDY REPORT TO CONGRESS

VOLUME V:

HEALTH EFFECTS OF MERCURY AND MERCURY COMPOUNDS

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U.S. Environmental Protection Agency

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LIST OF SYMBOLS, UNITS AND ACRONYMS

ATSDR	Agency for Toxic Substances and Disease Registry
BML	Biological monitoring level
bw	Body weight
CAA	Clean Air Act as amended in 1990
CHO	Chinese hamster ovary
C.I.	Confidence interval
CNS	Central nervous system
CRAVE	Carcinogen Risk Assessment Verification Endeavor
DDST	Denver Developmental Screen Test
DHHS	Department of Health and Human Services
DNA	Deoxyribonucleic acid
DWEL	Drinking water equivalent level
ECG	Electrocardiogram
EEG	Electroencephalogram
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
GABA	Gamma aminobutyric acid
Gd	Gestation day
HEC	Human equivalent concentration
Hg	Mercury
Hg•U	Urinary mercury
IgG	Immunoglobulin G
IRIS	Integrated Risk Information System
LC_{50}	Lethal concentration killing 50 percent of the animals tested (inhalation)
LD_{50}	Lethal dose killing 50 percent of the animals tested
LOAEL	Lowest-observed-adverse-effect level
MF	Modifying factor
MMAD	Mass median aerodynamic diameter
MMC	Methylmercuric chloride
MMH	Methylmercuric hydroxide
MRL	Minimal risk level
MTD	Maximum tolerated dose
NAG	N-acetyl-b-glycosaminidase
NADH	Reduced nicotinamide adenine dinucleotide
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NOAEL	No-observed-adverse-effect level
NS	Not specified
NTP	National Toxicology Program
PMA	Phenyl mercuric acetate
ppd	Postpartum day
RfD	Reference dose (oral)
RfD_{DT}	Reference dose for developmental toxicity
RfC	Reference concentration (inhalation)
SCE	Sister chromatid exchange
SGPT	Serum glutamic-pyruvic transaminase
SH	Sulfhydryl groups

LIST OF SYMBOLS, UNITS AND ACRONYMS (continued)

SMR	Standard mortality ratio
TOLD	Test of Language Development
TWA	Time-weighted average
UF	Uncertainty factor
UF_A	Uncertainty factor for interspecies extrapolation
UF_H	Uncertainty factor for intraspecies extrapolation (animal to human)
UF_L	Uncertainty factor for use of a LOAEL
UF_S	Uncertainty factor for use of a subchronic-duration study
WHO	World Health Organization

EXECUTIVE SUMMARY

Section 112(n)(1)(B) of the Clean Air Act (CAA), as amended in 1990, directs the U.S. Environmental Protection Agency (U.S. EPA) to submit to Congress a comprehensive study on atmospheric emissions of mercury. This document, which covers the human health effects of mercury and mercury compounds, is one volume of U.S. EPA's eight-volume Report in response to this directive.

Mercury is a naturally occurring element that is found in air, water and soil. It exists in any of three valence states: Hg^0 (elemental mercury), Hg_2^{2+} (mercurous mercury), or Hg^{2+} (mercuric mercury). Most of the population of the earth have some exposure to mercury as a result of normal daily activities. The general population may be exposed to mercury through inhalation of ambient air; consumption of contaminated food, water, or soil; and/or dermal exposure to substances containing mercury. In addition, some quantity of mercury is released from dental amalgam.

The health effects literature contains many investigations of populations with potentially high exposure to mercury, including industrial workers, people living near point sources of mercury emissions, people who consume large amounts of fish, and dental professionals. There also are numerous studies of populations unintentionally exposed to high levels of mercury, such as the Minamata poisoning episode in Japan. Volume IV (An Assessment Exposure to Mercury in the United States) presents measured and predicted mercury exposure for various U.S. populations.

The purpose of this volume, Volume V, is to summarize the available health effects information for mercury and mercury compounds and to present U.S. EPA's analysis for two critical pieces of the risk assessment paradigm described by the National Academy of Sciences in 1983. Specifically, this volume contains the hazard identification and dose-response assessments for three forms of mercury: elemental mercury, mercuric chloride (inorganic mercury),and methylmercury (organic mercury). In order to characterize risk for any populations, the evaluations presented in this volume must be combined with the assessment of exposure presented in Volume IV.

Volume V is not intended to be an exhaustive survey of the voluminous health effects literature available for mercury. Rather, the purpose is to present a brief survey of the studies relevant for assessing potential human health effects and to present more detailed information on those studies which form the basis for U.S. EPA's hazard identification and dose-response assessments. The three forms of mercury which are emphasized in this volume were selected based on data indicating that these are the predominant forms of mercury to which humans are exposed. In addition, examination of the published literature indicates that most health data are on these forms. It is acknowledged that certain populations can be exposed to many types of organic mercurials, such as antiseptics and pesticides. Volume V, however, deals with methylmercury except in cases where information on another organic is presented for illustrative purposes.

Toxicokinetics

The toxicokinetics (i.e., absorption, distribution, metabolism, and excretion) of mercury is highly dependent on the form of mercury to which a receptor has been exposed. Below is a brief summary of the toxicokinetics information for elemental mercury, mercuric chloride, and methylmercury. Chapter 2 contains a more complete summary of the toxicokinetics information available for mercury.

Elemental Mercury

The absorption of elemental mercury vapor occurs rapidly through the lungs, but it is poorly absorbed from the gastrointestinal tract. Once absorbed, elemental mercury is readily distributed throughout the body; it crosses both placental and blood-brain barriers. Elemental mercury is oxidized to inorganic divalent mercury by the hydrogen peroxidase-catalase pathway, which is present in most tissues. The distribution of absorbed elemental mercury is limited primarily by the oxidation of elemental mercury to the mercuric ion as the mercuric ion has a limited ability to cross the placental and blood-brain barriers. Once elemental mercury crosses these barriers and is oxidized to the mercuric ion, return to the general circulation is impeded, and mercury can be retained in brain tissue. The elimination of elemental mercury occurs via urine, feces, exhaled air, sweat, and saliva. The pattern of excretion is dependent on the extent to which elemental mercury has been oxidized to mercuric mercury.

Inorganic Mercury

Absorption of inorganic mercury through the gastrointestinal tract varies with the particular mercuric salt involved. Absorption decreases with decreasing solubility. Estimates of the percentage of inorganic mercury that is absorbed vary; as much as 20% may be absorbed. Available data indicate that absorption of mercuric chloride from the gastrointestinal tract results from an electrostatic interaction with the brush border membrane and limited passive diffusion. Increases in intestinal pH, high doses of mercuric chloride causing a corrosive action, a milk diet (e.g., neonates) and increases in pinocytotic activity in the gastrointestinal tract (e.g., neonates) have all been associated with increased absorption of inorganic mercury. Inorganic mercury has a limited capacity for penetrating the blood-brain or placental barriers. There is some evidence indicating that mercuric mercury in the body following oral exposures can be reduced to elemental mercury and excreted via exhaled air. Because of the relatively poor absorption of orally administered inorganic mercury, the majority of the ingested dose in humans is excreted through the feces.

Methylmercury

Methylmercury is rapidly and extensively absorbed through the gastrointestinal tract. Absorption information following inhalation exposures is limited. This form of mercury is distributed throughout the body and easily penetrates the blood-brain and placental barriers in humans and animals. Methylmercury transport into tissues appears to be mediated by the formation of a methylmercury-cysteine complex. This complex is structurally similar to methionine and is transported into cells via a widely distributed neutral amino acid carrier protein. Methylmercury in the body is considered to be relatively stable and is only slowly demethylated to form mercuric mercury in rats. It is hypothesized that methylmercury metabolism may be related to a latent or silent period observed in epidemiological studies observed as a delay in the onset of specific adverse effects. Methylmercury has a relatively long biological half-life in humans; estimates range from 44 to 80 days. Excretion occurs via the feces, breast milk, and urine.

Biological Monitoring/Pharmacokinetic Models

Chapter 2 provides information on biological monitoring of mercury as well as a summary of the development of pharmacokinetic models for mercury. The most common biological samples analyzed for mercury are blood, urine, and scalp hair. The methods most frequently used to determine the mercury levels in these sample types include atomic absorption spectrometry, neutron activation analysis, X-ray fluorescence, and gas chromatography.

Both simple and complex multi-compartmental models have been described in the literature. A recent report (Gearhart et al. 1995) presents an approach based upon data from human, rat, and monkey data that could be used for characterizing dose-response data both adults and neonates.

Biological Effects

Chapter 3 presents summary information on the toxicity of elemental mercury, mercuric mercury and methylmercury to various organ systems. The primary targets for toxicity of mercury and mercury compounds are the nervous system, the kidney, and the developing fetus. Other systems that may be affected include the respiratory, cardiovascular, gastrointestinal, hematologic, immune, and reproductive systems. For each form of mercury and each of the endpoints addressed, information from epidemiological studies, human case studies, and animal toxicity studies is summarized in tabular form. Critical studies are discussed in the accompanying text.

Elemental Mercury

A number of epidemiological studies have been conducted that examined cancer mortality and/or morbidity among workers occupationally exposed to elemental mercury. All of these studies, however, have limitations which compromise the interpretation of their results; these limitations include small sample sizes, probable exposure to other known lung carcinogens, failure to consider confounding factors such as smoking, and/or failure to observe correlations between estimated exposure and the cancer incidence. Only one animal study was identified that examined cancer incidence in animals exposed (by injection) to elemental mercury. While tumors were found at contact sites, the study was incompletely reported as to controls and statistics and, thus, considered inadequate for the purpose of risk assessment. Findings from genotoxicity assays are limited and do not provide supporting evidence for a carcinogenic effect of elemental mercury.

Effects on the nervous system appear to be the most sensitive toxicological endpoint observed following exposure to elemental mercury. Symptoms associated with elemental mercury-induced neurotoxicity include the following: tremors, initially affecting the hands and sometimes spreading to other parts of the body; emotional lability, often referred to as "erethism" and characterized by irritability, excessive shyness, confidence loss, and nervousness; insomnia; neuromuscular changes (e.g., weakness, muscle atrophy, muscle twitching); headaches; polyneuropathy (e.g., paresthesia, stocking-glove sensory loss, hyperactive tendon reflexes, slowed sensory and motor nerve conduction velocities); and memory loss and performance deficits in test of cognitive function. At higher concentrations, adverse renal effects and pulmonary dysfunction may also be observed.

A few studies have provided suggestive evidence for potential reproductive toxicity associated with exposure to elemental mercury. Data from two studies in rats demonstrate developmental effects of elemental mercury exposure. These were behavioral changes associated with both *in utero* and perinatal exposure.

Inorganic Mercury

There is no evidence in humans linking exposure to mercuric chloride with carcinogenic effects. Data in animals are limited. Focal hyperplasia and squamous cell papillomas of the forestomach as well as thyroid follicular adenomas and carcinomas were observed in male rats gavaged with mercuric chloride. In the same study, evidence for an increased incidence of squamous cell forestomach papillomas in female rats and renal adenomas and carcinomas in male mice were considered equivocal.

All increased tumor incidences were observed in excess of the maximum tolerated dose (MTD). In this context, the relevance of the tumors to human health evaluation has been questioned. Results from *in vitro* and *in vivo* tests for genotoxicity have been mixed and do not provide strong supporting data for carcinogenicity.

There are some data indicating that mercuric chloride may be a germ cell mutagen. Positive results have been obtained for chromosomal aberrations in multiple systems, and evidence suggests that mercuric chloride can reach female gonadal tissue.

The most sensitive general systemic adverse effect observed following exposure to inorganic mercury is the formation of mercuric mercury-induced autoimmune glomerulonephritis. The production and deposition of IgG antibodies to the glomerular basement membrane can be considered the first step in the formation of this mercuric-mercury-induced autoimmune glomerulonephritis.

Several studies in animals have evaluated the potential for developmental toxicity to occur following exposure to various inorganic salts. While the evidence suggests that developmental effects may occur, all of the studies have significant limitations.

Methylmercury

Three human studies that examined the relationship between methylmercury and cancer incidence were considered extremely limited because of study design inappropriate for risk assessment or incomplete data reporting. Evidence from animal studies provides limited evidence of carcinogenicity. Male ICR and B6C3F1 mice exposed orally to methylmercuric chloride were observed to have an increased incidence of renal adenomas, adenocarcinomas, and carcinomas. Renal epithelial cell hyperplasia and tumors, however, were observed only in the presence of profound nephrotoxicity suggesting that the tumors may be a consequence of reparative changes to the damaged kidneys. Tumors were observed at a single site, in a single species and sex.

Methylmercury appears to be clastogenic but not a potent mutagen. Studies have also shown evidence that methylmercury may induce mammalian germ cell chromosome aberrations. There are a number of studies in both humans and experimental animals that show methylmercury to be a developmental toxicant. Neurotoxicity in offspring is the most commonly observed effect and the effect seen at lowest exposures.

A significant body of human studies exists for evaluating the potential systemic toxicity of methylmercury. This data base is the result of studying two large scale poisoning episodes in Japan and Iraq as well as several epidemiological studies assessing populations that consume significant quantities of fish. In addition, much research on the toxicity of methylmercury has been conducted in animals including non-human primates.

The critical target for methylmercury toxicity is the nervous system. The developing fetus may be at particular risk from methylmercury exposure. Offspring born of women exposed to methylmercury during pregnancy have exhibited a variety of developmental neurological abnormalities, including the following: delayed onset of walking, delayed onset of talking, cerebral palsy, altered muscle tone and deep tendon reflexes, and reduced neurological test scores. Maternal toxicity may or may not have been present during pregnancy for those offspring exhibiting adverse effects. For the general population, the critical effects observed following methylmercury exposure are multiple central nervous system effects including ataxia and paresthesia.

A latent or silent period has been observed in some epidemiological and animal studies indicating a delay in the onset of adverse effects. It is hypothesized this delay may be related to methylmercury metabolism.

Sensitive Subpopulations

A susceptible population is a group that may experience more severe adverse effects at comparable exposure levels or adverse effects at lower exposure levels than the general population. The greater response of these sensitive subpopulations may be a result of a variety of intrinsic or extrinsic factors. For mercury, the most sensitive subpopulations may be developing organisms. Data are also available indicating that other factors may be associated with the identification of sensitive subpopulations including the following: age; gender; dietary insufficiencies of zinc, glutathione, or antioxidants; predisposition for autoimmune glomerulonephritis; and predisposition for acrodynia. More information on sensitive subpopulations is presented in Chapter 4.

Interactions

There are data demonstrating that a number of substances affect the pharmacokinetics and/or toxicity of mercury compounds. Of most interest is the potential interaction of selenium and mercury. Selenium is known to bioaccumulate in fish, so exposure to methylmercury from fish consumption may be associated with exposure to increased levels of selenium. There are data indicating that selenium co-administered with methylmercury can form selenium-methylmercury complexes. The formation of these complexes may temporarily prevent methylmercury-induced tissue damage but also may delay excretion of the methylmercury. Thus, formation of selenium-methylmercury complexes may not reduce methylmercury toxicity but rather may delay onset of symptoms. More information is needed to understand the possible interaction of selenium with methylmercury.

There is potential for interaction between various forms of mercury and ethanol, thiol compounds, tellurium, potassium dichromate, zinc, atrazine, and vitamins C and E.

Hazard Identification/Dose-Response Assessment

The available toxicological and epidemiological evidence was evaluated, and U.S. EPA risk assessment guidelines and methodologies were applied to hazard identification for various endpoints; namely, carcinogenicity, germ cell mutagenicity, developmental toxicity, and general systemic toxicity. Data supported quantitative assessments of systemic toxicity. For elemental mercury, an inhalation reference concentration (RfC[1]) was calculated; oral reference doses (RfD[1]) were calculated for inorganic mercury and methylmercury. Data for carcinogenicity of inorganic and methylmercury were judged to be inadequate in humans and limited from animal bioassays. The carcinogenicity data for all forms of mercury evaluated were not sufficient to support a quantitative assessment. No quantitative estimates were done for developmental toxicity. Table ES-1 summarizes the hazard identification and dose-response information for elemental mercury, inorganic mercury, and organic mercury. The bases for these decisions and the methodologies applied are presented in Chapter 6.

[1] The oral RfD and the inhalation RfC are estimates (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subpopulations) that is likely to be without an appreciable risk of deleterious health effects during a lifetime.

Table ES-1
Summary of U.S. EPA Hazard Identification/Dose-response Assessment for Mercury and Mercury Compounds

Form of Mercury	Oral RfD (mg/kg-day)	Inhalation RfC (mg/m^3)	Cancer Weight-of-evidence Rating	Cancer Slope Factor	Germ Cell Mutagenicity	Developmental Toxicity Data Base Characterization
Elemental	n/a[a]	0.0003[b]	D, not classifiable as to human carcinogenicity	n/a	Low weight of evidence	Insufficient human evidence; sufficient animal evidence
Inorganic	0.0003[c] (mercuric chloride)	Not[d] verifiable	C, possible human carcinogen	n/a	Moderate weight of evidence	Insufficient evidence
Organic	0.0001[e] (methyl-mercury)	n/a	C, possible human carcinogen	n/a	High weight of evidence	Sufficient human and animal data

[a] Not available; data do not support development of a value at this time.
[b] Critical effect is neurological toxicity (hand tremor; increases in memory disturbances; slight subjective and objective evidence of autoimmune dysfunction) in adults.
[c] Critical effect is renal toxicity resulting from an autoimmune disease caused by the accumulation of a hapten-mercury complex in the glomerular region of the kidneys.
[d] Data were judged insufficient for calculation of RfC.
[e] Critical effect is neurological toxicity in progeny of exposed women, RfD calculated using a benchmark dose (10%).

Ongoing Research

While much data has been collected on the potential toxicity of mercury and mercury compounds, much is still unknown. Two ongoing epidemiological studies are now providing critical information on the developmental toxicity of methylmercury. One study, being conducted in the Seychelles Islands, is evaluating dose-response relationships in a human population with dietary exposures (fish) at levels believed to be in the range of the threshold for developmental toxicity. The second study, conducted in the Faroe Islands, is assessing mercury exposure in a population that consumes a relatively large quantity of marine fish and marine mammals. Children exposed to methylmercury *in utero* and followed through 6 years of age have been assessed for mercury exposure and neurological developmental. Published data from these studies are summarized in Chapter 3. Implications of ongoing research is discussed along with uncertainties in risk assessments in Chapter 6.

Research Needs

Specifically, information is needed to reduce the uncertainties associated with the current oral RfDs and inhalation RfCs. More work with respect to both dose and duration of exposure would also allow for potentially assessing effects above the RfD/RfC. Limited evidence suggests that methylmercury and mercuric chloride are possible human carcinogens. Data are not sufficient to classify the potential carcinogenicity of elemental mercury. Research on mode of action in induction of tumors at high mercury dose will be of particular use in defining the nature of the dose response relationship for carcinogenicity. At this time data have been judged insufficient for calculation of quantitative developmental toxicity estimates for elemental and inorganic mercury; research toward this end should be encouraged. While some pharmacokinetic models have been developed additional work to ensure the

applicability of these to risk assessment should be pursued. In particular work aimed at validation of a fetal pharmacokinetic model and research in support of toxicokinetics will be useful.

Conclusions

The following conclusions progress from those with greater certainty to those with lesser certainty.

- The three forms of mercury discussed in this Report can present a human health hazard.
- Neurotoxicity is the most sensitive indicator of adverse effects in humans exposed to elemental mercury and methylmercury.
- Immune-mediated kidney toxicity is the most sensitive indicator of toxic effects of exposure to inorganic mercury. This judgement is largely based on results in experimental animals.
- Methylmercury is a developmental toxicant in humans.
- Methylmercury is likely to be a human germ cell mutagen. This judgement is based on data from human studies, genetic toxicology studies in animals and a consideration of the pharmacokinetics of methylmercury.
- An RfD for ingested methylmercury based on neurotoxic effects observed in Iraqi children exposed *in utero* is 1×10^{-4} mg/kg-day. The threshold estimate derived using a benchmark dose approach is not model dependent (polynomial vs. Weibull). The estimate is not much affected by data grouping, but is dependent on response classification and on parameters used in determination of ingestion relative to measured mercury in hair.
- An RfC for inhaled elemental mercury based on neurotoxic effects in exposed workers is 3×10^{-4} mg/m^3.
- An RfD for ingested inorganic mercury based on immune-mediated kidney effects in Brown-Norway rats is 3×10^{-4} mg/kg-day.
- Elemental mercury is a developmental toxicant in experimental animals. If the mechanisms of action producing developmental toxicity in animals occur in humans, elemental mercury is very likely to produce developmental effects in exposed human populations. U.S. EPA has made no estimate of dose response for developmental effects of elemental mercury.
- Methylmercury and inorganic mercury produce tumors in experimental animals at toxic doses. If the mechanisms of action which induced tumors in the animal models could occur in humans, it is possible that tumors could be induced in exposed humans by these forms of mercury. It is likely, however, that cancer would be induced only after mercury exposures in excess of those producing other types of toxic response.

There are many uncertainties associated with this analysis, due to an incomplete understanding of the toxicity of mercury and mercury compounds. The sources of uncertainty include the following:

- The data serving as the basis for the methylmercury RfD were from a population ingesting contaminated seed grain. The nutritional status of this group may not be similar to that of U.S. populations. The exposure was for a short albeit critical period of time. It is likely that there is a range of response among individuals to methylmercury exposure. The selenium status of the exposed Iraqi population is not certain, nor is it established the extent to which selenium has an effect on mercury toxicity.

- There was no NOAEL (no-observable-adverse-effect level) for estimation of a threshold for all developmental endpoints. A benchmark was estimated using a Weibull model on grouped data. Use of an estimate other than the 95% lower limit on 10% response provides alternate estimates. Other modeling approaches using data which have not been grouped provide similar estimates. Benchmark doses, NOAELs, LOAELs, from other human studies provide support for the benchmark used in the RfD.

- Ingestion levels of methylmercury associated with measured mercury in hair were estimated based on pharmacokinetic parameters derived from evaluation of the extant literature. Use of other plausible values for these parameters results in (relatively small) changes in the exposure estimate.

- While there are data to show that the developing fetus is more susceptible to methylmercury toxicity than adults, there are not sufficient data to support calculation of a separate RfD for children (vs. adults).

- The RfD for inorganic mercury is based on data in experimental animals; there is uncertainty in extrapolation to humans. It is thought that these animals constitute a good surrogate for a sensitive human subpopulation. The data were from less than lifetime exposures; there is uncertainty in extrapolation to a lifetime RfD. There was no NOAEL in the studies; there is uncertainty in extrapolation to a NOAEL or in estimation of a threshold for effects in animals.

- The RfC for elemental mercury was based on studies in exposed workers for which there is no reported NOAEL; there is uncertainty in estimating the no effect level in these populations. There is uncertainty as to whether reproductive effects could be occurring at lower exposure levels than those which produced the observed neurotoxicity.

- There are insufficient data to determine whether elemental mercury induces carcinogenic effects in experimental animals.

- Data are not sufficient to judge if elemental and inorganic mercury are germ cell mutagens.

- U.S. EPA did not formally evaluate data on mercury for reproductive effects.

To improve the risk assessment for mercury and mercury compounds, U.S. EPA would need the following:

- Results from ongoing studies in human populations with measurable exposure to methylmercury.
- Results for immune-mediated kidney effects from lifetime studies of sensitive animals exposed to inorganic mercury. Definitive data from human studies on effects of exposure to inorganic mercury.
- Data on inhalation effects of inorganic mercury exposure.
- Dose response data for developmental effects of elemental and inorganic mercury.
- Reproductive studies and analysis for all forms of mercury.
- Data on mode of action of inorganic and methylmercury tumor induction.
- Validated physiologically-based pharmacokinetic models for mercury which include a fetal component.

Based on the extant data and knowledge of developing studies, the following outcomes can be expected:

- Human populations exposed to sufficiently high levels of elemental mercury will have increased incidence of neurotoxic effects.
- Human populations exposed to sufficiently high levels of methylmercury either *in utero* or *post partum* will have increased incidence of neurotoxic effects.
- Human populations exposed to sufficiently high levels of inorganic mercury will have increased incidence of systemic effects including immune-mediated kidney effects.
- The RfDs and RfC calculated by U.S. EPA for systemic toxic effects of mercury are expected to be amounts of exposure that can be incurred on a daily basis for a lifetime without anticipation of adverse effects. This expectation is for populations including susceptible subpopulations.
- The RfDs are protective against carcinogenic effects; tumor induction in animals was observed only at doses likely to produce systemic toxic effects.

1. INTRODUCTION

Section 112(n)(1)(B) of the Clean Air Act (CAA), as amended in 1990, requires the U.S. Environmental Protection Agency (U.S. EPA) to submit a study on atmospheric mercury emissions to Congress. The sources of emissions that must be studied include electric utility steam generating units, municipal waste combustion units and other sources, including area sources. Congress directed that the Mercury Study evaluate many aspects of mercury emissions, including the rate and mass of emissions, their health and environmental effects, technologies to control such emissions and the costs of such controls.

In response to this mandate, U.S. EPA has prepared an eight-volume Mercury Study Report to Congress. The eight volumes are as follows:

I. Executive Summary
II. An Inventory of Anthropogenic Mercury Emissions in the United States
III. Fate and Transport of Mercury
IV. An Assessment of Exposure to Mercury in the United States
V. Health Effects of Mercury and Mercury Compounds
VI. An Ecological Assessment for Anthropogenic Mercury Emissions in the United States
VII. Characterization of Human Health and Wildlife Risks from Mercury Exposure in the United States
VIII. An Evaluation of Mercury Control Technologies and Costs

This volume (Volume V) addresses the potential human health effects associated with exposure to mercury. It summarizes the available human and animal studies and other supporting information relevant to the toxicity of mercury and mercury compounds in humans. It also summarizes U.S. EPA's current overall assessments of hazard and quantitative dose-response for various categories of toxic effects. This volume presents data relevant to assessment of potential effects on human health for elemental mercury, inorganic mercury and methylmercury. Organic mercury compounds other than methylmercury are generally not considered in this volume. Chapter 2 discusses the toxicokinetics of mercury, including information on absorption, distribution, metabolism and excretion. Chapter 3 is a summary of the toxicity literature for mercury. It is organized into three main subsections, corresponding to elemental mercury, inorganic mercury and methylmercury. Within each of these subsections, the study data are presented according to the effect type (e.g., death, renal toxicity, developmental toxicity, cancer). For each effect type, separate summary tables in similar formats are used to present the available data from human epidemiological studies, human case studies, and animal studies.

Chapter 6, Hazard Identification and Dose-Response Assessment, presents U.S. EPA's assessments of the hazard presented by various forms of mercury and, where possible, the quantitative dose-response information that is used in risk assessments of mercury. Chapters 4 and 5 briefly discuss populations with increased susceptibility to mercury and interactions between exposure to mercury and other substances. Ongoing research and research needs are described in Chapter 7, and Chapter 8 lists the references cited. Appendix A documents the dose conversion equations and factors used. Appendix B consists of RfD, RfC and cancer risk summaries for U.S. EPA's Integrated Risk Information System (IRIS). Appendix C lists the participants of a U.S. EPA-sponsored workshop on mercury issues held in 1987. Appendix D presents an analysis of uncertainty and variability in the methylmercury human effects threshold estimate.

2. TOXICOKINETICS

This chapter describes the toxicokinetics (i.e., absorption, distribution, metabolism and excretion) of mercury and mercury compounds in the body. Biomarkers of exposure and methods of analysis for measuring mercury levels in biological samples are discussed. The biotransformation of mercury in the environment is discussed in Volume III.

The absorption of elemental mercury vapor occurs rapidly through the lungs, but it is poorly absorbed from the gastrointestinal tract. Oral absorption of inorganic mercury involves absorption through the gastrointestinal tract; absorption information for the inhalation route is limited. Methylmercury is rapidly and extensively absorbed through the gastrointestinal tract.

Once absorbed, elemental mercury is readily distributed throughout the body; it crosses both placental and blood-brain barriers. Elemental mercury is oxidized to inorganic divalent mercury by the hydrogen peroxidase-catalase pathway, which is present in most tissues. The oxidation of elemental mercury to the inorganic mercuric cation in the brain can result in retention in the brain. Inorganic mercury has poor lipophilicity and a reduced capacity for penetrating the blood-brain or placental barriers. Once elemental mercury crosses the placental or blood-brain barriers and is oxidized to the mercuric ion, return to the general circulation is impeded, and mercury can retained in brain tissue. Recent studies indicate that transport and distribution of methylmercury is carrier-mediated. Methylmercury penetrates the blood-brain and placental barriers, can be converted to mercuric ion, and may accumulate in the brain and fetus.

The elimination of elemental mercury occurs via the urine, feces and expired air. Exposure to mercuric mercury results in the elimination of mercury in the urine and feces. Methylmercury is excreted primarily in the feces (mostly in the inorganic form) by humans.

2.1 Absorption

2.1.1 Elemental Mercury

2.1.1.1 Inhalation

Elemental mercury vapors are readily absorbed through the lungs. Studies in human volunteers have shown that approximately 75–85% of an inhaled dose of elemental mercury vapor was absorbed by the body (Nielsen-Kudsk 1965; Oikawa et al. 1982; Teisinger and Fiserova-Bergerova 1965; Hursh 1985; Hursh et al. 1985). The high lipid solubility of elemental mercury vapor relative to its vapor pressure favors its rapid diffusion across alveolar membranes and dissolution in blood lipids (Berlin et al. 1969b).

2.1.1.2 Oral

Liquid metallic mercury is very poorly absorbed from the gastrointestinal tract. In rats, less than 0.01% of an ingested dose of metallic mercury was absorbed (Bornmann et al. 1970). The release of mercury vapor from liquid elemental mercury in the gastrointestinal tract and the subsequent absorption of the released vapor is limited by reaction of the mercury with sulfur to form mercuric sulfide. The mercuric sulfide coats ingested metallic mercury, preventing release of elemental vapor (Berlin 1986).

2.1.1.3 Dermal

Elemental mercury vapor is absorbed through the skin of humans at an average rate of 0.024 ng Hg/cm^2 (skin) for every one mg/m^3 in the air (Hursh et al. 1989). This rate of dermal absorption is sufficient to account for less than 3% of the total amount absorbed during exposure to mercury vapor (greater than 97% of the absorption occurs through the lungs). Dermal absorption of liquid metallic mercury has been demonstrated in experimental animals (Schamberg et al. 1918); however, the extent of absorption was not quantified. Koizumi et al. (1994) measured mercury absorption through the skin of F344 rats exposed to solutions of industrially generated dust containing mercury. After 3 days mean blood concentration in dust-exposed rats was 15.5 μg/L compared to 3 μg/L for saline controls.

2.1.2 Inorganic Mercury

2.1.2.1 Inhalation

There is limited information suggesting that absorption occurs after inhalation of aerosols of mercuric chloride. Clarkson (1989) reported absorption to be 40% in dogs via inhalation. Inhalation exposure of rats to an aerosol of a 1% mercuric chloride solution for 1 hour/day, 4 days/week for 2 months resulted in retention of 5–6 μg HgCl/HR aerosol/100 g body weight or approximately 37-44 µg Hg/kg-day (Bernaudin et al. 1981). The authors prepared the aerosol "with reference to the maximum allowable air concentrations (0.10 mg Hg/m^3) for a man". Retention was defined at the end of exposure as total mercury in the rat carcass minus skin and hair. It is unknown to what extent the amount retained represented absorption through the lungs or absorption of material cleared from the respiratory tract by mucociliary activity and ultimately swallowed.

2.1.2.2 Oral

The absorption of mercuric mercury from the gastrointestinal tract has been estimated at approximately 7–15% in human volunteers following oral administration of radiolabeled inorganic mercury (Miettinen 1973; Rahola et al. 1973). Recent data from studies in mice, however, suggest that "true" absorption may be closer to 20% but appears lower due to intestinal pH, compound dissociation, age, diet, rapid biliary secretion and excretion in the feces (Kostial et al. 1978; Nielsen 1992). Because the excretion of absorbed mercury is rapid, mercury levels detected in the gastrointestinal tract most likely represent both unabsorbed and excreted mercury in the studies by Miettinen (1973) and Rahola et al. (1973). The absorption of mercuric chloride from the gastrointestinal tract is not believed to depend on any specific transport mechanism, reactive sulfhydryl groups, or oxidative metabolism (Foulkes and Bergman 1993). Rather, uptake appears to result from an electrostatic interaction with the brush border membrane and limited passive diffusion. Several factors have been identified that modulate absorption of mercuric mercury from the gastrointestinal tract. At high doses, the corrosive action of mercuric chloride may increase its uptake by breaking down membrane barriers between the ions and the blood. Increases in intestinal pH also increase absorption (Endo et al. 1990). Increased uptake also occurs in neonates (Kostial et al. 1978). The increased absorption in neonates is believed to be due in part to the milk diet of neonates (increased absorption was observed in adults given a milk diet) and in part to the increased pinocytotic activity in the gastrointestinal tract that occurs in the very young (Kostial et al. 1978). Diffusion through aqueous channels present in the immature brush border of neonates has also been suggested to account for the greater absorption in the very young (Foulkes and Bergman 1993).

Absorption of mercuric salts from the gastrointestinal tract varies with the particular salt involved. Absorption decreases with decreasing solubility (Endo et al. 1990). For example, the poorly

soluble salt mercuric sulfide is not absorbed from the gastrointestinal tract as well as the more soluble mercuric chloride salt (Sin et al. 1983).

Mercurous salts in the form of calomel (long in use as a therapeutic agent) are insoluble in water and are poorly absorbed from the gastrointestinal tract (Clarkson 1993a). Long term use of calomel, however, has resulted in toxicity in humans (Davis et al. 1974).

2.1.2.3 Dermal

Dermal absorption of mercuric chloride has been observed in treated guinea pigs (Skog and Wahlberg 1964). Approximately 2–3% of an applied dose was absorbed during a 5-hour period. Absorption was measured both by disappearance of the applied compound and by appearance in kidney, liver, urine and blood.

2.1.3 Methylmercury

2.1.3.1 Inhalation

Inhaled methylmercury vapors are absorbed through the lungs. Fang (1980) did not measure percent absorbed but showed a correlation between tissue mercury levels and both exposure level and duration in rats exposed to radioactively labelled methylmercury vapor.

2.1.3.2 Oral

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

Reports of the percentage of absorbed methylmercury distributed to the blood range from 1% to 10%. Following the ingestion of a single meal of methylmercury-contaminated fish, Kershaw et al. (1980) found that blood accounted for 5.9% of absorbed methylmercury, while Miettinen et al. (1971) found an initial value of 10%, decreasing to about 5% over the first 100 days. In a population that chronically ingested fish with high methylmercury levels, approximately 1% of the absorbed dose was distributed to the blood (Sherlock et al. 1982).

2.1.3.3 Dermal

Dermal absorption of the methylmercuric cation $(CH_3Hg)^+$ (as the dicyandiamide salt) has also been observed in treated guinea pigs (Skog and Wahlberg 1964). Approximately 3–5% of the applied dose was absorbed during a 5-hour period. Absorption was measured both by disappearance of the applied compound and by appearance in kidney, liver, urine and blood.

2.2 Distribution

2.2.1 Elemental Mercury

Because of its lipophilicity, absorbed elemental mercury vapor readily distributes throughout the body, crossing the blood-brain barrier in humans (Hursh et al., 1976; Nordberg and Serenius, 1969) and the placenta in rats and mice (Clarkson et al., 1972). The distribution of absorbed elemental mercury is limited primarily by the oxidation of elemental mercury to the mercuric ion and reduced ability of the mercuric ion to cross membrane barriers. The oxidation is sufficiently slow, however, to allow distribution to all tissues and organs. Once it is oxidized to the mercuric ion, it is indistinguishable from Hg^{2+} from inorganic sources (i.e., the highest levels of mercury accumulate in the kidneys) (Hursh et al. 1980; Rothstein and Hayes 1964). Based on an *in vitro* study by Hursh et al. (1988), oxidation of mercury in the blood is slow and, therefore, inhaled mercury reaches the brain primarily unoxidized (i.e., as dissolved vapor) and is available for rapid penetration into brain cells. Once in the brain, oxidation of elemental mercury to mercuric mercury in the brain enhances for the accumulation of mercury in these tissues (Hursh et al. 1988; Takahata et al. 1970). For example, ten years after termination of exposure, miners exposed to elemental mercury vapor had high concentrations of mercury ($\geq$120 ppm) in the brain (Takahata et al. 1970). A similar effect occurs when elemental mercury reaches the fetus and (after oxidation) accumulates in the tissues as inorganic mercury (Dencker et al. 1983).

In the blood, elemental mercury initially distributes predominantly to the red blood cells; at 20 minutes, 98% of the mercury in the blood is found in the red blood cells. Several hours following parenteral, oral or inhalation exposure, however, a stable ratio of red blood cell mercury to plasma mercury of approximately 1:1 is established (Gerstner and Huff, 1977; Clarkson, 1972; Cherian et al., 1978). The rise in plasma mercury levels was suggested to be due to binding to protein sulfhydryl groups by mercuric mercury formed when the elemental mercury was oxidized.

2.2.2 Inorganic Mercury

In contrast to elemental mercury vapor and methylmercury, mercuric mercury does not penetrate the blood-brain or placental barriers easily. Levels of mercury observed in the rat brain after injection of mercuric nitrate were 10-fold lower than after inhalation of an equivalent dose of elemental mercury vapor (Berlin et al. 1969a). Similarly, mercuric mercury shows only limited ability to penetrate to the fetus (Garrett et al. 1972). Mercuric mercury does, however, accumulate in the mouse placenta (Berg and Smith 1983; Mitani et al. 1978; Suzuki et al. 1984). In the blood, the mercuric ion is bound to sulfhydryl groups present in the plasma and erythrocytes. The ratio of human red blood cell mercuric mercury to plasma mercuric mercury is approximately 1:1 (0.53:1.20) (Hall et al. 1994). The half-life in blood for humans was reported to range from 19.7 to 65.6 days in a study of five subjects treated with i.v. mercuric nitrate (Hall et al. 1994). From the blood, mercuric mercury initially distributes to liver, but the highest levels are generally observed in the kidneys (Newton and Fry 1978). With time after exposure, accumulation in the kidneys may account for up to 90% of the total body burden (Rothstein and Hayes 1960). The mercury levels in the kidney are dose dependent, with increasing amounts occurring with higher administered dose levels (Cember, 1962). The highest concentration of mercuric mercury in the kidneys is found in the proximal tubules. Mercuric mercury induces metallothionein production in the kidneys (Piotrowski et al. 1974). The high metallothionein levels in the kidneys may contribute to the kidney's accumulation of mercuric mercury (Piotrowski et al. 1973).

In neonates, lower proportions of mercuric mercury distribute to the kidneys than in adult animals (Jugo 1976). This results in higher distribution to other tissues. The protective blood-brain

barrier is incomplete in fetal and neonatal animals, which may also contribute to the increased mercury levels in immature brain. For example, the higher levels in the neonatal brain of rats and guinea pigs are believed to be associated with the decrease in renal sequestration of the mercuric ion (Jugo 1976; Yoshida et al. 1989). The higher levels observed in the livers of rat neonates may be attributable to increased distribution to organs other than the kidney as well as to higher levels of neonatal hepatic metallothionein (Daston et al. 1986).

2.2.3 Methylmercury

Methylmercury is distributed throughout the body, easily penetrating the blood-brain and placental barriers in humans and animals (Clarkson 1972; Hansen 1988; Hansen et al. 1989; Nielsen and Andersen 1992; Soria et al. 1992; Suzuki et al. 1984). By contrast with elemental mercury, studies in rats indicate that methylmercury transport into tissues is mediated by the formation of a methylmercury-cysteine complex (Aschner and Aschner 1990; Tanaka et al. 1991, 1992; Kerper et al. 1992). The complex is structurally similar to methionine and is transported into cells via a widely distributed neutral amino acid carrier protein. Methylmercury associates with water-soluble molecules (e.g., proteins) or thiol-containing amino acids because of the high affinity of the methylmercuric cation $(CH_3Hg)^+$ for the sulfhydryl groups $(SH)^-$. Complexes of methylmercury with cysteine have been identified in blood, liver and bile of rats (Aschner and Aschner 1990).

Al-Shahristani and Shihab (1974) calculated a "biological half-life" of methylmercury in a study of 48 male and female subjects who had ingested seed grain contaminated by organic mercurials. The half-life ranged from 35 to 189 days with a mean of 72 days; it was determined from distribution of mercury along head hair.

The blood half-life is 49–164 days in humans (Aberg et al. 1969; Miettinen et al. 1971) and 10–15 days in monkeys (Rice et al. 1989). Smith et al. (1994) determined a blood half-life of 32–60 days in a study of seven adult males given i.v. methylmercury. In the blood, methylmercury is found predominantly in the red blood cells (Kershaw et al. 1980; Thomas et al. 1986). In humans, the ratio of red blood cell methylmercury to plasma methylmercury is approximately 20:1. This ratio varies in animal species; the ratio is approximately 20:1 in primates and guinea pigs, 7:1 in mice, greater than 100:1 in rats and 42:1 in cats (Hollins et al. 1975; Magos 1987).

The clinical significance of the differences in the distribution of various forms of mercury in the blood is that it permits diagnosis of the type of mercury to which an individual has been exposed. Short-chain alkyl mercury compounds such as methylmercury or ethyl mercury are very stable in the body, whereas long-chain compounds may be metabolized over time to the mercuric ion. The mercury distribution in the blood, therefore, may shift from a distribution characteristic of methylmercury to one more suggestive of inorganic mercury (Berlin 1986; Gerstner and Huff 1977).

Mercury has been found in the umbilical cord of human newborns at levels comparable to maternal blood levels (Grandjean et al. 1992a). For lactating mothers, the clearance of mercury from the blood appears to be faster than for non-lactating women. Lactating individuals have a blood half-life of 42 days compared to 75 days for non-lactating females among a group of people who had consumed contaminated seed grain (Greenwood et al. 1978). This finding may be due to excretion of mercury via the milk, increased food intake by mothers (which enhances biliary excretion) and/or altered hormonal patterns in lactating mothers (which affect the excretion pattern).

Methylmercury transport across the blood-brain barrier in rats may involve an amino acid carrier (Kerper et al. 1992). Following acute exposure to methylmercury, most of the mercury in the brain is in the organic form; however, with chronic exposures, a greater amount of the mercury in the brain is in the inorganic form, suggesting that the rate of demethylation increases with long-term exposure (Aschner and Aschner 1990). Rice (1989a, 1989b) demonstrated that tissue half-life in the brain may be significantly longer than the blood half-life for methylmercury.

The bioaccumulation of methylmercury can be affected by age and sex (Thomas et al. 1982, 1986, 1988). After administration of methylmercury to rats, the females had higher peak levels of mercury in the kidneys, primarily as methylmercury, compared to the males; inorganic mercury levels did not differ significantly between the sexes (Thomas et al. 1986). Accumulation of mercury in the body is also found to be higher in neonatal rats (Thomas et al. 1988) than in adult rats (Thomas et al. 1982). Ten days after administration of methylmercury, 94% of the dose was still detected in neonates while ≈60% was retained in adults (Thomas et al. 1988). The longer retention of mercury in the neonates may be attributed to various factors including the high amount of mercury accumulated in the pelt of the neonates due to lack of clearance (Thomas et al. 1988) and the lack of a fully developed biliary transport system in the neonates (Ballatori and Clarkson 1982).

2.3 Metabolism

2.3.1 Elemental Mercury

Elemental mercury dissolved in the blood is rapidly oxidized in red blood cells to mercuric mercury by catalase in the presence of hydrogen peroxide (Halbach and Clarkson 1978). Catalase is found in many tissues, and oxidation by this pathway probably occurs throughout the body (Nielsen-Kudsk 1973). The pathway is saturable, however, and hydrogen peroxide production is the rate-limiting step (Magos et al. 1989). Blood and tissue levels of mercuric mercury following exposure to high concentrations of elemental mercury are, therefore, lower than would be expected based on levels observed following exposure to low levels.

2.3.2 Inorganic Mercury

Several investigators have observed exhalation of elemental mercury vapor after oral administration of mercuric mercury to rats and mice, indicating that mercuric mercury in the body can be reduced to elemental mercury (Clarkson and Rothstein 1964; Dunn et al. 1981a, 1981b; Sugata and Clarkson 1979). The reduction of mercuric ion to elemental mercury may occur via cytochrome c, NADPH and NADH, or a superoxide anion produced by the xanthine-xanthine oxidase system (Ogata et al. 1987). There is no evidence that mercuric mercury is methylated to form methylmercury in mammalian cells. The studies of Rowland et al. on the intestinal flora of the Wistar rat show that microbes are responsible for at least a portion of mercuric chloride methylation in the gut.

Mercurous mercury is unstable in biological fluids and rapidly disassociates to one molecule of elemental mercury and one ion of mercuric mercury (Clarkson 1972).

2.3.3 Methylmercury

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

liver (Suzuki et al. 1984). *In vitro* demethylation has been reported to involve hydroxyl radicals produced by cytochrome P-450 reductase (Suda and Hirayama 1992) or hypochlorous acid scavengers (Suda and Takahashi 1992). Organic mercury compounds with longer alkyl chains are more readily metabolized over time to the mercuric ion (Berlin, 1986).

Methylmercury metabolism may be related to the latent or silent period observed in epidemiological studies from two methylmercury poisonings. During the latent period, both during and after the cessation of exposure, the patient feels no untoward effects. It is possible that a number of biochemical changes may take place in parallel during this period, and some may not be causatively related to the clinical outcome. Ganther (1978) has hypothesized that the carbon-mercury bond in methylmercury undergoes homolytic cleavage to release methyl free radicals. The free radicals are expected to initiate a chain of events involving peroxidation of lipid constituents of the neuronal cells. The onset of symptoms is delayed for the period of time that cellular systems are able to prevent or repair effects of lipid peroxidation. When the cellular defense mechanisms are overwhelmed, rapid and progressive degeneration of the tissue results. In the Iraqi poisoning incident, the latent period before toxic signs were noted varied from a matter of weeks to months. By contrast, in the Japanese poisoning incident, the latency was as long as a year or more. The difference in duration of the latent period may in part be due to the presence of selenium in the fish ingested by the Japanese population. The role of selenium in mercury toxicity is discussed further in Chapter 5.

2.4 Excretion

2.4.1 Elemental Mercury

Excretion of mercury after exposure to elemental mercury vapor may occur via exhaled air, urine, feces, sweat and saliva. The pattern of excretion of elemental mercury changes as elemental mercury is oxidized to mercuric mercury. During and immediately after an acute exposure, when dissolved elemental mercury is still present in the blood, glomerular filtration of dissolved mercury vapor occurs, and small amounts of mercury vapor can be found in the urine (Stopford et al. 1978). Mercury vapor present in the blood may also be exhaled; human volunteers exhaled approximately 7% of the retained dose within the first few days after exposure (Hursh et al. 1976). The half-life for excretion via the lungs is approximately 18 hours. Approximately 80% of the mercury accumulated in the body is eventually excreted as mercuric mercury. As the body burden of mercury is oxidized from elemental mercury to mercuric mercury, the pattern of excretion becomes more similar to mercuric mercury excretion. The majority of the excretion of mercuric mercury occurs in the feces and urine (Cherian et al. 1978). During the first few days after exposure of humans to mercury vapor, approximately four times more mercury was excreted in the feces than in the urine (Cherian et al. 1978). With time, as the relative mercury content of the kidneys increases, excretion by the urinary route also increases (Rothstein and Hayes 1964). Tissue levels of mercury decrease at different rates, but the half-life for excretion of whole-body mercury in humans (58 days) is estimated to be approximately equal to the half-life of elimination from the kidneys (64 days), where most of the body burden is located (Hursh et al. 1976). Excretion via the urine may be increased if mercury-induced damage of the renal tubular epithelium has happened and exfoliation of damaged mercury-containing cells occurs (Magos 1973).

Excretion via sweat and saliva are thought to contribute only minimally to total excretion under normal circumstances. In workers who have perspired profusely, however, the total amount of mercury excreted in the sweat during 90 minutes ranged from 50% to 200% of that found in a 16-hour composite sample of urine (Lovejoy et al. 1974).

2.4.2 Inorganic Mercury

Because of the poor absorption of orally administered mercuric mercury, the majority (≈85%) of an ingested dose in humans is excreted in the feces within a few days after administration (Miettinen 1973). Hall et al. (1994) showed that for five adult male volunteers given i.v. mercuric nitrate and evaluated for 70 days, 6.3-35% of the dose was excreted in urine and 17.9-38.1% in feces. For absorbed inorganic mercury, the half-life for excretion has been estimated to be ≈40 days (Rahola et al. 1973) and 67 days with a range of 49-96 days (Hall et al. 1994). Information on the routes of excretion for absorbed inorganic mercury are limited, but excretion would be expected to be similar to that of inorganic mercury formed in rats by the oxidation of elemental mercury (Rothstein and Hayes 1964). The majority of absorbed inorganic mercury is excreted in the urine (Berlin 1986).

Glomerular filtration is not thought to contribute substantially to urinary excretion of mercuric mercury (Cherian et al. 1978). Rather, mercuric mercury is excreted in the urine primarily as sulfhydryl conjugates (with cysteine or N-acetylcysteine) actively transported into the tubular lumen. Urinary levels correlate with renal mercury concentrations rather than blood mercury levels.

Fecal excretion of mercury occurs as the result of excretion in the saliva, secretion through the epithelium of the small intestines and colon and secretion in the bile (Berlin 1986). Secretion of mercuric mercury in the bile is believed to result from active transport of a mercury-glutathione complex across the canalicular membrane via the glutathione carrier (Ballatori and Clarkson 1982).

Mercuric mercury may also be excreted in breast milk during lactation (Yoshida et al. 1992). The levels in breast milk are proportional to the plasma content. In maternal guinea pigs, milk levels were approximately half of that found in plasma. After termination of exposure, however, mercury levels in milk decreased at a slower rate than plasma mercury levels.

2.4.3 Methylmercury

Like inorganic mercury, methylmercury has a relatively long half-life of approximately 70–80 days in the human body (Aberg et al. 1969; Bernard and Purdue 1984; Miettinen 1973). Recently a shorter half-life of 44 days was estimated by Smith et al. (1994) in their study of seven adult males treated i.v. with methylmercury. In this study methylmercury and inorganic mercury concentrations in blood and excreta were determined separately based on differential extractability into benzene. The predominant species in the blood was methylmercury; there was no detectable methylmercury in the urine.

The long half-life of methylmercury in the body is due, in part, to reabsorption of methylmercury secreted into the bile (hepato-biliary cycling) (Norseth and Clarkson, 1971). In this cycle, methylmercury forms a complex with glutathione in the hepatocyte, and the complex is secreted into the bile via a glutathione carrier protein (Clarkson, 1993b). The methylmercury-glutathione complex in the bile may be reabsorbed from the gallbladder and intestines into the blood. When microorganisms found in the intestines demethylate methylmercury to form mercuric mercury, this cycle is broken, and fecal excretion of mercury from methylmercury occurs (Rowland et al. 1980). Mercuric mercury is poorly absorbed from the intestines, and that which is not reabsorbed is excreted in the feces. In humans, approximately 90% of the absorbed dose of methylmercury is excreted in the feces as mercuric mercury. Excretion via the urine is minor but slowly increases with time; at 100 days after dosing, urinary excretion of mercury accounted for 20% of the daily amount excreted. The urinary excretion of mercury may reflect the deposition of demethylated mercury in the kidneys and its subsequent excretion.

In animals, the predominant route of methylmercury elimination also is the feces (Farris et al. 1993; Hollins et al. 1975; Thomas et al. 1987). As in humans, biliary excretion of methylmercury and its demethylation in gastrointestinal flora have been reported in rats (Farris et al., 1993). After a single oral dose of methylmercury, the major elimination route was the feces (65% of the administered dose as inorganic mercury and 15% of the administered dose as methylmercury) and the minor route was urine (1% of the administered dose as inorganic mercury and 4% of the administered dose as methylmercury) (Farris et al. 1993).

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

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

2.5 Biological Monitoring

This section describes the various biological media most frequently used when assessing mercury exposure. In addition, this section describes the available analytical methods for measuring mercury in biological samples. Reference values for mercury in standard biological media from the general population are shown in Table 2-1. These values represent total mercury, not individual mercury species. For hair and blood, these have been indexed to fish consumption as the most common route of exposure in humans.

2.5.1 Elemental Mercury

Blood and urinary mercury are common to assess occupational mercury exposure.

2.5.1.1 Blood

In workers chronically exposed to mercury vapor, a good correlation was observed between intensity of mercury vapor exposure and levels of mercury in the blood at the end of a workshift (Roels et al. 1987). The usefulness of blood as a biomarker for exposure to elemental mercury depends on the time elapsed since exposure and the level of exposure. For recent, high-level exposures, whole blood analysis may be used to assess exposure (Clarkson et al. 1988). Mercury in the blood peaks rapidly, however, and decreases with an initial half-life of approximately two to four days (Cherian et al. 1978). Thus, evaluation of blood mercury is of limited value if a substantial amount of time has elapsed since exposure. Also, dietary methylmercury contributes to the amount of mercury measured in blood. At low

levels of elemental mercury exposure, the contribution of dietary methylmercury to the total blood mercury may be high relative to that of the inhaled mercury, limiting the sensitivity of this biomarker. Several studies have

Table 2-1
Reference Values for Total Mercury Concentrations in Biological Media for the General Population

Medium	Mercury Concentration	Reference
Whole blood	1–8 µg/L	WHO (1990)
	2 µg/L	Nordberg et al. (1992)
Fish consumption:		Brune (1991)
No fish meals	2.0 µg/L	
2 meals/week	4.8 µg/L	
2-4 meals/week	8.4 µg/L	
more than 4 meals/week	44.4 µg/L	
Urine	4–5 µg/L	WHO (1990)
Scalp hair	2 µg/g	WHO (1990)
Fish consumption:		Airey (1983)
once/mo	1.4 µg/g	
once/2 wk	1.9 µg/g	
once/wk	2.5 µg/g	
once/day	11.6 µg/g	

separated whole blood into its plasma and erythrocyte fractions in order to evaluate potential confounding factors due to the presence of methylmercury (95% of methylmercury is found in the red blood cell). Some published values indexed to fish consumption are in Table 2-1.

2.5.1.2 Urine

Urinary mercury is thought to indicate most closely the mercury levels present in the kidneys (Clarkson et al. 1988). For most occupational exposures, urinary mercury has been used to estimate exposure. In contrast to blood mercury levels, urinary mercury peaks approximately 2–3 weeks after exposure and decreases at a much slower rate with a half-life of 40–60 days for short-term exposures and 90 days for long-term exposures (Barregard et al. 1992; Roels et al. 1991). The urine remains, therefore, a more appropriate indicator for longer exposures than blood samples. As little dietary methylmercury is excreted in the urine, the contribution of ingested methylmercury to the measured levels is not expected to be high. Good correlations have been observed between urinary mercury levels and air levels of mercury vapor; however, these correlations were obtained after correcting urinary mercury content for variations in the urinary excretion rate (using urinary creatinine content or specific gravity) and after standardizing the amount of time elapsed after exposure (Roels et al. 1987). Such steps are necessary because considerable intra- and interindividual variability has been observed in the urinary excretion rate (Barber and Wallis 1986; Piotrowski et al. 1975). Even when such precautions are taken, intraindividual variability remains at ≈18% (Barregard et al. 1992; Roels et al. 1987).

2.5.1.3 Exhaled Air

Exhaled air has been suggested as a possible biomarker of exposure to elemental mercury vapor because a portion of absorbed mercury vapor is excreted via the lungs. Excretion by this route has a half-

life of approximately 18 hours (Hursh et al. 1976). At low levels of exposure, however, mercury vapor released from dental amalgam may contribute substantially to the measured amount of mercury.

2.5.2 Inorganic Mercury

No information was identified in the literature that specifically assessed biological indicators for inorganic mercury exposure. The information presented above for detection of mercury in blood and urine after occupational exposure to elemental mercury vapor should also apply to inorganic mercury exposures because elemental mercury vapor is rapidly converted to mercuric mercury after absorption.

2.5.3 Methylmercury

Blood and scalp hair are the primary indicators used to assess methylmercury exposure.

2.5.3.1 Blood

Because methylmercury freely distributes throughout the body, blood is a good indicator medium for estimating methylmercury exposure. Because an individual's intake may fluctuate, blood levels may not necessarily reflect mercury intake over time (Sherlock et al. 1982; Sherlock and Quinn, 1988). At steady state, blood levels have been related to dose by the following equation (Kershaw et al. 1980):

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

Where:

C = concentration in blood (expressed in μg/L)
V = volume of blood (expressed as L)
b = the kinetic rate constant (day^{-1})
A = absorption rate (unitless)
F = fraction of dose that is present in blood
d = intake (μg/day)

It is useful to measure blood hematocrit and mercury concentrations in both whole blood and plasma. From these data, the red blood cell to plasma mercury ratio may be determined, and interference from exposure to high levels of elemental or inorganic mercury may be estimated (Clarkson et al. 1988).

2.5.3.2 Scalp Hair

Scalp hair can also be a good indicator medium for estimating methylmercury exposure (Phelps et al. 1980). Methylmercury is incorporated into scalp hair at the hair follicle in proportion to its content in blood. The hair-to-blood ratio in humans has been estimated as approximately 250:1 expressed as μg Hg/g hair to mg Hg/L blood, but some difficulties in measurements, interindividual variation in body burden, differences in hair growth rates, and variations in fresh and saltwater fish intake have led to varying estimates (Birke et al. 1972; Skerfving 1974). Once incorporated into the hair, the methylmercury is stable, and, therefore, gives a longitudinal history of blood methylmercury levels

(Phelps et al. 1980; WHO, 1990). Analysis of hair mercury levels may be confounded by adsorption of mercury vapor onto the hair strands (Francis et al. 1982).

2.5.4 Methods of Analysis for Measuring Mercury in Biological Samples

The most common methods used to determine mercury levels in blood, urine and hair of humans and animals include atomic absorption spectrometry (AAS), neutron activation analysis (NAA), X-ray fluorescence (XRF) and gas chromatography (GC). Table 2-2 identifies the major characteristics of these methods.

Table 2-2
Analytical Methods for the Detection of Mercury in Biological Samples

Method	Able to Distinguish Methylmercury?	Detection Limit (ppm)	References
NAA	No	0.1	Byrne and Kosta (1974) WHO (1976)
AAS	No No[a]	2 PPB range	Hatch and Ott (1968) Magos and Clarkson (1972)
GC — Electron capture	Yes	1.0	Von Burg et al. (1974) Cappon and Smith (1978)
XRF	No	"low ppm"	Marsh et al. (1987)

[a] The Magos and Clarkson method estimates methylmercury by subtracting the inorganic mercury content from the total mercury content.

2.6 Studies on Pharmacokinetic Models

2.6.1 Introduction

Pharmacokinetic modeling is a process by which administered dose, such as the amount of a compound instilled into the body via inhalation, ingestion or parenteral route is used to estimate measures of tissue dose which may not always be accessible to measurement by direct experimentation. A pharmacokinetic model is employed to predict relevant measures of tissue dose under a wide range of exposure conditions. In practice, the pharmacokinetic models used may incorporate features such as compartmental analysis and physiologically-based models.

Reports available on the *in vivo* distribution of several types of mercury compounds provide different physiokinetic relationships between the structure of mercury compounds and their behavior in living organisms because the studies reported have been carried out under different experimental conditions. Takeda et al. (1968) reported that in the rat, alkyl mercury compounds such as ethylmercuric chloride and butylmercuric chloride were excreted more slowly and were retained in higher concentration for a longer time in the body than mercuric chloride and phenylmercuric chloride. The distribution of mercury in the brain was found to depend on the structure of the mercury compounds; relatively high accumulation was observed for ethyl and n-butyl mercury compounds. Sebe and Itsuno (1962) reported that after oral administration methyl-, ethyl-, and n-propylmercury compounds were neurotoxic to rats;

n-butylmercury was not neurotoxic and thus presumably did not cross the blood-brain barrier. By contrast, Suzuki et al. (1963, 1964) reported that ethylmercuric acetate and n-butylmercuric acetate had similar patterns of distribution when subcutaneously administered to mice.

2.6.2 Inorganic mercury

Few controlled laboratory studies of pharmacokinetics of mercury in humans have been published (Hursh et al. 1976, Rahola et al. 1973). Rahola et al. (1973) examined mercury absorption and elimination after oral administration of mercuric nitrate to five male and five female volunteers, and reported very low and variable rate of gastrointestinal absorption (8 to 25% dose). They reported a half-time for inorganic mercury in human red blood cells of 16 days and whole body of 46 (32–60) days in males and somewhat lower values in females. Hursh et al. (1976) found half-times for mercury clearance from the body of 58 (35–90) days after exposure to mercury vapor. Whole body clearance from the Rahola et al. (1973) study appeared biphasic with half-times of 2.3 days for the fast compartment and 42 (39–45) days for the slow compartment.

Low and variable rates of absorption of orally administered inorganic mercury in the Rahola et al. (1973) study prompted Hall et al. (1994) to examine distribution of intravenously administered inorganic mercury in human volunteers. In order to describe retention of mercury after transient distributional effects, a one-compartment model was fit to the blood and body burden data after day 10, assuming first order kinetics. The half-lives observed in the single compartment model for blood and body burden were 30 (19.7–65.6 days) and 67 (48.6–95.5 days) days, respectively. The authors attempted closer agreement between observed and predicted values by structuring a multicompartment model. Measured mercury concentrations in blood, urine, feces, and whole body radioactive levels of mercury were used in an *a posteriori* fashion to develop a model comprising six blood compartments, one compartment each for feces and urine and a delayed compartment for feces. Inter-subject variability (temporal pattern of blood mercury) and the existence of a kinetically distinct plasma pool (three distinct compartments) for mercury resulted in equivocal predictions for blood, urine and feces; whether these findings point to uncertainties of measurement of body burden or incomplete collection of excreta or suggest other pathways of excretion, such as exhalation or sweating, is unknown. The authors concluded that this type of complex pattern of blood kinetics, although unusual, is not without precedent. Four kinetically distinct plasma pools of selenium has been reported after oral dosing with a stable isotopic tracer (Patterson and Zech 1992). Hall et al. (1994) noted that the apparently linear kinetics observed for the small tracer doses of i.v. inorganic mercury would likely change with toxicity associated with larger or more frequent doses.

2.6.3 Methylmercury

Methylmercury is structurally the simplest of the organic mercurials; it bioaccumulates in certain species of fish, some of which are important human and wildlife foods. In order to elucidate the mechanisms that influence the pharmacokinetics of both methylmercury and mercuric mercury and to extrapolate further both intra- and inter-species extrapolation of experimental data for these toxins, Farris and associates (1993) developed a physiological pharmacokinetic model for methylmercury and its metabolite, mercuric mercury. This was done in growing rats dosed orally with labeled methylmercury over a period of 98 days. Mercuric mercury accounted for less than 0.5% of total activity. Extensive sets of metabolism and distribution data were collected to understand the processes that influence the pharmacokinetics of both methylmercury and mercuric mercury. The model consisted of nine lumped compartments, each of which represented a major site of mercury accumulation, distribution or elimination. The carcass served as a residual compartment, which included all tissues and organs not

separately incorporated into the model. Model simulations in this study were made with experimentally determined concentrations of both inorganic and methylmercury in blood, brain, kidney and liver. The data showed bidirectional and symmetric transport of both chemical species between blood and tissues with relatively slow movement into and out of the brain. Some key parameters remained uncertain; for example, the rate constant for demethylation is one of the most critical in adopting the model to other species. This model, however, established a foundation for more complete understanding of methylmercury pharmacokinetics. With further refinements, it could be applied to other species including humans. To characterize health hazard from dietary methylmercury better, one needs to understand the distribution of methylmercury in the body, the extent to which it accumulates and the rate at which it is eliminated. Farris et al. (1993) noted that following methylmercury dosing there was a buildup of inorganic mercury in tissues and that excreted mercury was predominantly mercuric; methylmercury behaved as a single body pool, while mercuric mercury was handled differently in different tissues.

Smith and associates (1994) made further refinements to the Farris et al. (1993) model. They reported a multicompartment pharmacokinetic model for methylmercury and mercuric mercury in seven human volunteers. This model simulated the long-term disposition of methylmercury and inorganic mercury in humans following a single i.v. dose of radio-labeled methylmercury. This was a tracer amount to avoid toxic or saturation effects. The behavior of both methylmercury and inorganic mercury in the body was modeled with the simplest compartmental model which fitted the data; blood, urine and feces data were used to fit the model. In this model the tracer dose was delivered to the first blood compartment and subsequently distributed to two extra-vascular methylmercury compartments; two distinct compartments (urine and feces) for inorganic mercury were added features. This five-compartment model showed that inorganic mercury accumulated in the body and at longer times was the predominant form of mercury present. The biological half-life of methylmercury in the body was calculated to be 44 days, and 1.6% of the body burden was lost each day by both metabolism and excretion.

To characterize neurological impairments of prenatal methylmercury exposure in children, Gearhart and associates (1995) applied a more sophisticated multispecies pharmacokinetic model and statistical dose-response analysis to an epidemiological study of a large population in New Zealand (Kjellstrom et al. 1989) which featured relatively constant chronic exposure to methylmercury in fish. The model for methylmercury in this study consisted of an adult with 11 compartments representing both organ-specific and lumped tissues; eight compartments represented transport of methylmercury as flow-limited, and three other compartments represented transport as diffusion-limited. The flow-limited compartments were plasma, kidney, richly perfused, slowly perfused, brain-blood, placenta, liver and gut compartments; RBC, brain and fetus were the diffusion-limited compartments. There were also four other compartments in the model which were involved in methylmercury uptake and elimination: methylmercury in the urine; and methylmercury and inorganic mercury in the hair, feces and the intestinal lumen. The fetal sub-model for methylmercury consisted of four compartments: fetal plasma, RBCs, brain and the remaining fetal body. This modeling effort was designed to create a multispecies model that would be amenable to simulation of the kinetics of methylmercury by simply changing the species-specific parameters. Unlike Farris et al. (1993), separate red blood cell and plasma compartments were used to predict changes in kinetics of methylmercury across species due to differences in the red blood cell/plasma ratio. Different pharmacokinetic parameters, such as tissue/blood partition coefficients and volume distributions for humans, rats and monkeys, were taken from different studies published in the current literature. The authors provided a benchmark dose on results of a battery of neurobehavioral tests in 6-year-old children prenatally exposed to methylmercury in seafood. Their calculations suggested a NOAEL of 17 ppm Hg in maternal hair for the most sensitive

neurological event in children. The analysis of the pharmacokinetic model indicated that the fetal brain concentrations of methylmercury at this NOAEL were on the order of 50 ppb and were associated with maternal dietary intakes of methylmercury ranging from 0.8 to 2.5 μg/kg-day. These analyses provided support to the Iraqi data used in the development of the RfD for methylmercury, presented in the risk assessment chapter (Chapter 6) of this volume.

2.6.4 Discussion

Both simple and complex multi-compartment models have been reported by Hall et al. (1994), Farris et al. (1993), Smith et al. (1994) and Gearhart et al. (1995). The Hall et al. (1994) paper discussed a model which employed inorganic mercury data obtained from human studies; however, temporal patterns of blood mercury and the existence of kinetically distinct plasma pools for mercury present uncertainties which limit the use of this model in risk assessment. Farris et al. (1993) reported a multicompartment model using data obtained from rats exposed to methylmercury in diets over a period of 98 days. They observed a buildup of inorganic mercury in tissues and the conversion of methylmercury to inorganic mercury could not be accurately predicted by whole-body counting, which was also subjected to errors from low sensitivity and the inability to compensate for geometric changes due to redistribution of methylmercury or translocation of inorganic mercury to its target tissues. Smith and associates (1994) refined this model and presented a multicompartment model using data obtained from humans given a single i.v. dose of methylmercury. Uncertainties, however, persist in prediction of methylmercury exposures in food. Since methylmercury causes subtle neurotoxicity in children, this model may not be predictive of exposure in children. This potential neurotoxicity observed in prenatally exposed children prompted Gearhart et al. (1995) to develop multicompartment adult and fetal model using data from rat, monkey and humans. This model was applied to an epidemiology study on which benchmark dose analysis was used to better characterize the dose-response information rather than the traditional NOAEL approach. In the risk assessment chapter of this volume, U.S. EPA utilizes a benchmark dose approach for setting the RfD for methylmercury. A multispecies compartment model discussed in the Gearhart et al. (1995) report may provide a viable approach because it can use data from both adults and neonates. This approach can use adult and neonatal effects data from several animal and human studies to account for evidences of non-linearities in dose-responses. Research is needed to reduce uncertainties in racial, ethnic, and cultural differences which exist in epidemiological studies.

3. BIOLOGICAL EFFECTS

This chapter summarizes the available toxicity data on mercury compounds; the information is tabulated specifically for each form of mercury (i.e., elemental, inorganic and methylmercury) and each toxicity endpoint. Case studies in humans are distinguished from epidemiological studies and animal studies. In addition, critical studies for a given endpoint are briefly summarized in the narrative preceding the corresponding table.

The tables provide information on study design, observed effects, study limitations and any reported biological monitoring levels (BMLs) of mercury. To the extent possible, BML values have been reported in consistent units throughout this chapter (µg/L in body fluids, µg/g in tissue, µg/g creatinine in urine). It was not possible, however, to use completely consistent units because investigators measured mercury in different media (e.g., blood, urine, or tissue) or used different time frames (e.g., µg/L urine, µg/24 hour urine). In addition, some investigators normalized urine concentrations to the amount of creatinine, while most did not. An explanation is provided in Appendix A for any dose conversions required during review and evaluation of the toxicity and carcinogenicity studies reported in the discussions presented below.

3.1 Elemental Mercury

3.1.1 Critical Noncancer Data

This section describes studies evaluated by U.S. EPA for use in assessing general systemic health risks, primarily toxicity in exposed workers. Chapter 6 describes the derivation of an inhalation Reference Concentration (RfC) for elemental mercury based on neurotoxicity observed in several human occupational studies. For completeness, some of these studies are also presented in tabular form in succeeding sections.

Fawer et al. (1983) used a sensitive measure of intention tremor (tremors that occur at the initiation of voluntary movements) in workers occupationally exposed for an average of 26 years to metallic mercury vapor. A statistically significant difference was seen in the frequency of these tremors in mercury-exposed workers compared with unexposed workers. The concentration of metallic mercury in the air was measured, and a time-weighted-average (TWA) of 0.026 mg/m^3 over an average of 15.3 years was derived. This was based on the assumption that the workers were exposed to the same concentration of mercury for the duration of their employment. It should be noted that very little detail was presented with regard to the measurement of the exposure levels, and that it is likely that there were variations in the mercury air levels during the period of exposure. Furthermore, the tremors may have resulted from intermittent exposure to concentrations higher than the TWA.

Piikivi and Tolonen (1989) studied the effects of long-term exposure to mercury vapor on the electroencephalograms (EEG) of 41 chloralkali workers exposed for a mean of 15.6 ± 8.9 years by comparison to matched referent controls. They found that the exposed workers, who had mean blood mercury levels of 12 μg/L and mean urine mercury levels of 20 μg/L, tended to have an increased number of EEG abnormalities when analyzed by visual inspection only. When the EEGs were analyzed by computer, the exposed workers had significantly slower and attenuated EEGs as compared to the referents. These changes were observed in 15% of the exposed workers. The frequency of these changes correlated with cortical mercury content (measured in other studies); the changes were most prominent in the occipital cortex, less prominent in the parietal cortex and almost absent in the frontal cortex. The

authors extrapolated an exposure level associated with these EEG changes of 0.025 mg/m^3 from blood levels based on a conversion factor calculated by Roels et al. (1987).

Piikivi and Hanninen (1989) studied the subjective symptoms and psychological performances on a computer-administered test battery in 60 chloralkali workers exposed to mercury vapor for a mean of 13.7 ± 5.5 years as compared to matched referent controls. The exposed workers had mean blood mercury levels of 10 μg/L and mean urine mercury levels of 17 μg/L. A statistically significant increase in subjective measures of memory disturbance and sleep disorders was found in the exposed workers. The exposed workers also reported more anger, fatigue and confusion. No objective disturbances in perceptual motor, memory or learning abilities were found in the exposed workers. The authors extrapolated an exposure level associated with these subjective measures of memory disturbance of 0.025 mg/m^3 from blood levels based on a conversion factor calculated by Roels et al. (1987).

Both subjective and objective symptoms of autonomic dysfunction were investigated in 41 chloralkali workers exposed to mercury vapor for a mean of 15.6 ± 8.9 years as compared to matched referent controls (Piikivi 1989). The quantitative non-invasive test battery consisted of measurements of pulse rate variation in normal and deep breathing in the Valsalva maneuver and in vertical tilt, as well as blood pressure responses during standing and isometric work. The exposed workers had mean blood levels of 11.6 μg/L and mean urinary levels of 19.3 μg/L. The exposed workers complained of more subjective symptoms of autonomic dysfunction than the controls, but the only statistically significant difference was an increased reporting of palpitations in the exposed workers. The quantitative tests revealed a slight decrease in pulse rate variations, indicative of autonomic reflex dysfunction, in the exposed workers. The authors extrapolated an exposure level associated with these subjective and objective measures of autonomic dysfunction of 0.03 mg/m^3 from blood levels based on the conversion factor calculated by Roels et al. (1987).

Sensory and motor nerve conduction velocities were studied in 18 workers from a mercury cell chlorine plant (Levine 1982). Time-integrated urine mercury levels were used as an indicator of mercury exposure. Using linearized regression analysis, the authors found that motor and sensory nerve conduction velocity changes, (i.e., prolonged distal latencies) were correlated with the time-integrated urinary mercury levels in asymptomatic exposed workers and occurred when urinary mercury levels exceeded 25 μg/L. This study demonstrated that elemental mercury exposure can be associated with preclinical evidence of peripheral neurotoxicity.

Miller et al. (1975) investigated several subclinical parameters of neurological dysfunction in 142 workers exposed to inorganic mercury in either the chloralkali industry or a factory for the manufacture of magnetic materials. They found that there was a significant increase in average forearm tremor frequency in workers whose urinary mercury concentration exceeded 50 μg/L as compared to unexposed controls. Also observed were eyelid fasciculation, hyperactive deep tendon reflexes and dermatographia, but there was no correlation between the incidence of these findings and urinary mercury levels.

Roels et al. (1985) examined 131 male and 54 female workers occupationally exposed to mercury vapor for an average duration of 4.8 years. Urinary mercury (52 and 37 μg/g creatinine for males and females, respectively) and blood mercury levels (14 and 9 μg/L for males and females, respectively) were recorded, but atmospheric mercury concentration was not provided. Symptoms indicative of central nervous system (CNS) disorders were reported but were not related to mercury exposure. Minor renal tubular effects were detected in mercury-exposed males and females and were attributed to current exposure intensity rather than exposure duration. Male subjects with urinary

mercury levels of >50 μg/g creatinine exhibited preclinical signs of hand tremor. It was noted that females did not exhibit this effect, and that their urinary mercury never reached the level of 50 μg/g creatinine. A companion study (Roels et al. 1987) related air mercury levels to blood mercury (Hg·blood) and urinary mercury (Hg·U) values in 10 workers in a chloralkali battery plant. Duration of exposure was not specified. A high correlation was reported for Hg·air and Hg·U for pre-shift exposure ($r=0.70$, $p<0.001$) and post-shift ($r=0.81$, $p<0.001$) measurements. Based on these data and the results of their earlier (1985) study, the investigators suggested that some mercury-induced effects may occur when Hg·U levels exceed 50 μg/g creatinine and that this value corresponds to a mercury TWA of ≈0.04 mg Hg/m^3.

A survey of 567 workers at 21 chloralkali plants was conducted to ascertain the effects of mercury vapor inhalation (Smith et al. 1970). Mercury levels ranged from <0.01 to 0.27 mg/m^3, and chlorine concentrations ranged from 0.1 to 0.3 ppm in most of the working stations of these plants. Worker exposure to mercury levels (TWA) varied with 10.2% of the workers exposed to <0.01 mg Hg/m^3, 48.7% exposed to 0.01–0.05 mg Hg/m^3, 25.6% exposed to 0.06–0.10 mg Hg/m^3, and 4.8% exposed to 0.24–0.27 mg Hg/m^3 (approximately 85% were exposed to mercury levels ≤0.1 mg/m^3). The duration of employment for the examined workers ranged from one year (13.3%) to >10 years (31%) with 55.7% of the workers employed for 2 or 9 years. A group of 600 workers not exposed to chlorine served as a control group. A strong positive correlation ($p<0.001$) was found between the mercury TWAs and the reporting of subjective neuropsychiatric symptoms (nervousness, insomnia), occurrence of objective tremors and weight and appetite loss. A positive correlation ($p<0.001$) was also found between mercury exposure levels and urinary and blood mercury levels of test subjects. No adverse alterations in cardiorespiratory, gastrointestinal, renal, or hepatic functions were attributed to the mercury vapor exposure. Additionally, biochemical (hematologic data, enzyme activities) and clinical measurements (electrocardiogram, chest X-rays) were not different between the mercury-exposed and non-exposed workers. No significant signs or symptoms were noted for individuals exposed to mercury vapor concentrations ≤0.1 mg Hg/m^3. This study provides data indicative of a no-observed-adverse-effect level (NOAEL) of 0.1 mg Hg/m^3 and a lowest-observed-adverse-effect level (LOAEL) of 0.18 mg Hg/m^3. In a follow-up study conducted by Bunn et al. (1986), however, no significant differences in the frequency of objective or subjective findings such as weight loss and appetite loss were seen in workers exposed to mercury at levels that ranged between 50 and 100 mg/m^3. The study by Bunn et al. (1986) was limited, in that little information was provided regarding several methodological questions such as quality assurance measures and control of possible confounding variables.

Neurological signs and symptoms (i.e., tremors) were observed in 79 workers exposed to metallic mercury vapor; urinary mercury levels in affected subjects exceeded 500 μg/L. Short-term memory deficits were seen in workers whose urine levels were less than 500 μg/L (Langolf et al. 1978). Impaired performance in mechanical and visual memory tasks and psychomotor ability tests was reported by Forzi et al. (1978) in exposed workers whose urinary mercury levels exceeded 100 μg/L.

Decreased strength, decreased coordination, increased tremor, decreased sensation and increased prevalence of Babinski and snout reflexes were exhibited by 247 exposed workers whose urinary mercury levels exceeded 600 μg/L. Evidence of clinical neuropathy was observed at urinary mercury levels that exceeded 850 μg/L (Albers et al. 1988). Preclinical psychomotor dysfunction was reported to occur at a higher incidence in 43 exposed workers (mean exposure duration of 5 years) whose mean urinary excretion of mercury was 50 μg/L. In the same study, workers whose mean urinary mercury excretion was 71 μg/L had a higher incidence of total proteinuria and albuminuria (Roels et al. 1982). Postural and intention tremor was observed in 54 exposed workers (mean exposure duration of 7.7 years) whose mean urinary excretion of mercury was 63 μg/L (Roels et al. 1989). Verbeck et al. (1986)

observed an increase in tremor parameters with increasing urinary excretion of mercury in 21 workers exposed to mercury vapor for 0.5–19 years. The LOAEL for this effect was a mean urinary excretion of 35 μg/g creatinine.

The elemental mercury levels reported to be associated with preclinical and symptomatic neurological dysfunction are generally lower than those found to affect kidney function, as discussed below.

Piikivi and Ruokonen (1989) found no evidence of glomerular or tubular damage in 60 chloralkali workers exposed to mercury vapor for an average of 13.7 ± 5.5 years as compared to their matched referent controls. Renal function was assessed by measuring urinary albumin and N-acetyl-β-glucosaminidase (NAG) activity. The mean blood mercury level in the exposed workers was 14 μg/L, and the mean urinary mercury level was 17 μg/L. The authors extrapolated a NOAEL for kidney effects based on these results of 0.025 mg/m^3 from blood levels based on the conversion factor calculated by Roels et al. (1987).

Stewart et al. (1977) studied urinary protein excretion in 21 laboratory workers exposed to 0.01–0.05 mg/m^3 of mercury. Their urinary level of mercury was ~35 μg/L. Increased proteinuria was found in the exposed workers by comparison to unexposed controls. When preventive measures were instituted to limit exposure to mercury, proteinuria was no longer observed in the exposed technicians.

Lauwerys et al. (1983) found no change in several indices of renal function (e.g., proteinuria, albuminuria, urinary excretion of retinol-binding protein, aminoaciduria, creatinine in serum, β_2-microglobulin in serum) in 62 workers exposed to mercury vapor for an average of 5.5 years. The mean urinary mercury excretion in the exposed workers was 56 μg/g creatinine, which corresponds to an exposure level of ~0.046 mg/m^3 according to a conversion factor of 1:1.22 (air:urine [μg/g creatinine]) (Roels et al. 1987). Despite the lack of renal effects observed, 8 workers were found to have an increase in serum anti-laminin antibodies, which can be indicative of immunological effects. In a follow-up study conducted by Bernard et al. (1987), however, there was no evidence of increased serum anti-laminin antibodies in 58 workers exposed to mercury vapor for an average of 7.9 years. These workers had a mean urinary mercury excretion of 72 μg/g creatinine, which corresponds to an exposure level of ~0.059 mg/m^3.

Renal function in 100 chloralkali workers exposed to inorganic mercury vapor for an average of 8 years was studied (Stonard et al. 1983). No changes in the following urinary parameters of renal function were observed at mean urinary mercury excretion rates of 67 μg/g creatinine: total protein, albumin, α_1-acid glycoprotein, β_2-microglobulin, NAG and γ-glutamyl transferase. When urinary mercury excretion exceeded 100 μg/g creatinine, a small increase in the prevalence of higher activities of NAG and γ-glutamyl transferase were observed.

Rosenman et al. (1986) evaluated routine clinical parameters (physical exams, blood chemistry, urinalysis), neuropsychological disorders, urinary NAG, motor nerve conduction velocities and occurrence of lenticular opacities in 42 workers of a chemical plant producing mercury compounds. A positive correlation ($p<0.05$ to $p<0.001$) was noted between urinary mercury (levels ranged from 100–250 μg/L) and the number of neuropsychological symptoms, NAG excretions and decrease in motor nerve conduction velocities. Evidence of renal dysfunction was seen in 63 chloralkali workers. This included increased plasma and urinary concentrations of β-galactosidase, increased urinary excretion of high-molecular weight proteins and a slightly increased plasma β_2-microglobulin concentration. The

incidence of these effects increased in workers whose urinary mercury excretion exceeded 50 μg/g creatinine (Buchet et al. 1980).

Increased urinary NAG levels were found in workers whose urinary mercury levels exceeded 50 μg/L (Langworth et al. 1987). An increase in the concentration of urinary brush border proteins (BB-50) was observed in 20 workers whose mean urinary mercury excretion exceeded 50 μg/g creatinine (Mutti et al. 1985). Foa et al. (1976) found that 15 out of 81 chloralkali workers exposed to 0.06–0.30 mg/m^3 mercury exhibited proteinuria. An increased excretion of β-glutamyl transpeptidase, indicative of renal dysfunction, was found in 509 infants dermally exposed to phenylmercury via contaminated diapers (Gotelli et al. 1985).

The elemental mercury levels reported to be associated with preclinical and symptomatic neurological dysfunction and kidney effects are lower than those found to affect pulmonary function, as discussed below.

McFarland and Reigel (1978) described the cases of six workers who were acutely exposed (4–8 hours) to calculated metallic mercury vapor levels of 1.1–44 mg/m^3. These men exhibited a combination of chest pains, dyspnea, cough, hemoptysis, impairment of pulmonary function (reduced vital capacity), diffuse pulmonary infiltrates and evidence of interstitial pneumonitis. Although the respiratory symptoms resolved, all six cases exhibited chronic neurological dysfunction, presumably as a result of the acute, high-level exposure to mercury vapor.

Lilis et al. (1985) described the case of a 31-year-old male who was acutely exposed to high levels of mercury vapor in a gold extracting facility. Upon admission to the hospital, the patient exhibited dyspnea, chest pain with deep inspiration, irregular infiltrates in the lungs and reduced pulmonary function (forced vital capacity). The level of mercury to which he was exposed is not known, but a 24-hour urine collection contained 1,900 μg Hg/L. Although the patient improved gradually over the next several days, he still showed signs of pulmonary function abnormalities (e.g., restriction and diffusion impairment) 11 months after exposure.

Levin et al. (1988) described four cases of acute high-level mercury exposure during gold ore purification. The respiratory symptoms observed in these four cases ranged from minimal shortness of breath and cough to severe hypoxemia. The most severely affected patient exhibited mild interstitial lung disease both radiographically and on pulmonary function testing. One patient had a urinary mercury level of 245 μg/L upon hospital admission. The occurrence of long-term respiratory effects in these patients could not be evaluated since all but one refused follow-up treatment.

Schweinsberg (1994) evaluated mercury in hair, blood and urine of subjects who had amalgam fillings, who consumed fish or who had occupational exposure. The first group consisted of 67 males aged 16-72 yrs, mean age 36. The fish-eating population was 149 males and females (age 22-80, mean = 47) who either did or did not eat fish from the Rhine River. The workers were 105 male and female employees of a Thuringian thermometer plant (ages 19- 65, mean = 42). Both fish consumption and the presence of mercury amalgam fillings resulted in measurable mercury in blood or urine. The range of mercury in the workers was higher by about 100 fold. The authors present most of the data graphically; they report that persons without amalgam fillings who did not consume Rhine fish or work in the thermometer factory had blood mercury in the range of 0.2 µg/l (detection limit) and 0.4 µg/l (n not given). It appears that from Figure 5 of the paper that blood mercury levels ranged between 100-120 µg/l in 5 workers. The authors state that no changes in nerve conduction velocities or N-acetyl-β-D-glucosaminidase activities were observed (data not shown in paper).

3.1.2 Cancer Data

3.1.2.1 Human Data

A number of epidemiological studies were conducted that examined mortality among elemental mercury vapor-exposed workers. Conflicting data regarding a correlation between mercury exposure and an increased incidence of cancer mortalities have been obtained. All of the studies have limitations which compromise interpretation of their results. These studies are summarized in Table 3-1.

A retrospective cohort study examined mortality among 5,663 white males who worked between 1953 and 1958 at a plant in Oak Ridge, Tennessee, where elemental mercury was used for lithium isotope separation (Cragle et al. 1984). The workers were divided into three cohorts: exposed workers who had been monitored on a quarterly basis for mercury levels in urine (n=2,133); workers exposed in the mercury process section for whom urinalysis monitoring data were not collected (n=270); and unexposed workers from other sections of the nuclear weapons production facility (n=3,260). The study subjects worked at least 4 months during 1953–1958 (a period when mercury exposures were likely to be high); mortality data from death certificates were followed through the end of 1978. The mean age of the men at first employment at the facility was 33 years, and average length of their employment was greater than 16 years with a mean 3.73 years of estimated mercury exposure. Air mercury levels were monitored beginning in 1955, and during 1955 through the third quarter of 1956, air mercury levels were above 100 $\mu g/m^3$ in 30–80% of the samples. Thereafter, air mercury levels decreased to concentrations below 100 $\mu g/m^3$. The mortality experience [standard mortality ratio (SMR)] of each group was compared with the age-adjusted mortality experience of the U.S. white male population. Among exposed and monitored workers, there were no significant increases in mortality from cancer at any site, even after the level or length of exposure was considered. A significantly lower mortality from all causes was observed. There was an excess of deaths due to lung cancer in the exposed, monitored workers (42 observed, 31.36 expected) but also in the unexposed workers (71 observed and 52.93 expected). The SMR for each group was 1.34; the elevated incidence of lung cancer deaths was, therefore, attributed to some other factor at the plant and/or to lifestyle factors (e.g., smoking) common to both the exposed and unexposed groups. Study limitations include small cell sizes for cancer mortality, which limited the statistical stability of many comparisons.

Barregard et al. (1990) studied mortality and cancer morbidity between 1958 and 1984 in 1,190 workers from eight Swedish chloralkali plants that used the mercury cell process in the production of chlorine. The men included in the study had been monitored for urinary or blood mercury for more than one year between 1946 and 1984. Vital status and cause of death were ascertained from the National Population Register and the National Bureau of Statistics. The cancer incidence of the cohort was obtained from the Swedish Cancer Register. The observed total mortality and cancer incidences were compared with those of the general Swedish male population. Comparisons were not made between exposed and unexposed workers. Mean urinary mercury levels indicated a decrease in exposure between the 1950s and 1970s; the mean urinary mercury level was 200 µg/L during the 1950s, 150 µg/L during the 1960s and 50 µg/L in the 1970s. Mortality from all causes was not significantly increased in exposed workers. A significant increase in deaths from lung tumors with greater than 10 years of latency was seen in exposed workers (rate ratio, 2.0; 95% C.I. 1.0–3.8), but 9 of the 10 observed cases of lung cancer occurred among workers (457 of the 1,190) possibly exposed to asbestos as well as to mercury. No dose response was observed with respect to mercury exposure and lung tumors. This study is limited because no quantitation was provided on smoking status, and results were confounded by exposure to asbestos.

Table 3-1
Carcinogenic Effects of Elemental Mercury in Humans: Epidemiological Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/ 2,133 M	≥4 months (occup)	NS, but up to 80% of air samples in early years were >0.10; this declined to 1-10% in later years	No biologically significant increase in cancer mortality in workers at an isotope enrichment plant, compared with unexposed workers at the same plant, or with age-adjusted mortality of U.S. males. Lung cancer mortality was increased in exposed workers, but the increase was not statistically significant and a significant increase in lung cancer mortality was observed in unexposed workers. Limitations: small cell size for cancer mortality, limiting statistical power of comparisons BML not reported	Cragle et al. 1984
Human/376 cases of lung cancer (6 were hatmakers), 892 controls	NS (occup)	NS	Increased lung cancer incidence among female hat makers ($p = 0.01$). Controls were matched by age, sex and smoking history. Limitations: Hat makers were also exposed to arsenic BML not reported	Buiatti et al. 1985
Human/ cohort/3454 M, 5787 F	NS (occup)	NS	Increased incidence of glioblastomas among dental professionals. 95% C.I. = 1.3-3.4. Expected incidence was based on all employed people (Sweden), stratified by age, sex and county. Limitations: No information was provided on the duration or level of exposure; subjects were also exposed to chloroform and X-rays. BML not reported	Ahlbom et al. 1986
Human/9,912 M (369 silicotics, 9,543 nonsilicotics)	NS (occup)	NS	Increased lung cancer mortality among metal miners (95% C.I. = 0.94-2.90 for silicotics, 0.98-1.42 for nonsilicotics). Limitations: Miners were exposed to a variety of metals. Only 274 worked in mercury mines, and data were not reported separately for this group. Workers were also exposed to radon, increase may have been related to silicosis BML not reported	Amandus and Costello 1991
Human/ cohort/799 M	≥1 year (occup)	NS	Increased incidence of lung cancer among chloralkali workers, but there was no association with cumulative mercury dose, years of employment or latency (95% C.I. = 1.0-2.6). The increase could be partly explained by an assumed higher smoking incidence and asbestos exposure. Cancer mortality and incidence compared with age-adjusted Norwegian population. Limitations: Subjects were also exposed to chlorine and low levels of asbestos dust. Limited data available, since reported as an abstract. BML not reported	Ellingsen et al. 1992
Human/1190 M	at least one year	NS grouped by years x U-Hg; >1000 ug/L, 1000-2000 ug/L, >2000 ug/L	Cancer and mortality rates compared with general population; no increase in mortality; excess of lung cancers (rate ratio = 2.0; 95% CI 1.0-3.8) Limitations: co-exposure to asbestos	Barregard et al. 1990

Ahlbom et al. (1986) examined the cancer mortality during 1961 to 1979 of cohorts of Swedish dentists and dental nurses aged 20–64 and employed in 1960 (3,454 male dentists, 1,125 female dentists, 4,662 female dental nurses). Observed incidences were compared with those expected based on cancer incidence during 1961–1979 among all Swedes employed during 1960 and the proportion of all Swedes employed as dentists and dental nurses. Data were stratified by sex, age (5-year age groups), and county. The incidence of glioblastomas among the dentists and dental nurses combined was significantly increased (SMR, 2.1; 95% C.I. 1.3–3.4); the individual groups had elevated SMRs (2.0–2.5), but the 95% confidence intervals of these groups included unity. By contrast, physicians and nurses had SMRs of only 1.3 and 1.2, respectively. Exposure to mercury could not be established as the causative factor because exposure to other chemicals and X-rays was not ruled out.

Amandus and Costello (1991) examined the association between silicosis and lung cancer mortality between 1959 and 1975 in white male metal miners (n=9,912) employed in the United States between 1959 and 1961. Mercury exposures were not monitored. Exposures to specific metals among the silicotic and nonsilicotic groups were analyzed separately. Lung cancer mortality in both silicotic and nonsilicotic groups was compared with rates in white males in the U.S. population. Both silicotic (n=11) and nonsilicotic mercury miners (n=263) had significantly increased lung cancer mortality (SMR, 14.03, 95% C.I., 2.89–40.99 for silicotics; SMR, 2.66, 95% C.I. 1.15–5.24 for nonsilicotics). The analysis did not focus on mercury miners, and confounders such as smoking and radon exposure were not analyzed with respect to mercury exposure. This study is also limited by the small sample size for mercury miners.

A case-control study of persons admitted to a hospital in Florence, Italy with lung cancer between 1981-1983 was performed to evaluate occupational risk factors (Buiatti et al. 1985). Cases were matched with one or two controls (persons admitted to the hospital with diagnoses other than lung cancer or suicide) with respect to sex, age, date of admission, and smoking status. Women who had "ever worked" as hat makers had a significantly increased risk of lung cancer (p=0.01; determined using the Mantel-Haenszel Chi-square test). The duration of employment as a hat maker averaged 22.2 years, and latency averaged 47.8 years. Workers in the Italian hat industry were known to be occupationally exposed to mercury; however, the design of this study did not allow evaluation of the relationship between cumulative exposure and cancer incidence. In addition, interpretation of the results of this study is limited by the small sample size (only 6/376 cases reported this occupation) and by exposure of hat makers to other pollutants including arsenic, a known lung carcinogen.

Ellingsen et al. (1992) examined the total mortality and cancer incidence among 799 workers employed for more than 1 year in two Norwegian chloralkali plants. Mortality incidence between 1953 and 1988 and cancer incidence between 1953 and 1989 were examined. Mortality and cancer incidence were compared with that of the age-adjusted general male Norwegian population. No increase in total cancer incidence was reported, but lung cancer was significantly elevated in the workers (ratio, 1.66; 95% C.I. 1.0–2.6). No causal relationship can be drawn between mercury exposure and lung cancer because no correlation existed between cumulative mercury dose, years of employment, or latency time. Also, the prevalence of smoking was 10–20% higher in the exposed workers and many workers were also exposed to asbestos.

3.1.2.2 Animal Data

Druckrey et al. (1957) injected 0.1 mL of metallic mercury intraperitoneally into 39 rats (males and females; numbers of each not specified) of the BD III and BD IV strains. Among the rats surviving longer than 22 months, 5 out of 12 developed peritoneal sarcomas (three females and two males). All

sarcomas were observed to have droplets of mercury present. Although severe kidney damage was reported in all treated animals, there were no renal tumors or tumors at any site other than the peritoneal cavity.

3.1.3 Other Data

3.1.3.1 Death

Accidental exposure to high concentrations of elemental mercury vapor for short amounts of time has led to deaths in humans (Table 3-2). The cause of death in all available reports was respiratory failure. The onset of death occurred six hours to 23 days after exposure to mercury vapors (Campbell 1948; Kanluen and Gottlieb 1991; Rowens et al. 1991; Soni et al. 1992; Taueg et al. 1992). Urinary mercury concentrations indicated that body levels were up to 10 times higher than controls. Only acute-duration studies were found that directly linked elemental mercury vapor exposure to death.

Table 3-2
Lethality of Elemental Mercury in Humans: Case Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/1 F (4-month old)	5 hr	NS	Increased creatinine excretion; necrotic stomach mucosa; degeneration of convoluted tubules; death due to pulmonary edema Limitation: Limited exposure data BML not reported	Campbell 1948
Human/2 M, 2 F (adult, 2 elderly)	≈24 hr	NS	Respiratory distress; CNS alterations; nausea; tubular necrosis of proximal tubules in kidneys Limitation: Limited exposure data BML Range: 4.6-219 µg/L in urine	Kanluen and Gottlieb 1991; Rowens et al. 1991
Human/1 F (1-yr old)	<6 hr	NS	Breathing difficulty; distended abdomen Limitation: Limited exposure data BML not reported	Soni et al. 1992
Human/2 M, 2 F (adults)	NS (Acute)	≤0.91 at 11-18 days post-exposure	Dyspnea; respiratory failure; death at 11-24 days postexposure BML Range: 94-423 µg/L in urine	Taueg et al. 1992
Human/2 F (children)	Several months	0.01-0.04 several months after initial spill	Numbness in fingers and toes; absence of deep tendon reflexes; visual field defects; weakness BML not reported	Taueg et al. 1992

Animal studies reveal that pulmonary edema and asphyxiation result from acute high-concentration exposure to elemental mercury vapors (Table 3-3). Exposure to elemental mercury vapors for two hours at a concentration of 27 mg Hg/m^3 resulted in death of 20 of 32 rats (Livardjani et al. 1991). Rabbits exposed for 1 to 30 hours to 28.8 mg Hg/m^3 of elemental mercury vapors appeared to be

less affected. Death occurred in only one of two rabbits exposed for 30 hours (Ashe et al. 1953). Exposure to the same concentration for a shorter duration resulted in no deaths.

Table 3-3
Lethality of Elemental Mercury in Animals: Inhalation Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Rabbit/strain NS/14 (sex NS)	1-30 hr	28.8	LD_{50} for 30 hours; all rabbits exposed for shorter periods survived. Limitations: There was no control group, and details on effects were lacking. BML: 5,320 µg/L in blood	Ashe et al. 1953
Rat/Wistar/64 M/duration	1 or 2 hr	0, 30	Death was due to asphyxiation; pulmonary edema and fibrosis were observed. No animals exposed for 1 hour died by 15 days, and all animals exposed for 2 hours died within 5 days. Limitations: No control group; limited reporting of histology BML Range: 391-4,558 µg/L in blood at 1-15 days postexposure	Livardjani et al. 1991

3.1.3.2 Neurological

Case reports from accidental exposures to high concentrations of mercury vapors (Adams et al. 1983; Aronow et al. 1990; Barber 1978; Bluhm et al. 1992a; Fagala and Wigg 1992; Foulds et al. 1987; Friberg et al. 1953; Hallee 1969; Jaffe et al. 1983; Karpathios et al. 1991; Lilis et al. 1985; McFarland and Reigel 1978; Sexton et al. 1976; Snodgrass et al. 1981; Taueg et al. 1992) as well as studies of populations chronically exposed to potentially high concentrations (Ehrenberg et al. 1991; Friberg et al. 1953; Roels et al. 1982; Sexton et al. 1978) have provided considerable information about the neurotoxicity of elemental mercury vapor. These studies have shown effects on a wide variety of cognitive, sensory, personality and motor functions.

Occasionally, hearing loss, visual disturbances (visual field constriction), and/or hallucinations have also occurred. In general, symptoms have been observed to subside after removal from exposure. However, persistent effects (tremor, cognitive deficits) have been observed in occupationally exposed subjects 10 to 20 years after cessation of exposure (Albers et al. 1988; Ellingsen et al. 1993; Kishi et al. 1993).

Symptoms of Mercury Vapor-induced Neurotoxicity

The most prominent symptoms associated with mercury vapor-induced neurotoxicity include the following:

- tremors -- initially affecting the hands and sometimes spreading to other parts of the body
- emotional lability -- often referred to as "erethism" and characterized by irritability, excessive shyness, confidence loss and nervousness
- insomnia
- neuromuscular changes -- weakness, muscle atrophy, muscle twitching
- headaches
- polyneuropathy -- paresthesias, stocking-glove sensory loss, hyperactive tendon reflexes, slowed sensory and motor nerve conduction velocities
- memory loss and performance deficits in tests of cognitive function

Studies of workers exposed to elemental mercury vapor have reported frank neurotoxicity at exposure levels greater than 0.1 mg/m^3 (Smith et al. 1970) or at levels resulting in urinary mercury of greater than 300 µg in a 24-hour urine sample (Bidstrup et al. 1951). Several studies, however, have shown evidence of neurotoxicity at approximately 2- to 4-fold lower concentrations. Self-reported memory disturbances, sleep disorders, anger, fatigue, confusion and/or hand tremors were increased in workers chronically exposed to an estimated 0.025 mg/m^3 (blood levels of approximately 10 µg/L) (Langworth et al. 1992a; Piikivi and Hanninen 1989). Also, objective measures of cognitive and/or motor function in exposed populations have shown significant differences from unexposed controls (Ehrenberg et al. 1991; Fawer et al. 1983; Liang et al. 1993; Ngim et al. 1992; Piikivi and Tolonen 1989; Piikivi et al. 1984; Roels et al. 1982, 1989).

Table 3-4
Neurotoxicity of Elemental Mercury in Humans: Case Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/1 M (adult)	8-9 mo (occup)	0.02-0.45	Fatigue, irritability in an electrochemical industry worker Limitations: small sample size; concomitant exposure to chlorine; limited data reporting BML: 680-900 µg/L in urine	Friberg et al. 1953
Human/6 M	<8 hr	44.3 (est.)	Tremor; irritability; visual and hearing abnormalities Limitations: small sample size; limited data reporting BML Range: 1,060-3,280 µg/24 hr urine	McFarland and Reigel 1978
Human/5 M, 6 F (adults and children)/ 12 controls (sex NS)	51-176 d	0.1-1.0	Nervousness, insomnia and inattentiveness were more common than in controls; altered EEGs and personality changes also noted Limitations: small sample size BML: 183-620 µg/L in blood at first measure	Sexton et al. 1978
Human/2 M, 2 F (adults)	3 d	NS	Headache, slowed speech Limitation: Small sample size; limited exposure data BML: 82-5700 µg/24 hr urine	Snodgrass et al. 1981

Table 3-4 (continued)
Neurotoxicity of Elemental Mercury in Humans: Case Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/1 M (adult)	2 d	NS	Delayed neurotoxicity: paresthesias; muscle fasciculations; hyperactive deep tendon reflexes Limitation: small sample size; exposure data limited BML: 98.75 µg/L in urine 3.5 months after exposure	Adams et al. 1983
Human/1 F (8-month old)	≈1 d	NS	Seizures; weakness; short-term hearing deficit; cortical atrophy Limitations: Exposure data limited BML Range: 16-43 µg/24 hr urine	Jaffe et al. 1983
Human/1 M	~2 hr	NS	Dizziness, weakness Limitation: small sample size; limited exposure data BML: 1,900 µg/L urine on first day	Lilis et al. 1985
Human/1 F (child)	2 mo	NS	Lethargy; irritability Limitations: small sample size; limited reporting of symptoms; limited exposure data BML: 214 µg/L in 24 hr urine	Foulds et al. 1987
Human/1 M (child)	2 wk	NS	Tremor; sleep disturbance; anxiety; cold hands and feet Limitation: small sample size; limited exposure data BML: 130 µg/24 hr urine	Karpathios et al. 1991
Human/17-26 M	<16 hr	NS	Fatigue, headaches, irritability, depression, anxiety, tremor, impaired performance on visual-motor tests ($p<0.05$) reported in welders following accidental exposure. Limitation: Chronic exposure to other metals; exposure data limited BML: ~60 µg/L in blood at 20 d postexposure	Bluhm et al. 1992a
Human/1 F (child)	6 mo	NS	Peripheral neuropathy; erethism; dizziness; depression; irritability Limitation: small sample size; exposure data limited BML: 686 µg/24 hr urine	Fagala and Wigg 1992
Human/2 F (children)	Several months	0.01-0.04 several months after initial spill	Numbness in fingers and toes; absence of deep tendon reflexes; visual field defects; weakness BML not reported	Taueg et al. 1992

Table 3-5
Neurotoxicity of Elemental Mercury in Humans: Epidemiological Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/27 cases (sex NS)	3 mo-39 yr (occup)	0-1.67 (est.)	161 electric meter repair workers were examined, and 22 were found to be symptomatic; there were 5 index cases. Tremor; irritability; visual impairment were observed Limitation: Concomitant exposure to other chemicals is likely. BML Range: 1,495-7,950 µg/24 hr urine	Bidstrup et al. 1951
Human/3 M, 6 F exposed/10 M, 30 F controls	NS (occup)	NS	Neuropsychological tests showed irritability, tremor, memory loss, poor coordination, visual impairment; altered electrophysiology ($p<0.05$) in thermometer manufacturing employees Limitation: Exposure data limited BML Range: 4-1,101 µg/24 hr urine	Vroom and Greer 1972
Human/43 exposed/47 controls (sex NS)	>6 months Mean: 5.3 yr (occup)	NS	Objectively assessed tremor and eye-hand coordination tended to be higher in the exposed group, with a significant ($p<0.05$) difference on one test. There was a tendency toward a dose-response, but it was not statistically significant. Exposed group worked in amalgam or chloralkali plants; control workers were matched from the same plants, but unexposed. Limitation: Exposure data limited BML: 29.2 µg/L in blood (range: 5.3-135); 95.5 µg/g creatinine in urine (range 9.9-286)	Roels et al. 1982
Human/23 M exposed/22 M control	NS (occup)	NS	Decreased nerve conduction velocity ($p<0.05$); visual impairment ($p<0.01$); higher distress levels Of a sample of 298 dentists, the exposed group had "tissue" mercury levels in the top 20%; the controls were age-matched, with no detectable tissue mercury. Tissue mercury in the head and wrist was measured using x-ray fluorescence. Limitation: Exposure data limited BML: >20 µg/g in tissue	Shapiro et al. 1982
Human/12 exposed/12 controls (sex NS)	3 mo-8 yr (occup)	NS	In a battery of objective tests, the following findings were significant: tremor ($p<0.025$); decreased verbal intelligence; short- and long-term memory impairment ($p<0.01$); fatigue ($p<0.01$). The exposed group worked with amalgam (n=4) or were exposed to mercuric chloride, methoxyethyl mercuric chloride, methoxyethylmercuric acetate (n=8). Controls were matched by age, sex, education, ethnic background. Limitation: Exposure data limited; small sample size BML Range: <10-670 µg/L urine	Williamson et al. 1982
Human/26 exposed M/25 control M	Avg: 15.3 yr (occup)	0.026 (TWA) personal monitoring	Objectively assessed tremor was significantly ($p = 0.001$) elevated in the exposed group and correlated with exposure duration. Exposed group worked in fluorescent tube factories (n=7), chloralkali plants (n=12), or in acetaldehyde production. The control subjects worked at the same factories but had not been exposed to mercury. Mean BML: 8,280 µg/L in blood; 20 µg/g creatinine in urine	Fawer et al. 1983
Human/36 M exposed/36 controls	Avg: 16.9 yr (10-37 yr) (occup)	0.022–0.028 (est.)[a]	By comparison to age-matched controls, chloralkali workers had memory impairment, decreased verbal intelligence ($p<0.01$). BML: >15µg/L in blood; >56 µg/L in urine	Piikivi et al. 1984

Table 3-5 (continued)
Neurotoxicity of Elemental Mercury in Humans: Epidemiological Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/60 M exposed/60 M controls	Avg: 13.7 yr (5-28 yr) (occup)	0.025 (est.)	In a psychological and psychomotor test battery, there were statistically significant differences in subjective tests (memory disturbance, mood; $p<0.01$) and an objective test (hand-eye coordination, $p<0.001$). Subjects were chlorine-alkali workers and controls were age-matched. BML: 10.4 µg/L avg. in blood; 17.9 µg/g creatinine avg. in urine	Piikivi and Hanninen 1989
Human/41 M exposed/41 M controls	5–27 yr (occup)	0.025 (est.)	Attenuation of power density spectrum of EEG in chloralkali workers ($p<0.01$); controls were age-matched;slight increase in subjective symptoms of autonomic (cardiovascular) dysfunction and a slight decrease in pulse rate variations (cardiovascular reflex response). BML: 67.8 µg/L in blood; 20.6 µg/g creatinine in urine	Piikivi and Tolonen 1989
Human/54 M exposed/48 controls	Avg: 7.7 yr (1-20 y (occup)	NS	Chloralkali and amalgam workers had impaired eye-hand coordination ($p<0.001$) and hand steadiness ($p<0.02$) in objective tests, by comparison to unexposed matched controls from the same plants. Limitation: Exposure data limited Geometric mean BML: 24 µg/L blood; 63 µg/g creatinine	Roels et al. 1989
Human/10 M, 62 F exposed/9 M, 60 F control	Avg: 5 yr (occup)	0.076 (avg) 0.003-0.27 (range)	Neurological exam found difficulty with heel-to-toe gait ($p<0.05$) in thermometer manufacturers; control population worked at a nearby electronics manufacturer. Avg. BML: 73.2 µg/g creatinine in urine	Ehrenberg et al. 1991
Human/89 exposed/75 controls	>1 yr (occup)	0.025	Increased tiredness, memory disturbance, based on interviews ($p<0.001$), but no effect on psychometric tests or tremor in chloralkali workers. BML: 11 µg/L in blood; 25.4 µg/g creatinine in urine	Langworth et al. 1992a
Human/60 M, 38 F exposed/27 M, 27 F controls	10 hr/d 6 d/wk 0.7-24 yr (occup)	0.014 (TWA)	Impaired performance on neurobehavioral tests in dentists ($p<0.05$); severity of effect correlated with exposure. Limitations: Concomitant exposure to physical and vibration load (affecting dexterity tests); confounding exposure to folk medicine, BML: Mean 9.8 µg/L in blood; range: 0.63-57.3 µg/L in blood	Ngim et al. 1992
Human/77 M exposed/53 M controls	>1 yr Avg: 7.9 yr (occup)	0.059	In a study of ex-chloralkali workers (avg. 12.3 yr since last exposure) compared with age-matched controls, sensory nerve conduction velocity and visual evoked response correlated with mercury exposure ($p<0.05$) BML: 3,190 µg/g creatinine in urine current, 106 µg/L in urine during exposure	Ellingson et al. 1993
Human/117 M exposed/76 controls	389 min/d duration NS (occup)	1.5-3.3	Ex-mercury miners tested 18 years after the closure of the mine had lower scores on objective neuropsychological tests (motor coordination, reaction time, short-term memory) than age- and education-matched controls ($p<0.01$). 76 of the miners had a history of mercury poisoning, but subjective symptoms had generally decreased since exposure BML not reported	Kishi et al. 1993

Table 3-5 (continued)
Neurotoxicity of Elemental Mercury in Humans: Epidemiological Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/19 M, 69 F exposed/97 controls	≥2 yr Avg. 10.4 yr (occup)	0.033 (avg) 0.008-0.085 (range)	Increased fatigue and confusion; impaired performance on neurobehavioral tests in fluorescent lamp factory workers ($p<0.01$) Avg. BML: 25 µg/L in urine	Liang et al. 1993

[a] Estimate by extrapolating from urinary mercury levels (Roels et al. 1987).

In animals, as in humans, adverse neurological effects are observed after exposure to elemental mercury vapor. Effects observed in rabbits and mice after subchronic exposures included tremors, ataxia, paralysis, failure to respond to light and decreased conditioned avoidance responding (Fukuda 1971; Ganser and Kirschner 1985; Kishi et al. 1978). Pathologic changes (unspecified) were observed in the brains of rabbits at 0.86 mg Hg/m^3 (Ashe et al. 1953).

Table 3-6
Neurotoxicity of Elemental Mercury in Animals: Inhalation Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Rat/Albino/7 M exposed/6 M control	12-42 wk 5 d/wk 3 hr/d	0, 3	Tremor; decline in conditioned avoidance and conditioned escape responses. First significant effect at 20 weeks ($p<0.05$) Limitation: Only one level tested BML Range: 11.18-17.83 µg/g in cerebrum (wet weight)	Kishi et al. 1978
Mouse/ C57BL6J/ No. and sex NS	3.5 wk 5 d/wk 20–40 min/d	NS (saturated)	Ataxia; motor dysfunction Limitations: Poorly defined exposure conditions; limited data reporting on effects BML not reported	Ganser and Kirschner 1985
Rabbit/774 strain NS/ 31 (sex NS)	1-12 wk 5 d/wk 7 hr/d	0.86	Mild to moderate pathological changes in brains Limitations: One exposure level; limited data reporting BML: Brain level 1.2 µg/g	Ashe et al. 1953
Rabbit/strain NS/6 M	13 wk 4 d/wk 6 hr/d	0, 4	Tremor Limitation: No control BML: 0.8–3.9 µg/g wet weight (brain)	Fukuda 1971

3.1.3.3 Renal

The kidney is a sensitive target organ following inhalation exposure to elemental mercury. Acute accidental exposure in private homes or as a result of industrial accidents resulted in symptoms ranging from slight changes in urinary acid excretion to transient renal failure with proteinuria, nephrosis and necrosis of the proximal convoluted tubules (Bluhm et al. 1992b; Jaffe et al. 1983; Rowens et al. 1991; Tubbs et al. 1982). Proteinuria, proximal tubule damage and glomerulosclerotic changes were also reported in a workers occupationally exposed for up to 2.5 years; in two cases the exposure levels were measured at 0.02 to 0.45 mg/m^3 (Friberg et al. 1953; Kazantzis et al. 1962). Comparisons of exposed workers to unexposed controls found increased urinary N-acetyl-β-D-glucosaminidase in workers exposed to 0.025 mg/m^3 and increased incidence of proteinuria (Roels et al. 1982).

Table 3-7
Renal Toxicity of Elemental Mercury in Humans: Case Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/2 M (adult)	8-9 mo (occup)	0.02-0.45	Proteinuria and nephrosis in electrochemical industry workers Limitations: small sample size; concomitant exposure to chlorine BML: 160-900 µg/L in urine	Friberg et al. 1953
Human/3 M	4 mo-2.5 yr (occup)	NS	Heavy albuminuria; transient renal failure; proximal tubule damage; glomeruloscierotic changes Limitations: small sample size; concomitant exposure to other mercurials and other compounds; limited exposure data BML Range: 1,100-1,440 µg/L in urine	Kazantzis et al. 1962
Human/2 M/ 41 M controls	NS (occup)	NS	Proteinuria; glomerulonephritis in chemical plant workers Limitation: small sample size; concomitant exposure to other metals; limited exposure data BML Range: 174-548 µg/24 hr urine	Tubbs et al. 1982
Human/1 F (8-month old)	≈1 day	NS	Acute renal failure (proteinuria, glucosuria, granular casts) BML Range: 16 µg/24 hr urine	Jaffe et al. 1983
Human/ 2M, 2F	Once	NS	Necrosis of proximal tubule; increased serum urea nitrogen and creatinine in 2 subjects Limitations: small sample size; limited exposure data BML Range: 94–220 µg/L Hg in urine	Rowens et al. 1991
Human/11 M	<16 hr	NS	Hyperchloremia, low normal bicarbonate in urine in welders following accidental exposure Limitations: No information on pre-exposure range BML: ~60 µg/L in blood at 20 d postexposure	Bluhm et al. 1992b

Table 3-8
Renal Toxicity of Elemental Mercury in Humans: Epidemiological Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/21 NS	NS (occup)	0.01-0.05	Increased proteinuria in exposed pathology laboratory workers compared to unexposed controls. Proteinuria cleared when mercury exposure was limited. BML: ≈35 µg/L in urine	Stewart et al. 1977
Human/43 exposed/47 controls (sex NS)	>6 months Mean: 5.3 yr (occup)	NS	Proteinuria significantly elevated ($p<0.05$). Exposed group worked in amalgam or chloralkali plants; control workers were matched from the same plants but unexposed. Limitations: Exposure data limited BML: 29.2 µg/L in blood (range: 53-135); 95.5 µg/g creatinine in urine (range 9.9-286)	Roels et al. 1982
Human/ 62 M exposed, 60 M controls	1-25 yr (avg 5.5 yr) (occup)	0.046 (est.)	Among exposed chloralkali plant or zinc-amalgam factory workers, renal function parameters were not different from unexposed controls. Circulating anti-laminin antibodies found in eight exposed, 0 controls. No dose-effect relationship between blood or urine levels and occurrence of anti-laminin antibodies. Exposure level estimated using Roels et al (1987) conversion factor. BML: 16 µg/L (range 2.5-75.6) in blood; 56 µg/g creatinine (range 3-272) in urine	Lauwerys et al 1983
Human/100 M	8 yr (avg) (occup)	NS	Small increase in prevalence of higher activities of NAG and gamma-glutamyl transferase in chloralkali workers w/urinary mercury excretion >100 µg/g creatinine. No renal function changes in workers w/mean urine 67 µg/g creatinine Limitation: Exposure data limited BML: 67->100 µg/g creatinine in urine	Stonard et al. 1983
Human/58 M exposed	7.9 yr (avg.)	0.059 (est)	Follow-up to Lauwerys et al. (1983). In contrast to the earlier study, there was no evidence of anti-laminin antibodies in exposed workers BML: 72 µg/g creatinine in urine	Bernard et al. 1987
Human/41 M exposed, 41 M controls	1-20 yr (occup)	0, 0.025	Increased urinary N-acetyl-β-D-glucosaminidase in a group of chloralkali workers. Controls were age-matched. BML: 15.6 µg/L in blood	Barregard et al. 1988
Human/ 60 M exposed, 60 M controls	$13.7^{\pm}$ 5.5 yr (occup)	NS	No evidence of glomerular or tubular damage (effect on urinary albumin or N-acetyl-β-glucosaminidase activity) in chloralkali workers compared to controls. NOAEL of 25 mg/m^3 based on Roels et al. (1987) conversion factor. Limitation: Exposure data limited BML: 14 µg/L in blood; 17 µg/L in urine	Pilkivi and Ruokonen 1989

Only one study was found of kidney effects in animals from exposure to elemental mercury vapor (Ashe et al. 1953). The observed effects supported the human data, with kidney effects ranging from moderate unspecified pathological changes at shorter durations to necrosis and cellular degeneration at longer durations. Limited quantitative data were reported.

Table 3-9
Renal Toxicity of Elemental Mercury in Animals: Inhalation Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Rabbit/strain NS/14 (sex NS)	1-30 hr	28.8	Kidney pathology correlated with exposure duration, ranging from moderate changes at 1 hour to widespread necrosis at 30 hours. Limitations: No control group, limited data reporting BML Range: 20-5,320 µg/L in blood	Ashe et al. 1953

3.1.3.4 Respiratory

Respiratory toxicity in humans following exposure to elemental mercury vapors has been characterized by pulmonary edema and congestion, coughing, interstitial pneumonitis, respiratory failure and absence of air in lungs at time of histopathological examination (Bluhm et al. 1992a; Hallee 1969; McFarland and Reigel 1978; Milne et al. 1970; Snodgrass et al. 1981; Taueg et al. 1992). One case of occupational exposure to elemental mercury vapor occurred due to a faulty thermostat that heated to 450°F and vaporized the mercury it contained. Signs included cough, chest pains, reduced vital capacity and pneumonitis, which began within hours of the onset of exposure (McFarland and Reigel 1978). Accidental exposure to elemental mercury vapors in private homes has led to interstitial pneumonia, dyspnea, lung disease and respiratory failure (Hallee 1969; Snodgrass et al. 1981; Taueg et al. 1992). In each case, signs of toxicity persisted for days to months following acute exposure. No studies were identified regarding respiratory effects in humans following intermediate or chronic exposures to elemental mercury vapor.

Table 3-10
Respiratory Toxicity of Elemental Mercury in Humans: Case Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/1 M, 1 F (adults)	<12 hr	NS	Dyspnea; interstitial pneumonia; fibrosis; moderate restrictive lung disease Limitation: Case study BML Range: 191-557 µg/24 hr urine	Hallee 1969
Human/4 M	2.5-5 hr (occup)	1.1-1.7 (est.)	Cough; chest tightness occurred following an accidental exposure of electrochemical industry workers Limitation: Case study BML Range: 100-130 µg/L in urine 10-14 days postexposure	Milne et al. 1970

Table 3-10 (continued)
Respiratory Toxicity of Elemental Mercury in Humans: Case Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/6 M	<8 hr	44.3 (est.)	Pneumonitis; cough; chest pain Limitations: Case study; limited data reporting BML Range: 1,060-3,280 µg/24 hr urine	McFarland and Reigel 1978
Human/2 M, 2 F (adults)	3 d	NS	Cough; dyspnea Limitation: Case study BML: 82-5700 µg/24 hr urine	Snodgrass et al. 1981
Human/1 M	~2 hr	NS	Reduced vital capacity and dynamic lung volumes, shortness of breath Limitation: Case study BML: 1,900 µg/L urine on first day	Lilis et al. 1985
Human/17 M	<16 hr	NS	Congestion; dyspnea; lung infiltrates in up to 15/17 welders interviewed following accidental exposure Limitation: Limited data reporting of effects or exposure BML: ~60 µg/L in blood 20 d postexposure	Bluhm et al. 1992a
Human/2 M, 2 F (adults)	~24 hr	NS	Adult respiratory distress syndrome; respiratory failure BML Range: 4.6-219 µg/L in urine	Taueg et al. 1992

Rats exposed to 27 mg Hg/m^3 as elemental mercury vapor for one hour exhibited dyspnea, and exposure for two hours resulted in death by asphyxiation (Livardjani et al. 1991). Histopathological analyses revealed necrosis of the alveolar membrane, presence of hyaline membranes and evidence of pulmonary edema. Acute-duration studies with rabbits revealed degeneration and necrosis of the lungs (Ashe et al. 1953). Gage (1961) reported congestion and necrosis of the lungs following intermediate-duration exposure to elemental mercury vapor at a concentration of 1 mg Hg/m^3.

Table 3-11
Respiratory Toxicity of Elemental Mercury in Animals: Inhalation Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Rat/Wistar/ 6 F	7 wk 100 hr/wk 5 d/wk	1	Congestion; necrosis of lung Limitation: Limited data reporting BML: 10 µg/rat in lungs	Gage 1961
Rat/Wistar/64 M/ duration	1 or 2 hr	0, 27	Death by asphyxiation; lung edema; hyaline membranes; necrosis of alveolar epithelium BML Range: 391-4,558 µg/L in blood	Livardjani et al. 1991
Rabbit/strain NS/14 (sex NS)	1-30 hr	28.8	Pathology correlated with exposure duration and ranged from mild changes at 1 hour to marked cellular degeneration and necrosis at 30 hours. In another study, Ashe reported no respiratory damage in rats exposed to 0.1 mg/m3 for 72 weeks. BML Range: 20-5,320 µg/L in blood	Ashe et al. 1953

3.1.3.5 Cardiovascular

Signs of cardiovascular toxicity in humans after acute exposure to elemental mercury include tachycardia, elevated blood pressure and heart palpitations (Bluhm et al. 1992a; Snodgrass et al. 1981; Soni et al. 1992). Intermediate-duration exposure to elemental mercury vapors produced similar effects (i.e., tachycardia and elevated blood pressure) (Fagala and Wigg 1992; Foulds et al. 1987). Barregard et al. (1990) performed a study on chloralkali workers and showed that they had an increased risk of ischemic heart disease and cerebrovascular disease. These workers, however, were exposed to other chemicals and to magnetic fields which may have affected the results. Piikivi (1989) demonstrated a positive correlation between heart palpitations and urinary mercury concentrations in workers from a chloralkali plant. It is unclear from the available scientific literature, however, whether the effects on cardiovascular function (e.g., tachycardia, elevated blood pressure) are due to direct cardiac toxicity or to indirect toxicity (e.g., due to effects on neural control of cardiac function) of elemental mercury.

Table 3-12
Cardiovascular Toxicity of Elemental Mercury in Humans: Case Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/2 M, 2 F (adults)	3 d	NS	Elevated blood pressure; tachycardia Limitations: Case study; limited exposure data BML Range: 82-5,700 µg/24 hr urine	Snodgrass et al. 1981
Human/1 F (child)	2 mo	NS	Elevated blood pressure; tachycardia Limitations: Case study; limited exposure data BML Range: 214-296 µg/L in 24 hr urine	Foulds et al. 1987
Human/17 M	<16 hr	NS	Palpitations in 5/17 welders interviewed following accidental exposure Limitation: Exposure data limited BML: ~60 µg/L in blood 20 d postexposure	Bluhm et al. 1992a
Human/1 F (child)	6 mo	NS	Elevated blood pressure; tachycardia Limitations: Case study; limited exposure data BML: 686 µg/24 hr urine	Fagala and Wigg 1992
Human/1 M (3-yr old)	<6 hr	NS	Tachycardia Limitations: Case study; limited exposure data BML not reported	Soni et al. 1992

Table 3-13
Cardiovascular Toxicity of Elemental Mercury in Humans: Epidemiological Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/41 M exposed/41 M controls	16 yr (avg) 5-27 yr (occup)	0.03 (est.)	Palpitations in chloralkali workers ($p<0.05$); no significant effect on cardiovascular reflex responses compared to matched controls. BML Range: 3.5-52.5 µg/L in urine; avg 19.3 µg/L in urine	Piikivi 1989
Human/ 26 M	10 yr (avg) (occup)	Avg samples: 0.025-0.050	Increased mortality due to ischemic heart and cerebrovascular disease in chloralkali workers, compared to matched controls. Limitation: Possible confounding due to shift work BML: Decrease from 200 µg/L in urine in 1950's to <50 µg/L in 1990	Barregard et al. 1990

Few animal studies were located regarding cardiovascular effects after exposure to elemental mercury vapor. Studies in rabbits report unspecified cellular degeneration and necrosis of the cardiovascular system following both acute and intermediate exposure (Ashe et al. 1953). Ashe et al. (1953), however, concluded that the concentration of mercury is a better indicator of cardiovascular toxicity than the duration of exposure, especially at lower exposure levels.

Table 3-14
Cardiovascular Toxicity of Elemental Mercury in Animals: Inhalation Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Rabbit/strain NS/14 (sex NS)	1-30 hr	28.8	Pathology correlated with exposure duration and ranged from mild changes in the heart at 1 hour to marked cellular degeneration and necrosis at ≥12 hours. Limitations: No controls; limited data reporting; only one dose level tested BML Range: 20-5,320 µg/L in blood	Ashe et al. 1953
Rabbit/strain NS/16 (sex NS)	1-11 wk 5 d/wk 7 hr/d	6	Mild to moderate pathological changes of the heart. Pathological changes observed in subchronic studies were correlated with the exposure concentration but not exposure duration. Limitations: No controls; limited data reporting; only one dose level tested BML Range: 70-3,000 µg/L in blood	Ashe et al. 1953
Rabbit/strain NS/31 (sex NS)	12 wk 5 d/wk 7 hr/d	0.86	Most animals had mild heart pathology, but 2 animals each at 6 and 7 weeks had marked cellular degeneration and necrosis, with focal fibrosis. Limitations: Limited data reporting; only one dose level BML Range: 50-620 µg/L blood	Ashe et al. 1953

3.1.3.6 Gastrointestinal

Gastrointestinal effects have been reported by persons exposed to elemental mercury vapor. The most common sign of mercury poisoning is stomatitis (inflammation of the oral mucosa), which is usually reported following acute, high concentration exposure to elemental mercury vapors (Bluhm et al. 1992a; Snodgrass et al. 1981). Sexton et al. (1978), however, reported signs of bleeding gingiva in 12 people exposed to mercury vapors for two months after metallic mercury was spilled in two homes, and Schwartz et al. (1992) reported bleeding gums in a child exposed to mercury vapors for two to four weeks. In addition, Vroom and Greer (1972) documented mercury intoxication in nine workers at a thermometer manufacturing plant; the workers complained of sore gums and lesions on the oral mucosa after long-term exposure. Other commonly reported gastrointestinal effects include nausea, vomiting, diarrhea and abdominal cramps (Bluhm et al. 1992a; Campbell 1948; Lilis et al. 1985; Sexton et al. 1978; Snodgrass et al. 1981; Vroom and Greer 1972).

Table 3-15
Gastrointestinal Toxicity of Elemental Mercury in Humans: Case Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/1 F (4-month old)	5 hr	NS	Difficulty swallowing; abdominal pain; necrosis of stomach mucosa and duodenum Limitations: Case study; exposure data limited BML not reported	Campbell 1948
Human/3 M, 6 F	NS (occup)	NS	Sore gums; diarrhea in thermometer manufacturing employees Limitation: Exposure data limited BML Range: 4-1,101 µg/24 hr urine	Vroom and Greer 1972
Human/5 M, 6 F (adults and children)/12 controls (sex NS)	51-176 d	0.1-1.0	Nausea, vomiting, abdominal pain, anorexia, diarrhea, bleeding gingiva more common than in controls Limitations: Small sample size; no statistical analysis BML avg: 3.7 µg/L in urine; BML Range: 183-620 µg/L in blood	Sexton et al. 1978
Human/2 M, 2 F (adults)	3 days	NS	Nausea; vomiting; swelling of gums Limitation: Case study BML Range: 13-5,700 µg/24 hr urine	Snodgrass et al. 1981
Human/1 M	~2 hr	NS	Nausea; vomiting Limitations: Case study; limited reporting of symptoms; exposure data limited BML Range: 900-1,900 µg/L in urine (over 3 days)	Lilis et al. 1985
Human/17 M	<16 hr	NS	Diarrhea; cramps in up to 11/17 welders accidentally exposed Limitations: Case study; exposure data limited BML not reported	Bluhm et al. 1992a

Very little information is available concerning gastrointestinal toxicity after exposure to elemental mercury vapors. Ashe et al. (1953) exposed rabbits to mercury vapors for 1–30 hours at a concentration of 28.8 mg Hg/m^3 and found unspecified cellular degeneration and necrosis. When rabbits were exposed to 6 mg Hg/m^3 for 1–11 weeks, changes in the colon were seen during histopathological analysis (Ashe et al. 1953).

Table 3-16
Gastrointestinal Toxicity of Elemental Mercury in Animals: Inhalation Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Rabbit/strain NS/14 (sex NS)	1-30 hr	28.8	Earliest effect on colon (mild pathological changes) occurred at 2 hr; marked cellular degeneration and necrosis observed at 30 hr Limitations: No controls; limited data reporting BML Range: 20-5,320 µg/L in blood	Ashe et al. 1953
Rabbit/strain NS/16 (sex NS)	1-11 wk 5 d/wk 7 hr/d	6.0	No effects or mild histopathological changes in colon Limitations: No controls; limited data reporting BML Range: 70-3,600 µg/L blood	Ashe et al. 1953

3.1.3.7 Hepatic

Biochemical changes in hepatic enzymes were noted in a child who was exposed for approximately one day to an unspecified concentration of elemental mercury vapors (Jaffe et al. 1983). Serum glutamic-pyruvic transaminase (SGPT) and bilirubin levels were elevated, and synthesis of hepatic coagulation factors was reduced. No human studies were identified regarding the hepatic toxicity of mercury following intermediate or chronic exposures to elemental mercury vapors.

Table 3-17
Hepatic Toxicity of Elemental Mercury in Humans: Case Study

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/1 F (8-month old)	~1 d	NS	Elevated serum alanine amino-transferase and bilirubin Limitations: Case study; exposure data limited BML: 16 µg/24 hr urine	Jaffe et al. 1983

Ashe et al. (1953) performed histopathological analyses on rabbits after exposing them for one to 30 hours or for one to 11 weeks to elemental mercury vapors. The analyses revealed necrosis and cellular degeneration of the liver. No other animal studies were identified regarding the hepatic toxicity of mercury vapors following inhalation exposure.

Table 3-18
Hepatic Toxicity of Elemental Mercury in Animals: Inhalation Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Rabbit/strain NS/14 (sex NS)	1-30 hr	28.8	Pathology correlated with exposure duration. Moderate changes first occurred at 2 hr and widespread necrosis at 30 hr Limitations: No controls; limited data reporting BML Range: 20-5,320 µg/L in blood	Ashe et al. 1953
Rabbit/strain NS/16 (sex NS)	1-11 wk 5 d/wk 7 hr/d	6.0	Pathology was somewhat correlated with exposure duration and ranged from mild to marked cellular degeneration with necrosis. Limitations: No controls; limited data reporting BML Range: 70-3,600 µg/L blood	Ashe et al. 1953

3.1.3.8 Hematological

After acute-duration exposure to high concentrations of elemental mercury vapor, onset of "metal fume fever" may occur; this syndrome is characterized by leukocytosis with fever, chills and fatigue (Campbell 1948; Haddad and Stenberg 1963; Jaffe et al. 1983). Intermediate-duration exposure to mercury vapors led to an elevated white blood cell count in a 12-year-old female after exposure for six months (Fagala and Wigg 1992). Volunteers with dental amalgams had significantly decreased hemoglobin and hematocrit compared to controls without dental amalgams (Siblerud 1990).

Table 3-19
Hematological Toxicity of Elemental Mercury in Humans: Case Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/1 F (child)	6 mo	NS	Elevated white cell count Limitations: Case study; exposure data limited; skin lesions could have led to elevated count BML: 686 µg/24 hr urine	Fagala and Wigg 1992
Human/1 M (3.5 yr old)	2-4 wk	NS	Thrombocytopenia Limitation: Exposure data limited BML: 151 µg/L in blood	Schwartz et al. 1992

Table 3-20
Hematological Toxicity of Elemental Mercury in Humans: Epidemiological Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/47 (sex NS)	NS (occup)	<0.1	Decreased γ-aminolevulinic acid dehydratase and cholinesterase activity in erythrocytes, effects were significantly ($p<0.01$) correlated to urinary mercury BML Range: 2-472 µg/g of creatinine in urine.	Wada et al. 1969
Human/41 M exposed/55 controls	NS (occup)	Range: 0.106-0.783	Increased α2-macroglobulin and ceruloplasmin in mercury plant workers compared to unexposed controls ($p<0.001$) BML Range: 29-545 µg/L in urine	Bencko et al. 1990
Human/20 M, 30 F exposed/21 M, 30 F control	NS	NS	Subjects with amalgams had decreased ($p<0.02$) mean hemoglobin (14.66±1.09 g/dL in subjects vs. 14.88±1.14 g/dL in controls) and mean hematocrit (43.15±3.66% in subjects vs. 43.91±3.61% in controls). These reductions were significantly ($p<0.01$) correlated with increasing urine mercury in the subjects with amalgam. Limitations: Subjects identified through newspaper ads may have introduced self-selection bias; only mean data reported. BML avg: 3.7 µg/L in urine	Siblerud 1990

No animal studies were identified regarding the hematological toxicity of mercury vapors following inhalation exposure.

3.1.3.9 Immunological

The available evidence suggests that the immune reaction to elemental mercury exposure is idiosyncratic, with either increases or decreases in immune activity depending on genetic predisposition. Although there is evidence for an overall suppression of the humoral immune response among exposed workers (Moszczynski et al. 1990), this effect has not been consistently observed (Bencko et al. 1990; Langworth et al. 1992b). The failure to observe consistent decreases in antibody content of the serum may be due to small numbers of workers in each group who develop an autoimmune reaction upon exposure to mercury. For example, small numbers of workers exposed to elemental mercury vapors have had elevated levels of antiglomerular basement membrane and anti-DNA antibodies (Cardenas et al. 1993; Langworth et al. 1992b) or granular deposition of IgG and complement C3 in the renal glomeruli (Tubbs et al. 1982).

Table 3-21
Immunotoxicity of Elemental Mercury in Humans: Case Study

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/2 M	NS (occup)	<0.1	Deposition of IgG and C3 in glomeruli of chemical plant workers Limitation: Case study BML Range: 174-548 µg/24 hr urine	Tubbs et al. 1982

Table 3-22
Immunotoxicity of Elemental Mercury in Humans: Epidemiological Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/41 M exposed/55 controls	NS (occup)	Range: 0.106-0.783	Decreased IgG; increased IgA and IgM in mercury plant workers ($p<0.05$) BML Range: 29-545 µg/L in urine	Bencko et al. 1990
Human/50 exposed/50 controls	1.5-25 yr Avg: 11 yr (occup)	NS	Abnormally high anti-DNA antibody titre ($p<0.01$) Mean BML: 31.9 µg/L in urine	Cardenas et al. 1993

An autoimmune response to mercury has been produced in a susceptible strain of rats (Brown Norway) exposed to mercury vapor (Hua et al. 1993). In these rats, increased levels of serum IgE and antilaminin autoantibodies, deposition of IgG deposits in the renal glomeruli and proteinuria were observed.

Table 3-23
Immunotoxicity of Elemental Mercury in Animals: Inhalation Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Rat/BN/3-4 M, 3-4 F	5 wk 6 or 24 hr/d	0, 1	Increased serum IgE; anti-laminin autoantibody titre, IgG deposits along glomerular capillary walls ($p<0.001$) Mean BML: 90.3 µg/L in blood	Hua et al. 1993

3.1.3.10 Dermal

Exposure to elemental mercury vapors for acute or intermediate durations may elicit a response known as acrodynia or "pink disease", which is characterized by peeling palms of hands and soles of feet, excessive perspiration, itching, rash, joint pain and weakness, elevated blood pressure and tachycardia (Fagala and Wigg 1992; Karpathios et al 1991; Schwartz et al 1992). Children seem to be the most susceptible to acrodynia, although adults may be affected to a lesser degree (Warkany and Hubbard 1953). One man experienced a rash and stomatitis after inhalation exposure to mercury when repairing a cell in a chloralkali plant (Bluhm et al. 1992a); however, dermal exposure may have also occurred.

Table 3-24
Dermal Toxicity of Elemental Mercury in Humans: Case Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/1 M (child)	2 wk	NS	Red palms and soles; perspiration; rash Limitations: Case study; concomitant dermal exposure possible; exposure data limited BML: 130 µg/24 hr urine	Karpathios et al. 1991
Human/17 M	<16 hr	NS	Conjunctivitis; dermatitis in 8/17 welders exposed in an accident Limitation: Exposure data limited BML not reported	Bluhm et al. 1992a
Human/1 F (child)	6 mo	NS	Peeling skin on palms and soles Limitations: Case study; exposure data limited BML: 686 µg/24 hr urine	Fagala and Wigg 1992
Human/1 M (3-yr old)	2-4 wk	NS	Maculopapular whole body rash Limitations: Case study; exposure data limited BML: 151 µg/L in blood	Schwartz et al. 1992

No animal studies were identified regarding the dermal toxicity of mercury vapors following inhalation exposure.

3.1.3.11 Developmental

Although few reports have addressed the effects of maternal exposure to elemental mercury vapor on the developing fetus, the available information suggests that maternal exposure to sufficiently high concentrations of elemental mercury vapor may adversely affect the developing fetus. A study of the pregnancies of Polish dental professionals showed a high frequency of malformations of a nonspecified nature (Sikorski et al. 1987). In contrast, a study of Swedish dental professionals found no increases in malformations, abortions, or stillbirths (Ericson and Kallen 1989). An increase in low birth weight infants was noted in the offspring of female dental nurses (Ericson and Kallen 1989); however, in this same study similar effects were not observed for either dentists or dental technicians, and socioeconomic factors may have contributed to the effects observed. It is unknown to what extent discrepancies in the results of the above studies are attributable to differences in mercury exposure levels (only the study by Sikorski et al. (1987) attempted to assess exposure levels) or to other confounders.

Table 3-25
Developmental Toxicity of Elemental Mercury in Humans: Case Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/1 F	8 mo (occup)	NS	Infant death at birth; fetal hepatomegaly; spontaneous abortion in 2 successive pregnancies of a thermometer-manufacturing worker. Maternal toxicity included tremors, motor incoordination, hyperreflexivity, stomatitis. Limitations: Case study; exposure data limited; maternal toxicity also occurred. BML not reported	Derobert et al. 1950
Human/1 F	2 yr (occup)	NS	Delivery of viable infant at term to thermometer factory worker with mild peripheral neuropathy attributed to mercury. Maternal toxicity included slight decrease in sensory reflexes. Limitations: Case study; exposure data limited; no neurological assessment of infant; slight maternal toxicity also reported. BML: Mother: 875 µg/L in urine; Offspring: 2.5 µg/L in urine	Melkonian and Baker 1988
Human/1 F	≈17 wk	0.02-0.06	Delivery of normal child who met all developmental milestones. No maternal toxicity reported. Limitation: Case study; no psychodevelopmental testing BML: Mother: 230 µg/L in 24 hr urine at 17 wk, then declined; Offspring: 3,000 µg/g in hair	Thorp et al. 1992

The few animal studies that were identified indicate that inhalation of elemental mercury vapor may be toxic to the developing animal. In an abstract, Steffek et al. (1987) reported decreased fetal weight in offspring of rats exposed to elemental mercury vapor during gestation. Increased fetal and postnatal deaths were also reported by Baranski and Szymczyk (1973) among rats exposed to elemental mercury vapor for three weeks prior to mating and then again on gestation days 1–20, and increased resorptions in rats exposed on gestation days 10–15 or 1–20.

Pregnant Sprague-Dawley rats (12/group) were exposed on gestation days 11–14 and 17–20 to elemental mercury vapors (1.8 mg/m^3) for one or three hours/day (Danielsson et al. 1993). Litters were culled to 4 males and 4 females. Behavioral testing was done on one male and one female adult from each litter; the authors state that for behavioral testing 8 were tested for each group. There was no difference between controls and treatment groups for maternal weight gain. There was no obvious mercury toxicity in the dams. Offspring exposed *in utero* were no different from controls in the following measures: body weight; clinical signs; pinna unfolding; surface righting reflex development; tooth eruption; and results of a negative geotaxis test at days 7, 8 or 9 *post partum*. At 3 months of age, exposed male but not female rats showed significant decrements in four measures of spontaneous motor activity: locomotion, rearing, rearing time and total activity. By 14 months, the high-dose animals showed hyperactivity in the same test. Females were not evaluated in other adult behavioral tests. A test for habituation to novel environment at 7 months of age showed significant differences between controls and treated males on four measures. At 4 months, mercury-treated males had significantly higher latency in a maze learning test; at 15 months, there was no difference between controls and treated rats in a circular swim maze test.

Table 3-26
Developmental Toxicity of Elemental Mercury in Humans: Epidemiological Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/ 349 F exposed, 215 F controls	NS (occup)	NS	Rates of pregnancy and labor complications were high among women exposed to elemental mercury. Insufficient detail provided to evaluate dose-response relationship. Limitation: Lack of exposure or effect data BML not reported	Mishinova et al. 1980
Human/57 F	0.5-27 yr (occup)	NS	In a study of 57 dental professionals (117 pregnancies), reproductive failure (spontaneous abortion, stillbirth or congenital malformation--not described further) was higher than among unexposed controls, and the effect correlated with exposure level (p=0.004). No maternal toxicity reported. Limitations: Small study group; control group not described; exposure data limited BML avg: 0.527 µg/g in scalp hair	Sikorski et al. 1987
Human/8157 F exposed	NS (occup)	NS	Study of infants born to dental workers, compared with the general population. Based on medical registry, no increase in malformations, abortions, or stillbirths. Increased incidence of low birth weight infants among offspring of dental assistants (risk ratio 1.2, 95% C.I. 1.0-1.3), but the risk ratio was decreased for dentists, suggesting a socioeconomic effect. Case-control study of infants with neural tube defects found none born to dentists, but the expected number was only 0.5. No maternal toxicity reported. Limitation: Exposure data limited BML not reported.	Ericson and Kallen 1989

Early postnatal exposure (during a period of rapid brain growth) resulted in subtle behavioral changes when the rats were tested as young adults (Fredriksson et al. 1992). Eight litters/group, culled to 8 individuals, were exposed to 0.05 mg/m^3 for either 1 or 4 hr/day. Exposure was on days 11–17 of age. There were no signs of overt toxicity or changes in body weight. Spontaneous motor activity was evaluated at 2 and 4 months. The high-dose group showed increased rearing at the early test, but the repeat test indicated hypoactivity. The low-dose group was no different from controls at two months; at four months this group showed increased total activity and decreased rearing. In the spatial learning test administered at 6 months, low- dose rats had increased time to complete the task. High-dose animals were observed to have increases in time to complete the task and in numbers of errors. No information was given on the number of males and females tested or on any differences in behavior dependent on gender.

Table 3-27
Developmental Toxicity of Elemental Mercury in Animals: Inhalation Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Rat/Strain NS/23-24 F	Group I: 6 hr/d, 6-8 weeks before fertilization: Group II: 3 wk before mating and Gd 7-20	0, 2.5	Group I: Decreased number of live pups ($p<0.05$); decreased relative kidney ($p<0.01$) and liver weights ($p<0.05$) and increased ovaries ($p<0.05$) in 2-month-old pups. Group II: Mean number of live fetuses lower than in controls Limitations: Wide range in actual mercury concentration (0.5-4.8 mg/m^3); only one level tested; maternal toxicity BML not reported	Baranski and Szymczyk 1973
Rat/Sprague-Dawley/NS F	6 or 20d 24 hr/d Gd 10-15 or Gd 1-20	0, 0.1, 0.5, 1.0	Increased resorptions (LOAEL = 0.5 for Gd 10-15 and 1.0 for Gd 1-20); decreased maternal and fetal weights in group exposed to 1.0 on Gd 1-20. Limitations: Reported only as an abstract; limited study details; maternal toxicity BML not reported	Steffek et al. 1987
Rat/Sprague Dawley/ 4M, 4F	1 or 3 hr/day on Gd 11-14 plus 17-20	0, 1.8 mg/m^3	Hypoactivity at 3 months; hyperactivity at 14 months; decrement in habituation to novel environment at 7 months; retarded learning in radial arm maze at 4 months but no difference from controls in circular swim maze at 15 months. BML ranges for control through high dose group (mg Hg/kg in organs): 0.001-0.012 (brain); 0.004-0.112 (liver); 0.002-0.068 (kidney). Limitations: Limited testing of female offspring; no evaluation of differences between males and females; small numbers of rats/group.	Danielsson et al. 1993
Rat/Sprague-Dawley/8 F	7 d 1 or 4 hr/d on post-partum days 11-17	0, 0.05	Impaired spatial learning at 6 months ($p \leq 0.01$); increased locomotor activity in objective test ($p \leq 0.01$) BML Range: 0.017-0.063 µg/g in brain Limitations: No information on gender-specific behavioral effects; small number of animals/group.	Fredriksson et al. 1992
Squirrel monkey/10M, 1F	4-7 hr/day, 5 d/week, during gestation	0.5 or 1.0 (1304 to 4305 μg total)	Instability in lever-press durations and steady-state performance under concurrent schedules; aberrant transitions in treated animals. Five male monkeys born at same time served as controls; there were 5 treated M and one treated F. Maternal BML ranges 0.025 to 0.18 μg/g.	Newland et al. 1996.

3.1.3.12 Reproductive

Most studies that have examined the effects of occupational exposure to elemental mercury vapor on reproductive function have failed to find evidence of adverse effects (Alcser et al. 1989; Brodsky et al. 1985; Erfurth et al. 1990; Ericson and Kallen 1988; Heidam 1984; Lauwerys et al. 1985; McGregor and Mason 1991). A few studies have shown at least suggestive evidence that elemental mercury exposure may adversely affect reproductive function. In females exposed occupationally to metallic mercury vapor, a correlation was observed between scalp hair mercury and reproductive failure

or menstrual abnormalities (Sikorski et al. 1987). An increased incidence of pregnancy complications such as toxicosis or prolonged or hemorrhagic parturition was observed in exposed females when compared to unexposed controls (Mishonova et al. 1980). A slightly increased incidence of menstrual disorders in exposed females was reported by DeRosis et al. (1985); however, the statistical significance of this finding was not presented. No evidence for an effect on fertility was observed in exposed males, but one study of wives of exposed workers found an increased rate of spontaneous abortions (Cordier et al. 1991). It is possible that the wives were exposed to mercury as the result of handling contaminated clothing. None of the above studies presented information on exposure levels, and few presented biomonitoring data. Thus, it is difficult to compare findings in the various studies.

Table 3-28
Reproductive Toxicity of Elemental Mercury in Humans: Epidemiological Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/728 F exposed/1034 F controls	NS (occup)	NS	No increase in rate of spontaneous abortions in gardeners, dental assistants, painters, and factory workers. BML not reported	Heidam 1984
Human/29,514 M, 30,272 F	NS (occup)	NS	No correlation between mercury exposure (low and high) and rate of spontaneous abortions in dentists, dental assistants, or their wives. BML not reported	Brodsky et al. 1985
Human/153 F exposed/193 F controls	<5-17 yr (occup)	<0.01 TWA at study; >0.05 for 4 yr	Slightly increased prevalence of menstrual disorders in mercury lamp manufacturers, compared with workers subject to similar stresses but not exposed to mercury. Limitations: Subjective measures; no statistical analysis BML not reported	DeRosis et al. 1985
Human/103 M exposed/101 M controls	Avg: 5.9 yr (1–25 yr) (occup)	NS	No effect of paternal exposure on fertility of chloralkali, amalgam or electrical equipment workers, compared to controls with similar workloads. Avg BML: 52.4 µg/g creatinine in urine (range 5.1-272)	Lauwerys et al. 1985
Human/57 F exposed	0.5-27 yr (occup)	NS	In a study of 57 dental professionals (117 pregnancies), reproductive failure (spontaneous abortion, stillbirth or congenital malformation) was higher than among unexposed controls, and the effect was correlated with exposure level (extrapolated from hair Hg levels) ($p=0.004$). Irregular, painful, or hemorrhagic menses was correlated with exposure duration ($p=0.005$). Limitations: Small study size; control group not described; exposure data limited Avg BML: 0.527 µg/g in scalp hair	Sikorski et al. 1987
Human/8157 F	NS (occup)	NS	Based on medical registry, there was no increase in spontaneous abortions or stillbirths in pregnancies of dental professionals, compared to the general population. BML not reported	Ericson and Kallen 1988
Human/247 M exposed/255 M controls	4 mo–8 yr (occup)	NS	No association between paternal exposure and rate of miscarriages in Department of Energy plant workers. Limitation: Potential recall bias BML: reported only as value integrated over time	Alcser et al. 1989

Table 3-28 (continued)
Reproductive Toxicity of Elemental Mercury in Humans: Epidemiological Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/20 M exposed/21 M controls	2–18 yr (occup)	NS	No correlation between blood or urinary mercury, and male gonadotropic hormones of chloralkali workers, other industrially-exposed workers, or dentists and matched controls. BML: Avg. 46 µg/g creatinine in urine (workers); 2.3 µg/g creatinine (dentists)	Erfuth et al. 1990
Human/152 F exposed/374 F controls	NS (occup)	NS	Increased spontaneous abortions in women whose husbands were exposed to mercury vapors in chloralkali plants (rate doubled above 50 µg/L in urine; 95% C.I. = 0.99-5.23) Limitation: Exposure data limited Avg BML: 61.9 µg/L in urine (range 26.9–75.9 µg/L)	Cordier et al. 1991
Human/ 40 M exposed/ 63 M controls	2–20 yr (occup)	NS	No correlation between blood or urinary Hg and male gonadotropic hormones in workers from different industries (not specified) Avg BML: 103 µg/g creatinine in urine	McGregor and Mason 1991

In rats exposed to elemental mercury vapor, prolongation of estrous cycles was observed both when compared to either unexposed controls or preexposure rates of cycling (Baranski and Szymczyk 1973).

Table 3-29
Reproductive Toxicity of Elemental Mercury in Animals: Inhalation Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Rat/Strain NS/24 F	3 wk 5 d/wk 6 hr/d and Gd 7-20	2.5	Longer estrous cycles, but the effect was not statistically significant BML not reported	Baranski and Szymczyk 1973

3.1.3.13 Genotoxicity

Cytogenetic monitoring studies in populations exposed occupationally to elemental mercury vapor provide conflicting evidence for a clastogenic effect of elemental mercury. Early studies reported increased frequencies of chromosomal aberrations among exposed workers (Popescu et al. 1979; Verschaeve et al. 1976). These studies, however, were not well-controlled, and the results could not be reproduced in later studies (Mabille et al. 1984; Verschaeve et al. 1979). Popescu et al. (1979) compared two groups of men exposed to elemental mercury vapor (Group I, n=4; Group II, n=18) with an

unexposed group of ten individuals and found a statistically significant increase in incidence of chromosome aberrations in the exposed groups. Verschaeve et al. (1976) found an increase in aneuploidy in lymphocytes of 28 subjects exposed to low concentrations of mercury vapor (by comparison to seven controls), but these results were not repeated in later studies (Verschaeve et al. 1979). Mabille et al. (1984) did not find increases in structural chromosomal aberrations of lymphocytes of exposed workers.

More recently, Barregard et al. (1991) demonstrated a correlation between cumulative mercury exposure and induction of micronuclei among a group of chloralkali workers, suggesting a clastogenic effect. This study did not show significant differences in frequency or size of micronuclei between the exposed group to unexposed controls who were matched for age and smoking habits. Neither did they find a correlation between the induction of micronuclei and current mercury exposure as measured by blood or urine mercury levels. A correlation, however, was observed between cumulative exposure to mercury and micronuclei induction in T-lymphocytes in exposed workers suggesting a genotoxic effect.

Table 3-30
Genotoxicity of Elemental Mercury in Humans

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/ 8 M, 6 F (exposed)/ 3 M, 4 F (control)	NS (occup)	NS	Aneuploidy was significantly ($p<0.001$) increased in subjects exposed due to an unstated occupation or as a result of an accident at a university. Structural aberrations were not increased. Limitations: Small study size; smoking status not reported BML Range: 1-114 µg/L in urine	Verschaeve et al. 1976
Human/4 M exposed/10 controls (sex NS)	9.25 yr (avg) (occup)	Range: 0.15-0.44	Chromosome breaks (excluding gaps) were significantly ($p<0.001$) increased in whole blood cultures taken from chemical plant workers. There was no effect on numerical chromosome aberrations. Limitations: Small sample size; smoking status not reported. BML Range: 142-386 µg/L in urine at study	Popescu et al. 1979
Human/ 28 exposed/ 20 controls (sex NS)	1-11 yr (occup)	<0.05 at time of study	The incidence of structural and numerical chromosome aberrations in exposed chloralkali plant workers did not differ from controls. Eight of the controls were unexposed workers at the same plant and 12 were taken from the general population. Limitations: Small sample size; there were 12 smokers in the exposed group, 4/8 among the internal controls, and an unknown number of smokers in the external controls Avg BML: 35.4 µg/L in urine	Verschaeve et al. 1979
Human/ 22 exposed/ 25 controls (sex NS)	4 yr (avg) (0.3-15.3 yr) (occup)	NS	No increase in structural chromosome aberrations in zinc amalgam or chloralkali workers, compared to unexposed control workers at the same plant. Limitations: Small sample size; there were 15 smokers in the exposed group and 12 among the controls; limited exposure data Avg BML: 30.6 µg/L in blood; range: 7.5-105 µg/L	Mabille et al. 1984

Table 3-30 (continued)
Genotoxicity of Elemental Mercury in Humans

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/26 M	10 yr (avg) (min. 1 yr) (occup)	Avg samples: 0.025-0.050	The frequency of micronuclei in lymphocytes was correlated with cumulative exposure in Swedish chloralkali workers ($p = 0.0035$). There was no significant difference between the frequency in the exposed and control populations. Controls were matched by age; exposed and control groups each had 14 smokers Limitation: Small study size Avg BML: 9.6 µg/L in blood	Barregard et al. 1991

No studies were identified that examined the genotoxicity of elemental mercury in animals following inhalation exposure. Likewise no studies of genotoxic effects of mercury exposure *in vitro* were recovered.

3.2 Inorganic Mercury

Inorganic mercury occurs in numerous forms/compounds; the most common include mercuric chloride ($HgCl_2$), mercurous chloride (Hg_2Cl_2), mercuric oxide (HgO). The tables in this section include a notation in the dose column indicating the specific form of inorganic mercury involved in that study. Oral doses, shown in mg/kg-day, have been converted to mg Hg/kg-day using the method shown in Appendix A.

3.2.1 Critical Noncancer Data

This section describes studies evaluated by U.S. EPA for use in assessing general systemic health risks. Chapter 6 describes the derivation of an oral Reference Dose (RfD) for inorganic mercury based on several studies wherein kidney diseases consequent to immunological effects were observed. For completeness, some of these studies are also presented in tabular form in succeeding sections.

3.2.1.1 Human Data

Singer et al. (1987) studied nerve conduction velocity of the median motor, median sensor and sural nerves in 16 workers exposed to various inorganic mercury compounds (e.g., mercuric oxides and mercurial chlorides) for an average of 7.3 ± 7.1 years and compared to an unexposed control group using t-tests. They found a slowing of nerve conduction velocity in motor, but not sensory, nerves that correlated with increased blood and urine mercury levels and an increased number of neurologic symptoms. The mean mercury levels in the exposed workers were 1.4 μg/L and 10 μg/L for blood and urine, respectively. These urine levels are 2-fold less than those associated with peripheral neurotoxicity in other studies (e.g., Levine et al. 1982). There was considerable variability in the data presented by Singer et al. (1987), however, and the statistical analyses (t-test) were not as rigorous as those employed by Levine et al. (1982) who used linearized regression analysis. Furthermore, the subsections in the Levine et al. (1982) study were asymptomatic at higher urinary levels than those reported to be associated with subjective neurological complaints in the workers studied by Singer et al. (1987). These results, therefore, are not considered to be as reliable as those reported by Levine et al. (1982).

Kazantzis et al. (1962) performed renal biopsies in 2 (out of 4) workers with nephrotic syndrome who had been occupationally exposed to mercuric oxide, mercuric acetate and probably mercury vapors. The authors felt that the nephrotic syndrome seen in 3 of the 4 workers may have been an idiosyncratic reaction since many other workers in a factory survey had similarly high levels of urine mercury without developing proteinuria. This conclusion was strengthened by work in Brown Norway rats indicating a genetic (strain) susceptibility and that similar mercury-induced immune system responses have been seen in affected humans and the susceptible Brown Norway rats (U.S. EPA 1987b).

3.2.1.2 Animal Data

Bernaudin et al. (1981) reported that mercurials administered by inhalation or ingestion to Brown Norway rats resulted in the development of a systemic autoimmune disease. The mercuric chloride ingestion portion of the study involved the forcible feeding of either 0 or 3 mg/kg/week of mercuric chloride to male and female Brown Norway rats for up to 60 days. No abnormalities were reported using standard histological techniques in either experimental or control rats. Immunofluorescence histology revealed that 80% (4/5) of the mercuric-exposed rats were observed with a linear IgG deposition in the glomeruli after 15 days of exposure. After 60 days of mercuric chloride exposure, 100% (5/5) of the rats were seen with a mixed linear and granular pattern of IgG deposition in the glomeruli and granular IgG deposition in the arteries. Weak proteinuria was observed in 60% (3/5) of the rats fed mercuric chloride for 60 days. The control rats were observed to have no deposition of IgG in the glomeruli or arteries as well as normal urine protein concentrations.

Andres (1984) administered mercuric chloride (3 mg per kg of body weight in 1 mL of water) by gavage to five Brown Norway rats and two Lewis rats twice a week for 60 days. A sixth Brown Norway rat was given only 1 mL of water by gavage twice a week for 60 days. All rats had free access to tap water and pellet food. After 2–3 weeks of exposure, the Brown Norway mercuric chloride-treated rats started to lose weight and hair. Two of the mercuric chloride-treated Brown Norway rats died 30–40 days after beginning the study. No rats were observed to develop detectable proteinuria during the 60-day study. The kidneys appeared normal in all animals when evaluated using standard histological techniques, but examination by immuno-fluorescence showed deposits of IgG present in the renal glomeruli of only the mercuric-treated Brown Norway rats. The Brown Norway treated rats were also observed with mercury-induced morphological lesions of the ileum and colon with abnormal deposits of IgA in the basement membranes of the intestinal glands and of IgG in the basement membranes of the lamina propria. All observations in the Lewis rats and the control Brown Norway rat appeared normal.

The only chronic oral study designed to evaluate the toxicity of mercury salts was reported by Fitzhugh et al. (1950). In this study, rats of both sexes (20–24/group) were given 0.5, 2.5, 10, 40 or 160 ppm mercury as mercuric acetate in their food for up to 2 years. Assuming food consumption was equal to 5% body weight per day, the daily intake would have been 0.025, 0.125, 0.50, 2.0 and 8.0 mg Hg/kg for the five groups, respectively. At the highest dose level, a slight depression of body weight was detected in male rats only. The statistical significance of this body weight depression was not stated. Kidney weights were significantly ($p<0.05$) increased at the 2-and 8-mg Hg/kg-day dose levels. Pathologic changes originating in the proximal convoluted tubules of the kidneys were also noted with more severe effects in females than males. The primary weaknesses of this study were the lack of reporting (which adverse effects were observed with which dosing groups) and that the most sensitive strain, the Brown Norway rat, was not used for evaluating the mercury-induced adverse health effects.

NTP (1993) conducted subchronic and chronic gavage toxicity studies on Fischer 344 rats and B6C3F1 mice to evaluate the effects of mercuric chloride, and the kidney appeared to be the major organ

of toxicity. These studies were also summarized by Dieter et al. (1992). In the 6-month study, Fischer 344 rats (10/sex/group) were administered 0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day of mercuric chloride (0.23, 0.46, 0.92, 1.9, and 3.7 mg Hg/kg-day), 5 days/week, by gavage. Survival was not affected, although body weight gains were decreased in males at high dose and in females at or above 0.46 mg Hg/kg-day. Alkaline phosphatase and gamma-glutamyl transferase levels in the urine were significantly elevated in the females exposed to 3.7 mg Hg/kg-day at four and six months of exposure. Absolute and relative kidney weights were significantly increased in both sexes with exposure to at least 0.46 mg Hg/kg-day. The kidney weight changes were slightly dose-related in the females. Histopathology revealed corresponding changes in the kidneys. In males, the incidence of nephropathy was 80% in controls and 100% for all treated groups; however, severity was minimal in the two low-dose groups and minimal to mild in the 0.92-mg Hg/kg-day group and higher. In females, there was a significant increased incidence of nephropathy only at the high-dose group (4/10 with minimal severity). Nephropathy was characterized by foci of tubular regeneration, thickened tubular basement membrane and scattered dilated tubules containing hyaline casts. No treatment-related effects were observed in the other organs; however, histopathology on the other organs was performed only on control and high-dose rats.

B6C3F1 mice (10/sex/group) were administered gavage doses of 0, 1.25, 2.5, 5, 10, or 20 mg/kg-day mercuric chloride (0, 0.92, 1.9, 3.7, 7.4, or 14.8 mg Hg/kg-day) 5 days/week for 6 months (NTP 1993). There was a decrease in body weight gain in males at the highest dose tested. Significant increases occurred in absolute kidney weights of male mice dosed with 3.7 mg Hg/kg-day or more, and relative kidney weights were increased in male mice at the 7.4 and 14.8 mg Hg/kg-day doses. The kidney weight changes corresponded to an increased incidence of cytoplasmic vacuolation of renal tubule epithelium in males exposed to at least 3.7 mg Hg/kg-day. The exposed female mice did not exhibit any histopathologic changes in the kidneys.

In the 2-year NTP study, Fischer 344 rats (60 per sex per group) were administered 0, 2.5, and 5 mg/kg-day mercuric chloride (1.9 and 3.7 mg Hg/kg-day), 5 days a week, by gavage (Dieter et al. 1992; NTP 1993). After two years, survival was significantly reduced in the treated male rats compared to the controls. Mean body weights were significantly decreased in both treated males and females (9–10% and 14–15% decrease from control, respectively). At 15 months, relative kidney weights were significantly elevated (not dose-related) in all treated groups (15–20% increase from control), and relative brain weights were significantly elevated (slightly dose-related) in treated females (13–18%). The increased kidney weights were accompanied by an increase in severity of nephropathy. After two years, there was an increased incidence of nephropathy of moderate-to-marked severity and increased incidence of tubule hyperplasia in the kidneys of exposed males compared to the controls. The control males exhibited nephropathy, primarily of mild-to-moderate severity. Hyperparathyroidism, mineralization of the heart and fibrous osteodystrophy were observed and considered secondary to the renal impairment. There were no significant differences found in renal effects between exposed and control females. Other nonneoplastic effects included an increased incidence of forestomach hyperplasia in the exposed males and high dose females, increased incidence of nasal inflammation at the high-dose animals, slightly increased incidence of acute hepatic necrosis in the high-dose males and increased incidence of inflammation of the cecum in exposed males. Statistical analyses, however, were not performed on these histopathologic changes.

NTP (1993) also administered to B6C3F1 mice (60/sex/group) daily oral gavage doses of 0, 5, or 10 mg/kg mercuric chloride (0, 3.7 and 7.4 mg Hg/kg-day), 5 days a week, by gavage for 2 years. Survival and body weights of mice were slightly lower in mercuric chloride treated mice compared to controls. Absolute kidney weights were significantly increased in the treated males while relative kidney weights were significantly increased in high-dose males and both low- and high-dose females.

Histopathology revealed an increase in the incidence and severity of nephropathy in exposed males (mild severity in low dose and moderate-to-marked severity in high dose) and females (minimal severity in low dose and minimal-to-mild severity in high dose). Nephropathy was defined as foci of proximal convoluted tubules with thickened basement membrane and basophilic cells with scant cytoplasm. Some affected convoluted tubules contained hyaline casts. There was also an increase in nasal cavity inflammation (primarily infiltration of granulocytes in nasal mucosa) in the exposed animals.

In a 4-week oral study (Jonker et al. 1993), Wistar rats (5–10/sex/group) were fed a diet containing 15 and 120 ppm mercuric chloride (0.56 or 4.4 mg Hg/kg-day). A significant increase in relative kidney weight was reported for the low-dose females and high-dose males. There was also an increase in the incidence of high-dose males that had occasional basophilic tubules in the outer cortex of the kidneys. In the range-finding study by Jonker et al. (1993), rats were administered 75, 150, or 300 ppm mercuric chloride (2.8, 5.6, 11.1 mg Hg/kg-day) in the diet for four weeks. A significant increase in the relative kidney weights was observed in both sexes for all dose groups; the effect was dose related. Nephrosis and proteinaceous casts in the kidneys were reported in both sexes at the lowest dose. At 5.6 mg/kg-day, the body weight was significantly decreased in males and serum alkaline phosphatase levels were elevated in females. At 11.1 mg/kg-day, increased serum aspartate aminotransferase (both sexes), decreased urinary density (males), increased relative adrenal weight (males), increased serum sodium and phosphate levels (females) and decrease in body weight (females) were reported.

A series of studies (Boscolo et al. 1989; Carmignani et al. 1989, 1992) reported renal and cardiovascular changes in rats exposed to mercuric chloride in drinking water. These studies were limited due to the small number of animals and dose levels tested. Boscolo et al. (1989) evaluated the renal effects of mercuric chloride in two different rat strains. Male Sprague-Dawley rats (8/group) were administered 0 or 0.05 mg/mL mercury (0 or 7 mg Hg/kg-day), and male Wistar rats (8/group) received 0, 0.05, or 0.2 mg/mL mercury (0, 7, or 28 mg Hg/kg-day) in drinking water for 350 days. Increases in blood pressure and cardiac inotropism, without changes in heart rate, occurred in exposed rats of both strains. Hydropic degeneration and desquamation of the proximal tubular cells were exhibited in kidneys of Sprague-Dawley rats, with alterations and lysis of lysosomes in tubular cells and thickening of the basal membrane in the glomeruli. Wistar rats displayed tubular degeneration and membranous glomerulonephritis in 30% of the glomeruli at 7 mg/kg-day and all glomeruli at 28 mg/kg-day. Thickening of basal membrane and hypercellularity and alteration of the mesangial matrix in the glomeruli and hydropic degeneration of tubules were seen in Wistar rats. Similar findings of renal histopathology alterations and cardiovascular changes were reported by Carmignani et al. (1989) who administered 0 or 0.05 mg/L of mercury (7 mg/kg-day) to male Sprague-Dawley rats (8/group) for 350 days.

In Carmignani et al. (1992), male Sprague-Dawley rats (8/group) received 0 or 0.2 mg/mL of mercury (28 mg Hg/kg-day) as mercuric chloride in drinking water for a shorter duration (180 days). Similar renal changes were observed, as well as IgM deposition in the glomeruli (as shown by immunofluorescence). In addition, the treated group displayed significantly decreased urinary kallikrein and creatinine, decreased plasma renin and increased plasma angiotensin-converting enzyme. The cardiovascular effects were slightly different from Boscolo et al. (1989) and Carmignani et al. (1989); there was an increase in blood pressure but a decrease in cardiac inotropism in the exposed rats. The increase in blood pressure was suggested to be due to a vasoconstrictor effect, likely related to a greater release of noradrenaline from adrenergic neurons and to baroreflex hyposensitivity. The decrease in contractility was attributed to a direct toxic effect of the mercury on the cardiac muscle because of the high levels of mercury detected in the heart. The differences in the results of cardiovascular changes for the studies were not explained.

To evaluate the effect of mercuric chloride on the development of autoimmunity, female SJL/N mice (7/group) received 0.625, 1.25, 2.5, or 5 ppm mercuric chloride (0.07, 0.14, 0.28, or 0.56 mg Hg/kg-day) in drinking water ad libitum for 10 weeks (Hultman and Enestrom 1992). An increase in circulating antinucleolar antibodies was observed at 0.28 mg Hg/kg-day. The high-dose group had elevated granular IgG deposits in the renal mesangium and in vessel walls of glomerular capillaries, arteries and arterioles of the spleen and in intramyocardial arteries. Slight glomerular cell hyperplasia and discrete widening of the centrolobular zone were also exhibited in the 0.56-mg/kg-day group.

Agrawal and Chansouria (1989) administered 0, 2.6, 5.2, and 10.4 mg Hg/kg-day as mercuric chloride in drinking water to male Charles Foster rats for 60, 120, or 180 days (5/group). The relative adrenal gland weight was significantly increased for the dose groups at all durations compared to controls. Significant increases in adrenal and plasma corticosterone levels occurred in all dose groups at 60 and 120 days; however, changes were not seen after 180 days. The authors suggested that mercuric chloride may have acted as a chemical stressor in a dose- and duration-dependent manner. The study was limited because histopathology was not performed on the kidneys, and the adrenal gland was the only tissue evaluated.

Both male and female Brown Norway rats 7–9 weeks of age were divided into groups of 6–20 animals each (Druet et al. 1978). The numbers of each sex were not stated. The animals were injected subcutaneously with mercuric chloride 3 times weekly, for 8 weeks, with doses of 0, 0.07, 0.2, 0.4, 0.7, and 1.5 mg Hg/kg. An additional group was injected with a 0.04 mg/kg for 12 weeks. Antibody formation was measured by the use of kidney cryostat sections stained with a fluoresceinated sheep anti-rat IgG antiserum; urinary protein was assessed by the biuret method. Tubular lesions were seen at the higher dose levels. Proteinuria was seen at doses of 0.07 mg/kg and above, but not at 0.04 mg/kg. Proteinuria was considered a highly deleterious effect in that affected animals developed hypoalbuminemia and many died. Fixation of IgG antiserum was detected in all groups except controls.

3.2.2 Cancer Data

3.2.2.1 Human data

No data are available on the carcinogenic effects of inorganic mercury in humans.

3.2.2.2 Animal data

The results from a dietary study in rats and mice show equivocal evidence for carcinogenic activity in male mice and female rats and some evidence for carcinogenic activity in male rats. Two other dietary studies show negative evidence for carcinogenicity, but these studies are limited by inadequacies in the data and experimental design.

Mercuric chloride was administered by gavage in water at doses of 0, 2.5, or 5 mg/kg-day (0, 1.9 and 3.7 mg Hg/kg-day) to Fischer 344 rats (60/sex/group), 5 days a week, for over 104 weeks (NTP 1993). An interim sacrifice (10/sex/dose) was conducted after 15 months of exposure. Complete histopathological examinations were performed on all animals found dead, killed in extremis, or killed by design. Survival after 24 months was statistically significantly ($p<0.01$) lower in low- and high-dose males; survival was 43%, 17% and 8% in control, low-, and high-dose males, respectively, and 58%, 47%, and 50% in control, low-, and high-dose females, respectively. During the second year of the study, body weight gains of low- and high-dose males were 91% and 85% of controls, respectively, and body weight gains of low- and high-dose females were 90% and 86% of controls, respectively. At study termination, nephropathy was evident in almost all male and female rats including controls, but the

severity was much greater in treated males; the incidence of "marked" nephropathy was 6/50, 29/50, and 29/50 in control, low- and high-dose males, respectively.

Squamous cell papillomas of the forestomach showed a statistically significant ($p<0.001$) positive trend with dose by life table adjusted analysis; the incidences were 0/50, 3/50 and 12/50 in control, low-, and high-dose males, respectively. The incidence in female rats was 0/50, 0/49 and 2/50 in control, low- and high-dose groups, respectively. These neoplasms are rare neoplasms in rats and occurred in only 1 out of 264 historical controls. The incidence of papillary hyperplasia of the stratified squamous epithelium lining of the forestomach was statistically significantly ($p \leq 0.01$) elevated in all dosed males (3/49, 16/50 and 35/50 in control, low- and high-dose males, respectively) and in high-dose females (5/50, 5/49 and 20/50 in control, low- and high-dose females, respectively). The incidence of thyroid follicular cell carcinomas was marginally significantly ($p=0.044$ by logistic regression analysis; tumors not considered to be fatal) increased in high-dose males (1/50, 2/50 and 6/50 in control, low- and high-dose groups, respectively). The data, adjusted for survival, also showed a significant ($p=0.017$) positive trend in males. The combined incidence of thyroid follicular cell neoplasms (adenoma and/or carcinoma), however, was not significantly increased (2/50, 6/50 and 6/50 in control, low- and high-dose males, respectively). In female rats a significant decrease in the incidence of mammary gland fibroadenomas was observed (15/50, 5/48 and 2/50 in control, low- and high-dose females, respectively). Table 3-31 gives the incidences of lesions which were increased in treated animals.

The high mortality in both groups of treated males indicates that the maximally tolerated dose (MTD) was exceeded in these groups and limits the interpretation of the study. NTP (1993) considered the forestomach tumors to be of limited relevance to humans because the tumors did not appear to progress to malignancy. NTP (1993) also questioned the relevance of the thyroid carcinomas because these neoplasms are usually seen in conjunction with increased incidences of hyperplasia and adenomas, but increases in hyperplasia (2/50, 4/50 and 2/50 in control, low- and high-dose males, respectively) or adenomas (1/50, 4/50 and 0/50 in control, low- and high-dose males, respectively) were not observed.

In the same study, mercuric chloride was administered by gavage in water at doses of 0, 5, or 10 mg/kg-day (0, 3.7 and 7.4 mg Hg/kg-day), 5 days a week, for 104 weeks to B6C3F1 mice (60/sex/group) (NTP 1993). An interim sacrifice (10/sex/dose) was conducted after 15 months of exposure. Terminal survival of male mice was not affected by the administration of mercuric chloride; survival of high-dose females was slightly lower ($p=0.051$) than controls (41/60, 35/60 and 31/60 in control, low- and high-dose females, respectively). Body weight gain was not affected. Female mice exhibited a significant increase in the incidence of nephropathy (21/49, 43/50 and 42/50 in control, low- and high-dose females, respectively). Nephropathy was observed in 80–90% of the males in all groups. The severity of nephropathy increased with increasing dose (1.08, 1.74 and 2.51 in control, low- and high-dose males, respectively; 0.47, 1.02 and 1.24 in control, low- and high-dose females, respectively). The incidence of renal tubule hyperplasia was 1/50, 0/50 and 2/49 in control, low- and high-dose males.

Table 3-31
Incidence[a] of Selected Lesions in Rats in the NTP (1993) 2-Year Gavage Study

Tumor Site and Type	Dose Group (mg Hg/kg-day)					
	Males			Females		
	0	1.9	3.7	0	1.9	3.7
Forestomach						
Papillary hyperplasia	3/49	16/50[b]	35/50[b]	5/50	5/49	20/50[b]
Squamous cell papilloma	0/50	3/50	12/50[c]	0/50	0/49	2/50
Thyroid Follicular Cell[d]						
Adenoma	1/50	4/50	0/50	--	--	--
Carcinoma	1/50	2/50	6/50[e]	--	--	--
Adenoma or carcinoma	2/50	6/50	6/50	--	--	--

[a] Overall rate
[b] $p \leq 0.01$
[c] $p \leq 0.001$; trend test also $p<0.001$
[d] Data on thyroid follicular cell lesions were reported for males only.
[e] $p = 0.044$, logistic regression

As shown in Table 3-32, the combined incidence of renal tubule adenomas and adenocarcinomas was 0/50, 0/50 and 3/49 in control, low- and high-dose males, respectively. Although no tumors were seen in the low-dose group, a statistically significant (p=0.032) positive trend for increased incidence with increased dose was observed. These observations were considered important because renal tubule hyperplasia and tumors in mice are rare. The two-year historical incidence of renal tubule adenomas or adenocarcinomas in male mice dosed by gavage with water was 0/205, and only four of the nearly 400 completed NTP studies have shown increased renal tubule neoplasms in mice. NTP did not report a statistical comparison of the study data to historical control data. Analysis of the reported data with Fisher's Exact test, however, showed that the incidence of renal tubule adenomas or adenomas and carcinomas (combined) in the high-dose males was significantly elevated when compared to historical controls (Rice and Knauf 1994).

Table 3-32
Incidence[a] of Renal Tubule Tumors in Male Mice in the NTP (1993) 2-Year Gavage Study

	Dose Group (mg/kg-day)		
	0	5	10
Adenoma	0/50	0/50	2/49
Adenomacarcinoma	0/50	0/50	1/49
Adenoma or adenomacarcinoma	0/50	0/50	3/49[b]

[a] Overall rate
[b] p = 0.107; trend test p = 0.032

A 2-year feeding study in rats (20 or 24/sex/group; strain not specified) was conducted in which mercuric acetate was administered in the diet at doses of 0, 0.5, 2.5, 10, 40, and 160 ppm (0, 0.02, 0.1, 0.4, 1.7, and 6.9 mg Hg/kg-day) (Fitzhugh et al. 1950). Survival was not adversely affected in the study. Increases in kidney weight and renal tubular lesions were observed at the two highest doses. No statement was made in the study regarding carcinogenicity. This study was not intended as a carcinogenicity assay, and the number of animals/dose was rather small. Histopathological analyses were conducted on only 50% of the animals (complete histopathological analyses were conducted on only 31% of the animals examined), and no quantitation of results or statistical analysis was performed.

No increase in tumor incidence was observed in a carcinogenicity study using white Swiss mice (Schroeder and Mitchener 1975). Groups of mice (54/sex/group) were exposed until death to mercuric chloride in drinking water at 5 ppm mercury (0.95 mg Hg/kg-day). No effects on survival or body weights were observed. After dying, mice were weighed, dissected, gross tumors were detected, and some sections were made of the heart, lung, liver, kidney and spleen for microscopic examination. Mercuric chloride was nontoxic in the study. No statistically significant differences were observed in tumor incidences for treated animals and controls. This study is limited because complete histological examinations were not performed, only a single dose was tested, and the MTD was not achieved.

The increasing trend for renal tubular cell tumors in mice observed in the NTP (1993) study is supported by similar findings in mice after chronic dietary exposure to methylmercury (Hirano et al. 1986; Mitsumori et al. 1981, 1990). In these studies, dietary exposure to methylmercuric chloride resulted in increases in renal tubular tumors at doses where substantial nephrotoxicity was observed.

Table 3-33
Carcinogenic Effects of Inorganic Mercury in Animals: Oral Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations//BML	Reference
Rat/strain NS/20–24 M, 20-24 F	2 yr ad lib	0, 0.02, 0.1, 0.4, 1.7, 6.9[a]	No carcinogenicity reported Limitations: small number of animals/dose; complete histopathological examinations conducted on only 31% of animals; no statistical analyses	Fitzhugh et al. 1950
Rat/F344/ 60 M, 60 F	2 yr 5 d/wk 1 x/d (gavage)	0, 1.9, 3.7 ($HgCl_2$)	Thyroid follicular cell carcinomas in males at 3.7. Limitations: MTD exceeded (high mortality in treated males); limited relevance of lesions to humans	NTP 1993
Mouse/Swiss/54 M, 54 F	Lifetime ad lib	0.95 ($HgCl_2$)	No carcinogenicity reported Limitations: Complete histopathological examinations not performed; only single dose level tested; MTD not achieved	Schroeder and Mitchener 1975
Mouse/ B6C3F1/ 60 M, 60 F	2 yr 5 d/wk 1 x/d (gavage)	0, 3.7, 7.4 ($HgCl_2$)	Renal tubule tumors (adenoma or adenomacarcinoma) in 3/49 males at 7.4 (positive trend test, p=0.0032) Limitations: Severe nephropathy also observed in high-dose males.	NTP 1993

[a] Phenylmercuric acetate and mercuric acetate

3.2.3 Other Data

3.2.3.1 Death

The estimated lethal dose of inorganic mercury for a 70 kg adult is 10–42 mg Hg/kg (Gleason et al. 1957). Most deaths attributed to inorganic mercury occur soon after a person ingests a single large amount of mercury. Causes of death include cardiovascular failure, gastrointestinal damage and acute renal failure (Troen et al. 1951).

Table 3-34
Lethality of Inorganic Mercury in Humans: Case Study

Species/ No. per Sex	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Human/25 M, 29 F	Once	21-37 (est.) ($HgC1_2$)	Case studies of mercuric chloride poisonings in victims age 2-60 yr; 9 resulted in death (all adults). BML not reported	Troen et al. 1951

The estimated LD_{50} for rats following oral exposure to mercuric chloride is 25.9 mg Hg/kg; however, LD_{50} levels as high as 77.7 mg Hg/kg have been observed in rats (Kostial et al. 1978). Male rats appear to be more sensitive to the effects of mercuric chloride. This was demonstrated in a chronic-duration oral study with rats, in which 40/50 males and 21/49 females died at the low dose, 45/50 males and 20/50 females died at the high dose, compared to 24/50 males and 15/50 females in the controls (Dieter et al. 1992; NTP 1993). The increase in deaths in the male rats was statistically significant and were considered to be due to renal lesions. Mortality incidence was not significantly increased in exposed female groups.

Table 3-35
Lethality of Inorganic Mercury in Animals: Oral Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Rat/Albino (NS)/6 NS	Once (gavage)	NS (6 levels) ($HgCl_2$)	LD_{50} = 25.8 mg/kg for 2-week old pups; older rats had higher LD_{50} values. Limitation: Incomplete data reporting (i.e., doses not reported, toxic effects not specified) BML not reported	Kostial et al. 1978
Rat/F344/5 M, 5 F	14 d 5 d/wk 1 x/d (gavage)	0, 0.93, 1.9, 3.7, 7.4, 14.8 ($HgCl_2$)	2/5 males died at 14.8; no other animals died. BML: 45.4 µg/g in kidney of males, 43.3 µg/g in kidney of females	Dieter et al. 1992
Rat/F344/ 50 M, 50 F	2 yr 5 d/wk 1 x/d (gavage)	0, 1.9, 3.7 ($HgCl_2$)	40/50 males died at 1.9 mg/kg, vs. 24/50 control males; survival of dosed females was not significantly different from controls. BML not reported	Dieter et al. 1992
Mouse/ $B6C3F_1$/5 M, 5 F	14 d 5 d/wk 1 x/d (gavage)	0, 3.7, 7.4, 14.8, 29, 59 ($HgCl_2$)	9/10 died (LOAEL = 59). Limitations: small number of animals BML not reported	NTP 1993

3.2.3.2 Neurological

Limited studies are available concerning neurological toxicity following oral exposure to inorganic mercury. These studies are summarized below.

Table 3-36
Neurotoxicity of Inorganic Mercury in Humans: Case Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Human/2 F	6–25 yr	0.73 (Hg_2Cl_2)	Dementia, irritability, decreased cerebellar neurons, low brain weight Limitation: Case study BML: 3.4-4.7 µg/g in frontal cortex	Davis et al. 1974
Human/2 M (1 child), 1 adult F	3 mo	NS (Hg_2Cl_2) (HgS)	Drooling, dysphagia, irregular arm movements, impaired gait, convulsions following ingestion of patent medicines containing mercuric sulfide and mercurous chloride Limitation: Case studies; concomitant exposure to other metals; limited exposure data BML: 39-2800 µg/L in 24 hr urine	Kang-Yum and Oransky 1992

There are several animal studies in which inorganic mercury-induced neurotoxicity has been reported.

Table 3-37
Neurotoxicity of Inorganic Mercury in Animals: Oral Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Rat/ Holtzman/ 8 M exposed, 8 M control	11 wk	0, 0.74 ($HgCl_2$)	Weakening of hind legs, crossing reflex of limbs, ataxia; degenerative changes in neurons of dorsal root ganglia and Purkinje and granule cells of cerebellum Limitation: One dose level tested BML not reported	Chang and Hartmann 1972
Rat/Sprague Dawley/12 F exposed, 10 F controls	3 mo ad lib in feed	0, 2.2 ($HgCl_2$)	Inactivity and abnormal gait Limitation: One dose level tested BML not reported	Goldman and Blackburn 1979
Mouse/C57BL 6J/NS	17 mo ad lib in drinking water	0.74 for 110 d, then 7.4–14.8 for 400 d; 2.2 for 17 mo ($HgCl_2$)	No clinical signs of neurotoxicity; no effect on optic or peripheral nerve structure Limitation: Lack of statistical analyses due to insufficient number of animals tested; uncertainty of dosage due to large variation in water consumption BML not reported	Ganser and Kirschner 1985

3.2.3.3 Renal

The kidney appears to be the critical target organ for the effects of acute ingestion of inorganic mercury. Case studies of poisonings by mercuric chloride report acute renal failure, including

proteinuria, oliguria and hematuria, in people ingesting estimated doses of 3.5–37 mg Hg/kg (Afonso and deAlvarez 1960; Pesce et al. 1977; Troen et al. 1951). These effects are attributed to tubular and glomerular pathology.

Table 3-38
Renal Toxicity of Inorganic Mercury in Humans: Case Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Human/25 M, 29 F	Once	3.5-37 (est.) ($HgCl_2$)	Case studies of mercuric chloride poisonings in victims age 2-60 yr; 18 cases resulted in renal effects (albuminuria, anuria) BML not reported	Troen et al. 1951
Human/1 F (adult)	Once (tablet)	30 ($HgCl_2$)	Oliguria; proteinuria; hematuria following ingestion of mercuric chloride Limitation: Case study BML not reported	Afonso and deAlvarez 1960
Human/1 M	Once	21.4 ($HgCl_2$)	Proteinuria indicating glomerular and tubular damage Limitation: Case study BML: Avg 370 µg/L in blood	Pesce et al. 1977

There are numerous animal studies reporting kidney damage in rats and mice ingesting inorganic mercury. Acute exposures result in increased kidney weight with at least 0.46 mg Hg/kg-day and tubular necrosis at higher doses; males appear to have greater sensitivity for the histological changes than females (Fowler 1972; NTP 1993). Similarly, longer-term studies have found histopathologic effects affecting the tubules and glomeruli, including thickening of basement membranes and degeneration of tubular cells (Carmignani et al. 1989; Jonker et al. 1993; NTP 1993). A study monitoring kidney function reported ketonuria and proteinaceous casts (Jonker et al. 1993).

Table 3-39
Renal Toxicity of Inorganic Mercury in Animals: Oral Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Rat/Sprague Dawley/8 M	350 d ad lib in drinking water	0, 7 ($HgCl_2$)	Hydropic degeneration of tubular cells Limitation: Only one dose tested BML: 140 µg/g in kidney	Carmignani et al. 1989

Table 3-39 (continued)
Renal Toxicity of Inorganic Mercury in Animals: Oral Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Rat/Sprague-Dawley/8 M	180 d ad lib in drinking water	0, 28 ($HgCl_2$)	Hydropic degeneration of tubular cells, IgM deposition in glomeruli, decreased urinary kallikrein and creatinine, decreased plasma renin, increased plasma angiotensin-converting enzyme Limitation: Only one dose tested BML: 0.94 µg/g in blood	Carmignani et al. 1992
Rat/Wistar/5 M, 5 F exposed/10 M, 10 F controls	4 wk ad lib in feed	0, 0.56, 4.4 ($HgCl_2$)	Ketonuria in males at 4.4 mg/k-day; increased kidney weight in males and females (LOAEL = 0.56 in females, 4.4 in males) BML not reported	Jonker et al. 1993
Rat/Wistar/ 5 M, 5 F exposed/10 M, 10 F controls	4 wk ad lib in feed	2.8, 5.6, 11.1 ($HgCl_2$)	Ketonuria in males at all levels; increased relative kidney weight, increased nephrosis and proteinaceous casts in males and females (LOAEL = 2.8) BML not reported	Jonker et al. 1993
Rat/F344/ 5 M, 5 F	14 d 5 d/wk 1 x/d (gavage)	0, 0.93, 1.9, 3.7, 7.4, 14.8 ($HgCl_2$)	Increased absolute and relative kidney weight in males and females (LOAEL = 1.9); acute renal tubule necrosis at ≥3.7 mg/kg-day in both sexes BML: 43-46 µg/g in kidney at 14.8 mg/kg	NTP 1993
Rat/F344/ 10 M, 10 F	6 mo 5 d/wk 1 x/d (gavage)	0, 0.23, 0.46, 0.93, 1.8, 3.7 ($HgCl_2$)	Increased absolute and relative kidney weight in males and females (LOAEL = 0.46); increased severity of nephropathy in males (LOAEL = 0.93) BML: 86.2-89.6 µg/g in kidney at 0.93 mg/kg	NTP 1993
Rat/F344/ 60 M, 60 F	2 yr 5 d/wk 1 x/d (gavage)	0, 1.9, 3.7 ($HgCl_2$)	Increased severity of nephropathy in males (thickening of glomerular and tubular basement membranes; degeneration and atrophy of tubule epithelium) (LOAEL = 1.9) BML not reported	NTP 1993
Mouse/NMRI/20 (sex NS) exposed/10 controls	Once (gavage)	0, 5, 10, 20, 40 ($HgCl_2$)	Decreased selenium-dependent glutathione peroxidase activity in kidney; minor renal tubular damage (LOAEL = 10) BML: 260 µg/L in blood at 10 mg/kg	Nielsen et al. 1991
Mouse/NMRI/24 (sex NS)	Once (gavage)	0, 20 ($HgCl_2$)	Necrosis of proximal tubules BML not reported	Nielsen et al. 1991
Mouse/$B6C3F_1$/ 5 M, 5 F	14 d 5 d/wk 1 x/d (gavage)	0, 3.7, 7.4, 14.8, 29, 59 ($HgCl_2$)	Increased absolute and relative kidney weight (LOAEL = 3.7); acute renal tubular necrosis at 29 mg/kg-day in males and at 59 mg/kg-day in males and females BML: 116-171 µg/g in kidney at 29 mg/kg-day	NTP 1993
Mouse/$B6C3F_1$/ 10 M, 10 F	6 mo 5 d/wk 1 x/d (gavage)	0, 0.93, 1.9, 3.7, 7.4, 14.8 ($HgCl_2$)	Increased absolute and relative kidney weight of males (LOAEL = 3.7); Cytoplasmic vacuolation of tubule epithelium in males (LOAEL = 3.7) BML: 36.1-40.6 µg/g in kidney at 3.7 mg/kg-day	NTP 1993

Table 3-39 (continued)
Renal Toxicity of Inorganic Mercury in Animals: Oral Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Mouse/B6C3F$_1$/ 60 M, 60 F	2 yr 5 d/wk 1 x/d (gavage)	0, 3.7, 7.4 ($HgCl_2$)	Increased severity of nephropathy (foci of proximal tubules with thickened basement membrane; basophilic cells with scant cytoplasm (LOAEL = 3.7) BML not reported	NTP 1993

Bernaudin et al. (1981) exposed male and female Brown Norway rats (number not specified) to mercuric chloride via aerosols (4 hours/week) and intratracheal instillation for 2 months. The aerosol exposures resulted in a retention of 0.05–0.06 mg $HgCl_2$/kg/hour (based on radiolabeled mercury); the parameters of the aerosol were not well characterized (e.g., no mass median aerodynamic diameter (MMAD) or geometric standard deviation provided and the particle generation system was not adequately described). The autoimmune response was typified by a linear pattern of IgG conjugate fixation in kidney glomeruli and a granular pattern of fixation in kidney glomeruli and arteries, lung and spleen; evidence of autoimmune disease was noted at all but the lowest intratracheal exposure level (60 µg $HgCl_2$/kg/week). In two of three rats exposed to aerosols and examined when sacrificed, weak proteinuria (1, 28 and 47 mg/day) was detected. No significant proteinuria was observed in the animals administered mercuric chloride by intratracheal instillations.

Table 3-40
Renal Toxicity of Inorganic Mercury in Animals: Inhalation Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Rat/Brown Norway/ 3-8 both sexes	2 mo, 1x/wk (intra-tracheal)	0, 6, 11, 47, 79 mg/kg-day ($HgCl_2$)	Autoimmune effect in spleen at 6 mg/kg-day and in spleen, lung and kidney at higher doses BML not reported	Bernaudin et al. 1981
Rat/Brown Norway/5 both sexes	2 mo, 4 d/wk, 1 hr/d (aerosol)	1 ($HgCl_2/m^3$) (estimate of minimum air concentration)	Weak proteinuria; autoimmune effect in kidney, lung and spleen BML not reported	Bernaudin et al. 1981

3.2.3.4 Cardiovascular

No studies were located regarding the cardiovascular toxicity of inorganic mercury in humans following oral exposure.

Limited information was located regarding the cardiovascular toxicity of inorganic mercury following oral exposure in animals. Signs of cardiovascular toxicity in rats include increased blood pressure and varying changes in the contractility of the heart (Carmignani et al. 1989, 1992). These signs manifested after oral exposure to mercuric chloride in drinking water for 180 or 350 days. No other animal studies were located.

Table 3-41
Cardiovascular Toxicity of Inorganic Mercury in Animals: Oral Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Rat/Sprague Dawley/8 M	350 d ad lib in drinking water	0, 7 ($HgCl_2$)	Increased blood pressure; positive inotropic response ($p<0.05$) Limitation: Only one dose tested; small number of animals BML: 0.9 µg/g in heart	Carmignani et al. 1989
Rat/Wistar/8 M	180 d ad lib in drinking water	0, 28 ($HgCl_2$)	Increased blood pressure ($p<0.05$); negative inotropic response (not significant) Limitation: Only one dose tested; small number of animals BML: 940 µg/L in blood, 4.1 µg/g in heart	Carmignani et al. 1992

3.2.3.5 Gastrointestinal

Irritation of the gastrointestinal mucosa is a common outcome of mercury toxicity following ingestion of mercuric chloride (Murphy et al. 1979). Ingestion of inorganic mercury may also cause vomiting, nausea, severe abdominal pain and diarrhea (Afonso and deAlvarez 1960; Murphy et al. 1979). No studies were located regarding the gastrointestinal toxicity of inorganic mercury after ingestion in humans for intermediate or chronic durations.

Table 3-42
Gastrointestinal Toxicity of Inorganic Mercury in Humans: Case Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Human/25 M, 29 F	Once	3.5-37 (est.) ($HgCl_2$)	Case studies of mercuric chloride poisonings in victims age 2-60 yr; effects ranged from nausea to severe corrosive gastritis Limitation: exposure data limited BML not reported	Troen et al. 1951
Human/1 F (adult)	Once (tablets)	30 ($HgCl_2$)	Nausea; vomiting; abdominal cramps; diarrhea after ingestion of mercuric chloride Limitation: Case study BML not reported	Afonso and deAlvarez 1960

Similar signs of gastrointestinal irritation appear in mice after intermediate duration oral exposure to mercuric chloride (NTP 1993). Histopathologic analyses reveal inflammation and necrosis of the stomach tissue. Further damage occurs to the gastrointestinal tract with continued dosing (NTP 1993). The incidence of hyperplasia of the forestomach epithelium increases in high-dose rats fed mercuric chloride for two years (NTP 1993).

Table 3-43
Gastrointestinal Toxicity of Inorganic Mercury in Animals: Oral Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Rat/F344/60 M, 60 F	2 yr 5 d/wk 1 x/d (gavage)	0, 1.9, 3.7 ($HgCl_2$)	Forestomach epithelial hyperplasia (LOAEL = 1.9 in males, 3.7 in females). BML not reported	NTP 1993
Mouse/ B6C3F_1/5 M, 5 F	14 d 5 d/wk 1 x/d (gavage)	0, 3.7, 7.4, 14.8, 29, 59 ($HgCl_2$)	Stomach inflammation and necrosis (LOAEL = 59). BML: 116-171 µg/g in kidneys at 29 mg/kg-day Limitation: small number of animals	NTP 1993

3.2.3.6 Hepatic

Limited information is available regarding the hepatic toxicity of inorganic mercury in humans after oral exposure. Murphy et al. (1979) reported on a man who died after ingesting an unspecified amount of mercuric chloride. The man was jaundiced, and his liver enzymes were elevated prior to death. Histopathological analyses revealed a softened and enlarged liver.

Liver enzymes were increased in rats and mice that ingested mercuric chloride for 4 or 6 weeks (Dieter et al. 1983; Jonker et al. 1993; Rana and Boora 1992). In addition, liver weights were significantly increased in mice after exposure to mercuric chloride in drinking water (Dieter et al. 1983). No microscopic changes were seen, however, during the histopathological analyses. No other animal data were available regarding oral exposure to inorganic mercury.

Table 3-44
Hepatic Toxicity of Inorganic Mercury in Animals: Oral Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	**Reference**
Rat/Charles Foster/5 M	30 d 1 x/d feed	NS ($HgCl_2$)	Increased lipid peroxidation ($p<0.02$) Limitation: Only one dose (NS) tested BML not reported	Rana and Boora 1992
Rat/Wistar/ 5/sex exposed/10/sex controls	4 wk ad lib in feed	2.8, 5.6, 11.1 ($HgCl_2$)	Increased serum alkaline phosphatase in males and females (LOAEL = 5.6 in females, 11.1 in males) Limitation: small number of animals BML not reported	Jonker et al. 1993
Mouse/ $B6C3F_1$/10 M	1-7 wk ad lib in drinking water	0, 0.6, 2.9, 14.3 ($HgCl_2$)	Increased plasma cholinesterase (LOAEL = 2.9) Limitation: small number of animals tested BML: 0.6 µg/L in blood at 7 wk, at 2.9 mg/kg/d	Dieter et al. 1983

3.2.3.7 Immunological

In addition to the inorganic mercury-induced autoimmune glomerulonephritis discussed earlier (see discussion of renal effects in Section 3.2.1), several studies identified other immunotoxicity endpoints in animals after oral exposure to inorganic mercury.

3.2.3.8 Developmental

No studies were located regarding the developmental toxicity of inorganic mercury in humans after inhalation exposure.

The only information located regarding developmental toxicity in animals from inhalation exposure to mercuric mercury comes from a study in which mice were exposed to aerosols containing mercuric chloride during gestation (Selypes et al. 1984). Increases were observed in the incidence of delayed ossification and dead or resorbed fetuses; the statistical significance of these effects was not reported. In addition, at the highest concentration, a significant increase in weight retardation was also observed. Interpretation of this study is limited, however, because the aerosols were not well characterized, and it is not known to what extent the droplets were respirable or were cleared from the upper respiratory tract and swallowed.

Table 3-45
Immunotoxicity of Inorganic Mercury in Animals: Oral Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Rat/Brown-Norway/6 both sexes exposed/22 controls both sexes	2 mo 1 x/wk (gavage)	2.2 ($HgCl_2$)	IgG deposits in glomerular capillary wall of kidney and renal arteries, suggestive of autoimmune disease; similar deposits also observed in lungs and spleen; no deposits observed in controls Limitation: Only one dose tested; small number of animals tested BML not reported	Bernaudin et al. 1981
Mouse/ $B6C3F_1$/10 M	7 wk ad lib in drinking water	0, 0.6, 2.9, 14.3 ($HgCl_2$)	Suppression of lymphoproliferative response to T-cell, concavalin A and phytohemagglutinin (LOAEL = 2.9 mg/kg-day; $p<0.05$) BML: 600 µg/L in blood at 2.9 mg/kg-day	Dieter et al. 1983
Mouse/SJL or DBA/5 F	2 wk ad lib in drinking water	0, 0.7 ($HgCl_2$)	Increased lymphoproliferative response to concanavalin A and E. coli lipopolysaccharide ($p<0.02$) Limitations: only one dose tested; small number of animals tested BML not reported	Hultman and Johansson 1991
Mouse/SJL/7 F	10 wk ad lib in drinking water	0, 0.07, 0.14, 0.28, 0.56 ($HgCl_2$)	Increased antinucleolar antibodies in IgG class (LOAEL = 0.28, $p<0.05$) Limitation: small number of animals tested BML: 5.2 µg/g in kidney	Hultman and Enestrom 1992
Mouse/ $B6C3F_1$/5 M, 5 F	14 d 5 d/wk 1 x/d (gavage)	0, 3.7, 7.4, 14.8, 29, 59 ($HgCl_2$)	Decreased thymus weight (LOAEL = 14.8) Limitation: small number of animals tested BML: 116-171 µg/g in kidney at 29 mg/kg-day	NTP 1993

Table 3-46
Developmental Toxicity of Inorganic Mercury in Animals: Inhalation Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Mice/CFLP/ No. F NS	4 d 4 hr/d Gd 9-12	0, 0.17, 1.6 ($HgCl_2$)	Increased dead or resorbed fetuses; delayed ossification (LOAEL = 0.17) Limitations: Data were reported as number of embryos only, not as number of affected litters; no statistical analysis; aerosol exposure was not well characterized; maternal toxicity was not evaluated BML not reported	Selypes et al. 1984

Developmental effects have been reported in animals following oral exposure to inorganic mercury. These efforts include an increased incidence of abnormal fetuses in hamsters (Gale 1974), growth retardation in rats (Rizzo and Furst 1972) and decreased body weights in several rat studies.

Gale (1974) administered 0, 4, 8, 25, 35, 50, 75, or 100 mg mercuric acetate/kg (0, 2.5, 5, 16, 22, 32, 47, or 63 mg Hg/kg) to pregnant golden hamsters (10/exposed group; 3/control group) by gavage in distilled water on the 8th day of gestation. The pregnant animals were sacrificed on gestation day 12 or 14, and the uterine contents were examined. A statistically significant increase in the incidence of abnormal fetuses (combined incidence of small, retarded, edematous, and/or malformed fetuses) was observed at 16 mg Hg/kg. Statistically significant increases in the percentage of resorbed fetuses was observed at 22 mg Hg/kg and in the percentages of small, retarded and edematous fetuses observed at 32 mg Hg/kg. No treatment-related effects were observed on the fetuses at 5 mg Hg/kg. Toxic effects observed in maternal animals included weight loss, diarrhea, slight tremor, somnolence, tubular necrosis in the kidneys and cytoplasmic vacuolization of hepatocytes.

Rizzo and Furst (1972) administered ≈7 mg Hg/kg as mercuric oxide to pregnant Long-Evans rats (5/group) by gavage in peanut oil on gestation day 5, 12, or 19 in a pilot study. On gestation day 20 or 21, the rats were sacrificed, and the uterine contents were examined. Rats administered mercury on gestation day 5 had a higher percentage of fetuses with growth retardation and inhibition of eye formation (statistical significance not reported). Similar increases in these effects were not observed after administration on gestation day 12 or 19. No toxicity in maternal animals was reported.

McAnulty et al. (1982) administered 8, 12, 16, or 24 mg mercuric chloride/kg-day (6, 9, 12, or 18 mg Hg/kg-day) by gavage to pregnant rats (strain and number not specified) on gestation days 6–15 as reported in an abstract. The abstract did not report whether controls were used. Fetal and placental weights were decreased at 9 mg Hg/kg-day and above. At 12 and 18 mg Hg/kg-day, increased postimplantation losses were reported. These effects were attributed to maternal toxicity and decreased food intake. At 18 mg Hg/kg-day, increases in delayed ossification and malformations were reported. Statistical analyses were not reported.

Pritchard et al. (1982a) administered 4, 8, or 16 mg mercuric chloride/kg-day (3, 6, or 12 mg Hg/kg-day) by the oral route to pregnant rats (number and strain not specified) from gestation day 15 until postpartum day 25, as reported in an abstract. The abstract did not state whether controls were used. At 6 and 12 mg Hg/kg-day, pup weight was decreased on postpartum day 1. Subsequent weight gain in these groups was also decreased. No other effects on development or behavior were observed postpartum. Females at 6 and 12 mg Hg/kg-day had a decreased rate of weight gain, and gestation time was slightly extended. Statistical analyses were not reported.

Pritchard et al. (1982b) administered 12, 16, or 24 mg mercuric chloride/kg-day (9, 12, 18 mg Hg/kg-day) to female rats (strain and number not reported) by gavage before mating and during gestation. The abstract did not report whether controls were used. At 12 mg Hg/kg-day and above, females exhibited weight loss and appeared unhealthy, estrous cycles became irregular, and high preimplantation losses were observed. No effects on ovulation, estrous cycles, implantation, and fetal development were observed at 9 mg Hg/kg-day. Statistical analyses were not reported.

Table 3-47
Developmental Toxicity of Inorganic Mercury in Animals: Oral Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Rat/Long-Evans/5 F	Once Gd 5, 12, or 19 (gavage)	0, 2.0 (HgO)	Growth retardation; inhibition of eye formation in group treated on Gd 5, with some effect on Gd 12 group. Limitations: No statistical analysis; small number of litters in treated groups (and controls) BML not reported	Rizzo and Furst 1972
Rat/Strain NS/no. F NS	10 d 1 x/d Gd 6-15 (gavage)	6, 9, 12, 18 ($HgCl_2$)	Decreased fetal and placental weights (LOAEL = 9); malformations at 18. Limitations: Reported as an abstract; few details reported BML not reported	McAnulty et al. 1982
Rat/Strain NS/no. F NS	Approx. 32 d 1 x/d Gd 15-ppd 25 (gavage)	3, 6, 12 ($HgCl_2$)	Decreased pup weight and weight gain (LOAEL = 6); no effect in an unspecified developmental and behavioral testing battery. Limitations: Reported as an abstract; few details reported BML not reported	Pritchard et al. 1982a
Rat/Strain NS/no. F NS	Before mating and during gestation (gavage)	9, 12, 18 ($HgCl_2$)	High preimplantation loss (LOAEL = 12). Limitations: Reported as an abstract; few details reported BML not reported	Pritchard et al. 1982b
Hamster/ Golden/10 F exposed/3 F controls	Once Gd 8 (gavage)	0, 2.5, 5, 16, 22, 32, 47, 63 [$Hg(CH_3COO)_2$]	Increased incidence of abnormal (small, retarded, edematous, and/or malformed--exencephaly, encephalocele, ectrodactyly, etc.) fetuses (LOAEL = 16, $p<0.05$); maternal toxicity: weight loss, diarrhea, slight tremors, somnolence, tubular necrosis, hepatocellular necrosis. Limitation: Small sample size; smaller control group; insufficient detail about number of animals sacrificed at Gd 12 or Gd 14; single day of treatment; incomplete examinations reported (no visceral, only partial skeletal) BML not reported	Gale 1974

In addition to the oral and inhalation studies summarized above, several studies using other routes of administration (i.p., s.c., i.v.) provide evidence of developmental toxicity associated with exposure to mercury salts. These studies are summarized below.

Gale and Ferm (1971) injected anesthetized pregnant golden hamsters (6–19/group) intravenously with 0, 2, 3, or 4 mg mercuric acetate/kg (0, 1.3, 1.9, or 2.5 mg Hg/kg) on gestation day 8. Controls were injected with vehicle (demineralized water). Maternal animals were sacrificed on gestation day 12 or 14, and the uterine contents were examined. A significantly increased incidence of resorptions was observed at all doses. In addition, increased incidences of retarded and edematous fetuses were observed at all doses (statistical significance not reported). Toxic effects observed in maternal animals included weight loss, diarrhea, slight tremor, somnolence and kidney lesions; however, the report did not specify at which doses the maternal effects were observed.

Gale (1974) compared the embryotoxicity of mercuric acetate administered by different routes in pregnant golden hamsters (3–23/group). Subcutaneous administration of 0, 4, 8, 20, 35, or 50 mg mercuric acetate/kg (0, 2.5, 5, 13, 22, or 32 mg Hg/kg) on gestation day 8 resulted in a significant decrease in the percentage of normal embryos and a significant increase in the percentage of small embryos at 2.5 mg Hg/kg. At 5 mg Hg/kg, significant increases in resorptions, abnormal, retarded, edematous, and malformed fetuses were observed. Intraperitoneal administration of 0, 2, 4, or 8 mg mercuric acetate (0, 1.3, 2.5, or 5 mg Hg/kg) on gestation day 8 resulted in significant increases in the percentage of resorptions, abnormal, small, and edematous fetuses at 1.3 mg Hg/kg. Intravenous administration of 0 or 4 mg mercuric acetate/kg (0 or 2.5 mg Hg/kg) on gestation day 8 resulted in significant increases in resorptions, abnormal, small, retarded, edematous, and malformed fetuses at 2.5 mg Hg/kg. Comparison of the extent of the developmental toxicity demonstrated an effect of route of administration: i.p. > i.v. > s.c. > oral.

Gale (1981) compared the embryotoxicity of mercuric acetate in 6 strains of hamsters (LAK:LVG[SYR], CB/SsLak, LHC/Lak, LSH/SsLak, MHA/SsLak. PD4/Lak). Maternal animals of the various strains (3–9/group) were injected subcutaneously with 0 or 15 mg/kg mercuric acetate (0 or 9.5 mg Hg/kg) on gestation day 8. Controls were injected with demineralized distilled water. Maternal animals were sacrificed on gestation day 12 or 15, and the uterine contents were examined. The percentage of resorptions was significantly increased in all strains examined on days 12 and 15. Strain-specific variations were observed in the incidences of abnormal, edematous and retarded fetuses and in the incidences of ventral wall defects, distension of the pericardial cavity, cleft palate, hydrocephalus and cardiac abnormalities. Maternal toxicity was not described.

Kavlock et al. (1993) injected pregnant Sprague-Dawley rats (6–25/group) subcutaneously with 0, 1, 2, 3, or 4 mg mercuric chloride/kg (0, 0.7, 1.5, 2.2, or 3.0 mg Hg/kg) on gestation day 7, 9, 11, or 13. On gestation day 21, rats were sacrificed, and the uterine contents were examined. No increase in malformations was observed in fetuses from mercuric chloride-treated dams. Exposure on gestation day 7 resulted in a significant decrease in fetal weight and an increase in the number of supernumerary ribs (statistical significance not reported) at 2.2 mg Hg/kg. Exposure on gestation day 9 resulted in significantly decreased live fetuses/litter and increased resorptions at 3 mg Hg/kg. Exposure on gestation days 11 or 13 resulted in no significant differences in fetal parameters. Maternal toxicity (increased mortality, decreased body weight, increased kidney weight, increased urine osmolality, and/or increased serum urea or creatinine) were observed at 1.5 mg Hg/kg and above. No consistent correlations were observed between maternal and fetal toxicity.

Kajiwara and Inouye (1986) injected Kud:ddY mice (10/group) intravenously with 0, 0.5, 1.0, 1.5, 2.0, and 2.5 mg Hg/kg as mercuric chloride on gestation day 0. Controls were injected with vehicle (physiological saline). Maternal animals were sacrificed on gestation day 3.5, and the oviducts and uterus were flushed to obtain preimplantation embryos. At 1.5 mg Hg/kg and above, the number of abnormal embryos was significantly increased. Maternal animals at 1.5 mg Hg/kg and above exhibited a decrease in body weight (statistical significance not determined). The study authors suggested that fetal toxicity may have been related to maternal toxicity.

Kajiwara and Inouye (1992) injected Kud:ddY mice (5–15/group) intravenously with 0, 1, 2, or 2.5 mg Hg/kg as mercuric chloride on gestation day 0. Controls were injected with vehicle (physiological saline). Maternal animals were sacrificed on gestation day 5 or 12, and the oviducts and uterine contents were examined. The animals sacrificed at gestation day 5 showed statistically significant decreases in the number of embryos at all doses and an increase in blastocysts without decidua (delay of implantation) at 2 and 2.5 mg Hg/kg. The animals sacrificed at gestation day 12 showed a statistically significant decrease in the number of implants, number of living fetuses and

average fetal weight at 2 and 2.5 mg Hg/kg. Maternal toxicity was not well-described, but 7 females at 2.5 mg Hg/kg and 2 females at 2 mg Hg/kg died. The study did not determine whether the failure to implant was due to fetal toxicity or maternal uterine dysfunction.

A study by Bernard et al. (1992) was performed to assess whether prenatal and early postnatal exposure to inorganic mercuric salts can produce nephrotoxic effects. They found that s.c. injection of dams with just 1 mg/kg-day during pregnancy caused renal effects in the offspring. Of note is that these effects did not appear to be significantly different in the group of dams dosed throughout the gestation period compared to dams dosed only during the last 8 days of gestation.

3.2.3.9 Reproductive

A single case study was located concerning reproductive toxicity in humans exposed to inorganic mercury; however, it is not clear whether the effects were compound-related. No information was identified regarding the reproductive toxicity of inorganic mercury following inhalation exposure.

Table 3-48
Reproductive Toxicity of Inorganic Mercury in Humans: Case Study

Species/ No. per Sex	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Human/1 F	Once (tablet)	30 ($HgCl_2$)	Spontaneous abortion 13 days after ingestion of mercuric chloride Limitation: Case study; abortion may have been unrelated to mercury exposure BML not reported	Afonso and deAlvarez 1960

In animals orally exposed to inorganic mercury compounds, changes in the estrous cycle and ovulation and/or increased resorptions were reported (Pritchard et al. 1982b).

In male mice administered a single i.p. dose of 0.74 mg Hg/kg as mercuric chloride, fertility decreased between days 28 and 49 post-treatment with no obvious histological effects noted in the sperm (Lee and Dixon 1975). The period of decreased fertility indicated that spermatogonia and premeiotic spermatocytes were affected. The effects were less severe than those noted after treatment with a similar dose of methylmercury. A single i.p. dose of 1.5 mg Hg/kg as mercuric chloride administered 1–5 days prior to mating in female mice resulted in a significant decrease in the total number of implants, number of living embryos and a significant increase in the percentage of dead implants (Suter 1975). These effects suggest that mercury may be a weak inducer of dominant lethal mutations. In female golden hamsters administered 6.4 or 12.8 mg Hg/kg subcutaneously, there was no observed increase in chromosomal aberrations in metaphase II oocytes (Watanabe et al. 1982). At the first estrous cycle post treatment, there was a significant increase in the number of degenerated oocytes in animals at the high-dose group. At the second estrous cycle both treatment groups had increased numbers of degenerated oocytes, suggesting an effect of mercuric chloride on ovulation.

Table 3-49
Reproductive Toxicity of Inorganic Mercury in Animals; Oral Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Rat/strain NS/NS no. of F	NS "before mating and during gestation" (gavage)	9, 12, 18 ($HgCl_2$)	Irregular estrous cycles and high preimplantation loss (LOAEL = 12); decreased ovulation (LOAEL = 18) Limitations: Limited details; reported as an abstract; no statistical analysis reported BML not reported	Pritchard et al. 1982b

3.2.3.10 Genotoxicity

Two occupational studies (Anwar and Gabal 1991; Popescu et al. 1979) reported on workers inhaling inorganic mercury; the data were inconclusive regarding the clastogenic activity of inorganic mercury. Workers involved in the manufacture of mercury fulminate ($Hg[OCN]_2$) had a significant increase in the incidence of chromosomal aberrations and micronuclei in peripheral lymphocytes when compared to unexposed controls (Anwar and Gabal 1991). There was no correlation between urinary mercury levels or duration of exposure to the increased frequency of effects; the study authors concluded that mercury may not have been the clastogen in the manufacturing process. In a study by Popescu et al. (1979), 18 workers exposed to a mixture of mercuric chloride, methylmercuric chloride and ethylmercuric chloride had significant increases in the frequency of acentric fragments (chromosome breaks). The findings, however, are suspect because the control group was not matched for sex, smoking habits or sample size.

Table 3-50
Genotoxicity of Inorganic Mercury in Humans

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/29 M exposed/ 29 M control	20.8 yr (avg) (occup)	NS $Hg(OCN)_2$	Increased incidence of chromosomal aberrations ($p<0.001$) and micronuclei ($p<0.01$) in lymphocytes of workers exposed to mercury fulminate compared with age-matched controls; no correlation between frequency of chromosome and exposure duration or urinary mercury level. BML: 123.2 µg/L in urine (avg)	Anwar and Gabal 1991
Human/18 M exposed/ 10 control	10.5 yr (occup)	0.15-0.44 ($HgCl_2$)	Increased frequency of chromosomal breaks. Limitations: Workers also exposed to methylmercuric chloride and ethylmercuric chloride, and one worker had history of benzene poisoning; control group was not matched for sex, smoking habits, or sample size. BML: ≈890 µg/L in urine (avg)	Popescu et al. 1979

Exposure to inorganic mercury may produce an increase in chromosomal aberrations in mice following oral and inhalation exposure (Ghosh et al. 1991; Selypes et al. 1984). Mercuric chloride administered to mice by gavage induced a dose-related increase in chromosome aberrations and aberrant cells in the bone marrow (Ghosh et al. 1991); however, mice given i.p. doses of mercuric chloride have shown no increase in chromosomal aberrations in bone marrow cells (Poma et al. 1981) and no increase in aneuploidy in spermatogonia (Jagiello and Lin 1973). Similarly, an increased incidence of chromosomal aberrations (primarily deletion and numeric aberrations) was observed in livers of fetal mice exposed to mercury *in utero* as the result of maternal inhalation of aerosols of mercuric chloride (Selypes et al. 1984). Female golden hamsters injected s.c. with mercuric chloride were observed to have increased incidence of chromosome aberrations in bone marrow cells but not in metaphase II oocytes (Watanabe et al. 1982). Mercuric chloride concentrations in the ovaries were low but had an inhibiting effect on ovulation. Verschaeve et al. (1984) reported that *in vitro* exposure of human lymphocytes and muntjac fibroblasts to mercuric chloride resulted in segregation abnormalities; namely, c-mitotic figures. The effects of mercuric chloride on genetic material has been suggested to be due to the ability of mercury to inhibit of the formation of the mitotic spindle, which can result in c-mitotic figures. Mercuric chloride has also been shown to inhibit nucleolus organizing activity in human lymphocytes (Vershaeve et al. 1983).

Positive dominant lethal results have been obtained in studies in which rats were administered mercuric chloride orally (Zasukhina et al. 1983). Suter (1975) observed a small, but significant increase in the number of non-viable implants when female mice were administered mercuric chloride by intraperitoneal injection; this effect was not observed when males were treated. It was not clear whether the increase in non-viable implants was due to maternal toxicity or to a true dominant lethal effect of the treatment.

Sex-linked recessive lethal mutations were not observed as a consequence of exposure of male *Drosophila melanogaster* by either feeding or injection (NTP 1993).

As summarized in NTP (1993) and U.S. EPA (1985), mercuric chloride has produced some positive results for clastogenicity in a variety of *in vitro* and *in vivo* genotoxicity assays, but mixed results regarding its mutagenic activity have been reported. Mercuric chloride was negative in gene mutation tests with *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA102 with or without hepatic microsomal preparations (S9) (Arlauskas et al. 1985; Marzin and Phi 1985; Wong 1988). Mercuric chloride has shown evidence of DNA damage in the *Bacillus subtilis rec* assay (Kanematsu et al. 1980) but did not induce lytic phage in a lysogenic *E. coli* strain (Rossman et al. 1984).

DNA damage (single strand breaks) has also been observed in assays using rat and mouse embryo fibroblasts (Zasukhina et al. 1983) and Chinese hamster ovary (CHO) cells and human KB cells (Cantoni and Costa 1983; Cantoni et al. 1982, 1984a,b; Christie et al. 1984, 1986; Robison et al. 1982, 1984; Williams et al. 1987). Mercuric chloride also produced chromosome aberrations and sister chromatid exchange (SCE) in CHO cells (Howard et al. 1991) and SCE in human leucocytes (Morimoto et al. 1982). Negative results for chromosomal aberrations were reported for FM3A cells (from a mouse mammary carcinoma) (Umeda and Nishimura 1979) and for two human diploid lines, WI38 and MRC_5 (Paton and Allison 1972). Negative results for SCE were reported for don cells (Ohno et al. 1982) and for P388D, mouse cells and CHO cells (Anderson 1983). Evidence of gene mutations (considered weakly positive) was observed in L5178Y mouse lymphoma cells in the presence of microsomal preparations (Oberly et al. 1982).

NTP (1993) reached the following conclusions from their *in vitro* testing of mercuric chloride: not mutagenic for *Salmonella typhimurium* in preincubation protocols with and without rat and hamster

liver preparations; positive for L5178Y cells without addition of hepatic preparations; negative for SCE in CHO cells without addition of S9 but weakly positive when rat S9 was added; positive for chromosomal aberrations in CHO cells in the absence but not the presence of liver preparations; it was not clear what role was played by cytotoxicity in the generation of these chromosomal aberrations.

Table 3-51
Genotoxicity of Inorganic Mercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Mice/CFLP NS/No. F NS	4 d 4 hr/d Gd 9-12	0.17, 1.6 ($HgCl_2$)	Increased incidence of chromosomal aberrations in fetal hepatocytes Limitations: The number of mothers corresponding to the 10 fetuses examined was not reported; no statistical analysis BML not reported	Selypes et al. 1984

3.3 Methylmercury

Organic mercury compounds have been used as fungicides and as pharmaceutical agents (diuretics). Organic mercurials including Metaphen, Merthiolate and Mercurochrome still find use as topical antiseptics. Phenylmercury salts are used in pharmaceutical, ophthalmic and cosmetic preparations to control growth of microbial organisms (Joklik et al. 1984). Other organic mercury compounds include methylmercuric chloride (MMC), methylmercuric hydroxide (MMH) and phenylmercuric acetate (PMA). Nearly all of the available toxicity studies for organic mercury compounds, however, are for methylmercury. Unless otherwise noted, all studies summarized in tables in this section are for methylmercury. All oral doses were converted to mg Hg/kg-day, and all inhalation doses were converted mg Hg/m^3 using the method shown in Appendix A.

3.3.1 Critical Noncancer Data

This section provides descriptions of studies considered by U.S. EPA in evaluation of systemic health endpoints, largely neurotoxicity in exposed adults and in children exposed *in utero*. Chapter 6 describes the derivation of an RfD for methylmercury based on developmental neurologic abnormalities in human infants. For completeness some of these studies are also presented in subsequent sections in tabular form.

3.3.1.1 Human Data

Several studies of methylmercury poisonings in humans have been reported (see discussion on Neurologic Effects). CNS effects were observed in several studies summarized by Clarkson et al. (1976), Nordberg and Strangert (1976), and WHO (1976). CNS effects including ataxia and paresthesia have been observed in subjects with blood mercury concentrations as low as 200 µg/L.

The original epidemiologic report of methylmercury poisoning involved 628 human cases that occurred in Minamata, Japan, between 1953 and 1960. The overall prevalence rate for the Minamata region for neurologic and mental disorders was 59%. Among this group 78 deaths occurred, and hair concentrations of mercury ranged from 50–700 ppm. These hair mercury concentrations were determined through the use of less precise analytic methods than were available for later studies. The most common clinical signs observed in adults were paresthesia, ataxia, sensory disturbances, tremors, impairment of hearing and difficulty in walking. Examination of the brains of severely affected patients that died revealed marked atrophy of the brain (55% normal volume and weight) with cystic cavities and spongy foci. Microscopically, entire regions were devoid of neurons, granular cells in the cerebellum, golgi cells and Purkinje cells. Extensive investigations of congenital Minamata disease were undertaken, and 20 cases that occurred over a 4-year period were documented. In all instances the congenital cases showed a higher incidence of symptoms than did the cases wherein exposure occurred as an adult. Severe disturbances of nervous function were described, and the affected offspring were very late in reaching developmental milestones. Hair concentrations of mercury in affected infants ranged from 10 to 100 ppm. Hair mercury levels for the mothers during gestation were not available (Tsubaki 1977).

In 1971, an unknown number of people in Iraq were exposed to methylmercury-treated seed grain that was used in home-baked bread. Studies conducted on this population include that of Bakir et al. (1973), Marsh et al. (1987) and others. Toxicity was observed in many adults and children who had consumed this bread over a three-month period, but the population that showed greatest sensitivity were offspring of pregnant women who ate contaminated bread during gestation. The predominant symptom noted in adults was paresthesia, and it usually occurred after a latent period of from 16 to 38 days. In adults symptoms were dose-dependent, and among the more severely affected individuals ataxia, blurred vision, slurred speech and hearing difficulties were observed. Signs noted in the infants exposed during fetal development included cerebral palsy, altered muscle tone and deep tendon reflexes, as well as delayed developmental milestones (e.g., walking before 18 months and talking before 24 months). Some information indicated that male offspring were more sensitive than females. The mothers experienced paresthesia and other sensory disturbances but at higher doses than those associated with their children exposed *in utero*. Unique analytic features of mercury (analysis of segments of hair correlated to specific time periods in the past) permitted approximation of maternal blood levels that fetuses were exposed to *in utero*. The data collected by Marsh et al. (1987) summarized clinical neurologic signs of 81 mother and child pairs. From x-ray fluorescent spectrometric analysis of selected regions of maternal scalp hair, concentrations ranging from 1 to 674 ppm were determined, then correlated with clinical signs observed in the affected members of the 81 mother-child pairs. Among the exposed population there were affected and unaffected individuals throughout the dose-exposure range. (See also Bakir et al. (1973) in sections on Death and Neurological Effects).

McKeown-Eyssen et al. (1983) provided a report of neurologic abnormalities in four communities of Cree Indians in northern Quebec. A group of 247 children between 12 and 30 months of age was evaluated for clinical signs consistent with methylmercury exposure. A pediatric neurologist evaluated the children for the following: height, weight, head circumference, dysmorphic and congenital features and the presence of acquired disease. In addition to the DDST the following assessments were done: special senses, cranial nerve function, sensory function, muscle tone, stretch reflexes, co-ordination, persistence of Babinski response, and a summary of signs for absence or presence of neurologic abnormality. An attempt was made to account for possible confounding factors; the interviewers determined alcohol and tobacco consumption patterns among the mothers of affected children. Age of the mothers and multiparity was also taken into account in analysis of the data. Maternal hair mercury was used as the exposure measure. The average maternal hair mercury was the same for boys and girls (6 ppm); only 6% of the population had exposure above 20 ppm. The prevalence of multiple abnormal neurologic findings was about 4% for children of both sexes. The most frequently

observed abnormality was delayed deep tendon reflexes. This was seen in 11.4% of the boys and 12.2% of the girls. Abnormality of muscle tone or reflexes showed a significant positive association with maternal mercury exposure for boys, but not for girls. A consistent dose-response relationship for this effect was not observed; however, the greatest prevalence of the effect in boys occurred for those with mothers in the highest exposure group (13.0-23.9 ppm mercury in hair). No other measure of abnormal or decreased neurologic function or development showed a significant positive association with maternal hair mercury. The prevalence of abnormality of muscle tone or reflexes was found to increase 7 times with each increase of 10 ppm of the prenatal exposure index. There was possible influence of alcohol consumption and smoking among mothers on the effects observed in their children.

Studies performed in New Zealand investigated the development of children who had prenatal exposure to methylmercury (Kjellstrom et al. 1986a, 1989). A group of 11,000 mothers who regularly ate fish was initially screened by survey; of these, about 1000 had consumed 3 fish meals per week during pregnancy. Working from these two large groups, 31 matched pairs were established. A reference child matched for mother's ethnic group, age, child's birthplace and birth date was located for each child in the high fish consumption group. Mercury exposure during gestation was determined from maternal hair analysis. The average hair concentration for high exposure mothers was 8.8 ppm and for the reference group was 1.9 ppm. At 4 years of age, the children were tested using the Denver Developmental Screen Test (DDST). This is a standardized test of a child's mental development and can be administered in the child's home. It consists of four major function sectors: gross motor, fine motor, language and personal-social. A developmental delay in an individual item is scored when the child has failed in his/her response and at least 90% of children can pass this item at a younger age. The whole test is scored as abnormal, questionable, or normal. Standardized vision tests and sensory tests were also performed to measure development of these components of the nervous system. The prevalence for developmental delay in children was 52% for progeny of high mercury mothers and 17% for progeny of mothers of the reference group. The hair mercury concentrations of the mothers in this study were lower than those associated with CNS effects in children exposed in Japan and Iraq. The results of the DDST included 2 abnormal scores and 14 questionable scores in the high mercury-exposed group and 1 abnormal and 4 questionable scores in the control group. The results remained statistically significant after the 8 pairs where ethnic group matching was not successful and twins were excluded. Analysis of the DDST results by sector showed that developmental delays were most commonly noted in the fine motor and language sectors, but the differences between the experimental and control groups were not significant. The differences noted in performance of the DDST between high mercury-exposed and referent children could be due to confounding variables. Infants of the mercury-exposed group more frequently had low birth weights and were more likely to be born prematurely.

A second stage follow-up of the original Kjellstrom study was carried out when the children were 6 years old (Kjellstrom et al. 1986b). In this later study the high exposure children were compared with 3 control groups with lower prenatal mercury exposure. During pregnancy, mothers in two of these control groups had high fish consumption and average hair mercury concentrations of 3–6 ppm and 0–3ppm, respectively. The high exposure group was matched with controls for maternal ethnic group, age, smoking habits, residence, and sex of the child. For the second study, 61 of 74 high exposure children were available for study. Each child was tested with an array of scholastic, psychological and behavioral tests which included Test of Language Development (TOLD), the Wechsler Intelligence Scale for Children and McCarthy Scale of Children's Abilities. The results of the tests were compared between groups. Confounding was controlled for by a modelling procedure using linear multiple regression analysis. A principal finding was that normal results of the psychological test variables were influenced by ethnic background and social class. High prenatal methylmercury exposure decreased performance in the tests, but it contributed only a small part of the variation in test results. It was found that an average hair mercury level of 13–15 ppm during pregnancy was consistently associated with decreased test

performance. Size of the experimental groups limited the power of the study to determine if lower exposure levels might have had a significant effect on test results. The studies are limited for assessing methylmercury toxicity because the intelligence tests used may not be the most appropriate for defining the effects of methylmercury. Also, greater significance was seen in differences of cultural origins of the children than the differences in maternal hair methylmercury concentrations.

A prospective study (Marsh et al. 1995) of fetal exposure to methylmercury through maternal consumption of fish was conducted in the Peruvian fishing village of Mancora between 1981-1984. An account of the study was written in 1985 and subsequently published in 1995. Hair samples were obtained from 369 pregnant women, and neurologic examinations were performed on 194 of the children from these pregnancies. Of this cohort there were 131 mother-infant pairs with complete clinical data and hair samples that provided quantitative measures of methylmercury exposure during pregnancy. The peak hair mercury levels ranged from 1.2 to 30 ppm with a mean of 8.3 ppm. Testing of hair segments and statistical analyses confirmed that the women had reached a relatively steady state in terms of methylmercury. No significant correlation was shown between increasing maternal hair methylmercury and effects on the developmental determinations assessed in this study. Measures included the following: perinatal factors (labor or delivery difficulty, abnormal respiration or color), maternal paresthesia, speech retardation, muscle tone evaluation, determination of primitive and tendon reflexes, ataxia and mental and motor retardation.

There has been an increasing concern about the likelihood of exposure of people residing in the Amazonian watershed to methylmercury in fish and other sources. The Amazon River valley is the site of many small gold mining operations which use metallic mercury in the extraction process; it is estimated that 55-60% is released to the atmosphere and 40-45% enters the aquatic environment (Pfeiffer et al. 1989; Malm et al 1990). Lebel et al (1996) have published a study of 29 adult residents of two villages located on the Tapajos River, a tributary of the Amazon located about 200 Km from the mining sites. There were 14 women and 15 men aged 35 and younger who were randomly chosen from a previous survey. Total hair mercury ranged from 5.6 to 38.4 ppm; methylmercury constituted between 72.2% and 93.3% of the sampled mercury. A quantitative behavioral neurophysiologic battery was modified for administration to persons with minimal formal education in an area without electricity. For women only there was a decrease with increasing hair mercury in manual dexterity as measured in the Santa Ana test, Helsinki version. For both men and women there was a statistically significant decrease with increasing mercury in color discrimination capacity (as measured by the Lanthony D-15 desaturated panel). Near visual contrast sensitivity profiles (measured with the Vistech 6000) and peripheral visual field profile (Goldman Perimetry with Targets I and IV) were both reduced in the individuals with the highest hair mercuries. The authors noted that constriction of the visual field has been observed in other instances of mercury intoxication and that changes in contrast sensitivity has been noted in non-human primates exposed to methylmercury (Rice and Gilbert 1982; Rice and Gilbert 1990).

Lonky et al. (1996) have reported on studies of behavioral effects in newborns as a consequence of maternal consumption of fish from Lake Ontario in the U.S.. Fish from the U.S. Great Lakes have been shown to be contaminated with a number of environmental pollutants including PCBs and methylmercury. A total of 559 children were tested. Exposure was measured as fish consumption which was determined by interviews taking place during pregnancy. Information was collected on fish species, number of meals, serving size, and method of preparation. Exposure was scaled in PCB equivalent weights. Women were assigned to the high dose group if they reported eating at least 40 PCB-equivalent pounds of fish in their lifetime (n=152); the low fish consumption group numbered 243, and there were 164 no-fish-consumption controls. Infants born to the high fish consumption group scored significantly more poorly on the Reflex, Autonomic and Habituation clusters of the Neonatal Behavioral Assessment Scale. The authors do not attribute these developmental effects to exposure to any one compound;

analyses of cord blood and maternal hair are being done for PCBs, DDE, hexachlorobenzene, lead and mercury.

In 1981 a group of researchers in conjunction with the Seychelles Island government initiated a large study on developmental effects of low level methylmercury exposure from consumption of marine fish. The Seychelles is an island country in the Indian Ocean near the coast of Africa where fish are consumed by the population on a daily basis. A pilot study (essentially a cross-sectional study) was initiated which focused on all children born between February 1989 and February 1990. A total of 804 mother-infant pairs were enrolled, which was 48% of those eligible. The main study was designed to be prospective and involved 779 mother-child pairs. In both studies maternal hair samples and umbilical cord blood were measured for total mercury content using atomic absorption spectroscopy. Children were enrolled in the pilot study during the years 1987-1988; the main study enrolled children from 1989 to 1990. Both the pilot and main studies involved about 50% of all children born during the year, 804 and 779 children respectively. (Shamlaye et al. 1995)

The authors have noted that the pilot study was not as well-controlled as the main or longitudinal study: there were fewer covariates, medical records were not reviewed as carefully, there was less information on socio-economic status. Subsets of enrolled children were tested, and the main purpose of testing was to pilot the test batteries. Multiple hair samples collected for the pilot study during gestation had methylmercury ranging from 0.6 to 36.4 ppm with a median of 6.6 ppm (Myers et al. 1995b). The endpoints evaluated during the pilot study included a general neurologic evaluation and the DDST-R in addition to examinations of physical development. The age of each child was known; children were tested once between the ages of 5 and 109 weeks of age. In addition to DDST-R testing, a medical history was obtained, and general and neurologic examinations were also performed. Statistical analysis included the following covariates: gender, birth weight, one- and five-minute Apgar score, age at testing, and medical problems. Covariates for the mothers were age, tobacco and alcohol consumption during pregnancy and medical problems. An association between *in utero* mercury exposure was found for DDST-R abnormal plus questionable scores combined. (Myers et al 1995b)

A subset of the pilot cohort was evaluated at 66 months (Myers et al. 1995a). This group of 217 children was administered the McCarthy Scales of Children's Abilities, the Preschool Language Scale, and the Letter-Word Recognition and Applied Problems subtests of the Woodcock-Johnson Tests of Achievement that were appropriate to the children's age. The median maternal hair mercury for this group was 7.11 ppm. Mercury exposure (measured as maternal hair mercury) was negatively associated with four endpoints: The McCarthy General Cognitive Index and Perceptual Performance subscale; and the Preschool Language Scale Total Language and Auditory Comprehension subscale. When statistically determined outliers and points considered to be influential were removed from the analyses, statistical significance of the association remained only for auditory comprehension.

The prospective or main study, involved evaluation of children at 6.5, 19, 26 and 66 months of age. Age-appropriate tests administered included the following: Infantest (or Fagan's test of visual recognition memory), Bayley Scales of Infant Development (BSID), McCarthy Scales of Children's Abilities, the Preschool Language Scale and the DDST (6.5 months only). Maternal intelligence and home environment were also assessed (Marsh et al. 1995a). In the group (n = 740) evaluated at 6.5 months, median maternal hair mercury was 5.9 ppm with a range of 0.5 ppm to 26.7 ppm. No association with maternal hair mercury was found for any of six endpoints in six children tested at six months. (Myers et al. 1995c).

Evaluations at 19 and 29 months were done on groups of 738 and 736 individuals, respectively (Davidson et al. 1995). Median maternal hair mercury was 5.9 ppm and the range was 0.5 to 26.7 ppm.

Children were evaluated with the BSID at 19 months of age. At 29 months, children were administered the BSID as well as the Bayley Infant Behavior Record. Mean BSID mental indices were comparable to scores for children in the U.S. at both 19 and 29 months. The BSID Psychomotor Scale was reflective of the rapid development of motor skills by children reared in African cultures. No effects of mercury exposure were seen on outcome of five test endpoints at 19 months. At 29 months there was an association between mercury exposure and decreased activity level in male children only. The authors point out that the activity level observed during the testing session may not reflect the child's activity level in other settings. There was no association with maternal hair mercury in 15 other endpoints.

Delayed onset of walking and talking were among the measures of toxicity used in the evaluation of Iraqi children *in utero* (Marsh et al. 1987.) When the children in the Seychelles study reached the age of 19 months, caregivers were queried as to the time at which they walked and talked (n=760 and 680, respectively). Onset of walking was defined as walking without support and age at talking as the age at which the child said words other than "mama" or "dada." The mean age for walking was 10.7 months for girls and 10.6 for boys; for talking it was 10.5 for girls and 11.0 for boys. For female children, the mean age for walking was similar across all mercury exposures. In boys, the mean age for walking increased between 0.3 and 0.6 months from the lowest to highest mercury exposure groups. Statistical analyses adjusted covariates and outlying points data. With these adjustments there was no statistically significant association between maternal hair mercury and age at which the child walked or talked.

The overall conclusion of the studies published to date is that it is yet unclear whether an association exists between low level mercury exposure and neurologic deficits in children. The study does show a close correlation between maternal hair mercury and neonatal levels of mercury in brain tissue (Cernichiari et al. 1995). The authors cautioned in several papers that subtle neurologic and neurobehavioral effects are more likely to be detected in older rather than younger children. The overall conclusion of the authors is that their results require careful interpretation, and that an association between relatively low level mercury exposure *in utero* and neurologic deficits has not been conclusively demonstrated.

A large study was initiated in the Faroe Islands in 1986 on neurologic developmental effects of methylmercury and PCB exposure *in utero* (Grandjean et al. 1997). The population of the Faroes is relatively homogeneous. During pregnancy consumption of alcoholic beverages is uncommon. As with other fishing communities, seafood is large part of the Faroese diet. Increased mercury exposure, however, is largely attributed to the eating of pilot whale, which is traditionally hunted and shared among the population (Grandjean et al. 1992a). Subjects were a group of 917 (of an initial cohort of 1022) children born between 1986 and 1987 and evaluated at about 7 years of age. Mercury was measured in maternal hair and cord blood, and a subset of cords was evaluated for PCBs. Of the initial cohort the median blood mercury was 24.2 µg/L with 25% of the samples above 40 µg/L. The median maternal hair mercury concentration was 4.5 ppm, and 13% were greater than 10 ppm (Grandjean et al. 1992a). Cord blood mercury was found to be most closely associated with maternal hair mercury; the association with hair measurements in the children at 12 months and 7 years was not as strong (Grandjean et al. 1997).

At seven years children received a physical examination including a functional neurological examination which emphasized motor co-ordination and perceptual-motor performance. Visual acuity was measured using Snellen's board; contrast sensitivity was assessed using the Functional Acuity Contrast Test. Standard hearing tests were done. Neurophysiological tests included the following: pattern-reversal visual evoked potentials; brainstem auditory evoked potentials; postural sway under four conditions; and coefficient of variation for R-R intervals on electrocardiogram as a measure of autonomic nervous system function. Neuropsychological tests included these: motor tests--the Neurobehavioral

Evaluation System (NES) finger tapping test and the NES Hand-Eye Coordination test; tactile processing -- Tactual Performance Test; vigilance/attention--NES Continuous Performance Test; attention and tracking -- Wechsler Intelligence Scale for Children-revised (WISC-r) digit span forward; reasoning and cognitive flexibility--WISC-r similarities; visuospatial--WISC-r Block Designs and Bender Visual Motor Gestalt Test; language--Boston Naming Test; memory--California Verbal Learning Test; and mood--Nonverbal Analogue Profile of Mood States.

Three neurological tests were found to be difficult for 7-year-old children; fewer than 60% of the children performed optimally. On one of these tests, finger opposition, the group of 465 with optimal performance had a geometric mean cord blood mercury of 21.8 µg/L of 21.8 by contrast to 23.9 µg/L for the 425 children with questionable or deficient performance; this was a statistically significant difference. The neurophysiological tests showed no indication of mercury-associated dysfunction. Significant negative associations were seen on several neuropsychological tests. Even with inclusion of covariates with uncertain influence on these tests results, multiple regression analysis indicated that 9/20 measures showed mercury related decrements ($p < 0.05$, one tailed). Application of a Peters-Belson adjustment resulted in significant mercury associations for 11/20 measures.

Pilot whale fat (blubber) is consumed in the Faroe Islands, and this could result in increased exposure to PCBs. PCB concentrations in Faroese breast milk has been shown to be higher than in other Scandinavian countries (Grandjean et al. 1995b). PCB determinations were done on a total of 436 cord bloods, and PCB exposure was included as a covariate in the regression analyses. This had an effect only on the regression for the Boston Naming Test. The authors concluded that *in utero* exposure to methylmercury affects several domains of cerebral function. After exclusion of children with maternal hair mercury concentrations above 10 ppm, the association between mercury exposure and neuropsychological dysfunction remained unchanged. The authors, therefore, concluded that adverse effects are observed at exposures below 10 ppm maternal hair (Grandjean et al. 1997).

3.3.1.2 Animal Data

Rice (1989b) dosed five cynomolgus monkeys (*Macaca fascicularis*) from birth to 7 years of age with 0.05 mg Hg/kg-day as methylmercuric chloride and performed clinical and neurologic examinations during the dosing period and for an additional 6 years. As a sensitive indicator of the latent effects of methylmercury, neurologic examinations performed at the end of the observation period revealed insensitivity to touch and loss of tactile response. In the later stages of the observation period monkeys dosed with methylmercury were clumsier and slower to react when placed in the exercise cage than were unexposed monkeys.

Gunderson et al. (1986) administered daily doses of 0.04–0.06 mg Hg/kg as methylmercuric hydroxide to 11 crab-eating macaques (*Macaca fascicularis*) throughout pregnancy, resulting in maternal blood levels of 1080–1330 µg/L in mothers and 1410–1840 µg/L in the offspring. When tested 35 days after birth, the infants exhibited visual recognition deficits.

Groups of 7 or 8 female crab-eating macaques (*Macaca fascicularis*) were dosed with 0.05 or 0.09 mg/kg-day of methylmercury through 4 menstrual cycles (Burbacher et al. 1984). They were mated with untreated males, and clinical observations were made for an additional 4 months. Two of 7 high-dose females aborted, and 3 did not conceive during the 4-month mating period. The other two females delivered live infants. Two of 7 females exposed to 0.05 mg/kg-day aborted; the remaining 5 females delivered live infants. All control females conceived, and 6 delivered live infants. These reproductive results approached but did not reach statistical significance. Reproductive failure within dose groups could be predicted by blood mercury levels. The dams did not show clinical signs of methylmercury

poisoning during the breeding period or gestation, but when females were dosed with 0.09 mg/kg-day for a year, 4 of 7 did show adverse neurologic signs.

Bornhausen et al. (1980) has reported a decrease in operant behavior performance in 4-month-old rats whose dams had received methylmercuric chloride on gestation days 6–9. A statistically significant effect was seen in offspring whose dams had received 0.01 and 0.05 mg/kg five times during gestation. The authors postulated that more severe effects of *in utero* exposure would be seen in humans since the biological half-time of mercury in the brain of humans is 5 times longer than the rat. In addition, much longer *in utero* exposure to mercury would occur in humans since gestation is much longer.

Groups of Wistar rats (50/sex/group) were administered daily doses of 0.002, 0.01, 0.05, and 0.25 mg Hg/kg-day as methylmercuric chloride for 26 months (Munro et al. 1980). Female rats that received 0.25 mg/kg-day had reduced body weight gains and showed only minimal clinical signs of neurotoxicity; however, male rats that received this dose did show overt clinical signs of neurotoxicity, had decreased hemoglobin and hematocrit values, had reduced weight gains and showed increased mortality. Histopathologic examination of rats of both sexes receiving 0.25 mg/kg-day revealed demyelination of dorsal nerve roots and peripheral nerves. Males showed severe kidney damage, and females had minimal renal damage. This study showed a NOAEL of 0.05 mg/kg-day and a LOAEL of 0.25 mg/kg-day.

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

Ultrastructural renal changes were also observed in rhesus monkeys treated with 0.08–0.12 mg/kg of methylmercury although clinical changes were not observed (Chen et al. 1983).

3.3.2 Cancer Data

3.3.2.1 Human Data

Three studies were identified that examined the relationship between methylmercury exposure and cancer. No persuasive evidence of increased carcinogenicity attributable to methylmercury exposure was observed in any of the studies. Interpretation of these studies, however, was limited by poor study design and incomplete descriptions of methodology and/or results.

Tamashiro et al. (1984) evaluated the causes of death in 334 subjects from the Kumamoto Prefecture who had been diagnosed with Minamata disease and died between 1970 and 1980. Minamata disease was used as a surrogate for methylmercury exposure. The cases were fishermen and their families who had been diagnosed with methylmercury poisoning (Minamata disease); thus, Minamata disease was used as a surrogate for methylmercury exposure. The controls were selected from all deaths that had occurred in the same city or town as had the cases and were matched on the basis of sex, age at

death (within 3 years) and year of death; two controls were matched to each case. Malignant neoplasms were designated as the underlying cause of death in 14.7% (49/334) of the cases and 20.1% (134/668) of the controls. For 47 cases in which Minamata disease was listed as the underlying cause of death, the investigators reanalyzed the mortality data and selected one of the secondary causes to be the underlying cause of death in order to allow examination of the cases and controls under similar conditions and parameters. The three cases for which Minamata disease was listed as the only cause of death were excluded from further analysis. Using the Mantel-Haenzel method to estimate odds ratios, no significant differences were observed between the cases and controls with respect to the proportion of deaths due to malignant neoplasms among males, females, or both sexes combined. The estimated odds ratios and 95% confidence intervals were 0.84 (0.49–1.43), 0.58 (0.28–1.21), and 0.75 (0.50–1.11) for males, females, and both sexes combined. Similarly, no increases were observed among the cases relative to the controls when malignant neoplasms were identified as a secondary cause of death or were listed on death certificates as one of multiple causes of death. These data suggest that cancer incidence is not increased in persons with overt signs of methylmercury poisoning when compared to persons for whom no diagnosis of methylmercury poisoning had been made. Interpretation is limited, however, by potential bias in designating the cause of death among patients with known Minamata disease and by the uncertainty regarding the extent of methylmercury exposure and undiagnosed Minamata disease among the controls.

In a subsequent study, Tamashiro et al. (1986) compared the mortality patterns (between 1970 and 1981) among residents of Fukuro and Tsukinoura districts (inhabited mainly by fishermen and their families) in the Kumamoto Prefecture with age-matched residents of Minamata city (also in the Kumamoto Prefecture) who died between 1972 and 1978. In this study, high exposure to methylmercury was inferred from residence in a district believed to have higher intake of local seafood. By contrast, in the 1984 study described above, high methylmercury exposure was inferred from a diagnosis of Minamata disease. A total of 416 deaths were recorded in the Fukuro and Tsukinoura districts in 1970–1981, and 2,325 deaths were recorded in Minamata City in 1972–1978. No statistically significant increase in the overall cancer mortality rate was observed; however, an increase in the mortality rate due to liver cancer was observed (SMR, 207.3; 95% C.I. 116.0–341.9). Analysis of mortality by sex showed a statistically significant increase in the rate of liver cancer only among males (SMR, 250.5; 95% C.I. 133.4–428.4). Males also had statistically significantly higher mortality due to chronic liver disease and cirrhosis. The authors note that these results should be interpreted with caution because the population of Fukuro and Tsukinoura districts had higher alcohol consumption and a higher prevalence of hepatitis B (a predisposing factor for hepatocellular cancer). Interpretation of these results is also limited by an incomplete description of the methodology used to calculate the SMRs; it is unclear whether the study authors used appropriate methods to compare mortality data collected over disparate time frames (i.e. 12 years for exposed and seven years for controls).

In a study from Poland, Janicki et al. (1987) reported a statistically significant ($p<0.02$) increase in the mercury content of hair in leukemia patients (0.92 ± 1.44 ppm; n=47) relative to that in healthy unrelated patients (0.49 ± 0.41 µg/g; n=79). Similarly, the mercury content in the hair of a subgroup of leukemia patients (0.69 ± 0.75 µg/g; n=19) was significantly ($p<0.05$) greater than that in healthy relatives who had shared the same residence for at least 3 years preceding the onset of the disease (0.43 ± 0.24 µg/g; n=52). When patients with specific types of leukemia were compared with the healthy unrelated subjects (0.49 ± 0.41 µg/g; n=79), only those with acute leukemia (type not specified; 1.24 ± 1.93 µg/g; n=23) had a significantly increased hair mercury content. No significant differences in hair mercury content were observed in 9 patients with chronic granulocytic leukemia or 15 patients with chronic lymphocytic leukemia when compared to the unrelated, healthy controls. This study is of limited use for cancer risk assessment because of the following: small size of population studied; inadequate description of the leukemia patients or healthy controls (e.g., age distribution, length of residence in the

region, criteria for inclusion in the study); uncertainty regarding the source of mercury exposure (the authors presumed that exposure was the result of use of methylmercury-containing fungicides); uncertainty regarding the correlation between the chronology of incorporation of mercury in the hair and onset of the disease; and the failure to address exposure to other chemicals or adjust for other leukemia risk factors. Furthermore, the variability of hair mercury content was large, and the mean hair mercury levels were within normal limits for all groups. One cannot rule out the likelihood that the observed correlation of leukemia incidence with mercury in hair is due to chance alone.

The carcinogenic effects of organomercury seed dressing exposure were investigated in a series of case-control studies for incidence of soft-tissue sarcomas (Eriksson et al. 1981; Hardell and Eriksson, 1988; Eriksson et al. 1990) or malignant lymphomas (Hardell et al. 1981). These studies were conducted in Swedish populations exposed to phenoxyacetic acid herbicides or chlorophenols (the exposures of primary interest in the studies), organomercury seed dressings, or other pesticides. Exposure frequencies were derived from questionnaires and/or interviews. Control groups from the same region of the country were matched to cases based on vital status. There were 402 total cases of soft-tissue sarcoma, and (among persons not exposed to phenoxyacetic acid herbicides) there were 128 cases of malignant lymphoma. In each study, the odds ratio for exposure to organomercury in seed dressings and sarcoma or lymphoma was either less than 1.0, or the range of the 95% confidence interval for the odds ratio included 1.0; therefore, no association was indicated for organomercury exposure and soft-tissue sarcoma or malignant lymphoma. The conclusions from these studies are limited, however, due to the study subjects' likely exposures to the other pesticides and chemicals.

Table 3-52
Carcinogenic Effects of Methylmercury in Humans: Epidemiological Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Human/334 exposed (M+F), 668 control	NS	NS	No increase in cancer mortality among Minamata exposure victims (i.e., with overt methylmercury poisoning). Minamata disease was used as a surrogate for methylmercury exposure. Limitations: Exposure levels or number of undiagnosed cases among controls not known.	Tamashiro et al. 1984
Human/412 exposed (M+F)	NS	NS	Increased incidence of liver cancer in males living in the vicinity of Minamata Bay. Limitations: "Exposed" districts had higher alcohol consumption and higher prevalence of hepatitis B.	Tamashiro et al. 1986
Human/47 w/ leukemia (sex not specified) control 79	NS	NS	Increased mercury in hair of leukemia patients; however, mean hair mercury levels in the leukemia patients was within the normal range. Limitations: Small study population; source of methylmercury exposure not clear; failure to address other leukemia risk factors or exposure to other chemicals.	Janicki et al. 1987

3.3.2.2 Animal Data

The results from three dietary studies in two strains of mice indicate that methylmercury is carcinogenic. A fourth dietary study in mice, three dietary studies in rats and a dietary study in cats failed to show carcinogenicity of methylmercury. Interpretation of two of the positive studies was complicated by observation of tumors only at doses that exceeded the MTD. Interpretation of four non-positive studies was limited because of deficiencies in study design or failure to achieve an MTD.

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

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

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

Table 3-53
Carcinogenic Effects of Methylmercury in Animals: Oral Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Rat/strain NS/ 25 M, 25 F	2 yr ad lib in feed	0, 0.004, 0.02, 0.1	Tumors at comparable incidence in all groups Limitations: Small sample size; failure to achieve MTD BML avg: 850 µg/L in blood at 0.004, 6,500 µg/L at 0.02, and 36,000-39,000 µg/L at 0.1	Verschuuren et al. 1976
Rat/ Sprague Dawley/56 M, 56 F	130 wk ad lib in FEFD	0.011, 0.05, 0.28 (M); 0.014, 0.064, 0.34 (F)	No increase in tumor incidence	Mitsumori et al. 1983, 1984
Mice/Swiss/ 54 M, 54 F	weaning until death in drinking water	0, 0.19, 0.19-0.95 (MMA)	No increase in gross tumor incidence Limitation: Histological examination not performed.	Schroeder and Mitchener 1975
Mouse/ICR/ 60 M, 60 F	78 wk ad lib in feed	0, 1.6, 3.1	Increased incidence renal adenomas and adenocarcinomas in low-dose males. Limitations: Very poor survival in both male dose groups.	Mitsumori et al. 1981
Mouse/ICR/ 60 M, 60 F	104 wk ad lib in feed	0, 0.02, 0.03, 0.11, 0.15, 0.6, 0.73	Incidence of renal epithelial adenocarcinoma significantly increased in males at 0.73; not invasive. Limitations: MTD exceeded (including severe renal damage in high-dose males)	Hirano et al. 1986
Mouse/ B6C3F1/ 60 M, 60 F	2 yr ad lib in feed	0.03, 0.14, 0.69 (M); 0.03, 0.13, 0.6 (F)	Renal epithelial carcinomas and adenomas in males at 0.69. Limitation: MTD exceeded in high-dose males.	Mitsumori et al. 1990

Table 3-53 (continued)
Carcinogenic Effects of Methylmercury in Animals: Oral Exposure

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Mice/Swiss/ NS	15 wk ad lib in drinking water	0, 0.03, 0.07, 0.27 (MMC)	Number of lung adenomas/mouse and tumor size/mouse increased with dose	Blakley 1984
Cat/domestic/ 4-5 M, 4-5 F	2 yr ad lib in feed	0, 0.0084, 0.02, 0.046, 0.074, 0.176	No increase in tumor incidence Limitations: Small group size, short exposure duration, no pathological data for 3 lowest doses.	Charbonneau et al. 1976

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

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

No increase in tumor incidence or decrease in tumor latency was observed in another study using rats (strain not specified) (Verschuuren et al. 1976). Groups of 25 female and 25 male rats were

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

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

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

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

Blakley (1984) administered methylmercuric chloride to female Swiss mice (number/group not specified) in drinking water at concentrations of 0, 0.2, 0.5 or 2.0 mg/L for 15 weeks. This corresponded to approximately 0, 0.03, 0.07 and 0.27 mg Hg/kg-day. At the end of week 3, a single dose of 1.5 mg/kg of urethane was administered intraperitoneally to 16–20 mice/group. No effects on weight gain or food consumption were observed. Lung tumor incidence in mice not administered urethane (number/group

not specified) was less than 1 tumor/mouse in all groups. Statistically significant trends for increases in the number and size of lung adenomas/mouse with increasing methylmercury dose were observed; the tumor number/mouse was 21.5, 19.4, 19.4 and 33.1 in control, low-, mid- and high-dose mice, respectively, and the tumor size/mouse was 0.70, 0.73, 0.76 and 0.76 mm in control, low-, mid- and high-dose mice, respectively. The study authors suggest that the increase in tumor number and size may have been related to immunosuppressive activity of methylmercury. It should be noted that this is considered a short term assay and that only pulmonary adenomas were evaluated.

3.3.3 Other Data

3.3.3.1 Death

Methylmercury is a potent toxin that causes impairment of the CNS and developmental toxicity in humans. Ingestion of methylmercury from treated seed grain or contaminated fish has resulted in death. An outbreak of methylmercury poisoning in Iraq caused deaths in people who consumed methylmercury from bread made with grain treated with a fungicide (Al-Saleem and the Clinical Committee on Mercury Poisoning 1976; Bakir et al. 1973). The deaths were attributed to impaired CNS function. A syndrome known as Minamata disease has been characterized by nervous system impairment following consumption of methylmercury-contaminated fish from Minamata Bay in Japan. Symptoms of Minamata disease include the following: prickling; tingling sensation of extremities; impaired peripheral vision, hearing, taste and smell; slurred speech; muscle weakness; irritability; memory loss; depression; and sleeping difficulties (Kutsuna 1968; Takeuchi et al. 1962; Tsubaki and Takahashi 1986). Deaths from Minamata disease can be broken into two categories: deaths occurring from the beginning of the outbreak (1954) to 1969, and deaths occurring from 1970 to 1980 (Tamashiro et al. 1984). Over half of the deaths in the first group were attributed to Minamata disease and/or noninflammatory disease of the central nervous system, or pneumonia, whereas deaths in the second group were attributed to cerebrovascular disease with underlying Minamata disease. The mean age at death for the first group was 45.4 years for males and 26.4 years for females, and the mean age at death for the second group was 70.0 years for males and 72.7 years for females (Tamashiro et al. 1984).

Table 3-54
Lethality of Methylmercury in Humans: Case Study of Oral Exposure

Species/ No. per Sex	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Human/6,530 both sexes	43-68 d (feed)	0.71-5.7 (est.)	Of 6,350 cases admitted to hospitals, 459 died after eating bread made from grain treated with methylmercury fungicide BML: <100-5,000 µg/L in blood	Bakir et al. 1973
Human/1,422 both sexes	NS	NS	Of 1,422 patients from the Minamata disease outbreak in 1959, 378 died by 1980. Limitation: exposure data limited BML not reported	Tamashiro et al. 1984

Very little information regarding death after inhalation exposure to methylmercury was located. One study reported a man who died after being exposed for three years to alkylmercury particles from seed dressings (Hook et al. 1954). Prior to death, the man experienced increased symptoms of neurotoxicity. A case study reported on the deaths of two women exposed to diethylmercury vapors for

3–5 months (Hill 1943). Gastrointestinal effects and neurological symptoms occurred prior to deaths. No other human studies were located regarding death after inhalation exposure to methylmercury.

Table 3-55
Lethality of Methylmercury in Humans: Case Studies of Inhalation Exposure

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/2 F	3-5 mo (occup)	NS	Death following exposure to diethylmercury vapors Limitation: Case study; concomitant dermal exposure likely; limited exposure data BML not reported	Hill 1943
Human/1 M	3 yr (occup)	NS	Death following exposure to pesticide containing methylmercury Limitations: Case study; concomitant dermal exposure likely; limited exposure data Range: 500-640 µg/L in urine	Hook et al. 1954

Mice given a single oral dose of methylmercury had an increased incidence of death compared to controls (Yasutake et al. 1991). Male mice appear to be more sensitive to the effects of methylmercury than females, possibly due to the effect of mercury on the male kidneys. Mice exposed for 26 weeks to 3.1 mg Hg/kg-day as methylmercury in drinking water also showed an increase in mortality compared to controls (51/60 males and 59/60 females of exposed group died versus 1/60 males and 1/60 females in controls) (Mitsumori et al. 1981). Longer studies (78 and 104 weeks) confirm that methylmercury causes significantly increased mortality in mice compared to controls (Mitsumori et al. 1981, 1990). No animal studies were located on death after inhalation exposure to methylmercury.

Table 3-56
Lethality of Methylmercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Mouse/ICR/60 M, 60 F	78 wk ad lib in feed	0, 1.6, 3.1 (MMC)	51/60 males and 59/60 females receiving 3.1 mg/kg/d died by week 26, vs 7 males and 6 females at 1.6, and 1 control males and 1 control female; death at 52 wk was also elevated at 1.6 Limitation: No statistical analysis BML not reported	Mitsumori et al. 1981
Mouse/ $B6C3F_1$/60 M, 60 F	104 wk ad lib in feed	0, 0.03, 0.13, 0.14, 0.60, 0.69 (MMC)	50/60 males treated with 0.69 mg/kg/d died vs. 31/60 control males; survival of females and males at lower doses was unaffected BML not reported	Mitsumori et al. 1990

Table 3-56 (continued)
Lethality of Methylmercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Mouse/ C57BL/6 M, 6 F	Once	4, 8, 16, 24, 32, 40	4/6 males died (LOAEL = 16); LOAEL for females was 40 (4/6 died); no statistical analysis or LD_{50} calculated Limitation: small number of animals tested BML: 2.45 µg/g in kidney of males at 16 mg/kg	Yasutake et al. 1991

3.3.3.2 Neurological

The nervous system is the primary target organ for methylmercury toxicity. Information from the large-scale poisonings in Japan (Niigata and Minamata) and Iraq provide substantial information regarding the neurotoxicity of methylmercury in humans (Bakir et al. 1973, 1980; Berglund et al. 1971; Harada 1978; Marsh et al. 1987; Rustam and Hamdi 1974). In Japan, poisonings occurred between 1953 and 1960 when people consumed seafood that had been contaminated by methylmercury released by a chemical plant into Minamata Bay and the Agano river near Niigata. In Iraq, poisonings occurred in the winter of 1971 to 1972 when people ate bread made from seed grain that had been treated with a mercury-containing fungicide. In all of these episodes, neurotoxicity was the most prominent effect observed in the exposed populations. In the Iraqi incident, more than 6000 patients were hospitalized, and more than 500 deaths occurred, usually due to CNS failure.

The least severely affected persons from the poisonings in Japan and Iraq experienced numbness or tingling (paresthesia) of the extremities and/or perioral area. Additional symptoms frequently experienced by more severely affected individuals included the following: ataxia (gait impairment ranging from mild incoordination or unsteadiness to complete inability to walk); blurred vision; constriction of visual fields (in extreme cases blindness); slurred speech; and hearing difficulties (deafness in extreme cases). Less frequently observed symptoms associated with the methylmercury poisonings included tremors, muscular weakness, abnormal reflexes, increased muscle tone, and clouded memory or stuperousness. A long latent period (16–38 days in the Iraqi episode and up to several years in the Japanese episodes) between exposure and onset of symptoms of neurotoxicity was observed. The cause for the latent period is unknown. It is thought that latency may be related to cellular repair mechanisms dealing with damage from lipid peroxidation. At the point when repair processes are overwhelmed tissue damage and accompanying symptoms become apparent. The possible ameliorating effect of selenium in the diet has also been hypothesized to play a part in latency.

Similar neurological symptoms have been observed in persons ingesting meat contaminated with ethylmercuric chloride (Cinca et al. 1979). Two boys who ultimately died from exposure exhibited neurological signs including gait disturbance, ataxia, dysarthria, dysphagia, aphonia, hyperactive tendon reflexes, hypotonia, mydriasis and agitation. In the surviving members of the family, ataxia, gait impairment, spasticity, drowsiness, intention tremor, agitation, speech difficulties and visual disturbances were reported. All effects except the narrowing of the visual fields disappeared after exposure termination.

Histopathologic analyses of nervous system tissue taken from poisoning victims show neuronal degeneration in the cerebrum and cerebellum (Bakir et al. 1980; Swedish Expert Group 1971; Takeuchi et al. 1962). In the cerebral cortex, the calcarine area was most regularly affected with varying degrees of damage in the pre- and postcentral cortices, superior temporal gyrus, and basal ganglia. In the cerebellar cortex, granule cell loss predominated, but this was usually less severe than cerebral damage. An autopsy of two boys who ingested ethyl mercury contaminated meat revealed nerve cell loss and glial proliferation in the cerebral cortex, demyelination, granule cell loss in the cerebellum, and motor neuron loss in the ventral horns of the spinal cord (Cinca et al. 1979). Less information is available regarding the histopathology of peripheral nerve involvement, but sural nerves taken from two victims of the Minamata episode showed evidence of peripheral nerve degeneration and regeneration (Miyakawa et al. 1976). Fourteen Iraqi patients who developed ataxia and "pins and needles" and could not perform heel-to-toe walk were examined for impaired peripheral nerve function (Von Burg and Rustam 1974a, 1974b). Determinations of motor and sensory conduction velocities, sensory threshold and latency, reflex of the tibial nerve and myoneural transmission were performed, but there were no statistical significances between exposed and unexposed control groups; the mean values of the experimental group, however, were somewhat lower than those of the controls. There was also no consistent correlation between clinical or electrophysiological observation on the peripheral nervous system and blood mercury levels. In two patients who were hospitalized 10 days after ingestion of ethyl mercury-contaminated meat, sensory nerve conduction velocity was decreased immediately after admission but was found to be normal six months later (Cinca et al. 1979).

Table 3-57
Neurotoxicity of Methylmercury in Humans: Case Studies of Oral Exposure

Species/ No. per Sex	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Human/14 cases	NS	NS	Ataxia; impaired heel-to-toe walk; complaints of "pins and needles". Sensory and motor peripheral nerves were not affected. Clinical and electrophysiological observations did not correlate with blood concentration Limitation: Exposure concentration and duration not known BML: blood Hg levels were 138-878 µg/L	Von Burg and Rustam 1974a, 1974b
Human/No. NS	NS	NS	Paresthesia/numbness; constriction of visual field; incoordination; difficulty speaking; tremor in consumers of contaminated fish Limitation: Limited details reported BML not reported	Harada 1978
Human/2 M, 2 F	Once	NS (ethyl mercury chloride)	Gait disturbance, ataxia, dysarthria, speech difficulties, visual disturbances, hyperactive tendon reflexes, mydriasis, agitation, coma; nerve degeneration in cerebral cortex, cerebellum, and ventral horns of spinal cord; decreased sensory nerve conduction velocity. Ingestion of ethyl mercury chloride-contaminated meat. Limitation: Exposure concentration not known BML: hair Hg levels of 152-542 µg/g	Cinca et al. 1979
Human/6530 cases both sexes	43-68 d	0.71-5.7 (est.)	Paresthesia/numbness in extremities and perioral area; ataxia; constriction of visual field or blindness; slurred speech; hearing difficulties following ingestion of grain contaminated with methylmercury. Incidence and severity of effect correlated with blood concentration BML: Total body burden ≥50 mg at time of onset	Bakir et al. 1973, 1980
Human/81 F	<5 mo	NS (MMC)	Paresthesia and "other neurological symptoms" BML Range: 1-674 µg/g Hg in hair; one woman with 14 µg/g (maximum in strand) had paresthesia and a woman with 10 µg/g had other symptoms. However, others with levels as high as 600 µg/g had no symptoms. (This is a follow-up study to Bakir et al. 1980)	Marsh et al. 1987

There are two case studies that report neurotoxicity in humans following inhalation of methylmercury (Hook et al. 1954; Hunter et al. 1940); however, no quantitative data were available. The two studies described in Table 3-58 demonstrated the spectrum of neurotoxic effects that occur following occupational exposure to methylmercury. Weiss and Simon (1975) have suggested that such changes in function in the general population, particularly at relatively low doses, may not be clinically detectable as a loss of function but may be unmasked by the normal processes of aging.

Table 3-58
Neurotoxicity of Methylmercury in Humans: Case Studies of Inhalation Exposure

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitation/BML	Reference
Human/5 M	5 mo-2 yr (occup)	NS	Tingling of limbs; unsteady gait; difficulty performing fine movements; constricted visual field following exposure to methylmercury nitrate, methylmercury iodide, of methylmercury phosphate in chemical factories Limitations: Case studies; concomitant dermal exposure and exposure to other chemicals likely; limited exposure data BML not reported	Hunter et al. 1940
Human/1 M	3 yr (occup)	NS	Weakness in arms and legs; irregular EEG; sensory and speech disorders following exposure to pesticide containing methylmercury Limitations: Case study; concomitant dermal exposure likely; limited exposure data BML Range: 500-640 µg/L in urine	Hook et al. 1954

As a result of the methylmercury poisonings in Japan and Iraq, substantial information on the neurotoxicity of methylmercury has been generated from animal studies. Relatively brief, high level exposures in rats have been shown to cause characteristic signs of neurotoxicity (flailing and hindlimb crossing when the animal is lifted by the tail) and neuronal degeneration in the cerebellum, cerebral cortex and dorsal root ganglia (Inouye and Murakami 1975; Leyshon and Morgan 1991; Magos et al. 1985; Yip and Chang 1981). As with humans there is a latency period; the effects frequently are not observed or do not show maximal severity until several days after the cessation of dosing. In an acute study, exposure of rats to a single gavage dose of 19.9 mg Hg/kg as methylmercuric chloride resulted in impaired open-field tests such as decreases in standing upright, area traversed and activity compared to the control group (Post et al. 1973). Animals were lethargic and ataxic initially, but symptoms disappeared within 3 hours.

Longer-term, lower-level exposures revealed that evidence of neuronal degeneration may be observed prior to the onset of overt signs of toxicity. Degeneration in the cerebellum was found in rats given 10 mg Hg/kg as methylmercuric chloride once every 3 days for 15 days (Leyshon and Morgan 1991) while severe degenerative changes in the dorsal root fibers were observed in rats given 1.6 mg Hg/kg-day as methylmercuric chloride for 8 weeks (Yip and Chang 1981). Munro et al. (1980) observed demyelination of dorsal nerve roots and damage in sciatic nerves with oral exposure to 0.25 mg Hg/kg-day as methylmercuric chloride for up to 26 months. In mice given 1.9 mg Hg/kg-day as methylmercury, cerebellar lesions were observed as early as eight days after the start of dosing, but changes in motor activity did not develop until 24 weeks of exposure (MacDonald and Harbison 1977). Similarly, cats receiving methylmercury in the diet for 11 months displayed degenerative changes in the cerebellum and cerebral cortex, but incoordination or weakness was observed in only a small number of the animals with histopathological changes (Chang et al. 1974).

The molecular basis for methylmercury neurotoxicity is likely to be complex and multifactorial. The broad affinity of mercury for -SH groups leads to membrane, enzyme and cytoplasmic organelle interaction. Major mechanistic pathways have been proposed to include the following: inhibition of macromolecular metabolism, especially that of protein translation and nucleic acid biogenesis; oxidative

injury; disturbance in Ca^{2+} hemostasis; aberrant protein phosphorylation. The mechanisms underlying inhibition of protein and RNA synthesis are multiple. Depending upon the systems used with *in vitro*, *in vivo* or neuronal cell suspensions, evidence for inhibition of translation associated with a change in ATP/ADP concentration has been found. On the other hand, direct inhibition of elongation was documented secondary to the selective inhibition of certain aminoacyl-tRNA synthetase (Cheung and Verity, 1985). Syversen (1977) investigated the effects of methylmercury on protein synthesis in rats using techniques which allowed analysis of different cell populations from the central nervous system. Results of this study indicated selective irreversible damage to granule cells of the cerebellum, whereas damage to the other neurons, such as Purkinje cells was reversible. Such selectivity of toxicity is a feature of the neuronal loss seen in human and experimental disease. Methylmercury has also been suggested to cause neuronal degeneration by promoting the formation of reactive oxygen species (Ali et al. 1992; Le Bel et al. 1990, 1992; Verity and Sarafian 1991). While contributory, such oxidative injury does not appear primary to the site of toxicity as appropriate protective measures blocking oxidative stress and lipoperoxide formation are only minimally cytoprotective.

A recent review by Atchison and Hard (1994) discusses several proposed mechanisms of action of methylmercury on Ca^{2+} hemostasis and ion channel function. Individual studies have demonstrated that the neuromuscular actions of methylmercury occur predominantly at the presynaptic site (Atchison et al. 1984). Methylmercury may interfere with acetylcholine neurotransmitter release and subsequently synaptic transmission (Atchison et al. 1986; Barrett et al. 1974; Schafer et al. 1990; Schafer and Atchison 1989, 1991). Finally, Sarafian (1993) demonstrated that the methylmercury-induced stimulation of protein phosphorylation in cerebellar granule cell culture is coupled to Ca^{2+} uptake, changed intracellular Ca^{2+} hemostasis and inositol phosphate metabolism. These latter observations invoke the activation of the protein kinase C pathway.

Cats and monkeys appear to be more sensitive to the neurotoxic effects of methylmercury than rodents. Long-term studies in primates and in cats have shown neurological impairment at doses as low as 0.05 mg Hg/kg-day. In cats, mild impairment of motor activity and decreased pain sensitivity was observed at 0.046 mg Hg/kg-day as methylmercury after 60 weeks of exposure (Charbonneau et al. 1976). In cynomolgus monkeys given methylmercury from birth until approximately 7 years of age, impairment of spatial visual function was observed after 3 years, and decreased fine motor performance, touch and pinprick sensitivity and impaired high frequency hearing were observed six to seven years after cessation of dosing (Rice 1989b; Rice and Gilbert 1982, 1992). Exposure of cynomolgus monkeys to 0.03 mg Hg/kg-day as methylmercury for approximately 4 months caused no detectable changes in motor activity or effects on vision or hearing, but degenerative changes were observed in neurons of the calcarine cortex and sural nerve when these were examined electron microscopically (Sato and Ikuta 1975). At higher doses (0.08 mg Hg/kg-day), slight tremor, motor incoordination and blindness were observed in *Macaca fascicularis* monkeys after four months of exposure (Burbacher et al. 1988).

The developing organism is generally at higher risk of neurotoxicity than adults. The section on developmental effects of methylmercury lists studies wherein animals were observed with neurological or neurobehavioral deficits as a consequence of *in utero* or perinatal methylmercury exposure.

Table 3-59
Neurotoxicity of Methylmercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Rat/Wistar/ 10 F	0–12 or 12–20 d, 1x/d (gavage)	2, 4 (MMC)*	Hindlimb crossing (LOAEL = 4) after 0–12 days BML not reported	Inouye and Murakami 1975
Rat/Wistar/ 50 F, 50 M	up to 26 mo ad lib in feed	0.002, 0.01, 0.05, 0.25 (MMC)*	Ruffled fur, loss of balance, hindlimb crossing, paralysis (LOAEL = 0.25) after 6 mo (males more affected); demyelination of dorsal nerve roots and damage in teased sciatic nerves at 0.25 Avg. BML at 0.25: 115 ppm in blood	Munro et al. 1980
Rat/Charles River/6 M	8 wk 7 d/wk 1 x/d (gavage)	0, 1.6 (MMC)*	Degeneration of dorsal root fiber BML not reported	Yip and Chang 1981
Rat/Wistar/24 M, 18 F	5 d 1 x/d (gavage)	8 (MMC)*	Cerebellar granule cell and dorsal root ganglion cell degeneration; flailing and hind leg crossing following administration of methylmercuric chloride Limitations: Only one level tested; no controls Avg BML: 150,000 µg/L in blood	Magos et al. 1985
Rat/Wistar/15 M	5 x/15 d (gavage)	0, 10 (MMC)*	Granule cell degeneration in cerebellum BML: 60 µg/g dry cerebellar weight	Leyshon and Morgan 1991
Swiss origin Mouse M	28 wk (ad lib drinking water)	1.9, 9.5 (MMC)*	Ataxia; degenerative changes of Purkinje cells; granule cell loss in cerebellum; (LOAEL = 1.9) BML not reported	MacDonald and Harbison 1977
Cat/Breed NS/15-16 both sexes	11 mo (ad lib in feed)	0, 0.015 (MM)	Degeneration of cerebellum and cerebral cortex; necrosis of dorsal root ganglia of kittens fed mercury-contaminated tuna BML not reported	Chang et al. 1974
Cat/Breed NS/8-10 NS	2 yr 7 d/wk (feed)	0.003, 0.008, 0.020, 0.046, 0.074, 0.176 (MMC)*	Impaired hopping reaction; decreased pain sensitivity; degeneration of dorsal root ganglia (LOAEL = 0.046) Avg BML: 9,000 µg/L in blood at 0.046 mg/kg-day	Charbonneau et al. 1976
Monkey/ *Macaca fascicularis*/1-2 both sexes	36–132 d 1 x/d (feed)	0.02, 0.03, 0.04, 0.07, 0.21	Atrophy of neurons in calcarine cortex; focal degeneration in sural nerves (LOAEL=0.03); ataxic gait, myoclonic seizures at 0.21 mg/kg-day Limitation: small number of animals tested BML: Maximal at 0.03 mg/kg-day of 460 µg/L in blood and 62 µg/g in hair	Sato and Ikuta 1975
Monkey/ *Macaca artoides*, *Macaca nemestrina*/2 both sexes	90-270 d 1 x/wk (gavage)	1 for 5 doses, then 0.4, 0.5, 0.6	Tremor; visual impairment (LOAEL = 0.5 mg/kg) Limitations: Small number of animals tested, limited description of effects Avg BML: 2,900 µg/L in blood	Evans et al. 1977

Table 3-59 (continued)
Neurotoxicity of Methylmercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Monkey/ *Macaca fascicularis*/5 exposed, 2 control (sex NS)	3-4 yr 7 d/wk 1 x/d (NS)	0, 0.05 (MMC)*	Spatial visual impairment Limitation: One dose level tested BML: 600-900 µg/L in blood	Rice and Gilbert 1982
Monkey/ *Macaca fascicularis*/7-8 F	~3 yr 1 x/d (oral route NS)	0, 0.04, 0.06, 0.08 (MMC)*	Slight tremor; motor incoordination; blindness (LOAEL = 0.04) following administration of methylmercury hydroxide; time to onset was 177-395 d Avg BML: 2,030 µg/L in blood at highest dose	Burbacher et al. 1988
Monkey/ *Macaca fascicularis*/4 M, 1 F exposed, 1 M, 2 F controls	6.5-7 yr 7 d/wk 1 x/d (capsule; gavage)	0, 0.05 (MMC)*	Six years after end of dosing (follow-up study to Rice and Gilbert 1982): decreased fine motor performance; diminished touch and pinprick sensitivity; impaired high frequency hearing ($p<0.05$) Limitations: small number of animals tested; one dose level tested BML: Not detectable at time of testing	Rice 1989b; Rice and Gilbert 1992

*MMC = methylmercuric chloride

3.3.3.3 Renal

No studies were located regarding the renal toxicity of methylmercury in humans following oral exposure. Renal histopathology and decreased function have been observed following acute or chronic oral exposure of rats and mice to methylmercury. Renal tubule vacuolation was observed in rats receiving 8 mg Hg/kg-day for 5 days (Magos et al. 1985), and decreased phenolsulfonphthalein excretion occurred in male mice receiving a single dose of 16 mg Hg/kg-day or greater and females at 32 mg Hg/kg-day or greater as methylmercuric chloride (Yasutake et al. 1991). Chronic nephropathy, including epithelial degeneration of proximal tubules and interstitial fibrosis, was observed at longer durations (Fowler 1972; Hirano et al. 1986; Mitsumori et al. 1990). Males were more sensitive than females to renal effects (Mitsumori et al. 1990).

Table 3-60
Renal Toxicity of Methylmercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Rat/Wistar/3 M, 6 F exposed/16 controls (sex NS)	12 wk ad lib in feed	0, 0.08 (M) 0, 0.09 (F) (MMC)	Cytoplasmic mass in proximal tubule cells Limitation: Only one level tested; small number of treated animals BML not reported	Fowler 1972
Rat/Wistar/24 M, 18 F	5 d 1 x/d (gavage)	8	Renal tubule vacuolation and dilation Limitation: One level tested, no controls Avg. BML: 150,000 µg/L in blood	Magos et al. 1985
Mouse/ICR/60 M, 60 F	26 wk ad lib in feed	0, 0.03, 0.15, 0.72 (M); 0.02, 0.11, 0.62 (F)	Toxic epithelial degeneration of renal proximal tubules (LOAEL = 0.62 F; 0.72 M) BML not reported	Hirano et al. 1986
Mouse/ $B6C3F_1$/60 M, 60 F	104 wk ad lib in feed	0, 0.03, 0.14, 0.68 (M); 0.03, 0.13, 0.6 (F) (MMC)	Chronic nephropathy (epithelial cell degeneration, regeneration of proximal tubules, interstitial fibrosis) in males at $\geq$0.14 and in females at 0.60 ($p<0.01$) BML not reported	Mitsumori et al. 1990
Mouse/ C57BL/6 M, 6 F	Once (gavage)	4, 8, 16, 24, 32, 40 (MMC)	Decreased phenolsulfonphthalein excretion and increased serum creatinine in males (LOAEL = 16 in males, 32 in females); swollen epithelial cells in proximal tubules Limitation: No statistical analysis; small number of treated animals BML: 2.45 µg/g in kidneys of males and 1.9 µg/g in kidneys of females at 16 mg/kg	Yasutake et al. 1991

3.3.3.4 Cardiovascular

Only one study was located regarding the cardiovascular toxicity of methylmercury in humans. Hook et al. (1954) reported two men with elevated blood pressure after inhalation exposure to organic mercury particulates from seed dressings. Other neurotoxic effects were also present at the time of examination, and one man subsequently died.

Table 3-61
Cardiovascular Toxicity of Methylmercury in Humans: Case Study

Species/ No. per Sex	Exposure Duration	Dose (mg/m^3)	Effects/Limitations/BML	Reference
Human/1 M	3 yr (occup)	NS	Elevated blood pressure Limitations: Case study; concomitant dermal exposure likely BML Range: 500-640 µg/L in urine	Hook et al. 1954

Very little information was located regarding the effects of oral methylmercury exposure on the cardiovascular system. Rats given two daily doses of methylmercuric chloride exhibited decreases in heart rates (Arito and Takahashi 1991). Rats treated with methylmercuric chloride for one month had increased systolic blood pressures beginning 42 days after cessation of dosing (Wakita 1987). This effect persisted for more than a year.

Table 3-62
Cardiovascular Toxicity of Methylmercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitation/BML	Reference
Rat/Wistar/10 (sex NS)	23-28 d 7 d/wk (gavage)	0.4, 1.2 (MMC)	Increased systolic pressure beginning 42 d after the end of treatment ($p<0.05$) BML not reported	Wakita 1987
Rat/Sprague-Dawley/5-6 (sex NS)	2 d 1 x/d (gavage)	12 (MMC)	Decreased heart rate ($p<0.05$) Limitation: Only one dose tested for this parameter BML: 10 µg/g in brain	Arito and Takahashi 1991

3.3.3.5 Gastrointestinal

No information was located regarding the gastrointestinal toxicity of methylmercury in humans. Only one study was located regarding the gastrointestinal toxicity of methylmercury following oral exposure in animals. Mitsumori et al. (1990) reported an increased incidence of stomach ulceration in mice following a 2-year exposure to 0.69 mg Hg/kg-day as methylmercuric chloride in drinking water.

Table 3-63
Gastrointestinal Toxicity of Methylmercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Mouse/ $B6C3F_1$/60 M, 60 F	104 wk ad lib in feed	0, 0.03, 0.14, 0.69 (M); 0.03, 0.13, 0.6 (F) (MMC)	Stomach ulceration in males at 0.69 ($p<0.05$) BML not reported	Mitsumori et al. 1990

3.3.3.6 Immunological

Suppression of the humoral and cellular immune responses have been observed in animals after oral exposure to methylmercury or methylmercuric chloride. Both decreases in the production of antibody-producing cells and decreased antibody titre in response to inoculation with immune-stimulating agents (such as sheep red blood cells) have been observed (Blakley et al. 1980; Koller et al. 1977; Ohi et al. 1976). Decreases in natural killer T-cell activity have been observed in animals after exposure to methylmercury (Ilback 1991).

Table 3-64
Immunotoxicity of Methylmercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Rat/Brown Norway/6 both sexes exposed/22 both sexes/controls	NS x/wk 2 mo	0, 4.8 (MMC)	IgG deposits along the glomerular capillary wall of the kidney, not in arteries, suggestive of an autoimmune disease; no effect seen in controls. Limitation: only one level tested BML not reported	Bernaudin et al 1981
Mouse/ICR/6 M	5 d 1 x/d (gavage)	0.27, 2.7 (MMC)	Decreased production of antibody-producing cells (LOAEL = 2.7; $p<0.01$). Limitation: small number of animals, only males tested BML not reported	Ohi et al. 1976
Mouse/Swiss/8-10 M	3 wk ad lib in drinking water	0.076, 0.3, 1.52 (MMC)	Decreased production of antibody-producing cells and decreased antibody titer (LOAEL = 0.076; $p<0.01$). Limitation: small number of animals, only males tested BML not reported	Blakley et al. 1980

Table 3-64 (continued)
Immunotoxicity of Methylmercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Mouse/Balb/c CUM/ 8 F	12 wk ad lib in feed	0, 0.5	Reduced natural killer T-cell activity; decreased thymus weight and cell number ($p<0.01$). Limitation: small number of animals treated, only females tested BML not reported	Ilback 1991
Rabbit/New Zealand white/10 M, 10 F	14 wk 1 x/d in feed	0.04, 0.4, 0.8 (MMC)	Decreased antibody titer (LOAEL = 0.4) (26% of the animals at 0.4 and no controls died by wk 14). Limitations: No statistical analysis BML: 2,240 µg/L in blood at 0.4 mg/kg/d at wk 14	Koller et al. 1977

3.3.3.7 Dermal

Al-Mufti et al. (1976) studied the effects of methylmercury in humans who ate contaminated bread; a correlation between bread consumption and a history of rash was reported. No other information was located regarding dermal effects of organic mercury following oral exposure.

Table 3-65
Dermal Toxicity of Methylmercury in Humans: Epidemiological Study

Species/ No. per Sex	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Human/415 exposed/1012 controls (sex NS)	≈1-3 mo (feed)	NS (MMC)	"History of rash" in 14% of exposed group, compared with <1% of unexposed Limitations: Effects poorly described; no statistical analysis BML not reported	Al-Mufti et al. 1976

3.3.3.8 Developmental

Methylmercury readily crosses the placental barrier, and marked developmental toxicity has been observed in both humans and animals after gestational exposures. Infants exposed to methylmercury through the mother's milk or during gestation had elevated blood mercury levels, as did their mothers (Amin-Zaki et al. 1976). Human data from epidemic poisonings that occurred in Japan (Harada 1978) and Iraq (Amin-Zaki et al. 1974), as well as isolated exposures (Snyder and Seelinger 1976) indicate that methylmercury predominantly affects the developing nervous system. Infants born to mothers who ingested fish contaminated with methylmercury from Minamata Bay in Japan between 1953 and 1960

appeared normal at birth but within several months exhibited mental retardation, retention of primitive reflexes, cerebellar symptoms, dysarthria, hyperkinesia, hypersalivation, strabismus and pyramidal symptoms (Harada 1978). Similarly, infants born to mothers who had ingested bread made with seed grain treated with methylmercury-containing fungicides in Iraq during 1971 to 1972 exhibited symptoms ranging from delays in speech and motor development to mental retardation, reflex abnormalities and seizures (Amin-Zaki et al. 1974, 1978). Histopathologic analyses of brain tissues from infants that died in the Iraqi (Choi et al. 1978) and Minamata (Harada 1978) episodes showed atrophy and hypoplasia of the cerebral cortex, corpus callosum and granule cell layer of the cerebellum; dysmyelination of the pyramidal tracts; and/or abnormal neuronal cytoarchitecture characterized by ectopic cells and disorganization of cellular layers.

A number of studies have attempted to evaluate developmental neurotoxicity in populations with elevated methylmercury exposure from consumption of fish as a major component of the diet but for whom massive poisonings have not been reported. Kjellstrom et al. 1989) observed a higher incidence of abnormal scoring on tests designed to assess intelligence and development among children from New Zealand whose mothers had high levels of hair mercury. Also a study by McKeown-Eyssen et al. (1983) of a Cree population from northern Quebec revealed a correlation between maternal exposure (as determined using hair levels) and abnormal muscle tone or reflexes in male children. A dose-response for this effect was not observed.

Dose-response analyses of human data from the Iraqi epidemic of 1971 to 1972 have indicated correlations between maximal maternal hair levels during pregnancy and the severity of the neurological deficits seen in the children (Cox et al. 1989; Marsh et al. 1981, 1987). An evaluation of a calculated threshold for response is presented in Section 6.3.1 of this volume.

Table 3-66
Developmental Toxicity of Methylmercury in Humans: Case Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Human/8 M, 7 F infants	~2 mo. (feed)	NS	Assessment of 15 mother-infant pairs where the mothers ate grain treated with methylmercury fungicide during pregnancy. Motor and mental development were impaired (blindness, impaired hearing) in 6 infants; there were no congenital malformations. BML: Affected infants: ~3,000 µg/L in blood at 2 months; Affected mothers: ≥400 µg/L in blood	Amin-Zaki et al. 1974
Human/1 F	6 mo. 3 mo. postcoital-term (feed)	NS	Severe neurological impairment (blindness, myoclonic seizures, spastic quadriparesis) of male infant born to a mother eating meat from pigs that had eaten grain treated with methylmercury fungicide. Limitation: Case report BML not reported	Snyder and Seelinger 1976

Table 3-67
Developmental Toxicity of Methylmercury in Humans: Epidemiologic Studies

Species/ No. per Sex	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Human/220 F	NS (food)	NS	Mental retardation, atrophy of brain and degeneration of cerebellum in offspring. Of 220 infants born in Minamata (to mothers eating contaminated fish), 13 had severe symptoms; the number with less severe symptoms was not reported. Limitations: Few details on methods or results BML not reported	Harada 1978
Human/84 mother-child pairs	few days to several mo. (food)	NS	Assessment of mother-infant pairs where mothers ate grain treated with methylmercury fungicide during pregnancy (same Iraqi population as reported by Amin-Zaki et al. 1974). Severe psychomotor retardation in infants. BML Range: 37-293 µg/g in hair (maximum in segment of maternal hair)	Marsh et al. 1981
Human/243 exposed (sex NS) aged 12-30 mo.	Gestation and lactation (food)	NS	Abnormal tendon reflexes or muscle tone in male offspring correlated with methylmercury exposure ($p<0.05$). Conducted as a case-control study after potential affected measures were identified. Limitation: Author reported that the statistical method could have led to an association by chance. BML avg: 6 µg/g in maternal hair	McKeown-Eyssen et al. 1983
Human/81 mother-child pairs	few days to several mo. (food)	NS	Assessment of mother-infant pairs where mothers ate grain treated with methylmercury fungicide during pregnancy (same Iraqi population as reported by Amin-Zaki et al. 1974). Delayed walking and talking; seizures; mental retardation. BML Range: ~18-598 µg/g (maximum in strand) in hair of mothers of affected infants	Marsh et al. 1987

The developmental toxicity of oral exposure to methylmercury has been extensively studied in animals. In rodents exposed *in utero*, a spectrum of effects has been observed ranging from decreases in fetal weight and skeletal ossification and increases in skeletal variations and malformations (brain lesions, hydrocephalus, cleft palate, micrognathia, edema, subcutaneous bleeding, hydronephrosis, hypoplasia of the kidneys, dilation of the renal pelvis) to increased resorptions and fetal deaths (Fuyuta et al. 1978, 1979; Inouye and Kajiwara 1988a; Inouye and Murakami 1975; Khera and Tabacova 1973; Nolen et al. 1972; Reuhl et al. 1981; Yasuda et al. 1985). The severity of the effects generally increased with dose, and the incidence of malformations increased with exposures that occurred later in gestation (Fuyuta et al. 1978; Inouye and Murakami 1975). Brain lesions have been observed in a variety of areas including the brain mantle, corpus callosum, caudate putamen and cerebellum. In guinea pigs, early gestational exposures (weeks 3–5 of pregnancy) resulted primarily in developmental disturbances of the brain (smaller brains, dilated lateral ventricles and reduced size of caudate putamen), whereas later gestational exposures (>week 6 of pregnancy) resulted in widespread neuronal degeneration (Inouye and Kajiwara 1988b).

In addition to structural changes, functional changes have been observed in animals after gestational exposures. Such functional effects include abnormal tail position during walking; flexion;

hindlimb crossing; decreased locomotor activity, responding in an avoidance task and righting response; increased passiveness, startle-response and sensitivity to pentylenetetrazol-induced convulsions; and impaired maze performance, operant behavior, swimming behavior, tactile-kinesthetic function, visual recognition memory, temporal discrimination, and subtle learning deficits such as insensitivity to changing reinforcement contingencies (Bornhausen et al. 1980; Buelke-Sam et al. 1985; Burbacher et al. 1990; Elsner 1991; Geyer et al. 1985; Gunderson et al. 1988; Hughes and Annau 1976; Inouye et al. 1985; Musch et al. 1978; Olson and Boush 1975; Rice 1992; Rice and Gilbert 1990; Stoltenburg-Didinger and Markwort 1990; Suter and Schon 1986; Newland et al. 1994).

Overt neurological impairment is the endpoint used to document methylmercury poisonings; however, as shown in animal studies, methylmercury may produce more subtle neurodevelopmental effects such as impairment of sensory or cognitive systems. Schreiner et al. (1986) exposed rats to 0, 0.2 or 0.6 mg Hg/kg-day as methylmercuric chloride *in utero* and during lactation to evaluate pup performance on visual discrimination reversal task. While no overt signs of neurotoxicity were evident, subtle differences between the control and high-dose group were observed during more difficult tasks. A stressful or highly demanding situation appears to be necessary for the expression of these sensory effects, wherein the decreased ability to adapt to the altered conditions became manifest. Spyker et al. (1972) reported that although no signs of neurological toxicity was observed in mouse pups exposed to methylmercury *in utero*, open field and swimming tests revealed subtle neurological effects in the exposed pups. Newland et al. (1994) administered methylmercury by gavage to pregnant squirrel monkeys between weeks 11 and 14.5 of gestation. Doses were adjusted to maintain 0.7 to 0.9 ppm Hg in the maternal blood. There were three controls and three methylmercury-treated offspring. Offspring were evaluated at 5-6 on a lever pressing test which required discrimination between degrees of reinforcement. At steady state, monkeys exposed to methylmercury *in utero* were less sensitive to differences in reinforcement rates. When reinforcement rates changed, exposed animals either changed their behavior slowly in response to the altered reinforcement or not at all.

The developmental toxicity of methylmercury may be attributable to the ability of methylmercury to bind to sulfhydryl-rich tubulin (a protein component of microtubules) and cause its depolymerization (Falconer et al. 1994; Sager et al. 1983). Both cell division and cell migration require intact microtubules for normal functioning. Disruption of microtubule function could result in the derangement of cell migration (Choi et al. 1978; Falconer et al. 1994; Matsumoto et al. 1965) and arrested cell division (Reuhl et al. 1994; Sager et al. 1984).

Table 3-68
Developmental Toxicity of Methylmercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Rat/Charles River/20 F	9 d Gd 6-14 ad lib in drinking water	0, 0.02, 0.2, 4	Increased number of fetuses with soft tissue variations of the urinary system and incomplete ossification or calcification (LOAEL = 4; $p<0.05$). BML not reported	Nolen et al. 1972

Table 3-68 (continued)
Developmental Toxicity of Methylmercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Rat/Wistar/35 F	52 d ad lib in feed	0, 0.002, 0.01, 0.05, 0.25 (MMC)	Increased incidence of eye defects (in harderian and lachrymal glands) and salivary glands in fetuses (LOAEL = 0.25); significant dose response (p = 0.01). Mothers were treated from immaturity through weaning or later. Limitations: Incomplete reporting; of results BML not reported	Khera and Tabacova 1973
Rat/Wistar/10 F	8, 12, or 20 d 1 x/d Gd 12-20, 0-12, or 0-20 (gavage)	2, 4 (MMC)	Increased brain lesions and generalized edema (Gd 0-20) (LOAEL = 2). Limitations: Limited data reporting; no statistical analysis; small number of treated animals BML not reported	Inouye and Murakami 1975
Rat/Holtzman/5 F	during gestation, during lactation, or postnatal days 21-30 in drinking water	0, 2.5 (MMC)	Decreased visual evoked potential latencies for peaks N1 ($p \leq 0.05$), P1 ($p \leq 0.01$) and P2 ($p \leq 0.01$) in 30-day old pups exposed during gestation, during lactation, or during postnatal days 21-30. BML not reported	Zenick 1976
Rat/Charles River CD/20 F	47 d prior to and during gestation ad lib in drinking water	0.42, 0.7, 1.4 (MMH)	Ultrastructural changes, dose-related decrease in biochemical activity in mitochondria of fetal hepatocytes ($p<0.01$) following administration of methylmercury hydroxide to mothers (LOAEL = 1.4). BML: 40 µg/g (organic and inorganic) in liver of fetuses at 1.4 mg/kg-day	Fowler and Woods 1977
Rat/Long-Evans/4 exposed, 6 control	Once Gd 7 (gavage)	0, 4 (MMC)	Increased P1-N1 amplitudes and decreased P2 and N2 latencies of cortically visual evoked potential ($p<0.05$). BML not reported	Dyer et al. 1978
Rat/Wistar/20 F	8 d 1 x/d Gd 7-14 (gavage)	0, 2, 4, 6 (MMC)	At 6 mg/kg-day, decreased maternal weight gain, increased resorptions and fetal deaths ($p<0.001$); decreased fetal body weight increased skeletal and visceral malformations (hydrocephaly, wavy ribs). (LOAEL = 4; $p<0.01$) BML not reported	Fuyuta et al. 1978
Rat/Wistar-Neuherberg/ No. F. NS	4 d Gd 6-9 (gavage)	0, 0.04, 1.6 (MMC)	Impaired ability to perform operant conditioning procedures (number of responses on lever required in specified period of time) (LOAEL = 0.05). Limitation: Statistical analyses not reported BML not reported	Musch et al. 1978
Rat/Wistar/10 F	4 d 1 x/d Gd 6-9 (gavage)	0, 0.004, 0.008, 0.035 (MMC)	Reduction in behavioral performance in offspring of treated mice following operant conditioning (LOAEL = 0.008; $p<0.01$). BML not reported	Bornhausen et al. 1980

Table 3-68 (continued)
Developmental Toxicity of Methylmercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Rat/Sprague-Dawley/No. F NS	Once Gd 8 (gavage)	0, 6.3 (MMC)	Shorter avoidance latency in 60-day old offspring (LOAEL = 6.3). BML not reported	Cuomo et al. 1984
Rat/Sprague-Dawley/No. F NS	10 d 1 x/d Gd 6-15 (gavage)	0, 0.2, 1, 2, 4 (MMC)	Delayed sexual development (vaginal patency and testes descent), reduced pivoting, delayed surface righting, partially retarded swimming development, increased activity in center of open field, impaired startle reflex response. Reduced maternal weight gain and litter weight. No live offspring were produced at 4 mg/kg-day (LOAEL = 2; $p<0.05$). BML not reported	Geyer et al. 1985
Rat/Sprague-Dawley/ 15-19 F	4 d 1 x/d Gd 6-9 (gavage)	0, 1.6, 4.8 (MMC)	Delayed vaginal patency, delayed surface righting, retarded swimming development, lower activity, impaired complex water maze performance. Increased mortality of pups at 1-21 days of age (LOAEL = 4.8; $p<0.05$). BML not reported	Vorhees 1985
Rat/Wistar/ 38 M, 38 F	during gestation and lactation ad lib in drinking water	0, 0.2, 0.6 (MMC)	Increase in response latency in male ($p<0.05$) and female pups ($p<0.01$) and in passiveness ($p<0.05$) in visual discrimination reversal task at 0.6 mg/kg-day (LOAEL = 0.6). BML not reported	Schreiner et al. 1986
Rat/HAN-Wistar/10 F	13 days prior to mating until post-natal day 21 in drinking water	0, 0.2, 0.6, 1.7 (MMC)	Reduced weight gain, ataxia and inability to give birth in dams at 1.7. High mortality in pups at 1.7. Impaired swimming behavior and righting reflex, delayed sexual maturity (vaginal opening and testes descent) at 0.2 and 0.6. (LOAEL = 0.2; $p \leq 0.05$). BML = 9,700-191,000 µg/L in dams and 10,000-127,000 µg/L in pups at birth	Suter and Schon 1986
Rat/Wistar/No. F NS	4 d 1 x/d Gd 6-9 (gavage)	0, 0.02, 0.04, 0.4, 4 (MMC)	Increased startle response; impaired swimming behavior, decreased locomotor and nose-poking behavior; alteration of dendritic spine morphology (LOAEL = 4). Limitations: Limited data reporting; no statistical analysis BML not reported	Stoltenburg-Didinger and Markwort 1990
Rat/Wistar/16 F	2 wk prior to mating through weaning ad lib in drinking water	0, 0.08-0.38, 0.34-0.95 (MMC)	Impaired tactile-kinesthetic function ($p \leq 0.05$) (LOAEL = 0.08-0.38). BML not reported	Elsner 1991

Table 3-68 (continued)
Developmental Toxicity of Methylmercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Rat/Sprague-Dawley/No. and sex NS	Once Gd 15 (gavage)	0, 6.4 (MMC)	Increased $GABA_A$ receptors in prenatally exposed pups sacrificed at 14 or 21 days postpartum; increased behavioral depression after diazepam. Limitations: Only one treatment level; no data on number of animals BML not reported	Guidetti et al. 1992
Mouse/SvSl/ No. F NS	Once Gd 7 or 9 (i.p.)	0, 0.16 mg MMD/20 g	Impaired swimming ability and open-field behavior ($p<0.05$) in 30-day old pups. Dose administered as methylmercury dicyandiamide (MMD) BML not reported	Spyker et al. 1972
Mouse/CFW/No. F NS	Once Gd 8 (i.v.)	0, 1, 2, 3, 5, 10 (MMH)	Increased number of trials to criterion ($p<0.05$) and increased number that failed to attain criterion in 2-way avoidance test conducted on 56-day old pups (LOAEL = 3). BML not reported	Hughes and Annau 1976
Mouse/ 129/Svsl/ No. F NS	Once Gd 10 (s.c.)	0, 5, 7, 10	Longer center square latency at 10 (once) and 3.5 (3 d), decreased rearings and increased backings at 3.5; decreased locomotor activity at 7 and 10; postnatal growth retardation at 7 and 10 (LOAEL = 7; $p<0.05$). BML not reported	Su and Okita 1976
Mouse/C57BL/ 10 F	8 d 1 x/d Gd 6-13 (gavage)	0, 2, 4, 4.8, 6 (MMC)	Increased resorptions and fetal deaths at 4.8 and 6 ($p<0.01$); increased malformations (cleft palate, fused vertebrae) at 2 and higher ($p<0.05$); increased skeletal variations; decreased maternal weight gain at 4.8 mg/kg-day (LOAEL = 2). Limitation: small number of treated animals BML not reported	Fuyuta et al. 1978
Mouse/ DUB/ICR/8 F exposed, 7 F controls	Once Gd 12 (gavage)	0, 8	Arrest of brain cells during mitosis ($p<0.01$). Limitations: Only one dose tested; small number of animals tested BML not reported	Rodier et al. 1984
Mouse/ C3H/HeN/10 F	Once Gd 13, 14, 15, 16, or 17 (gavage)	0, 16 (MMC)	Decreased neonatal survival and weight gain; impaired righting response; decreased locomotor activity; abnormal gait; crossing of hindlimbs; decreased brain weight in groups treated on Gd 13 or 14 ($p<0.01$); dilated lateral ventricles; slightly simplified cerebellar pattern. Effects were seen in groups dosed on all days, but somewhat stronger in those treated on Gd 13 or 14. Limitations: Incomplete reporting of data; most parameters were not analyzed statistically; only one dose tested BML: ~20 µg/g in brain of fetuses	Inouye et al. 1985

Table 3-68 (continued)
Developmental Toxicity of Methylmercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Guinea pig/ Hartley/5-9 F	Once Gd 21, 28, 35, 42, or 49 (gavage)	9.4-15 7.5 mg/animal (wt 500-800 g) (MMC)	Aborted litters and retarded fetal brain development at all treatment times. Limitations: No statistical analysis; small number of treated animals, only 1 day of dosing Avg BML over treatment time: Fetal: 2,600 µ/L in blood; Maternal: 1,800 µg/g in blood	Inouye and Kajiwara 1988b
Hamster/ Golden/10 F	Once at Gd 10, or 6 d 1 x/d Gd 10-15 (gavage)	0, 1.6, 8 (MMC)	Degeneration of cerebellar neurons in rats born to mothers treated with 1.6 mg/kg/d on Gd 10-15 or a single dose of 8 mg/kg on Gd 10 and sacrificed neonatally or as adults. Limitation: small number of treated animals BML not reported	Reuhl et al. 1981
Monkey/*Macaca fascicularis*/9 F exposed, 8 F control	approx. 1-3 yr 1 x/d prior to mating through gestation (in apple juice)	0, 0.04, 0.06	Impaired visual recognition memory (data pooled from both groups of infants of exposed mothers) compared to unexposed controls; test performed at 50-60 days of age. Limitation: small number of treatment animals BML Range: 880-2,450 µg/L in blood of infants at birth; 280-830 µg/L at testing	Gunderson et al. 1988
Monkey/*Macaca fascicularis*/ 12 F exposed, 13 F control	approx. 4 mo to 2 yr 1 x/d prior to mating through gestation (in apple juice)	0, 0.04	Decrease in social play behavior and concomitant increase in nonsocial passive behavior compared to unexposed controls; tests performed at 2 weeks to 8 months of age. Limitation: small number of treatment animals BML Range: 1,565 µg/L in blood of infants at birth	Gunderson et al. 1988
Monkey/*Macaca fascicularis*/5 mothers	4-4.5 yr 1 x/d in utero and postnatally (gavage)	0, 0.01, 0.025, 0.5 (MMC)	Spatial visual impairment (LOAEL = 0.01). Limitation: Small number of infants (5 high-dose; 2 mid-dose; 1 low-dose) BML not reported	Rice and Gilbert 1990
Monkey/ *Macaca fascicularis*/4 M, 1 F exposed, 1 M, 2 F controls	6.5-7 yr 7 d/wk 1 x/d (capsule; gavage)	0, 0.05 (MMC)	Six years after end of dosing (follow-up study to Rice and Gilbert 1982); decreased fine motor performance; diminished touch and pinprick sensitivity; impaired high frequency hearing ($p<0.05$). Limitations: small number of animals tested; one dose level tested BML: Not detectable at time of dosing	Rice 1989b; Rice and Gilbert 1992
Monkey/*Macaca fasicularis*/13 total	4-4.5 yr 1xd *in utero* and postnatally (gavage)	0, 0.01, 0.025, or 0.05	Monkeys tested as juveniles showed no gross intellectual impairment; some indication of decreased temporal discrimination. BML in treated animals at birth averaged 0.46, 0.93, or 2.66 ppm; decreased to steady-state of 0.20, 0.25 or 0.60 ppm.	Rice 1992.

Table 3-68 (continued)
Developmental Toxicity of Methylmercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Monkey/*Macaca fascicularis*/23 F	unspecified period prior to mating through gestation	0.04, 0.06, 0.08	No effect on spatial memory of adult offspring of animals treated with methylmercury hydroxide (data pooled from 24 animals, all treated groups). BML Range: 1,040-2,460 µg/L in blood of infants at birth	Gilbert et al. 1993
Monkey/*Saimiri sciureus*/3 F	week 11 or 14.5 until parturition (gavage)	0.7 to 0.9 ppm methylmercury in maternal blood	Monkeys exposed *in utero* tested (on learned lever pulling activity) at ages 5-6 yr. Methylmercury treatment resulted in decreased sensitivity to degrees in reinforcement; change in reinforcement degree resulted in either no behavior change or slow change by comparison to controls. Limitations: small number of animals tested; incomplete reporting on treatment.	Newland et al. 1994

3.3.3.9 Reproductive

Although no data were located regarding the reproductive effects of oral exposure to methylmercury in humans, animal data suggest that, at sufficiently high doses, methylmercury may adversely affect reproductive function in both males and females. When male rats were given methylmercury for several days prior to mating, mated females were observed with increased preimplantation losses (Khera 1973). Exposure of male monkeys to methylmercury for longer durations has been shown to adversely affect sperm motility and speed and to result in increased incidences of sperm tail defects (Mohamed et al. 1987). Decreases in spermatogenesis and tubular atrophy of the testes have been observed upon histopathological analyses of the testes of mice exposed to methylmercury chronically (Hirano et al. 1986; Mitsumori et al. 1990).

Less information is available regarding the effects of methylmercury on female reproductive function. Exposure of female monkeys to methylmercury for 4 months prior to mating produced no effects on the length of the menstrual cycle but resulted in decreased conceptions and increased early abortions and stillbirths (Burbacher et al. 1988). Several studies have shown increased rates of resorptions and abortions after exposure during gestation (Fuyuta et al. 1978; Hughes and Annau 1976; Inouye and Kajiwara 1988a); however, it is unclear from these studies whether the effects observed are the result of maternal reproductive failure or fetal toxicity.

Table 3-69
Reproductive Toxicity of Methylmercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Rat/Wistar/10-20 M	7 d 1 x/d (gavage)	0, 1, 2.5, 5 (MMC)	Reduced mean litter size after male exposure (LOAEL = 5; $p<0.01$) in sequential mating trials with unexposed females BML not reported	Khera 1973
Rat/Wistar/14-19 M	95-125 d 1 x/d	0.1, 0.5, 1 (MMC)	Males were mated to unexposed females concurrent with dosing. Reduced mean litter size (LOAEL = 0.5) BML not reported	Khera 1973
Mouse/Swiss Webster/10-20 M	5-7 d 1 x/d (gavage)	0, 1, 2.5, 5 (MMC)	No effect on number of viable embryos, dead embryos, or percent pregnancy (NOAEL = 5) BML not reported	Khera 1973
Mouse/ICR/60 M, 60 F	104 wk ad lib in feed	0, 0.03, 0.15, 0.72 (M); 0.02, 0.11, 0.62 (F) (MMC)	Significantly decreased spermatogenesis (LOAEL = 0.73; significance level not reported) BML not reported	Hirano et al. 1986
Mouse/B6C3F_1/60 M, 60 F	104 wk ad lib in feed	0, 0.03, 0.14, 0.68 (M); 0.03, 0.13, 0.6 (F) (MMC)	Tubular atrophy of the testes (LOAEL = 0.69; $p<0.01$) BML not reported	Mitsumori et al. 1990
Monkey/*Macaca fascicularis*/3 M	20 wk 7 d/wk 1 x/d (gavage)	0, 0.047, 0.065	Decreased sperm motility and speed; increased sperm tail defects (LOAEL = 0.065; $p<0.05$) BML: ~2200 µg/L in blood at 0.065 mg/kg-day, approaching steady state	Mohamed et al. 1987
Monkey/*Macaca fascicularis*/7-9 F	4 mo prior to mating 1 x/d (gavage)	0, 0.04, 0.06, 0.08 (MMH)	Abortion; stillbirth; decreased conception in exposed females (LOAEL = 0.06); no effect on menstrual cyclicity Avg. BML: 1,600 µg/L in blood at equilibrium at 0.06 mg/kg	Burbacher et al. 1988

3.3.3.10 Genotoxicity

Data from several studies in humans suggest that ingesting methylmercury may cause chromosomal aberrations and sister chromatid exchange (Skerfving et al. 1970, 1974; Wulf et al. 1986; Franchi et al. 1994).

A study of nine Swedish subjects who consumed mercury-contaminated fish and 4 controls showed a statistically significant rank correlation between blood mercury and percentage of lymphocytes with chromosome breaks (Skerfving et al. 1970). An extension of this study (Skerfving et al. 1974) included 23 "exposed" (5 females and 18 males) and 16 "controls" (3 females and 13 males). The authors

reported a significant correlation between blood mercury level and frequency of chromatid changes and "unstable" chromosome aberrations; there was no correlation with "stable" chromosome aberrations.

The Wulf et al. (1988) study was of 92 Greenlander Eskimos. Subjects were divided into three groups based on intake of seal meat (6 times/week; 2-5 times/week; once/week or no consumption of seal meat). Higher frequency of SCE in lymphocytes was correlated with blood mercury concentration; an increase of 10 µg Hg/L in blood was associated with an increase of 0.3 SCE/cell. Positive correlations were also found for smoking, diet, living district and cadmium exposure.

Franchi et al. (1994) evaluated formation of micronuclei in peripheral blood lymphocytes of Mediterranean fishers, a group with presumed high exposure to methylmercury. Fifty-one subjects were interviewed on age, number of seafood-based meals/week and habits such as smoking and alcohol consumption. Total blood mercury was measured; the range was 10.08 – 304.11 ng/g with a mean of 88.97 $\pm$ 54.09 ng/g. There was a statistically significant correlation between blood mercury concentration and micronucleus frequency and between age and micronucleus frequency.

Table 3-70
Genotoxicity of Methylmercury in Humans: Case Study

Species/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Human/6 M, 3 F exposed; 3 M, 1 F control	>5 yr ≥3 x/wk	NS	Correlation between blood mercury concentration and chromosome breaks in lymphocytes cultured from people who ate mercury-contaminated fish Limitation: Small sample size; limited exposure data BML Range: 4-650 µg/L in blood	Skerfving et al. 1970

Table 3-71
Genotoxicity of Methylmercury in Humans: Epidemiology Study

Species/ No. per Sex	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Human/24-63 (both sexes)	NS	NS	Incidence of sister chromatid exchanges (SCEs) in cultured peripheral lymphocytes correlated with intake of seal meat in an Eskimo population (as a surrogate for mercury intake); $p = 0.001$. Other factors also correlated with SCEs, but multiple regression analysis found that some of the effect was attributable to mercury. Limitation: Limited exposure data BML not reported	Wulf et al. 1986

Table 3-71 (continued)
Genotoxicity of Methylmercury in Humans: Epidemiology Study

Species/ No. per Sex	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Human / 51 M	measured as seafood meals/ week. Range 2 - 14.	NS	Incidence of micronuclei positively correlated with blood mercury concentration and with age. No correlation with smoking or number of seafood meals /week. Limitation: no control group. BML range: 10.08 - 403.11 μg/g blood.	Franchi et al. 1994.
Human/18M exposed/10 control	10.5 yr (occup)	0.15-0.44 ($HgCl_{2)}$	Increased frequency of chromosomal breaks. Limitations: Workers also exposed to mercuric chloride and one worker had history of benzene poisoning; control group was not matched for sex, smoking habits, or sample size. BML: ≈890 μg/L in urine (avg)	Popescu et al. 1979

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

Table 3-72
Genotoxicity of Methylmercury in Cats

Species/ Strain/ No. per Sex per Group	Exposure Duration	Dose (mg/kg-day)	Effects/Limitations/BML	Reference
Cat/Breed and sex NS	39 mo 7 d/wk	0.008, 0.020, 0.046	No dose-related changes in unscheduled DNA synthesis in cultured lymphocytes or frequency of chromosomal aberrations in bone marrow of cats fed mercury-contaminated fish or a fish diet supplemented with methylmercuric chloride Limitations: No positive control; no assessment of cytotoxicity BML Range: 500-13,500 µg/L Hg in blood	Miller et al. 1979

Strain-specific differences exist with respect to the ability of methylmercury to produce dominant lethal effects in mice (Suter 1975). When (SEC x C_{57}Bl)F_1 males were injected with 10 mg/kg methylmercury hydroxide, there was a slight reduction in the total number of implantations and a decrease in the number of viable embryos. This was not observed when (101 x C_3H)F_1 males were

exposed in a similar fashion. When female (10 x C_3H)F_1 mice were treated with methylmercuric hydroxide, no increase in the incidence of dead implants was observed (unlike the case for mercuric chloride). Changes in chromosome number but no increase in chromosome aberrations were observed in oocytes of Syrian hamsters treated with one i.p injection of 10 mg/kg methylmercuric chloride (Mailhes 1983). Methylmercury was administered s.c. to golden hamsters at doses of 6.4 mg or 12.8 mg Hg/kg/body weight. Polyploidy and chromosomal aberrations were increased in bone marrow cells, but there was no effect on metaphase II oocytes. There was an inhibitory effect on ovulation which the authors noted was not as severe as that induced by mercuric chloride in the same study (Watanabe et al. 1982). Non-dysjunction and sex-linked recessive lethal mutations were seen in *Drosophila melanogaster* treated with methylmercury in the diet (Ramel 1972).

As reviewed in WHO (1990), methylmercury is not a potent mutagen but is capable of causing chromosome damage in a variety of systems. *In vitro* studies have generally shown clastogenic activity but only weak mutagenic activity. Methylmercuric chloride and dimethylmercury were both shown to induce chromosome aberrations and aneuploidy in primary cultures of human lymphocytes; methylmercuric chloride was the more potent clastogen at equally toxic doses (Betti et al. 1992). Both methylmercury and mercuric chloride induced a dose dependent increase in SCE in primary human lymphocytes and muntjac fibroblasts; methylmercury was about five time more effective in this regard (Verschaeve et al. 1984; Morimoto et al. 1982).

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

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

4. SUSCEPTIBLE POPULATIONS

A susceptible population is a group who may experience more severe adverse effects at comparable levels or adverse effects at lower exposure levels than the general population. The greater response of these sensitive subpopulations may be a result of a variety of intrinsic or extrinsic factors. Volume V describes populations that may be at increase risk because of higher exposure to mercury and mercury compounds. Additional factors that may be important include, but are not limited to, the following: an impaired ability of the detoxification, excretory, or compensatory processes in the body to protect against or reduce toxicity; differences in physiological protective mechanisms (e.g., blood brain barrier); or unique toxic reactions that are specific to the genetic makeup, developmental stage, health status, gender or age of the individual.

The nervous and renal systems are the primary targets for mercury-induced toxicity. Data are also available indicating some effects to the respiratory, cardiovascular, gastrointestinal, hematologic, immune, and reproductive systems. The developing organism appears to be particularly sensitive to methylmercury exposure. In addition, it is probable that individuals with preexisting damage or disease in target organs for mercury-induced toxicity may experience more severe effects upon exposure to mercury. The populations listed below may be highly susceptible to mercury toxicity.

- Developing Organisms. Data from epidemic poisonings in Japan (Harada 1978) and Iraq (Marsh et al. 1987) indicate that infants exposed *in utero* to methylmercury developed marked neurological development delays while their mothers experienced little or no overt signs of toxicity. Data indicate that the developing fetus may be 5 to 10 times more sensitive than the adult (Clarkson, 1992). This difference in sensitivity is believed to be due, in part, to the high sensitivity of developmental processes (i.e., cellular division, differentiation, and migration) to disruption by mercury (Choi et al. 1978; Sager et al. 1982). One factor that may account for this difference in sensitivity is the presence of an incomplete blood brain barrier in the fetus. Another important factor may be the lack of methylmercury excretion in the fetus (Grandjean et al. 1994).

- Age - Infants and Other Age Groups. Available data indicate that neonates are at increased risk to inorganic mercury and methylmercury. Both inorganic and organic forms of mercury are excreted in breast milk (Sundberg and Oskarsson 1992; Yoshida et al. 1992; Grandjean et al. 1994); thus, neonates in an exposed population may experience increased mercury exposure. Animal data for rats indicate that suckling infants retain a higher percentage of ingested inorganic mercury than do adults (Kostial et al. 1978). The most significant difference in organ retention (neonates > adults) was methylmercury in the brain following exposure to methylmercury (Yang et al. 1973; Kostial et al. 1978) and inorganic mercury retained in the kidney following exposure to elemental mercury (Yoshida et al. 1992). These differences may be associated with an increased absorption of mercury with a milk diet, a decrease in excretion, or an incomplete blood brain barrier (Kostial et al. 1978, Grandjean et al. 1994).

  Signs of toxicity may begin to be manifested several years after the cessation of dosing, possibly related to subclinical effects being unmasked by aging. Rice (1989b) dosed monkeys with methylmercury from birth to 6.5-7 years of age. Although there were no overt signs of neurotoxicity during dosing, neurological deficits were observed at 13

years of age, 6-7 years following cessation of exposure. Similarly, a small human population with Minamata disease has been identified in Japan as experiencing new or worsening neurological effects a few years following termination of mercury exposure. This late-onset Minamata disease may be related to several factors including aging (Igata 1993).

- Gender. Sex-related differences in mercury toxicokinetics and sensitivity to mercury have been observed, although data indicate that the more sensitive sex may differ by species and strain. Using death as the critical endpoint, in one strain of mice, C57BL/6N, males were less sensitive to methylmercury following daily dosing than females while, in contrast, male mice were more sensitive than females in another strain, BALB/cA (Yasutake and Hirayama 1988). In humans, although the ratio of males to females with Minamata disease has been reported to be 1.2:1, the ratio of deaths was recorded at 1.8:1 (Tamashiro et al. 1984).

  Other studies are in general agreement that male rats (Thomas et al. 1986) and mice (Nielsen and Andersen 1991a, 1991b) eliminate mercury faster and have lower tissue levels than females following dosing with methylmercury. Part of the difference in whole-body retention of mercury in methylmercury-exposed mice has been associated with varying degrees of deposition of mercury in the carcass, including the skin and hair (Nielsen and Andersen 1991b). This difference is thought to be due in part to differences in glutathione metabolism and renal excretion of mercury, which is affected by the hormonal status of testosterone (Nielsen et al. 1994). Hirayama et al. (1987) have reported that the toxicokinetics of methylmercury in castrated male mice was very similar to that in female mice, and that the male pattern of methylmercury toxicokinetics could be restored by testosterone treatment. Such differences were not observed in a small set of similarly tested human volunteers (Miettinen et al. 1971).

- Dietary Insufficiencies of Zinc, Glutathione, or Antioxidants. Mercury has been suggested to cause tissue damage by increasing the formation of reactive oxygen species and activation of lipoperoxidation, calcium-dependent proteolysis, endonuclease activity, and phospholipid hydrolysis (Ali et al. 1992; LeBel et al. 1990, 1992; Gstraunthaler et al. 1983; Verity and Sarafian 1991). Zinc, glutathione, and antioxidant deficiencies would be expected to exacerbate mercury-induced damage by limiting cellular defenses against the oxidative processes. Animal data support the importance of zinc, glutathione, and antioxidants in limiting mercury-induced damage (Fukino et al. 1992; Girardi and Elias 1991; Yamini and Sleight 1984) (see also Section 5, Interactions).

- Predisposition for Autoimmune Glomerulonephritis. Autoimmune glomerulonephritis is a form of renal toxicity characterized by proteinuria, deposition of immune material (i.e., autoantibodies and complement C3) in the renal mesangium and glomerular blood vessels and glomerular cell hyperplasia (Bigazzi 1992; Goldman et al. 1991; Mathieson 1992). Limited human data suggest that certain individuals may develop this autoimmune response when exposed to inorganic or elemental mercury (Cardenas et al. 1993; Langworth et al. 1992b; Tubbs et al. 1982). While the etiology of this syndrome has not been completely elucidated, data from susceptible and resistant strains of animals indicate that susceptibility is governed by both major histocompatibility complex (MHC)

genes and non-MHC genes (Aten et al. 1991; Druet et al. 1978; Hultman and Enestrom 1992; Hultman et al. 1992; Michaelson et al. 1985; Sapin et al. 1984).

- Predisposition for Acrodynia. Acrodynia, also known as "pink disease," is a hypersensitive response following exposure to elemental or inorganic mercury and is characterized by the following signs and symptoms: irritability; marked mood swings; restlessness; itching; flushing, swelling, and/or desquamation of the palms of the hands and soles of the feet (the tip of the nose, ears, and cheeks may also be affected); excessive perspiration; loss of appetite; tachycardia; hypertension; joint pains and muscle weakness; photophobia; and sleeplessness. Acrodynia, which is more likely related to exposure level rather than any inherent, genetic sensitivity, rarely occurs in the general population.

  Limited reports indicate that acrodynia has been almost exclusively observed in children, affecting approximately 1 in 500 exposed children (Blondell and Knott 1993; Warkany and Hubbard 1953). This disease was recently observed in a 4-year-old Michigan boy who was exposed to mercury vapor released from paint in which mercury had been used as a fungicide (Aronow et al. 1990). In this case, family members (i.e., both parents and two siblings) were also exposed to the mercury vapors but remained asymptomatic (Aronow et al. 1990). This case study supports the hypothesis that there is no genetic predisposition to acrodynia.

  Acrodynia was more frequently observed in the past when mercury-containing laxatives, worming medications, teething powders and diaper rinses were widely used (Gotelli et al. 1985; Warkany and Hubbard 1953). The physiological basis for this hypersensitivity has not been identified. It does not appear, however, to be an allergic reaction to mercury or to occur in the most highly exposed individuals (Warkany and Hubbard 1953).

5. INTERACTIONS

A number of interactions have been identified for chemicals that affect the pharmacokinetics and/or toxicity of mercury compounds. Table 5-1 summarizes interactions in which potentiation or protection from the toxic effects of mercury have been observed. Interactions that affect mercury toxicokinetics are also shown. The effect on toxicity, however, can not be predicted based on changes in distribution or excretion. For example, zinc pretreatment increases renal mercury levels but decreases toxicity because it alters the distribution within the kidney (Zalups and Cherian 1992).

Only the interaction of selenium with mercury will be discussed in detail here. Selenium is known to bioaccumulate in fish, so exposure to methylmercury in fish is associated with exposure to increased levels of selenium. Where the main source of dietary mercury is fish, the diet is naturally enriched with selenium relative to mercury. Increased selenium has been suspected of providing some degree of protection, either by preventing oxidative damage or by forming a methylmercury-selenium complex (Grandjean 1992a). It does not appear that the population in the Iraqi poisoning incident was selenium deficient. Animal studies have demonstrated that simultaneous ingestion of selenium may be protective against toxicity of methylmercury based upon its antioxidant properties (see Table 5-1). This may explain why the latent period in Japan, where the population was exposed to methylmercury in fish, was longer than that in Iraqi, where the exposure was to methylmercury in grain.

A common association between the metabolism of selenium and methylmercury is the thiol-containing peptide glutathione (GSH). The metabolic cycling and oxidation-reduction of GSH are integral processes coupled to the activation and metabolism of selenium (Hill and Burke 1982) and the metabolism and detoxification of methylmercury (Ballatori and Clarkson 1982; Thomas and Smith 1982).

There are data to indicate that selenium co-administered with methylmercury can form selenium-methylmercury complexes (Magos et al 1987). The formation of these complexes appeared temporarily to prevent methylmercury-induced tissue damage but also apparently delayed excretion of the methylmercury in the urine. Thus, formation of selenium-methylmercury complexes may not reduce methylmercury toxicity but may rather delay the onset of symptoms.

In support of the protective role of biological selenium, several investigators have found that a diet supplemented with seafood high in selenium delayed the onset of methylmercury intoxication in rats (Ganther 1980; Ohi et al. 1976). Ganther (1980) has observed that rats given selenium plus methylmercury show increased body burdens of both selenium and methylmercury without signs of toxicity. The accumulation of these elements may lead to mutual detoxification, but such coaccumulation is not always linked to protection. Fair and associates (1985) have examined renal ultrastructure changes along with changes in gamma glutamyl transferase activity in mice coadministered both selenium and methylmercury in diet for 7 or 20 days or given a single i.p. dose. The results of this study indicated that dietary selenium had only an initial protective effect against mercury accumulation in the kidney; injected selenium offered longer protection.

Selenium has been shown to protect against the developmental toxicity of methylmercury in mice (Nishikido et al. 1987; Satoh et al. 1985) and protects against oxidative damage by free radicals (Cuvin-Aralar and Furness 1991; DiSimplicio et al. 1993; Ganther 1978). Further studies reported by Fredricksson et al. (1993) indicated that dietary selenium supplementation during pregestation through lactation in rats resulted in reduction of some adverse effects (hypoactivity) in neonates of the

methylmercury administered to mothers by gavage during organogenesis period. Significant increases in glutathione peroxidase activity were noticed in animals fed selenium supplemented diet.

Kosta et al. (1975) have observed a coaccumulation of mercury and selenium in the organs and tissues of mineworkers at an approximate molar ratio of 1:1. In these circumstances, the abnormally high mercury levels detected in the tissues were without apparent adverse effects on the miners. After exposure to mercury in the mines, several of the miners had been retired 10–16 years when the study was conducted. The selenium intake from the diet was not reported but was said not to be abnormally high, suggesting that the co-accumulation with mercury is a natural and autoprotective effect. It is plausible that in areas naturally low in selenium, individuals would be at greater risk from methylmercury poisoning than those in areas of high selenium concentration.

A group of 21 workers with no previous history of mercury exposure were monitored for urinary mercury and selenium after their employment in the demolition of a chlor-alkali plant. Pre-exposure urinary mercury ranged from 0.3 - 1.9 nmol/ mmol creatinine (mean = 0.8); urinary selenium was 13.9 - 89.5 (mean = 39.1). Post-exposure urinary mercury was significantly increased; 1.2 - 10.0 nmol/ mmol creatinine (mean = 4.8). Selenium in the urine was decreased post exposure to 10.1 - 52.9 nmol/ mmol creatinine (mean = 29.0). The authors did not speculate on the biological significance of the change in urinary selenium.

Co-administration of methylmercury and selenium apparently results in decreased methylmercury concentrations in kidney; mercury levels in brain and liver, however, are increased (Suzuki and Yamamoto, 1984; Brzenicka and Chmielnicka, 1985; Komsta-Szumska *et al*., 1983). Selenium has also been observed to increase methylmercury staining in spinal cord and nerve cell bodies (Møller-Madsen and Danscher, 1991). A positive correlation between brain mercury and selenium levels was observed in monkeys exposed to methylmercury with no additional exposure to selenium other than that in a standard diet (Björkman *et al*., 1995). The apparent protective effect of selenium against overt high-dose methylmercury toxicity has been attributed to the decreased accumulation of methylmercury in kidney in the presence of selenium (Stillings *et al*., 1974). It is doubtful that this effect on kidney is relevant at environmental levels of methylmercury. It has also been suggested that the formation of bis (methylmercury) selinide may render methylmercury less toxic (Naganuma and Imura, 1980), but there is no direct evidence for this. In addition, although increased fish consumption was associated with a very modest increase in (cord) blood selenium levels in a fish-eating population, the blood mercury levels increased much more dramatically (Grandjean *et al*., 1992). Grain may also contain substantial levels of selenium, depending on the soil in which it is grown. Based on the questionable relevance of any protective effect of selenium against high-dose methylmercury nephrotoxicity, the fact that increased selenium intake results in increased brain mercury levels following methylmercury ingestion, and a complete lack of data on the selenium status of the Iraqi population exposed to methylmercury via grain, there is no reason to postulate that ingestion of methylmercury in a fish matrix would result in decreased toxicity compared to ingestion in a non-fish matrix. Indeed, the study in cats addressed this directly found no difference in toxicity or tissue levels when methylmercury was administered as contaminated fish or added to a non-fish meal (Charbonneau *et al*., 1974).

Table 5-1
Interactions of Mercury with Other Compounds

Compounds	Effects Observed	Proposed Underlying Mechanism(s)	References
Diethylmaleate and inorganic mercury	Increased renal toxicity	Diethylmaleate causes depletion of nonprotein sulfhydryls	Girardi and Elias 1991
Ethanol and methylmercury	Potentiated toxicity Increased mortality, increased severity of neurotoxicity, renal toxicity Decreased time to onset of neurotoxicity	Unknown; increased mercury concentrations were observed in brain and kidneys, but changes in mercury content were insufficient to fully explain the potentiated toxicity	Rumbeiha et al. 1992 Tamashiro et al. 1986 Turner et al. 1981
Ethanol and elemental mercury	No data on toxicity Decreased mercury absorption Increased mercury levels in liver and in fetus	Inhibition of oxidation of metallic mercury to mercuric mercury by catalase The effect on toxicity can not be predicted, due to the opposing effects.	Nielsen-Kudsk 1965 Magos and Webb 1979 Khayat and Dencker 1982, 1984b
Ethanol and inorganic mercury	No data on toxicity Increased mercury exhalation	Elemental mercury was exhaled, suggesting that ethanol increased the activity of an unidentified enzyme that reduces mercuric mercury to elemental mercury. Because elemental mercury, but not mercuric mercury, can cross the blood brain barrier and the placenta, toxicity to the brain and the developing fetus may be increased	Dunn et al. 1981b
Thiol compounds [e.g., *N*-acetylpenicillamine, penicillamine, 2-mercapto propanol (BAL)] and inorganic mercury	Protection from renal toxicity	Competition for protein binding sites; subsequent increases in urinary excretion of mercury	Magos and Webb 1979
Selenium and mercury (simultaneous exposure)	Increased survival Decreased or delayed renal, developmental toxicity	Mercuric mercury and selenium form a complex with a high molecular weight protein Methylmercury forms a bismethylmercury selenide complex Potential mechanisms for protection: -redistribution from sensitive targets -competition of selenium for mercury binding sites associated with toxicity -increased selenium available for selenium-dependent glutathione peroxidase (prevention of oxidative damage)	Parizek and Ostadolva 1967 Satoh et al. 1985 Naganuma and Imura 1981 Mengel and Karlog 1980 Cuvin-Aralar and Furness 1991 Imura and Naganuma 1991 Nylander and Weiner 1991
Tellurium and elemental or inorganic mercury	Decreased toxicity (effect unspecified) Retention in body increased	Complexation of tellurium with mercury, by analogy to the chemically-related selenium	Magos and Webb 1979 Khayat and Dencker 1984a

Table 5-1 (continued)
Interactions of Mercury with Other Compounds

Compounds	Effects Observed	Proposed Underlying Mechanism(s)	References
Potassium dichromate and inorganic mercury	Decreased renal function (measured as inhibition of *p*-aminohippurate transport)	Unknown; both chemicals are toxic to the renal proximal tubule	Baggett and Berndt 1984
Zinc pre-treatment and inorganic mercury	Some protection from nephrotoxicity of inorganic mercury	Zinc pretreatment induces metallothionein binding in kidneys Mercury binds preferentially to metallothionein, so that less mercury is available to cause oxidative damage in the proximal tubules	Zalups and Cherian 1992
Zinc-deficiency and inorganic mercury	Exacerbation of renal toxicity	Zinc-deficiency and mercury both independently increase renal oxidative stress Together, the protective mechanisms of the kidney are overwhelmed and oxidative damage is compounded	Fukino et al. 1992
Atrazine and methylmercury	Early onset of neurotoxicity	Atrazine causes depletion of nonprotein sulfhydryls	Meydani and Hathcock 1984
Vitamin C deficiency and methylmercury	Increased severity of neurological damage	Antioxidant properties of Vitamin C and protection against oxidative damage caused by mercury	Yamini and Sleight 1984
Vitamin E and methylmercury	Increased survival and decreased toxicity	Protection is possibly related to antioxidant properties of Vitamin E affording protection against oxidative damage caused by mercury	Welsh 1979
Potassium dichromate and mercuric chloride	Synergistic inhibition of renal transport	Mercuric chloride and potassium dichromate are both toxic to renal proximal tubule	Baggett and Berndt 1984

6. HAZARD IDENTIFICATION AND DOSE-RESPONSE ASSESSMENT

6.1 Background

Risk assessments done by U.S. EPA follow the paradigm established by the National Academy of Sciences (NRC 1983). This entails a series of interconnected steps including hazard identification, dose response assessment, exposure assessment and risk characterization. Two processes, hazard identification and dose response are the focus of this chapter. Volume IV of this Report presents the assessment of exposure to mercury emissions in the atmosphere, and Volume VII covers the risk characterization.

Hazard identification poses the following questions: is the agent in question likely to pose a hazard to human health; and what types of adverse effects could be expected as a consequence of the exposure to the agent. Dose-response assessment uses available human, experimental animal and *in vitro* data to estimate the exposure level or dose which is expected to produce and adverse health effect. In accomplishing the aims of risk assessment U.S. EPA applies published Guidelines for Risk Assessment.

6.1.1 Hazard Identification

U.S. EPA has published Guidelines for hazard identification in three areas: developmental effects, germ cell mutagenicity, and carcinogenic effects. Guidelines for assessment of reproductive effects were finalized while this Report to Congress was in the process of review; these have not been applied to the Mercury Study. The specific categorizations for each of those endpoints described in published guidelines are discussed below. For general, systemic noncancer effects, there is no structured process resulting in a categorization; instead, the hazard identification step is included in the dose-response assessment process, wherein a critical effect is selected.

6.1.1.1 Developmental Effects

Guidelines for hazard identification in the area of developmental effects were developed by U.S. EPA in 1986 and subsequently revised (U.S. EPA 1989, 1991). The Guidelines direct that data from all available relevant studies be considered, whether the studies indicate a potential hazard or not. Preferred data are from human studies, when available, and animal studies. The revised guidelines do not use an alphanumeric scheme such as that given in the carcinogenicity guidelines. Instead two broad categories are used to characterize the health-related data base: Sufficient Evidence and Insufficient Evidence. The Guidelines define Sufficient Human Evidence as follows:

> "...data from epidemiologic studies (e.g., case control and cohort) that provide convincing evidence for the scientific community to judge that a causal relationship is or is not supported. A case series in conjunction with strong supporting evidence may also be used".

Sufficient Experimental Animal Evidence/Limited Human Data is described in the following way:

> "The minimum evidence necessary to judge that a potential hazard exists generally would be data demonstrating an adverse developmental effect in a single, appropriate, well-conducted study in a single experimental animal species. The minimum evidence

needed to judge that a potential hazard does not exist would include data from appropriate, well-conducted laboratory animal studies (at least two) which evaluated a variety of the potential manifestations of developmental toxicity, and showed no developmental effects at doses that were minimally toxic to the adult."

6.1.1.2 Germ Cell Mutagenicity

The U.S. EPA (1986) has published Guidelines for classification of potential hazard of mutagenic effects in human germ cells. Evidence from human and animal *in vivo* and *in vitro* systems is considered in the judgement as to which of eight numerical classes of concern most clearly defines the data on an environmental agent. In general, the hierarchy of preference for data type is the following:

- Data on germ cells are preferred to data on somatic cells;
- In *vivo tests* are preferred to *in vitro*;
- Data from tests in eukaryotes are preferred to data from prokaryotes.

The weight-of-evidence categories are these, presented in order of decreasing strength of evidence for human germ cell mutagenicity.

1. Positive data derived from human germ cell mutagenicity studies.
2. Valid positive results from studies on heritable mutational events (any kind) in mammalian germ cells.
3. Valid positive results from mammalian germ cell chromosome aberration studies that do not include an intergeneration test.
4. Sufficient evidence for a chemical's interaction with mammalian germ cells, together with valid positive mutagenicity test results from two assays systems, at least one of which is mammalian. The positive results may be both for gene mutations or both for chromosome aberrations; if one is for gene mutations and the other for chromosome aberrations, both must be from mammalian systems.
5. Suggestive evidence for a chemical's interaction with mammalian germ cells, together with valid positive mutagenicity evidence from two assay systems as described under 4.
6. Positive mutagenicity test results of less strength than defined under 4, combined with suggestive evidence for a chemical's interaction with mammalian germ cells.
7. Non-mutagenic. Although definitive proof of non-mutagenicity is not possible, a chemical could be classified operationally as a non-mutagen for human germ cells, if it gives valid negative results for all endpoints of concern.
8. Not classifiable based on inadequate evidence bearing on either mutagenicity or chemical interaction with mammalian germ cells.

These categories are intended as guidance in assessing a level of concern for an agent's likelihood to be a germ cell mutagen. The three forms of mercury are discussed in Section 6.2.2 in terms of level of concern rather than an assigned numerical category.

6.1.1.3 Carcinogenic effects

U.S. EPA categorizes the carcinogenic potential of a chemical, based on the overall weight-of-evidence, according to the following scheme.

Group A: Human Carcinogen. Sufficient evidence exists from epidemiology studies to support a causal association between exposure to the chemical and human cancer.

Group B: Probable Human Carcinogen. There is sufficient evidence of carcinogenicity in animals with limited (Group B1) or inadequate (Group B2) evidence in humans.

Group C: Possible Human Carcinogen. There is limited evidence of carcinogenicity in animals in the absence of human data.

Group D: Not Classified as to Human Carcinogenicity. There is inadequate human and animal evidence of carcinogenicity or no data are available.

Group E: Evidence of Noncarcinogenicity for Humans. There is no evidence of carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies.

For specific guidance as to the use of human, animal and supporting data in the above categorization for cancer, consult the Risk Assessment Guidelines of 1986 (U.S. EPA 1987a).

U.S. EPA has been in the process of revising its Guidelines for cancer risk assessment. The revised Guidelines will implement the use of narrative categorization. The new guidelines also encourage greater use of mechanistic data, including information which can be gained from genetic toxicology. Data which elucidate the mode of action of an agent will also have a direct impact on the dose response assessment for carcinogenicity. In the past a default procedure for dose response assessment was most often followed; that of linear low dose extrapolation using an upper bound on the low dose term of a linearized multistage mathematical model. The revised Guidelines dictate that the type of low dose extrapolation to be used, if any, be guided by information on the carcinogen's mode of action. Evidence of genetic toxicity has now become key in making decisions about dose response assessment.

While the Mercury Study Report to Congress was in preparation, revised Carcinogen Risk Assessment Guidelines were published in the Federal Register. As the approval process was not final, it was necessary to apply the existing Guideline's alphanumeric categories; however, an expanded narrative was done, and the weight of evidence judgement followed closely the revised format for expanded consideration of mechanistic data.

An application of the proposed revisions to the Carcinogenic Risk Assessment Guidelines was presented at the 1996 meeting of the Society for Risk Analysis (Schoeny 1996). An abstract of that presentation is given at the end of section 6.

6.1.2 Dose-response Assessment

6.1.2.1 Systemic Noncancer Effects

In the quantification of systemic noncarcinogenic effects, an oral reference dose (RfD), an inhalation reference concentration (RfC) or both may be calculated. The oral RfD and inhalation RfC are estimates (with uncertainty spanning perhaps an order magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious health effects during a lifetime. The RfD and RfC are derived from a no-observed-adverse-effect level (NOAEL), or lowest-observed-adverse-effect level (LOAEL), identified from a subchronic or chronic study and divided by an uncertainty factor(s) times a modifying factor. The RfD or RfC is calculated as follows:

$$RfD = \frac{(NOAEL\ or\ LOAEL)}{[Uncertainty\ \ Factor\ (s)\ x\ Modifying\ \ Factor]} = \_\_ \ mg/kg-day$$

$$RfC = \frac{(NOAEL_{HEC}\ or\ LOAEL_{HEC})}{[Uncertainty\ \ Factor\ (s)\ x\ Modifying\ \ Factor]} = \_\_ \ mg/m^3$$

The methodologies used to derive the RfD or inhalation RfC require the adjustment of NOAEL and LOAEL values (whether from experimental animal or human studies) to lifetime exposure conditions (i.e., 24 hours per day for a lifetime of 70 years). Inhalation RfC methods further require conversion by dosimetric adjustment or the use of a physiologically-based pharmacokinetic model from NOAELs and LOAELs observed in laboratory animal experiments to human equivalent concentrations (HEC). Different default adjustments are made based on whether the observed toxicity is in the upper or lower respiratory tract or at remote sites, and a $NOAEL_{(HEC)}$ or $LOAEL_{(HEC)}$ is derived for use in the equation above (U.S. EPA 1990).

Selection of the uncertainty factor (UF) to be employed in the calculation of the RfD/RfC is based upon professional judgment which considers the entire data base of toxicologic effects for the chemical. In order to ensure that UFs are selected and applied in a consistent manner, the U.S. EPA (1994) employs a modification to the guidelines proposed by the National Academy of Sciences (NAS 1977, 1980), as shown in the box on the next page.

As noted in the box, the standard UF for extrapolating from animals to humans has been reduced to three for the derivation of inhalation RfCs. A factor of three was chosen because, assuming the range of the UF is distributed log normally, the reduction of a standard 10-fold UF by half (i.e. $10^{0.5}$) results in three. Other UFs can be reduced to three if the situation warrants, based on the scientific judgement of the U.S. EPA RfD/RfC Work Group (an Agency peer review group). Considerations in the selection of UFs and/or a modifying factor include, but are not limited to, pharmacokinetics/pharmacodynamics, concomitant exposures, relevance of the laboratory animal models, species sensitivity, severity of the effect, potential for recovery, slope and shape of the dose-response curve, exposure uncertainties, quality of the critical study, and data gaps.

Uncertainty Factors Used in RfD/RfC Calculations

Standard Uncertainty Factors (UFs)

Use a 10-fold factor when extrapolating from valid experimental results from studies using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among the members of the human population. [10_H]

Use an additional 10-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to risks for humans. [10_A] A 3-fold uncertainty factor is used for extrapolating from inhalation studies on experimental animals to humans for the derivation of an inhalation RfC. This difference is because dosimetric adjustments reduce the uncertainty associated with extrapolation between experimental animals and humans.

Use an additional 10-fold factor when extrapolating from less than chronic results on experimental animals when there are no useful long-term human data. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs. [10_S]

Use an additional 10-fold factor when deriving an RfD from a LOAEL instead of a NOAEL. This factor is intended to account for the uncertainty in extrapolating from LOAELs to NOAELs. [10_L]

Modifying Factor (MF)

Use professional judgment to determine another uncertainty factor (MF) that is greater than zero and less than or equal to 10. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and data base not explicitly treated above, e.g., the completeness of the overall data base and the number of species tested. The default value for the MF is 1.

From the RfD, a Drinking Water Equivalent Level (DWEL) can be calculated. The DWEL represents a medium-specific (i.e., drinking water) lifetime exposure at which adverse, noncarcinogenic health effects are not anticipated to occur. The DWEL assumes 100% exposure from drinking water. The DWEL provides the noncarcinogenic health effects basis for establishing a drinking water standard. For ingestion data, the DWEL is derived as follows:

$$DWEL = \frac{(RfD) \times (Body\ weight\ in\ kg)}{Drinking\ Water\ Volume\ in\ L/day} = \_\_ mg/L$$

where:

Body weight = assumed to be 70 kg for an adult
Drinking water volume = assumed to be 2 L/day for an adult

6.1.2.2 Developmental Effects

For agents considered to have sufficient evidence for developmental toxicity it is appropriate to consider calculation of a quantitative dose-response estimate. In general, a threshold is assumed for the dose-response curve for agents producing developmental toxicity. This is "based on the known capacity

of the developing organism to compensate for or to repair a certain amount of damage at the cellular level. In addition, because of the multipotency of cells at certain stages of development, multiple insults at the molecular or cellular level may be required to produce an effect on the whole organism" (U.S. EPA 1991).

Due to the paucity of human data, dose-response assessment of developmental toxicity is most often done using animal data. The assessment includes the identification of dose levels associated with observed developmental effects as well as those doses which apparently produce no adverse effects. The critical effect is ascertained from the available data. A critical effect is defined as the most sensitive developmental effect from the most appropriate and/or sensitive mammalian species; LOAEL and NOAEL determinations are then made. The NOAEL is defined as "the highest dose at which there is no statistically or biologically significant increase in the frequency of an adverse effect in any of the possible manifestations of developmental toxicity when compared with the appropriate control group in a data base characterized as having sufficient evidence for use in risk assessment" (U.S. EPA 1991). The LOAEL is defined in the following manner: "The LOAEL is the lowest dose at which there is a statistically or biologically significant increase in the frequency of adverse developmental effects when compared with the appropriate control group in a data base characterized as having sufficient evidence".

Because of the limitations associated with the use of the NOAEL/LOAEL approach, U.S. EPA is investigating the use of alternative methods employing more data in a dose-response assessment. One such approach is the estimation of a benchmark dose (BMD). This approach is based on the use of a mathematical model to derive an estimate of an incidence level (e.g., 1%, 5%, 10%, etc). This is done by applying a model to data in the observed range, selecting an incidence level at or near the observed range (typically 10%), and then determining an upper confidence limit on the modeled curve. The value of the upper limit, for a 10% incidence, is then used to derive the BMD, which is the lower confidence limit on dose for that incidence level.

The last step in dose-response assessment is the calculation of a reference dose or reference concentration for developmental toxicity (RfD_{DT} or RfC_{DT}). This is done by applying appropriate uncertainty factors to the LOAEL, NOAEL, or BMD. Uncertainty factors generally include the following:

- 10 for interspecies variation (animal to human)
- 10 for intraspecies variation

Additional factors may be applied to account for other areas of uncertainty, such as identification of a LOAEL in the absence of a NOAEL. In this case, the factor may be as much as 10 fold, depending on the sensitivity of the endpoints evaluated in the data base. An uncertainty factor is generally not used to account for duration of exposure when calculating the RfD_{DT}. If developmental toxicity is the critical effect for the chronic RfD, an additional uncertainty factor (for study duration) may be used. Modifying factors may be used to deal with the degree of confidence in the data base for the agent being evaluated. For a discussion of application of uncertainty factors to BMDs, see U.S. EPA (1991).

6.1.2.3 Germ Cell Mutagenicity

According to U.S. EPA (1986), a dose-response assessment of an agent's potential for human germ cell mutagenicity can presently be done using only data from *in vivo* heritable germ cell tests. This will remain the case until such time as other assays are demonstrated to have an equivalent predictability for human effects. The usable tests are, thus, limited to the following: morphological specific locus and

biochemical specific locus assays; and heritable translocation tests. Data from such assays are generated from exposures much higher than those expected for humans as a consequence of environmental exposure. Estimation of extent of human risk is done by extrapolating the observed mutation frequency or phenotypic effects downward to the expected human exposure range. Available data and mechanistic considerations are used in the choice of the dose-response model and extrapolation procedure.

6.1.2.4 Carcinogenic Effects

Mathematical models can be used, if data are sufficient, to calculate the estimated excess cancer risk associated with either the ingestion or inhalation of the contaminant if toxicologic evidence leads to the classification of the contaminant as one of the following: A, Known Human Carcinogen; B, Probable Human Carcinogen; or C, Possible Human Carcinogen. The data used in these estimates usually come from lifetime exposure studies using animals. In order to estimate the potential cancer risk for humans from animal data, animal doses must be converted to equivalent human doses. This conversion includes correction for noncontinuous exposure for less-than-lifetime exposure studies and for differences in size. The factor to compensate for the size difference should be determined from appropriate experimental data. In the absence of such data, a default value should be used, such as the cube root of the ratio of the animal and human body weights. A default assumption is that the average adult human body weight is 70 kg, that the average water consumption of an adult human is 2 L of water per day, and that the average adult breathes 20 m^3 of air per day.

For contaminants with a carcinogenic potential, chemical levels are correlated with a carcinogenic risk estimate by employing a cancer potency (unit risk) value together with the assumption for lifetime exposure. The cancer unit risk has generally been derived by assuming low dose linearity and applying a mathematical model such as a linearized multistage model with a 95% upper confidence limit. Cancer risk estimates have also been calculated using other models such as the one-hit, Weibull, logit and probit. There is little basis in the current understanding of the biologic mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than any other. Because each model is based upon differing assumptions, the estimates derived for each model can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of cancer risk rate levels has an inherent uncertainty that is due to the systematic and random errors in scientific measurement. In most cases, only studies using experimental animals have been performed. Thus, there is uncertainty when the data are extrapolated to humans. When developing cancer risk rate levels, several other areas of uncertainty exist, such as the incomplete knowledge concerning the health effects of contaminants in environmental media, the impact of the experimental animal's age, sex and species, the nature of the target organ system(s) examined and the actual rate of exposure of the internal targets in experimental animals or humans. Dose-response data usually are available only for high levels of exposure and not for the lower levels of exposure closer to where a standard may be set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible interactive effects.

6.2 Hazard Identification for Mercury

Because there are no U.S. EPA guidelines for hazard identification of systemic noncancer effects, this section does not include a discussion of systemic noncancer effects.

6.2.1 Developmental Effects

6.2.1.1 Elemental Mercury

Data for developmental effects of elemental mercury are detailed in Section 3.1.3.11 (Tables 3-25, 3-26 and 3-27). Human studies are inconclusive. The study by Mishinova et al. (1980) provided insufficient experimental detail to permit evaluation of an exposure-response relationship. Sikorski et al. (1987) found an increase in reproductive failure among 57 dental professionals by comparison to controls. This reproductive failure (described as spontaneous abortions, stillbirths or congenital malformations) was significantly correlated with exposure level. Maternal toxic signs were not reported. The study was limited by the small population and the lack of description of the control group. These findings were not reproduced in Ericson and Kallen's 1989 study of 8157 infants born to dental professionals in Sweden. When compared to the general population, there was no increase in malformations, abortions or stillbirths. Exposure data were limited in this study.

There are four animal studies evaluating potential developmental effects associated with exposure to elemental mercury. In Baranski and Szymczyk (1973), female rats (strain not specified) were exposed to 2.5 mg/m^3 mercury vapor for 6 to 8 weeks before fertilization or for 3 weeks prior to mating and on days 7–20 of gestation. In the first experiment, mortality among pups was increased in the exposed group, and there were changes in pup organ weights (decreased kidney and liver weight and increased ovary weight). In the second exposed group, mean number of live pups was decreased; mortality among pups was 100% by day 6 *post partum*. There were signs of frank toxicity in the dams including spasms, tremors and death. Information is taken from an English translation of this Polish paper.

Steffek et al. (1987) is reported in abstract. Rats (strain not specified) were exposed to 0.1, 0.5 or 1.0 mg/m^3 mercury for either the entire gestation period or for days 10–15. No effects on resorption or gross abnormality were seen in the low-dose group. Exposure to the mid and high doses for days 10–15 resulted in increased numbers of resorption (5/41 and 7/71, respectively; denominators are presumed to be numbers of litters -- not specified in text); exposure for the entire gestational period resulted in gross defects in 2/115 fetuses in the low dose and increased resorption (19/38) in the high dose. Maternal and fetal weight was decreased in the group exposed to 1.0 mg/m^3 for the entire gestation period. No statistical analyses were reported in the abstract.

Two studies in rats focused on behavioral changes consequent to inhalation of elemental mercury during development. In the first, Danielsson et al. (1993) exposed pregnant Sprague-Dawley rats to mercury vapor at 1.8 mg/m^3 for either 1 or 3 hours on gestation days 11–14 and 17–20. There were no signs of toxicity in the dams and offspring of treated animals were no different from controls on the following measures: body weight; clinical signs; pinna unfolding; surface righting reflex development; tooth eruption; and results of a negative geotaxis test at days 7, 8 or 9 *post partum*. Male rats exposed *in utero* were significantly hypoactive by comparison to controls at 3 months and hyperactive at 14 months. Exposed males were impaired in a test of habituation to novel environments and showed decreased ability to learn a maze. They were not different from controls in a circular swim test administered at 15 months of age. Females were tested only in the spontaneous motor activity tests; treated females were no different from controls on this measure.

These results were similar to those reported by Fredriksson et al. (1992). In this instance rats were exposed postnatally on days 11–17 of age to 0.05 mg Hg/m^3 for either 1 or 3 hours/day. High-dose rats showed increased activity (rearing) at 2 months but had decreased activity by comparison to controls

at 4 months. Low-dose rats were no different from controls at 2 months; at 4 months this group showed increased total activity and decreased rearing. In the spatial learning test administered at 6 months low-dose rats showed increases in time to complete the task. High-dose animals were observed to have increases in both time to complete the task and in numbers of errors. Data were not reported on gender differences in behavior as a result of exposure to mercury vapor.

Both of these studies involved exposure during critical developmental periods, one pre-natal and one post-natal prior to sexual maturity. Both showed differences from controls (by ANOVA) on one of four major manifestations of developmental effects listed in the Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA 1991); namely, functional deficits, in this case in locomotion and learning. In the Danielsson et al. (1993) paper, these deficits were observed in male offspring in the absence of maternal toxicity, which according to the Guidelines raises the level of concern. The studies suggest that the observed effects are not reversible. Latency to reach a platform in the circular swim maze was significant in the high-dose group at 15 months but not at 7 months, and total activity was decreased in the low-dose group and increased in the high-dose group at 14 months.

The Guidelines specify that for a judgement of Sufficient Experimental Animal Evidence/Limited Human Data the minimum data set is the following:

> " The minimum evidence necessary to judge that a potential hazard exists generally would be data demonstrating an adverse developmental effect in a single, appropriate, well conducted study in a single experimental animal species."

As the data set for elemental mercury consists of two appropriate studies albeit with minimal group sizes and two incompletely reported studies suggestive of effect, the judgement of Sufficient Experimental Animal Evidence/Limited Human Data is the most appropriate.

6.2.1.2 Inorganic Mercury

Data on developmental effects of mercuric chloride are found in Section 3.2.3.8 (Tables 3-46 and 3-47). There is one study in mice of developmental effects of inhaled mercuric chloride and none in humans. Selypes et al. (1984) reported increases in delayed ossification and dead or resorbed fetuses as a consequence of exposure of CFLP/N mice to 0.17 and 1.6 mg Hg/m^3 as mercuric chloride in an aerosol for 4 hours on days 9–12 of gestation. There were no statistical analyses, reporting of blood mercury levels or evaluation of maternal toxicity.

Developmental effects following oral exposure to methylmercury are reported in five oral studies in rats and hamsters (Table 3-47). McAnulty et al. (1982) is reported in abstract. Oral (not further specified) mercuric chloride was administered on days 6–15 of gestation at doses of 6, 9, 12, or 18 mg Hg/kg-day. This resulted in decreased fetal and placental weights in fetuses in the 6 mg/kg-day group and malformation at the highest dose. The authors concluded that inorganic mercury was a developmental toxicant only at doses which were maternally toxic.

Rizzo and Furst (1972) treated Long Evans rats with 0 or 2 mg Hg/kg-day as mercuric oxide on gestation days 5, 12 or 19. Effects noted were growth retardation and inhibition of eye formation in the group treated on day 5. No statistical analyses were reported, nor were blood mercury levels given.

Pritchard et al. (1982a) reported in abstract results of treating rats (strain not specified) with mercuric chloride at 3.0, 6.0 or 12.0 mg Hg/kg-day for about 32 days (gestation day 15 until 25 days

postpartum). Effects included decreased pup weight and weight gain with a LOAEL of 6.0. In another experiment reported in an abstract, Pritchard et al. (1982b) exposed female rats to mercuric chloride doses of up to 18 mg Hg/kg-day before mating and during gestation. High implantation loss was observed with exposure to 9 mg Hg/kg-day and higher. Embryonic and fetal development was reported to be unaffected with doses up to 9 mg Hg/kg-day. The abstracts presented insufficient details, and there was no reporting of statistical analyses.

Gale (1974) gavaged female hamsters (10/group) on gestation day 8 with mercuric acetate at the following doses: 2.5, 5.0, 16.0, 22.0, 32.0, 47.0, or 63.0 mg Hg/kg-day. There were 3 control animals. A variety of malformations and growth effects were noted in animals treated with 16 mg/kg-day or higher. The authors also treated hamsters via other routes. Their evaluation of efficacy in production of fetal effects was i.p. > i.v. > s.c. > oral. Maternal toxicity included weight loss, diarrhea, slight tremor, somnolence, tubular necrosis and hepatocellular necrosis (dose levels not specified). There was insufficient detail reported for determination of a NOAEL for dams.

In addition to studies of oral or inhalation administration of inorganic mercury there are several studies which indicate that mercury salts cause developmental toxicity when delivered i.p., s.c. or i.v. routes (Bernard et al. 1992; Gale and Ferm 1971; Gale 1974, 1981; Kajiwara and Inouye 1986, 1992; Kavlock et al. 1993). In Gale (1981), wherein exposure was of six strains of hamster to mercuric acetate, s.c., there was no description of maternal toxicity. In Kavlock et al. (1993) (s.c., rats, mercuric acetate) fetal effects were noted at doses above the lowest observed maternally toxic dose. Kajiwara and Inouye (1986) reported their opinion that in mice injected i.v. with mercuric chloride, fetal toxicity was related to maternal toxicity. In their 1992 study, there was no determination whether implantation loss in mercuric chloride exposed dams was due to fetal toxicity or to maternal uterine dysfunction. The effects reported by Bernard et al. (1992) can be better characterized as a transitory nephrotic effect rather than a developmental deficit.

Each of these studies is limited in its usefulness for assessment of the risk of inorganic mercury to cause human developmental toxicity. The data base as a whole suggests an effect of inorganic mercury at doses as low as 2 mg Hg/kg-day. The data, however, are considered insufficient for risk assessment based on any single study or on the database as a whole (Insufficient Evidence, in the language of the Guidelines).

6.2.1.3 Methylmercury

Data for developmental effects of methylmercury are presented in Section 3.3.3.8 (Tables 3-66, 3-67 and 3-68); studies are primarily by the oral route and none by the inhalation route. Human studies of developmental effects include evaluation of children born to mothers exposed to contaminated grain in Iraq (Amin-Zaki et al. 1976; Marsh et al. 1981, 1987) and contaminated fish in Japan (Harada 1978). Effects noted in the Iraqi children included delays in speech and motor development, mental retardation, reflex abnormalities and seizures. Infants born to mothers ingesting fish from the contaminated Minamata Bay in Japan appeared normal at birth. Within several months, however, the following effects were noted: mental retardation, retention of primitive reflexes, cerebellar symptoms, dysarthria, hyperkinesia, hypersalivation, strabismus and pyramidal symptoms. Histologic examination of brain tissues of infants from both populations showed a number of signs of pathology. Kjellstrom et al. (1989), in a study of a population in New Zealand, has observed an inverse correlation between IQ in children and hair mercury levels in their mothers. In a group of Cree Indians in Quebec, maternal hair mercury level was correlated with abnormal muscle tone in male children (McKeown-Eyssen et al. 1983).

Numerous animal studies have demonstrated a variety of developmental effects occurring in rats, mice and monkeys exposed orally to methylmercury and are presented in Chapter 3 (Table 3-68). Developmental effects have been observed in offspring of rats of three strains treated orally with methylmercury. Developmental effects have also been seen in two strains of mice as well as in guinea pigs, hamsters and monkeys.

In rodents exposed *in utero*, decreased fetal weight and increased fetal malformations and deaths have been reported (Fuyuta et al. 1978, 1979; Inouye and Kajiwara 1988a; Inouye and Murakami 1975; Khera and Tabacova 1973; Nolen et al. 1972; Reuhl et al. 1981; Yasuda et al. 1985).

Methylmercury exposure during gestation as well as during the lactation period produces neurodevelopmental effects (structural and functional alterations) in the exposed pups. Structural effects include lesions in the brain mantle, corpus callosum, caudate putamen, and cerebellum. In guinea pigs, early gestational exposures (weeks 3–5 of pregnancy) resulted primarily in developmental disturbances of the brain (smaller brains, dilated lateral ventricles, and reduced size of caudate putamen), whereas later gestational exposures (>week 6 of pregnancy) resulted in widespread neuronal degeneration (Inouye and Kajiwara 1988b). Functional changes include abnormal tail position during walking, flexion, hindlimb crossing, decreased locomotor activity, increased passiveness and startle-response, impaired maze performance, operant behavior, swimming behavior, tactile-kinesthetic function, visual recognition memory, and temporal discrimination (Bornhausen et al. 1980; Buelke-Sam et al. 1985; Burbacher et al. 1990; Elsner 1991; Geyer et al. 1985; Gunderson et al. 1988; Hughes and Annau 1976; Inouye et al. 1985; Musch et al. 1978; Olson and Boush 1975; Rice 1992; Rice and Gilbert 1990; Stoltenburg-Didinger and Markwort 1990; Suter and Schon 1986).

While there are limitations to some of these studies (e.g., lack of information on BML, small study size), the totality of the data base supports a judgment of Sufficient Human and Animal Data for Developmental Toxicity of methylmercury.

6.2.2 Germ Cell Mutagenicity

6.2.2.1 Elemental Mercury

Data for genotoxicity of elemental mercury are described in Section 3.1.3.13 (Table 3-30). Results for an association of somatic cell chromosomal effects with occupational exposure to elemental mercury are variable. Popescu et al. (1979) and Verschaeve et al. (1976) reported increased incidence of aberrations or aneuploidy. Most recently Barregard et al. (1991) showed a significant correlation between cumulative exposure to elemental mercury and micronuclei induction in T-lymphocytes. Negative results were reported by Verschaeve et al. (1979) and Mabille et al. (1984). No studies of mutagenic effect are reported.

Elemental mercury once absorbed is widely distributed throughout the body; there are no data, however, on elemental mercury in gonadal tissue. Based on both positive and negative findings for somatic cell chromosomal aberrations in workers, elemental mercury is placed in a group of low confidence for potential as a human germ cell mutagen.

6.2.2.2 Inorganic Mercury

Data for genotoxic effects of inorganic mercury are described in Section 3.2.3.10 (Table 3-50). There are no data on inorganic mercury from human germ cell mutagenicity studies or from studies on heritable mutational events in animals. Anwar and Gabal (1991) reported a statistically significant increase by comparison to age-matched controls in both chromosomal aberrations and micronuclei in lymphocytes of workers exposed to mercury fulminate. There was a correlation between frequency of aberrations and exposure duration. Elemental mercury has been shown to be clastogenic both *in vivo* and *in vitro.* Results of tests for mutagenicity have been variable; generally test results in prokaryotes are negative for mutagenicity (but may be positive for DNA damage), and results in eukaryotes are positive. Suter (1975) observed a small, but statistically significant increase in non-viable implants when female mice were administered mercuric chloride intraperitoneally; the authors were not certain whether this was a true dominant lethal effect or was attributable to maternal toxicity.

Chromosome aberrations were observed in somatic cells in occupationally exposed humans (Anwar and Gabal 1991), in somatic cells of mice exposed by gavage (Ghosh et al. 1991), and in Chinese Hamster Ovary cells treated *in vitro* (NTP 1993; Howard et al. 1991). Sex-linked recessive mutations were not observed in *Drosophila* (NTP 1993), and positive results in a dominant lethal test were compromised by maternal toxicity (Suter 1975). There are other data for DNA damage and limited data for gene mutation. Inorganic mercury is less well-distributed in the body than is elemental mercury; it does not readily pass blood-brain or placental barriers. In one reported study (Jagiello and Lin 1973), mice treated intraperitoneally were not shown to have an increased incidence of aneuploidy in spermatogonia. Watanabe et al. (1982), however, showed that while hamsters injected s.c with mercuric chloride had no increase in aberrations in metaphase II oocytes, there was detectable mercuric chloride in ovaries and some inhibition of ovulation.

The totality of available data indicates a moderate weight of evidence for germ cell mutagenicity: sex-linked recessive and dominant lethal results were compromised, but there are positive results for chromosomal aberrations in multiple systems (including *in vivo* exposure) and evidence that mercuric chloride can reach female gonadal tissue.

6.2.2.3 Methylmercury

Summaries of data for genotoxicity of methylmercury are presented in Section 3.3.3.10 (Tables 3-70, 3-71 and 3-72).

Methylmercury appears to be clastogenic but not a potent mutagen. Methylmercury is widely distributed in the body, breaching both blood-brain and placental barriers in humans. There are data indicating that methylmercury administered i.p. reaches germ cells and may produce adverse effects. Suter (1975) observed a slight reduction in both numbers of implantations and viable embryos in (SEC x C57Bl)F_1 females which had been mated to treated males. This was not noted in (101 x C3H)F_1 mice. When Syrian hamsters were treated intraperitoneally with methylmercury, aneuploidy but not chromosomal aberrations was seen in oocytes. Sex-linked recessive lethal mutations were increased in *Drosophila melanogaster* given dietary methylmercury. Watanabe et al. (1982) noted some decrease in ovulation in hamsters treated s.c. with methylmercury, further indication that methylmercury is distributed to female gonadal tissue.

Studies have reported increased incidence of chromosome aberrations (Skerfving et al. 1970, 1974) or SCE (Wulf et al. 1968) in lymphocytes of humans ingesting mercury-contaminated fish or

meat. Chromosome aberrations have been reported in cats treated *in vivo* and in cultured human lymphocytes *in vitro*. Evidence of DNA damage has been shown in a number of *in vitro* systems.

As there are data for mammalian germ cell chromosome aberration and limited data from a heritable mutation study, methylmercury is placed in a group of high concern for potential human germ cell mutagenicity. All that keeps methylmercury from the highest level of concern is lack of positive results in a heritable mutation assay.

6.2.3 Carcinogenic Effects

This section presents the critical carcinogenicity studies evaluated by the U.S. EPA for the weight-of-evidence classification of elemental, inorganic (mercuric chloride) and organic (methylmercury) forms of mercury. These studies are discussed more completely in Chapter 3 and summarized in Tables 3-1, 3-31, 3-32, 3-33, 3-52, and 3-53.

6.2.3.1 Elemental Mercury

Human data regarding the carcinogenicity of inhalation of elemental mercury are insufficient to determine whether such exposures may result in increased cancer incidence. Several studies report statistically significant increases in lung cancer mortality among groups of exposed workers (Amandus and Costello 1991; Barregard et al. 1990; Buiatti et al. 1985; Ellingsen et al. 1992). The interpretation of these studies is limited by small sample sizes, probable exposure to other known lung carcinogens, failure to consider confounders such as smoking and failure to observe correlations between estimated exposure and the cancer incidence. A study of dental professionals found a significant increase in the incidence of glioblastomas (Ahlbom et al. 1986). It is not known whether exposure to mercury, X-rays, or other potential carcinogens in the workplace contributed to the effects observed. No increase in cancer mortality was observed among workers exposed to mercury vapor in a nuclear weapons facility (Cragle et al. 1984), but this study was also limited by the small sample size. No studies were identified that examined cancer incidence in animals exposed chronically to elemental mercury vapor. These studies are presented in greater detail in Section 3.1.2.

The overall findings from cytogenetic monitoring studies of workers occupationally exposed to mercury by inhalation provide very limited evidence of genotoxic effects. Popescu et al. (1979) compared four men exposed to elemental mercury vapor with an unexposed group and found an increased number of chromosomal aberrations. Verschaeve et al. (1979) found an increased incidence of aneuploidy after exposure to low concentrations.

In summary, human epidemiological studies failed to show a correlation between exposure to elemental mercury vapor and increased cancer incidence, but the studies are limited by confounding factors. Only one study in animals is reported (Druckrey et al. 1957); tumors were found only at contact sites, and the study is incompletely reported as to controls and statistics. Animal data are, thus, also inadequate. Findings from assays for genotoxicity are limited and provide no convincing evidence that mercury exposure has an effect on the number or structure of chromosomes in human somatic cells. The most appropriate category is, thus, Group D, not classifiable as to human carcinogenicity.

This classification was reviewed by the Carcinogen Risk Assessment Verification Endeavor (CRAVE), an Agency Peer Review Work Group. The classification was accepted as appropriate on March 3, 1994.

6.2.3.2 Inorganic Mercury

There are no data available on the carcinogenic effects of inorganic mercury (mercuric chloride) in humans. In animals, there is equivocal evidence of carcinogenicity in rats and mice. In rats gavaged with mercuric chloride for two years (NTP 1993), survival was significantly reduced in males (17% and 8% survival in low and high-dose males versus 43% survival in controls), indicating that the maximally tolerated dose (MTD) was exceeded. There was an increased incidence of forestomach squamous cell papillomas (0/50, 3/50, 12/50 in control, low, and high-dose males, respectively; 0/50, 0/49 and 2/50 in control, low and high-dose females, respectively). Papillary hyperplasia of the forestomach was also significantly elevated in both male dose groups and in high-dose females. In addition, the incidence of thyroid follicular cell carcinomas in treated males (1/50, 2/50 and 6/50 in control, low- and high-dose males, respectively) showed a significantly positive trend. There were, however, no increases in thyroid hyperplasia of adenomas; it is not clear that the increase in thyroid carcinomas is a treatment-related effect. The NTP also considered the forestomach tumors to be of limited relevance to humans; there was no evidence that these contact site tumors progressed to malignancy.

In a companion study in mice (NTP 1993), there was a significantly increasing trend for renal tubular cell tumors (adenomas and adenoma carcinomas). No dose groups were statistically significantly different from the control by pair-wise comparison, although the incidence in the high-dose group was elevated. There was a significant increase in severe nephropathy in treated animals. The NTP studies and two nonpositive bioassays are summarized in Section 3.2.2.

In summary, there are no data in humans linking mercuric chloride with carcinogenic effects. Data in animals are limited. Focal hyperplasia and squamous cell papillomas of the forestomach as well as thyroid follicular adenomas and carcinomas were observed in male rats gavaged with mercuric chloride. In the same study, evidence for increased incidence of squamous cell forestomach papillomas in female rats and renal adenomas and carcinomas in male mice were considered equivocal. All increased tumor incidences were observed at what were considered high doses (in excess of the MTD). In this context, the relevance of the thyroid tumor to human health evaluation has been questioned; these tumors are considered to be secondary to the hyperplastic response. Results from *in vitro* and *in vivo* tests for genotoxicity have been mixed with no clear indication of a strong somatic cell genotoxic effect of mercuric chloride exposure.

Based on the absence of human data and limited data for carcinogenicity in animals, mercuric chloride is classified as Group C, possible human carcinogen. This classification was reviewed by CRAVE on March 3, 1994 and found to be appropriate.

6.2.3.3 Methylmercury

The available human data are inconclusive regarding the carcinogenicity of methylmercury in humans exposed by the oral route. A study of leukemia patients from a rural area in Poland showed a significantly higher mercury content in hair in the leukemia patients than in healthy unrelated patients or healthy relatives (Janicki et al. 1987). The population studied was small, and the study did not adjust for other leukemia risk factors. In addition, two studies of larger populations exposed to methylmercury during the Minamata incident failed to show increases in leukemia or total cancer incidence (Tamashiro et al. 1984, 1986). Although one of these studies showed a significant increase in liver cancer incidence, factors other than mercury exposure were likely contributors to the increase. These epidemiological studies are presented in greater detail in Section 3.3.2.1.

Animal studies show some evidence of carcinogenicity in two strains of mice, but studies in rats have not shown similar results. Male ICR mice given methylmercuric chloride in the diet for up to two years had significantly increased incidences of renal epithelial adenomas and/or adenocarcinomas (Hirano et al. 1986; Mitsumori et al. 1981). Similarly, male B6C3F1 mice given methylmercuric chloride in the diet for up to two years had significantly increased incidences of renal epithelial carcinomas and adenomas (Mitsumori et al. 1990). In contrast, Sprague-Dawley rats administered methylmercury in the diet for up to 130 weeks exhibited no increase in tumor incidence (Mitsumori et al. 1983, 1984). Although the dose was lower in the rats than in the mice, a maximally tolerated dose was achieved in the rat study as evidenced by an approximately 20–30% decrease in body weight gain and by significant increases in renal and neuronal toxicity in both male and female rats at the highest dose tested. Other studies also failed to show increases in tumor incidence after chronic exposure to methylmercury (Schroeder and Mitchener 1975; Verschuuren et al. 1976), but these studies were limited by small sample sizes, failure to achieve a maximally tolerated dose and/or incomplete histopathological examinations. These studies are presented more completely in Section 3.3.2.2.

In summary, data for carcinogenicity from human studies are considered inadequate. Three studies that examined the relationship between methylmercury exposure in humans and increased incidence of cancer were limited by poor study design or incomplete description of methodology or results. Data from animal studies are considered to provide limited evidence of carcinogenicity. Male ICR and B6C3F1 mice exposed to methylmercuric chloride in the diet were observed with increased incidence of renal adenomas, adenocarcinomas and carcinomas. Tumors were observed at a single site, in a single species and sex. Renal epithelial cell hyperplasia and tumors were observed only in the presence of profound nephrotoxicity; tumors were suggested to be consequent to reparative changes in the affected organs. Although genotoxicity test data suggest that methylmercury is clastogenic, there are also negative tests.

The limited data in animals above support a categorization of Group C, possible human carcinogen. The CRAVE Work Group accepted this weight-of-evidence judgment as appropriate at its March 3, 1994 meeting.

6.2.3.4 Application of proposed revision of the Guidelines for Carcinogen Risk Assessment

Data described in the above three sections were re-evaluated using criteria described in the proposed revisions to the Guidelines for Carcinogen Risk. Among the changes in the revised guidelines are emphasis on use of data describing the mode of action of the putative carcinogen, both in weight of evidence judgements and in decisions as to the most appropriate type of low dose extrapolation. The revised Guidelines encourage consideration of relevance to human health risk of route and magnitude of exposure in animal bioassays. Both of these considerations were part of a re-evaluation of the data for carcinogenicity of the three forms of mercury. Results of a presentation (Schoeny, 1996) on the application of the revised Guidelines are summarized below.

Elemental mercury

The likelihood of elemental mercury to be a human carcinogen cannot be determined; data in humans and animal bioassays are inadequate. Epidemiologic studies, though confounded showed no correlation between exposure to elemental mercury vapor and carcinogenicity. Animal data were not positive, but the published study was considered inadequate. The study was done by a route (intramuscular injection) not relevant to human exposure (inhalation). Genetic toxicity data are limited and equivocal.

Inorganic (mercuric chloride)

The data for inorganic mercury indicate that it is not likely to be a human carcinogen under conditions of exposure generally encountered in the environment. There are no data on carcinogenicity in humans. Findings in animals included squamous cell papillomas of the forestomach and thyroid follicular cell adenomas and carcinomas in gavaged rats, and renal adenomas and adenocarcinomas in male mice. All increased tumor incidences were at high doses (in excess of the MTD). Genetic toxicity data gave a mix of positive and negative response for chromosomal breakage and were equivocal for somatic cell point mutations. The mode of action for forestomach tumors appears to be a high dose effect related to irritation and cytotoxicity. The mode of action for thyroid tumors is not clear; there was no treatment-related increase in incidence of hyperplasia. There was high mortality in rats from renal toxicity. Renal toxicity in male mice was less severe; the mode of action of inorganic mercury in producing renal neoplasms is not clear. Human exposure (other than occupational or accidental poisoning) is likely to be to low levels of inorganic mercury in water or food plants.

Methylmercury

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

6.3 Dose-Response Assessment For Mercury

6.3.1 Systemic Noncancer Effects

6.3.1.1 Oral Reference Doses (RfDs)

Elemental mercury

Metallic mercury is only slowly absorbed by the gastrointestinal tract (~0.01%) and because of this is thought to be of no toxicological consequence (Klaassen et al. 1986) when ingested. Further discussion of an RfD for this form of mercury is not presented.

Inorganic mercury (mercuric chloride)

An RfD for inorganic mercury of $3x10^{-4}$ mg/kg-day has been verified by the RfD/RfC Work Group. The critical effect serving as the basis for the RfD is kidney toxicity due to an auto-immune disease caused by the accumulation of IgG antibodies in the glomerular region of the kidneys.

On October 26 and 27 of 1987, a panel of mercury experts met at a Peer Review Workshop convened by U.S. EPA for the purpose of reviewing outstanding issues concerning the health effects and risk assessment of inorganic mercury (U.S. EPA 1987). The panel participants are listed in Appendix C. Five consensus conclusions and recommendations were agreed to as a result of this workshop; these are presented in Table 6-1. The RfD was determined using data on autoimmune glomerulonephritis observed in rats. Based on three studies using the Brown-Norway rat, a DWEL value was determined using studies described below. The Brown-Norway rat is very sensitive to this mercuric mercury-induced autoimmune effects, although this effect has also been demonstrated in other strains of rats and other species of experimental animals (Andres 1984; Bernaudin et al. 1981; Hultman and Enestrom 1992). The Brown-Norway rat is believed to be a good surrogate for the study of mercury-induced kidney damage in sensitive humans (U.S. EPA 1987b). The glomerulonephritis is characterized by deposition of anti-glomerular basement membrane antibodies (IgG) in renal glomeruli and after prolonged exposure is often accompanied by proteinuria and, in some cases, nephrosis (Druet et al. 1978).

LOAEL values were identified from three individual studies. In Druet et al. (1978), Brown-Norway rats were exposed to mercuric chloride via subcutaneous injection, 3 times/week, for 8 weeks. The dose levels administered were 0, 0.1, 0.25, 0.5, 1.0 and 2.0 mg Hg/kg, and there were 6–20 animals/group. An additional group of animals received 0.05 mg Hg/kg for 12 weeks. (The number of animals/sex was not stated.) Druet and colleagues measured antibody formation (using a fluoresceinated sheep anti-rat IgG antiserum) and urinary protein levels. Proteinuria occurred at doses $\geq$0.1 mg/kg (LOAEL); the proteinuria was considered a highly deleterious effect, as it frequently led to hypoalbuminemia and even death. A LOAEL for lifetime exposure was calculated to be 0.226 mg/kg-day, using the following conversion:

$$0.05 \text{ mg/kg} \times 3 \text{ days}/7 \text{ days} \times 0.739\ [HgCl_2 \rightarrow Hg^{2+}] \times 100\% \text{ absorption}/7\%$$
$$= 0.226 \text{ mg Hg/kg-day}$$

In a 60-day study conducted by Bernaudin et al. (1981), Brown-Norway rats (5/group) were force-fed 0 or 3 mg/kg/week mercuric chloride. At the end of the 60 days, there were no classic histological abnormalities in the kidneys of treated animals. Using immunofluorescence, however, IgG deposition was evident in all of the treated rats, and weak proteinuria was noted in 3/5 dosed animals. A lifetime LOAEL was calculated to be 0.317 mg Hg/kg-day. Dose conversion was done in the following manner:

$$3 \text{ mg/kg} \times 1 \text{ day}/7 \text{ days} \times 0.739\ [HgCl_2 \rightarrow Hg^{2+}]$$
$$= 0.317 \text{ mg Hg/kg-day}$$

Table 6-1
Consensus Decisions of Peer Review Panel

- The most sensitive adverse effect for mercury risk assessment is formation of mercuric mercury-induced autoimmune glomerulonephritis. The production and deposition of IgG antibodies to the glomerular basement membrane can be considered the first step in the formation of this mercuric mercury-induced autoimmune glomerulonephritis.
- The Brown-Norway rat should be used for mercury risk assessment. The Brown-Norway rat is a good test species for the study of Hg^{2+}-induced autoimmune glomerulonephritis. The Brown-Norway rat is not unique in this regard (i.e., this effect has also been observed in rabbits).
- The Brown-Norway rat is a good surrogate for the study of mercury-induced kidney damage in sensitive humans. For this reason, the uncertainty factor (for interspecies variability) used to calculate criteria and health advisories (based on risk assessments using the Brown Norway rat) should be reduced by 10-fold.
- Hg^{2+} absorption values of 7% from the oral route and 100% from the subcutaneous route should be used to calculate criteria and health advisories.
- A DWEL of 0.010 mg/L was recommended based on the weight of evidence from the studies using Brown Norway rats and limited tissue data.

Similar results were obtained by Andres (1984). Five Brown-Norway rats were exposed to 3 mg/kg mercuric chloride via gavage 2 times/week for 60 days. In this same study, Lewis rats (n=2) were also exposed using the same dosing regimen. After 60 days, the kidneys of all treated animals appeared normal histologically, and no proteinuria was reported in any treated animals; IgG deposition in the renal glomeruli was demonstrated using immunofluorescence in Brown-Norway rats. No antibody deposition was noted in the Lewis rats. The lifetime LOAEL was determined to be 0.633 mg Hg/kg-day. Dose conversion was done in the following manner:

$$3 \text{ mg/kg} \times 2 \text{ days}/7 \text{ days} \times 0.739 \; [HgCl_2 \rightarrow Hg^{2+}]$$
$$= 0.633 \text{ mg Hg/kg-day}$$

As the result of intensive review of these and other studies, as well as the discussions of the panel of mercury experts convened for this purpose, a recommended DWEL of 0.01 mg/L was derived from the LOAELs above, and, subsequently, the oral RfD value was back-calculated:

$$RfD = \frac{DWEL \times 2 \; L/day}{70 \; kg \; bw}$$

$$RfD = 0.010 \text{ mg/L} \times 2\text{L/day}/70 \text{ kg bw}$$
$$= 0.0003 \text{ mg/kg bw/day}$$

The RfD for inorganic mercury was reviewed by the RfD/RfC Work Group which reached consensus for verification on November 16, 1988. The Work Group agreed to application of an uncertainty factor of 1000 to the LOAELs above (which ranged from 0.23 to 0.63 mg Hg/kg-day). The uncertainty factor was composed of a 10-fold each for subchronic to chronic and LOAEL to NOAEL extrapolation, and an additional 10-fold factor for both animal to human and sensitive populations. The resulting RfD of $3x10^{-4}$ mg/kg-day was given high confidence based on the weight of the evidence from the studies using Brown Norway rats and the entirety of the data base.

A literature search for the years 1988 to 1994 has been conducted and recently reviewed (September 1994). The NTP (1993) study was among those considered. A rat NOAEL of 0.23 mg Hg/kg administered dose has been identified for renal effects for the 6-month portion of the study. A description of the NTP gavage study has been included in the summary information for IRIS. U.S. EPA concluded that no change in the RfD for inorganic mercury is needed at this time.

Methylmercury

U.S. EPA has on two occasions published RfDs for methylmercury which have represented the Agency consensus for that time. These are described in the sections below. The original RfD of 0.3 µg/kg/day was determined in 1985. The current RfD of 0.1 µg/kg/day was established as Agency consensus in 1995. At the time of the generation of the Mercury Study Report to Congress, it became apparent that considerable new data on the health effects of methylmercury in humans were emerging. Among these are large studies of fish or fish and marine mammal consuming populations in the Seychelles and Faroes Islands. Smaller scale studies are in progress which describe effects in populations around the U.S. Great Lakes. In addition, there are new evaluations, including novel statistical approaches and application of physiologically-based pharmacokinetic (PBPK) models, to published work described in section 3.3.1.1 of this volume.

As much of this new data has either not yet been published or have not yet been subject to rigorous review, it was decided that it was premature for U.S. EPA to make a change in the 1995 methylmercury RfD at this time. This decision was approved by the Science Advisory Board (SAB), a public advisory group providing extramural scientific information and advice to the Administrator and other officials of the Environmental Protection Agency. The SAB is structured to provide balanced, expert assessment of scientific matters relating to problems facing the Agency. Their report makes the following statement.

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

The SAB report continues:

"Investigators conducting two new major prospective longitudinal studies--one in the Seychelles Islands the other in the Faeroe Islands--have recently begun to publish findings in the literature and are expected to continue releasing their findings during the next 2-3 years. These studies have advantages over those cited in the previous paragraph in that they have much larger samples sizes, a larger number of developmental endpoints, potentially more sensitive developmental endpoints, and control a more extensive set of potential confounding influences. On the other hand, the studies have some limitations in terms of low exposures (to PCBs in the Faeroes) and ethnically homogenous societies. Since only a

small portion of these new data sets have been published to date and because questions have been raised about the sensitivity and appropriateness of the several statistical procedures used in the analyses, the Subcommittee concluded that it would be premature to include any data from these studies in this report until they are subjected to appropriate peer review. **Because these data are so much more comprehensive and relevant to contemporary regulatory issues than the data heretofore available, once there has been adequate opportunity for peer review and debate within the scientific community, the RfD may need to be reassessed in terms of the most sensitive endpoints from these new studies.**"

An inter-agency process, with external involvement will be undertaken for the purpose of reviewing these new data, their evaluations, and the evaluations of existing data. An outcome of this process will be an assessment by U.S. EPA of its RfD for methylmercury to determine if a change is warranted.

Former RfD

A hazard identification and dose-response assessment was proposed for methylmercury in 1980 (U.S. EPA 1980) and later verified by the RfD/RfC Work Group on December 2, 1985. This assessment was subsequently included on U.S. EPA's Integrated Risk Information System (IRIS). The critical effects were multiple central nervous system (CNS) effects including ataxia and paresthesia in populations of humans exposed to methylmercury through consumption of contaminated grain (summarized by Clarkson et al. 1975, Nordberg and Strangert 1976 and WHO 1976); see study descriptions in Section 3.3.

The RfD for methylmercury was determined to be $3x10^{-4}$ mg/kg-day, based on a LOAEL of 0.003 mg/kg-day (corresponding to 200 µg/L blood concentration) and an uncertainty factor of 10 used to adjust the LOAEL to what is expected to be a NOAEL. An additional uncertainty factor of 10 for sensitive individuals for chronic exposure was not deemed necessary at the time of the RfD's verification, as the adverse effects were seen in what was regarded as a sensitive group of individuals, namely adults who consumed methylmercury-contaminated grain.

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

Since the time of verification, several submissions to IRIS have questioned the value of this RfD, and, specifically, whether or not this RfD is protective against developmental effects. Subsequent to the RfD verification, the effects in Iraqi children of *in utero* exposure to methylmercury were reported by Marsh et al. (1987). Discussion of the methylmercury RfD by the RfD/RfC Work Group was reported in 1992 and 1994. Consensus for verification of the RfD described below was reached in January of 1995.

Current U.S. EPA RfD

Determination of critical effect

Marsh et al. (1987) was chosen as the most appropriate study for determination of an RfD protective of a putative sensitive subpopulation; namely infants born to mothers exposed to methylmercury during gestation. This paper describes neurologic abnormalities observed in progeny of women who consumed bread prepared from methylmercury-treated seed grain while pregnant (See Chapter 3 for study description). Among the signs noted in the infants exposed during fetal development were cerebral palsy, altered muscle tone and deep tendon reflexes as well as delayed developmental milestones (i.e., walking by 18 months and talking by 24 months). Each child in the study was examined by two neurologists who scored observed effects on a scale for severity ranging from 0 to 11. The data collected by Marsh et al. (1987) summarize clinical neurologic signs of 81 mother and child pairs. From x-ray fluorescent spectrometric analysis of selected regions of maternal scalp hair, concentrations ranging from 1 to 674 ppm mercury were determined, then correlated with clinical signs observed in the affected members of the mother-child pairs. Among the exposed population there were affected and unaffected individuals throughout the exposure range.

Method employed for determination of critical dose

In order to quantitate an average daily mercury ingestion rate for the mothers, hair concentrations were determined for periods during gestation when actual methylmercury exposure had occurred. This procedure is possible since hair grows an average rate of 1 cm/month (Al-Shahristani et al. 1976) and since Iraqi women wear their hair very long; appropriate samples were, thus, available for the period of gestation when exposure occurred.

A number of laboratory studies support a correlation between hair concentrations and concurrent blood concentrations. Some variation in the ratio exists; a ratio of 250:1 (µg mercury/mg in hair:µg mercury/ml of blood) was used to derive the RfD critical dose. A more complete discussion for the choice of this ratio is provided in the next section.

The hair concentration at a hypothetical NOAEL for developmental effects was determined by application of a benchmark dose approach (see subsequent section for discussion of methods and data used). The analysis used the combined incidence of all neurological effects in children exposed *in utero* as reported in the Marsh et al. (1987) study. A Weibull model for extra risk was used to determine the benchmark dose of 11 ppm mercury in maternal hair (11 mg/kg hair). This was converted to 44 μg/L blood using the above 250:1 ratio.

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

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

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

Where:

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

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

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

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

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

The dose d (1.1 μg/kg-day) is the total daily quantity of methylmercury that is ingested by a 60 kg individual to maintain a blood concentration of 44 μg/L or a hair concentration of 11 ppm.

The rationales for use of specific values for equation parameters follow.

Hair to blood concentration ratio. The hair:blood concentration ratio for total mercury is frequently cited as 250:1 expressed as μg mercury/g hair to μg mercury/ml of blood. Ratios reported in the literature range from 140 to 416, a difference of about a factor of 3. Table 6-2 provides the results of 12 recent studies in which hair to blood ratios were calculated for a variety of human populations. Differences in the location of hair sampled (head versus chest and distance from scalp) may contribute to the differences observed. Variability in the hair-blood relationship for mercury concentration can also be attributed to the fact that unsegmented hair analysis gives a time-weighted average of mercury exposure, while analysis of mercury in blood reflects a much shorter period average of exposure. As much as a 3-fold seasonal variation in mercury levels was observed in average hair levels for a group of individuals with moderate to high fish consumption rates, with yearly highs occurring in the fall and early winter (Phelps et al. 1980; Suzuki et al. 1992). The relatively high ratio reported by Tsubaki (Table 6-2) may have reflected the fact that mercury levels were declining at the time of sampling so that the hair levels reflect earlier, higher blood levels. Cernichiari et al. (1995a) reported a maternal hair:blood ratio of 416:1 for residents of the Seychelles Islands. The authors remarked that while this ratio was high, statistical uncertainties do not permit a judgement as to whether it is truly outside the range reported in WHO (1990) recapitulated in Table 6-2. Phelps (1980) obtained multiple blood samples and sequentially analyzed lengths of hair from individuals. Both hair and blood samples were taken for 339 individuals in Northwestern Ontario. After reviewing the various reports for converting hair concentrations to blood concentrations, the analysis in the Phelps (1980) paper was selected by the Agency RfD/RfC Work Group because of the large sample size and the attention to sampling and analysis that was made. The

ratio Phelps observed between the total mercury concentration in hair taken close to the scalp and simultaneous blood sampling for this group was 296:1. To estimate the actual ratio the authors assumed that blood and hair samples were taken following complete cessation of methylmercury intake. They also assumed a half-life of methylmercury in blood of 52 days and a lag of 4 weeks for appearance of the relevant level in hair at the scalp. Phelps also determined that 94% of the mercury in hair is methylmercury. Based on these assumptions, they calculated that if the actual hair:blood ratio were 200:1, they would have observed a ratio of 290. Based on these and other considerations, Phelps states that the actual ratio is "probably higher than 200, but less than the observed value of 296." As the authors point out, one-third of the study population was sampled during the rising phase of seasonal variation (and two-thirds or more in the falling phase). Phelps et al. (1980) had assumed that all were sampled in the falling phase. This fact would tend to result in a lower observed ratio; therefore, the actual average is likely to be greater than 200. It was concluded by U.S. EPA that a midpoint value of 250 is acceptable for the purpose of estimating average blood levels in the Iraqi population.

Fraction of mercury in diet that is absorbed (A). After administration of radiolabeled methylmercuric nitrate in water to 3 healthy volunteers, uptake was reported to be >95%. (Aberg et al. 1969). This value is supported by experiments in human volunteers conducted by Miettinen et al. (1971). These researchers incubated fish liver homogenate with radiolabeled methylmercury nitrate to produce methylmercury proteinate. The proteinate was then fed to fish for a week; the fish were killed, cooked and fed to volunteers after confirmation of methylmercury concentration. Mean uptake exceeded 94%. Based upon these experimental results an absorption factor of 0.95 was used in these calculations.

Fraction of the absorbed dose that is found in the blood (f). There are three reports of the fraction of absorbed methylmercury dose distributed to blood volume in humans. Kershaw et al., (1980) reported an average fraction of 0.059 of absorbed dose in total blood volume, based on a study of 5 adult male subjects who ingested methylmercury-contaminated tuna. In a group of 9 male and 6 female volunteers who had received ^{203}Hg-methylmercury in fish, approximately 10% of the total mercury body burden was present in one liter of blood in the first few days after exposure; this dropped to approximately 5% over the first 100 days (Miettinen et al. 1971) In another study, an average value of 1.14% for the percentage of absorbed dose in one kg of blood was derived from data on subjects who consumed a known amount of methylmercury in fish over a 3-month period (Sherlock et al. 1984). Average daily intake in the study ranged from 43 to 233 µg/day, and there was a dose-related effect on percentage of absorbed dose that ranged from 1.03% to 1.26% in one liter of blood. Each of these values was multiplied by 5 to yield the total amount in the blood compartment, as there are approximately 5 liters of blood in an adult human body (0.01 x 5 = 0.05). The value 0.05 has

Table 6-2
Available Data on Hair: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[1]	140	50	5-270	1-57	--	--
Soria et al., 1992	218	16	2.4-9.1	0.15-20	--	at scalp
Tejning, 1967[1]	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[2]	260	45	2-800	20-325	--	--
Birke et al., 1972[2]	280[3]	12	4-650	1-180	5	at scalp
Den Tonkelaar et al., 1974	280	47	1-40.5	<0.5-13.2	--	--
Kershaw et al., 1980	292[4]	5	--	--	5	at scalp
Phelps et al., 1980	296	339	1-60	1-150	10	at scalp
Sherlock et al., 1982	367	98	1.1-42.3	0.2-21	24	--
Cernichiari et al., 1995	416	740	0.5-26.7		10	at scalp
Tsubaki, 1971a[1]	370	~25	--	--	"longer tuft"[1]	--

[1] As cited in Berglund et al. 1971
[2] As cited in WHO, 1976
[3] Ratio of methylmercury in hair to methylmercury in blood
[4] 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

been used for this parameter in the past by other groups; e.g.., Berglund et al. (1971) and WHO (1990). A value of 0.05 was used for "f" in the above equation.

Elimination constant (b). Several studies reported clearance half-times for methylmercury from blood or hair in the range of 35-189 days (Miettinen 1972; Kershaw et al. 1980; Al-Shahristani et al. 1974; Sherlock et al. 1984). Two of these studies included the Iraqi population exposed during the 1971−1972 incident. A value reported in Cox et al. (1989) was derived from the study group which included the mothers of the infants upon which this risk assessment is based. The average elimination constant of the 4 studies is 0.014; the average of individual values reported for 20 volunteers ingesting from 42 to 233 µg mercury/day in fish for 3 months (Sherlock et al. 1982) is also 0.014. A value of 0.014 $days^{-1}$ was, thus, used for term "b" in the above equation.

Volume of blood in the body (V). That blood volume is 7% of body weight has been determined by various experimental methods. There is an increase of 20% to 30% (to about 8.5 to 9%) during

pregnancy (Best 1961). Specific data for the body weight of Iraqi women were not found. Assuming an average body weight of 58 kg and a blood volume of 9% during pregnancy, a blood volume of 5.22 liters was derived. In the equation on page 6-19, term "V" was taken to be 5 liters.

Body weight. While the critical endpoint for the RfD is developmental effects in offspring, the critical dose is calculated using parameters specific to the mothers who ingested the mercury contaminated grain. Data on body weights of the subjects were not available. A default value of 60 kg (rounded from 58) for an adult female was used.

Grouping of data

Data used in the U.S. EPA benchmark dose calculation were excerpted from the publication, *Seafood Safety* (NAS 1991). The tables of incidence of various clinical effects in children that were provided in this document readily lent themselves to the benchmark dose modeling approach. The continuous data for the Iraqi population that were reported in Marsh et al. (1987) were placed in five dose groups, and incidence rates were provided for delayed onset of walking, delayed onset of talking, mental symptoms, seizures, neurological scores above 3, and neurological scores above 4 for affected children. Neurologic scores were determined by clinical evaluation for cranial nerve signs, speech, involuntary movement, limb tone strength, deep tendon reflexes, plantar responses, coordination, dexterity, primitive reflexes, sensation, posture, and ability to sit, stand and run. Table 6-3 shows the input data for the modeling procedure for effects found in children. Incidence data for each of the adverse effects in children were taken directly from Table 6-11, *Seafood Safety* (NAS, 1991). The effects of late walking, late talking, and neurologic scores greater than 3 were also combined for additional analysis. Table 6-4 shows the incidence data for each of the effects observed in adults as grouped in Table 6-13 of the *Seafood Safety* document.

Adjustments for background incidence

As an adjustment for background rates of effects, the benchmark dose estimates for methylmercury were calculated to estimate the dose associated with "extra risk." Another choice would have been to calculate based on "additional risk." Additional risk (AR) is defined as the added incidence of observing an effect above the background rate relative to the entire population of interest, AR = [P(d)-P(0)]/1. In the additional risk calculation, the background rate is subtracted off, but still applied to the entire population, including those exhibiting the background effect, thus in a sense "double counting" for background effects. It can be seen that extra risk (ER) is always mathematically greater than or equal to additional risk, ER = [P(d)-P(0)]/[1-P(0)], and is thus a more conservative measure of risk (whenever the background rate is not equal to zero). Conceptually, extra risk is the added incidence of observing an effect above the background rate relative to the proportion of the population of interest that is not expected

Table 6-3
Incidence of Effects in Iraqi Children By Exposure Group[a]

Effect	1.37	10	52.53	163.38	436.60
Late Walking	0	2	2	3	12
Late Talking	2	1	3	4	11
Mental Symptoms	1	0	1	3	4
Seizures	0	0	1	2	4
Neurological Scores >3	3	1	4	3	9
Neurological Scores >4	0	1	2	2	6
All Endpoints	4	3	6	8	14
N	27	14	13	12	15

[a] From Table 6-11 of Seafood Safety; Dose is geometric mean in ppm maternal hair.

Table 6-4
Incidence of Effects in Iraqi Adults by Exposure Group[a]

Effect	50	350	750	1500	2500	3500	4500
Paresthesia	2	1	8	10	20	14	7
Ataxia	1	0	2	8	15	17	7
Visual Changes	0	0	4	9	14	10	6
Dysarthria	1	1	1	4	6	13	6
Hearing Defects	0	0	1	0	3	6	5
Deaths	0	0	0	0	0	3	2
N	21	19	19	17	25	17	7

[a] From Table 6-13 of Seafood Safety; Dose is geometric mean in ppb blood.

to exhibit such an effect. Extra risk is then more easily interpreted than additional risk, because it applies the additional risk only to the proportion of the population that is not represented by the background rate.

Extra risk has been traditionally used in U.S. EPA's cancer risk assessments (Anderson et al. 1983) and is discussed in detail in a report on the benchmark dose by U.S. EPA's Risk Assessment Forum (U.S. EPA 1995).

Derivation of a benchmark dose

Benchmark dose estimates were made by calculating the 95% lower confidence limits on doses corresponding to the 1%, 5% and 10% extra risk levels using a quantal Weibull model (K.S.Crump Division of ICF Kaiser International). The Weibull model was chosen for the benchmark dose calculations for the methylmercury data as recent research suggests it may be the best model for developmental toxicity data (Faustman et al., 1994). The form of the quantal Weibull that was used is the following:

$$P(d) = A0 + (1 - A0)(1 - \exp[-A1 * d^{A2}]),$$

where d = dose,
A0 = background rate = 0.12468,
A1 = slope = 9.47×10^{-3}, and
A2 = shape parameter = 1.000.

For each endpoint and for the combined endpoints, the incidence of response was regressed on the dose. A Chi-squared test of goodness-of-fit was used to test the null hypothesis (Ho) that the predicted incidence was equal to the observed incidence, so that Ho would be rejected for p-values less than 0.05.

Results for individual effects and all effects combined for children exposed *in utero* are given in Table 6-5; results for adults are given for comparison in Table 6-6. For calculation of the lower bound on the 10% risk level, A0 = 0.12468, A1 = 9.470230 x 10^{-3}, A2 = 1.00000. The RfD/RfC Work Group chose the benchmark (lower bound on the dose for 10% risk) based on modeling of all effects in children. Recent research (Allen et al. 1994a, b) suggests that the 10% level for the benchmark dose roughly correlates with a NOAEL for developmental toxicity data. Note that this conclusion was based on controlled animal studies and on calculation of additional risk. Both the polynomial and Weibull models place a lower 95% confidence limit on the dose corresponding to a 10% risk level at 11 ppm hair concentration for methylmercury. The benchmark dose rounded to 11 ppm was used in the calculation of the RfD.

Dose groupings other than those used in *Seafood Safety* were also done and benchmark doses run as above for comparison. Both density-based grouping and uniform concentration intervals were used.

The local density of observations relative to the mercury level in hair was analyzed using a density estimation algorithm (smooth function in S-PLUS for Windows, Ver. 3.1; S-PLUS Guide to Statistical and Mathematical Analysis). The function estimates a probability density for the distribution of a variable by calculating a locally-weighted density of the observations. That is, the function estimates the probability that an observation will be near a specific value based on how the actual values are clustered. In this case, the function was used to estimate the probability density for an observation in the neighborhood of any given maternal hair mercury concentration. The density plot is shown in Figure 6-1. The peaks represent relatively greater numbers of data points than the troughs in the vicinity of the associated hair mercury concentrations.

Table 6-5
Methylmercury Benchmark Dose Estimates (ppm hair)
Maximum Likelihood Estimates and 95% Lower Confidence Limits from Weibull Model
Incidence of Effects in Children (Marsh et al. 1987)

Effect	0.01		0.05		0.10		G-O-F[a] P-Value
	MLE	95% CL	MLE	95% CL	MLE	95% CL	
Late Walking	3.3	2.1	16.7	10.9	34.3	22.4	0.16
Late Talking	4.7	2.4	22.1	12.3	43.8	25.3	0.79
Mental Symptoms	12.0	6.4	61.0	32.8	125.4	67.5	0.63
Seizures	11.8	6.7	60.4	34.3	124.2	70.5	0.86
Neuro Score >3	5.6	3.3	28.8	17.0	59.1	34.9	0.58
Neuro Score >4	8.1	4.6	41.4	23.7	84.9	48.7	0.48
All Endpoints	1.6	1.1	8.3	5.4	17.1	11.1	0.94

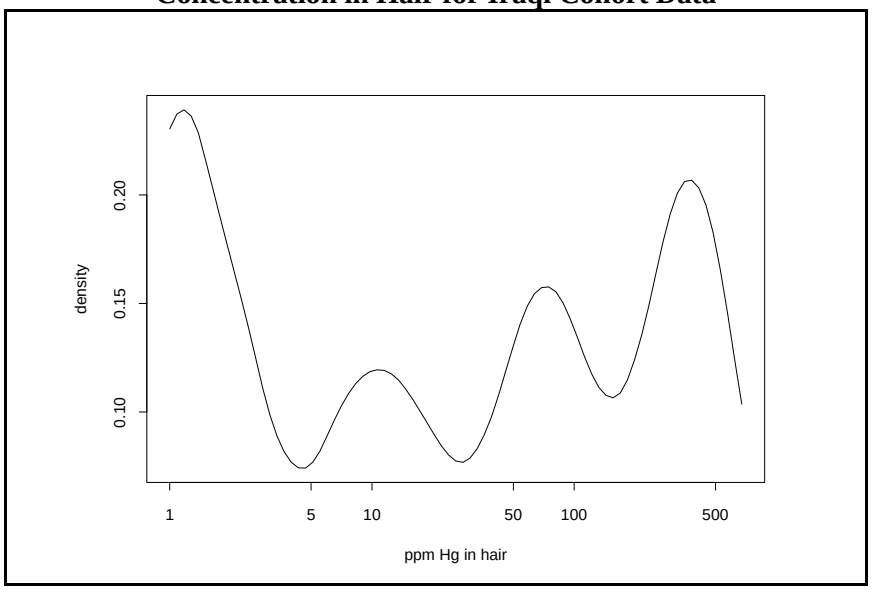
[a] Goodness-of-fit p-value for testing the null hypothesis, Ho: Predicted Incidence = Observed Incidence.

Table 6-6
Methylmercury Benchmark Dose Estimates (ppb blood)
Maximum Likelihood Estimates and 95% Lower Confidence Limits from Weibull Model
Incidence of Effects in Adults (Bakir et al. 1987)

Effect	0.01		0.05		0.10		G-O-F[a] P-Value
	MLE	95% CL	MLE	95% CL	MLE	95% CL	
Paresthesia	45.3	14.3	169.0	73.2	302.2	150.5	0.36
Ataxia	330.4	140.8	652.9	369.7	882.0	564.7	0.22
Visual Changes	64.1	25.4	249.1	129.9	453.6	266.9	0.26
Dysarthria	728.9	235.9	1265.4	621.8	1614.4	949.8	0.41
Hearing Defects	1462.9	535.2	2137.5	1202.8	2527.1	1705.6	0.53
Deaths	2226.3	1106.8	3007.2	2167.3	3434.2	2797.0	0.83

[a] Goodness-of-fit p-value for testing the null hypothesis, Ho: Predicted Incidence = Observed Incidence.

Figure 6-1
Density of Data Points Relative to Mercury Concentration in Hair for Iraqi Cohort Data



The density distribution is characterized by four distinct peaks. Exposure dose groups were defined as trough-to-trough intervals with the peak values taken as the nominal value for each interval. The nominal dose-group value, concentration ranges, and incidence of combined developmental effects are given in Table 6-7. A benchmark dose was calculated from the incidence of all effects as grouped in Table 6-7. The lower 95% confidence interval on the benchmark dose for the 10% response is 13 ppm, compared to the 11 ppm value used as the basis for the RfD.

Another alternative dose grouping approach was to divide the entire exposure range into four equal log-dose intervals. The geometric midpoint of each interval was taken as the nominal value for the interval. The nominal dose-group value, concentration ranges, and incidence of combined developmental effects are given in Table 6-8. The benchmark calculated as the lower bound on the 10% incidence for all effects is 10.3 ppm, compared to the 11 ppm used for the RfD.

Table 6-7
Density-Based Dose Groupings

Nominal Dose (ppm)	Dose Range (ppm)	Incidence
1.18	1 - 4	5/27
10.6	5 - 28	3/16
78.8	29 - 156	10/17
381	157 - 674	18/21

Table 6-8
Uniform Dose Groupings

Nominal Dose (ppm)	Dose Range (ppm)	Incidence
2.25	1 - 5	5/28
11.5	6 - 25	3/14
58.6	26 - 132	9/17
299	133 - 674	19/22

Two other analyses were done. In the first, data on males and females were grouped as published in *Seafood Safety*, and the Weibull model was as for the data in Table 6-9. The lower 95% confidence interval on the benchmark dose for the 10% response for males only was 10ppm and for females only, 11ppm. The last analysis consisted of fitting all data (for males and females) on all endpoints in Table 6-9 without grouping . When the model was restricted such that the Weibull power would drop below 1, the lower 95% bound on the dose for the 10% response was 11ppm. For an unrestricted model the benchmark dose was. Table 6-9 lists all equivalent benchmarks (lower 95% bounds on a 10% effect level for all measured endpoints) calculated by U.S.EPA on the data of Marsh et al. (1987).

Table 6-9
Benchmark Dosed Calculated on Data from Marsh et al. (1987)

Data Grouping	Benchmark Dose (ppm maternal hair)
Grouping in *Seafood Safety*	11
Density-based grouping	13
Uniform concentration intervals	10
Males only (*Seafood Safety* groups)	10
Females only (*Seafood Safety* groups)	10
Individual data points (restricted model)	11

Uncertainty and modifying factors

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

Calculation of the oral RfD for methylmercury

In this instance the RfD was calculated using the following equation:

$$RfD = \frac{Benchmark\ Dose}{UF\ x\ MF}$$

$$= \frac{1.1\ ug/kg-day}{10}$$

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

Confidence in the oral RfD for methylmercury

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

An analysis of uncertainty in an RfD based on the Iraqi data is found in Appendix D of this volume. Discussions of areas of uncertainty can also be found in Volume VII, Risk Characterization.

Choice of Benchmark or NOAEL as the basis for the RfD

Estimates of threshold levels for neurotoxicity have been performed by WHO (1990) using data from the Niigata episode and the Iraqi poisoning. In the exposures in Japan, hair levels associated with thresholds for neurotoxicity were estimated by WHO to be approximately 100 ppm. Estimates of threshold levels associated with paresthesia in the Iraqi episode indicate that the threshold level for paresthesia is approximately 25 to 40 mg (total body burden). This corresponds to blood levels of approximately 250 to 400 μg/L and hair levels of approximately 50 μg/g. Thresholds (total body burden) estimated by Bakir et al. (1973) for other neurotoxic signs were 55 mg for ataxia, 90 mg for dysarthria (difficulty with speech), 170 mg for deafness, and 200 mg for death.

A number of additional studies of human populations generally support the dose range of the benchmark dose level for perinatal effects. The designs for these studies as well as summaries of results are given in section 3.3.1.1. A few of the studies have data suitable for calculation of benchmark doses as was done for the results of Marsh et al. (1987). Table 6-10 compares one published benchmark dose (on the New Zealand population) as well as several others calculated for the Mercury Study Report to Congress. Table 6-12 provides a compilation of NOAELs and LOAELs determined from inspection of human data sets as well as other published comparable measures.

A recent analysis of the Kjellstrom (Kjellstrom et al. 1986a, b,1989) data was published by Gearhart et al. (1995). In this analysis the authors used a PBPK model which incorporated a fetal

compartment. They calculated a benchmark dose on all 28 tests included in the initial study design by Kjellstrom; this was done assuming values of 1 and 5% for background deficiency in test scores. The range of benchmark doses calculated was 10 to 31 ppm maternal hair mercury. The authors' preferred benchmark was 17 ppm, for an estimated background incidence of 5% and the lower bound on the 10% risk level (Table 6-10).

Table 6-10
Summary of Benchmark Doses (BMD) Estimated for Methylmercury

Study site	Duration of Exposure	n[a]	Endpoint	Benchmark (ppm maternal hair mercury)	Reference[b]
Iraq	short term (~ 3 mo)	81	all developmental effects reported in Marsh et al.(1987)	11	Marsh et al (1987); this document
Iraq	short term (~ 3 mo)	81	all effects except late walking, late talking	15	Marsh et al (1987); this document
Seychelles	long term	789	DDST, abnormal plus questionable scores	16	Meyers et al. (1995); this document
New Zealand	long term	237	all measures in 28 tests	17	Kjellstrom et al. (1986a, b, 1989); Gearhart et al. (1995)

a n=number of subjects in analysis
b first reference is to source of data; second is to source of BMD calculation.

Data have recently been published from the pilot (or cross sectional) study and testing of children from up to age 29 months in the main or prospective study in the Seychelles (Myers et al. 1995a,b,c,d; Davidson et al. 1995). The range of maternal hair mercury levels for the pilot study was 0.6 to 36.4 ppm; the range for the main study was 0.5 to 26.7 ppm. The Among tests administered to the 60 month old children in the cross-sectional study was the Denver Developmental Screen Test (DDST) (Myers et al. 1995b) which was included largely to provide a point of comparison with other population studies (e.g. Kjellstrom et al. 1986a, b, 1989). The frequency of abnormal plus questionable scores was analyzed by multiple logistic regression which showed an association for increased frequency of non-normal scores with increasing maternal hair mercury. Results for the abnormal plus questionable scores (Table 6-11) were used to calculate a benchmark dose as a lower 95% limit on a 10% effect level. The resulting estimate is 16 ppm maternal hair. The study authors have commented that the main study, which was done under more controlled circumstances, did not show any relationship between DDST-R results and maternal hair mercury.

Table 6-11
Results of Revised Denver Developmental Screening Test (DDST)
Administered to Seychellois Children in Cross-sectional Study[a]

Result	Maternal Hair Mercury, ppm					
	0-3	>3-6	>6-9	>9-12	>12	total
normal	114 (92.7)	209 (92.5)	169 (91.4)	107 (93.0)	122 (87.1)	721 (91.4)
abnormal	0	1	1	0	1	3
questionable	9	16	15	8	17	65
abnormal plus questionable	9 (7.3)	17 (7.5)	16 (8.7)	8 (7.0)	18 (12.9)	68 (8/6)

a Data from Myers et al. (1995b)

In a recent publication, Gearhart et al. (1995) proposed a RfD in the range of 0.8 to 2.5 µg/kg-day based on their analysis of effects in a population of children in New Zealand. This population was assumed to be exposed *in utero* to methylmercury as a consequence of high fish consumption by their mothers. Gearhart et al. (1995) estimated that a maternal intake of methylmercury in the range of 0.8 to 2.5 µg/kg-day corresponded to a NOAEL for developmental effects. These results support the U.S. EPA estimate of maternal intake of 1 µg/kg-day methylmercury corresponding to a benchmark dose of 11 ppm mercury in hair for developmental effects, prior to applying an uncertainty factor. The primary area of disagreement between Gearhart et al. (1995) and the U.S. EPA is in the use of an uncertainty factor. The authors felt that no uncertainty or modifying factors were needed as the NOAEL was calculated on effects in a sensitive subpopulation. U.S. EPA applied a 10-fold uncertainty factor to account for interindividual variation in the human population (particularly in hair to blood mercury ratio) and for lack of certain types of data.

The NOAEL was implicitly defined by Gearhart et al. (1995) as the lower 95% confidence interval on the dose associated with a 10% change in the test scores from the New Zealand study (Kjellstrom et al. 1989). That is, the benchmark dose calculation was performed on continuous variables by contrast to the binary variables from the Iraqi study (Marsh et al. 1987) used by the U.S. EPA. The qualitative equivalence of the two kinds of benchmark doses has not been established; both presumably represent a minimum risk level as does a NOAEL.

Using a hockey stick parametric dose response analysis of the data on delayed walking in the Iraqi children, Cox et al. (1989) concluded that the "best statistical estimate" of the threshold for health effects was 7.3 ppm mercury in hair with a 95% range of uncertainty between 0 and 14. A more recent analysis of the same data (Cox et al. 1995) focuses on the importance of four data points termed influential for the estimation of the population threshold. The newer analysis indicates that when a background response rate of 4% is assumed, the threshold estimate is 9 ppm maternal hair (Table 6-12). The authors indicate that dose-response analyses based on the “late walking” endpoint are unreliable because it relies on four influential observations in the data set from Marsh et al. (1978). The data points in question are the only responders below 150 ppm (mercury in hair). In particular, Cox et al. (1995) state that the four observations are isolated from the remainder of the responders and would be expected to have considerable influence on threshold estimate. This conclusion is based on a visual interpretation of a plot of the data (Figure 2 in Cox et al. 1995). Based on visual inspection of the same figure, an argument could

Table 6-12
Estimates of No Observed Adverse Effect Levels (NOAELs) and Lowest Observed Adverse Effects Levels (LOAELs) from Human Studies

Study Site	Exposure	n	Endpoint	Estimate type[a]	Estimate in ppm mercury in hair	Reference[b]
Iraq	*in utero* and *post partum* /short term	84	All developmental	NOAEL	13	Marsh et al. (1981); Marsh et al. (1981)
Iraq	*in utero* /short term	81	All developmental	NOAEL	7-10	Marsh et al. (1987); this document
Iraq	*in utero* /short term	81	delayed walking	LOAEL	14	Marsh et al. (1987); this document
Iraq	*in utero* /short term	81	delayed walking	best estimate of threshold	7.3	Marsh et al. (1987); Cox et al. (1989).
Iraq	*in utero* /short term	81	delayed walking	best estimate of threshold	9	Marsh et al. (1987); Cox et al. (1995)
New Zealand	*in utero* /long term	237	IQ tests (WISC-R, TOLD)	threshold	5-15	Kjellstrom et al. (1986a, b, 1989); Lipfert (1994)
Canada (Cree population)	*in utero* /long term	247	deep tendon reflex	LOAEL	2-15	McKeowin - Eyssen (1983); Lipfert (1994).
Amazon	adult/long term	29	visual discrimination	LOAEL	20	Lebel et al. (1995); this document
Niigata	adult women/ long term	430	Minimata disease	threshold	25	Kinjo et al. (1995); this document

Table 6-12 (continued)
Estimates of No Observed Adverse Effect Levels (NOAELs) and
Lowest Observed Adverse Effects Levels (LOAELs) from Human Studies

Study Site	Exposure	n	Endpoint	Estimate type[a]	Estimate in ppm mercury in hair	Reference[b]
Iraq, Cree, New Zealand	*in utero* /short and long term		multiple effects	LOAEL	10-20	Marsh et al. (1981), McKeowin -Eyssen (1983), Kjellstrom et al (1986a, b, 1989); Hoover et al 1997.
Seychelles (pilot, 66 month old children)	*in utero* /long term	217	Preschool Language Scale (auditory comprehension)	LOAEL	12 - 36	Myers et al. (1995a); this document
Seychelles (main, 29 month old children)	*in utero* /long term	736	BSID	NOAEL	12- 26.7	Davidson et al. 1995; this document
Seychelles (main, 29 month old children)	*in utero* /long term	736	Activity level, boys	LOAEL	12- 26.7	Davidson et al. 1995; this document
Faroes	*in utero* /long term	917	Neuropsychologica l tests	LOAEL	<10	Grandjean et al. (1997); Grandjean et al. (1997)

a Threshold and best estimate defined by author of estimate.
b First reference is to source of data; second is to source of analysis.

be made that the separation is not that marked considering the first eight responders. No quantitative sensitivity analysis was performed to investigate the effect of removing one or more of these data points. Cox et al. (1995) point out that if the four points are assumed to represent background, then the threshold for late walking would be greater than 100 ppm. It would seem unlikely, however, that these observations represent background given that no responses were observed in the 37 individuals with lower levels of exposure. It should be noted that the U.S. EPA benchmark dose was done on incidence of all effects, rather than on late walking only.

Crump et al. (1995) reanalyzed data from the Iraqi methylmercury poisoning episode presented in Marsh et al. (1987). In their analysis, Crump et al. (1995) reported that the statistical upper limit of the threshold could be as high as 255 ppm. Furthermore, the maximum likelihood estimate of the threshold using a different parametric model was presented as virtually zero. These and other analyses demonstrated that threshold estimates based on parametric models exhibit high statistical variability and model dependency, and are highly sensitive to the precise definition of an abnormal response.

Using a statistical analysis for trend that does not require grouping of the data, Crump et al. (1995) demonstrated that the association between health effects and methylmercury concentrations in hair is statistically significant at mercury concentrations in excess of about 80 ppm. In addition, Crump et al. calculated benchmark doses by applying dose-response models to each of the three endpoints: late walking, late talking and neurological score. Unlike the benchmark calculations made by the U.S. EPA (1994), these analyses did not involve grouping of the data into discrete dose groups, nor did they require dichotomizing continuous responses like age first walked into "late walking" or "no walking." Their calculation of the 95% lower bounds on the hair concentration corresponding to an additional risk of 10% ranged from 54 ppm to 274 ppm mercury in hair. Crump et al. (1995) concluded that the trend analyses and benchmark analyses provided a sounder basis for determining RfDs than the type of hockey stick analysis presented by Cox et al. (1989). They felt that the acute nature of the exposures, as well as other difficulties with the Iraqi data, present limitations in the use of these data for a chronic RfD for methylmercury.

The Cox et al. (1995) and Crump et al. (1995) analyses deal primarily with one endpoint, late walking. This appears to be the most sensitive of the endpoints described in Marsh et al. (1978). Both Cox et al. and Crump et al., as well as the U.S. EPA analysis in Appendix D of Volume V, show considerable uncertainty in thresholds estimated from the data on late walking. The peculiar nature of the uncertainty, in this case, makes it difficult to distinguish between 7 ppm maternal hair mercury and 114 ppm as a best (maximum likelihood) estimate for the threshold. Cox et al. (1995) attribute this bimodal uncertainty to four influential observations between 14 ppm and 60 ppm isolated from the remainder of the responders beginning at 154 ppm; they do not present arguments, other than visual, for censoring these data. Crump et al. (1995) show that changing the definition of late walking from greater than 18 months to 18 months or greater eliminates the bimodal uncertainty with a best threshold estimate of 230 ppm. The implication in both analyses is that the background incidence of late walking, as reported in other studies, is not consistent with the lower thresholds. While this is true, the use of historical controls for this analysis may not be appropriate, given the relatively large number of observations at low exposure levels in the Iraqi cohort; 33% of the observations were at hair mercury concentrations considered to be background levels (3 ppm or less).

Late walking, as assessed in the exposed Iraqi population (Marsh et al. 1978), is almost certainly a valid indicator of methylmercury toxicity but may be unreliable as the sole basis for detailed dose-response analysis. The primary reason for this may be the uncertainty in maternal recall for both birth date and date of first walking. The uncertainty in this particular case could be quite large, given the lack of recorded information. The primary impact of this kind of uncertainty would be on the response classification of individuals at the upper bound of normal (18 months for first walking) and at the lower bound of abnormal. The lowest abnormal first walking time presented in Marsh et al. (1978) was 20 months. The impact of assuming uncertainty in the classification of the observations in these two groups is large given the large number of observations in the two groups (19 data points at 18 months and 8 data points at 20 months). The analysis in Appendix D to Volume V of the Mercury Study Report to Congress shows that thresholds estimated for late walking are unstable when classification uncertainty is considered. The same kind of subjective uncertainty is applicable to the late talking endpoint, as well. The thresholds for late talking, however, are much more stable, statistically, as there are fewer observations that are near the normal/abnormal threshold value of 24 months.

McKeown-Eyssen et al. (1983) observed a positive association between abnormal tendon reflexes in boys and increasing maternal hair mercury. This was a study of 234 Cree children between the ages of 12 to 30 months residing in northern Quebec communities. Average maternal hair mercury for boys and girls was 6 ppm; the maximum was 24 ppm and 6% of the population had hair mercury levels in excess of 20ppm. A LOAEL of between 2-15 ppm maternal hair mercury was calculated by Lipfert et al. (1996). These authors felt that the McKeown-Eyssen et al. (1983) study provides only marginal support for a LOAEL above 10 ppm; they concluded that part of the significance of the adverse effects rests on the in (in their opinion) inappropriate inclusion of two observations of increased muscle

tone in the 2-15 ppm group. In a later paper, Hoover et al. (1997) reported that a LOAEL range of 10-20 ppm from this study was warranted.

Marsh et al. (1995) have published results of a study conducted between 1981 and 1984 in residents of coastal communities of Peru. The prospective study was of 131 child-mother pairs; testing for potential effects of fetal methylmercury exposure was patterned after the study of children exposed *in utero* in Iraq. Peak maternal hair methylmercury ranged between 1.2 to 30 ppm with a geometric mean of 8.3 ppm. These authors showed no effects of methylmercury on measures similar to those performed on the Iraqi children (including time of first walking and talking). A NOAEL (in the absence of a LOAEL) from this study would be 30 ppm maternal hair mercury (Table 6-12).

Lebel et al. (1996) published a study of 29 young adults (less than 35 years of age) who resided in villages on the Tapajos River, about 200 Km downstream of Amazonian gold-mining sites. Hair mercury ranged from 5.6 ppm to 38.4 ppm; methymercury constituted between 72.2% and 93.3% of the total. The authors found that decreases in several measures of visual acuity (color discrimination loss, contrast sensitivity and visual field reduction) were related to increased hair mercury. The authors note that constriction of the visual field has been reported in other instances of mercury intoxication. Inspection of the data presented as charts indicates a LOAEL of about 20ppm.

Kinjo et al.(1995) have published an analysis of the relationship between hair mercury concentration and the incidence of Minamata disease in Niigata, Japan. Hair samples were collected in 1965 and analyzed by a colorimetric procedure. The population used in the statistical analyses consisted of 147 males and 430 females. The authors felt that the colorimetric method used to determine hair mercury was not reliable at levels below 20 ppm. Data on individuals with hair mercury less than 20 ppm were excluded from the analysis; these data, however were included in Figure 3 of the paper. Minamata disease was defined by criteria established for receipt of compensation by the Japanese government medical committee (Tamashiro et al 1985). Both hockey stick and logit models were applied to obtain a range of threshold values of 24.7 to 49.3 for females; inspection of the graphed data in Figure 3 indicates a threshold for Minamata disease in females between 20 and 30 ppm. For males the threshold estimates are between 43 and 48 ppm.

A pilot study (essentially a cross-sectional study) of developmental effects in a seafood consuming population in the Seychelles Islands focused on all children born between February 1989 and February 1990. A total of 804 mother-infant pairs were observed to have maternal hair mercury in the range of 0.6 to 36.4 ppm with a median of 6.6 ppm (Myers et al. 1995b). Children were tested once between the ages of 5 and 109 weeks of age. An association was found for *in utero* mercury exposure and DDST-R abnormal plus questionable scores combined (Myers et al 1995b). A benchmark dose was derived on this data set as had been done on the Kjellstrom et al. (1986a, b, 1989) data. The BMD (95% lower bound on the 10% effect level) is 16 ppm maternal hair mercury (Table 6-10).

A subset of the pilot cohort (217 children) was evaluated at 66 months using the McCarthy Scales of Children's Abilities, the Preschool Language Scale, and tests from the Woodcock-Johnson Tests of Achievement that were appropriate to the children's age (Myers et al. 1995a). The median maternal hair mercury for this group was 7.11 ppm. Mercury exposure (measured as maternal hair mercury) was negatively associated with four endpoints: the McCarthy General Cognitive Index and Perceptual Performance subscale; and the Preschool Language Scale Total Language and Auditory Comprehension subscale. When statistically determined outliers and points considered to be influential were removed from the analyses, statistical significance of the association remained only for auditory comprehension. If a decrease in the scores on the auditory comprehension test is considered an adverse effect, a LOAEL from this study would be in the range of 12-36 ppm maternal hair mercury.

The main study was designed to be prospective; children were evaluated at 6.5, 19, 29 and 66 months of age (data on the 66 month old children have not yet been published) (Shamlaye et al, 1995) .

In the group evaluated at 6.5 months, median maternal hair mercury was 5.9 ppm with a range of 0.5 ppm to 26.7 ppm. No association with maternal hair mercury was found for any endpoint tested. (Myers et al. 1995c). Evaluations at 19 and 26 months were done on groups of 738 and 736 individuals, respectively (Davidson et al. 1995). Median maternal hair mercury was 5.9 ppm, and the range was 0.5 to 26.7 ppm. Children were evaluated with the BSID at 19 months of age. No effects of mercury exposure were seen on outcome of tests administered at 19 months. At 29 months, children were administered the BSID as well as the Bayley Infant Behavior Record. At 29 months there was an association between mercury exposure and decreased activity level in male children only. If the decrease in activity level is considered an adverse effect, a LOAEL for the study would be in the range of 12-26.7 ppm maternal hair mercury (Table 6-12). If this study is considered non-positive a NOAEL would be in the range of 12-26.7 ppm maternal hair mercury.

The overall conclusion of the studies published to date is that it is yet unclear whether an association exists between low level mercury exposure and neurologic deficits in children. The study shows a close correlation between maternal hair mercury and neonatal levels of mercury in brain tissue (Cernichiari et al. 1995). The authors cautioned in several papers that subtle neurologic and neurobehavioral effects are more likely to be detected in older rather than younger children. The overall conclusion of the authors is that their results require careful interpretation, and that an association between relatively low level mercury exposure *in utero* and neurologic deficits has not been demonstrated.

In 1986 a large study was initiated in the Faroe Islands on neurologic developmental effects of methylmercury and PCB exposure *in utero* (Grandjean et al. 1997). Subjects were a group of 917 children born between 1986 and 1987 and examined at about 7 years of age. Mercury was measured in maternal hair and cord blood, and a subset of cords was evaluated for PCBs. The median maternal hair mercury concentration was 4.5 ppm, and 13% were greater than 10 ppm (Grandjean et al 1992a). The geometric mean cord blood mercury concentration for the group of children who completed the evaluation was 22.8 ppm. Significant negative associations were seen for several neuropsychological tests. Even after inclusion of covariates with uncertain influence on these tests, multiple regression analysis indicated that 9/20 measures showed mercury related decrements ($p < 0.05$, one tailed). Application of a Peters-Belson adjustment resulted in significant mercury associations for 11/20 measures. PCB determinations were done on a total of 436 cords, and PCB exposure was included as a covariate in the regression analyses. This had an effect only on the regression for the Boston Naming Test. After exclusion of children with maternal hair mercury concentrations above 10 ppm, these associations remained almost unchanged. The authors concluded that *in utero* exposure to methylmercury at levels below 10 ppm maternal hair mercury affects several domains of cerebral function; in particular, attention, language and memory (Grandjean et al. 1997). Table 6.12 thus lists a LOAEL for this study as less than 10 ppm maternal hair.

With the exception of the estimates for threshold for Minimata disease, the NOAEL, BMD and threshold estimates (nine altogether) fall in a narrow range of 5-26.7 ppm maternal hair mercury. This is a large degree of overlap with the 11ppm BMD and provides a great deal of support for this estimate based on the Iraqi data.

Chronic rodent (e.g. Bornhausen et al. 1980) and nonhuman primate studies (e.g. Burbacher et al. 1984; Gunderson et al. 1986; Rice et al. 1989a,b) provide data to support estimated NOAELs and LOAELs for developmental end points. The endpoints measured in these animal studies are relevant to the types of toxicity which have been reported in children and they have been induced by dosing protocols that are relevant to human exposures. Experiments in nonhuman primates have identified adverse effects of methylmercury exposure in these areas: sensory (visual, somatosensory, auditory), cognitive (learning under concurrent schedules, recognition of faces), social play, and schedule-controlled operant behavior. The sensory, cognitive, and motor deficits appear reliable over a consistent range of doses in nonhuman primates exposed to methylmercury during development.

Table 6-13 lists NOAELs and LOAELs from animal studies. As the RfD for methylmercury is based on effects in children who were exposed *in utero*, it is particularly useful to consider developmental effects in animals exposed to methylmercury. NOAELs and LOAELs from selected developmental studies of methylmercury in animals can be found in Table 6-14; a more complete compilation is in section 3.3.3.8.

Table 6-13
Estimates of NOAELs and LOAELs from Animal Studies

Species/ Strain/ No. per Sex per Group	Exposure Duration	NOAEL, LOAEL (mg/kg-day)	Effects	Reference
Rat/Wistar/ 10 F	0–12 or 12–20 d, 1x/d	NOAEL =2; LOAEL=4	Hindlimb crossing after 0–12 days	Inouye and Murakami 1975
Rat/Wistar/ 50 F, 50 M	up to 26 mo	NOAEL = 0.05; LOAEL=0.25	Ruffled fur, loss of balance, hindlimb crossing, paralysis after 6 mo (males more affected); demyelination of dorsal nerve roots and damage in teased sciatic nerves at 0.25	Munro et al. 1980
Swiss origin Mouse M	28 wk	LOAEL = 1.9	Ataxia; degenerative changes of Purkinje cells; granule cell loss in cerebellum	MacDonald and Harbison 1977
Cat/Breed NS/15-16 both sexes	11 mo	LOAEL = 0.015	Degeneration of cerebellum and cerebral cortex; necrosis of dorsal root ganglia of kittens fed mercury-contaminated tuna	Chang et al. 1974
Cat/Breed NS/8-10 NS	2 yr 7 d/wk	NOAEL = 0.020; LOAEL = 0.046	Impaired hopping reaction; decreased pain sensitivity; degeneration of dorsal root ganglia	Charbonneau et al. 1976
Monkey/ *Macaca fasciculari s*/1-2 both sexes	36–132 d 1 x/d	NOAEL = 0.02; LOAEL =, 0.03	Atrophy of neurons in calcarine cortex; focal degeneration in sural nerves	Sato and Ikuta 1975
Monkey/ *Macaca artoides, Macaca nemestrina* /2 both sexes	90-270 d 1 x/wk	NOAEL= 0.4; LOAEL = 0.5	Tremor; visual impairment	Evans et al. 1977

Table 6-13 (continued)
Estimates of NOAELs and LOAELs from Animal Studies

Species/ Strain/ No. per Sex per Group	Exposure Duration	NOAEL, LOAEL (mg/kg-day)	Effects	Reference
Monkey/ *Macaca fascicularis*/5 exposed, 2 control (sex NS)	~4 yr 7 d/wk 1 x/d	LOAEL= 0.05	Spatial visual impairment	Rice and Gilbert 1982
Monkey/ *Macaca fascicularis*/7-8 F	~3 yr 1 x/d	LOAEL = 0.04	Slight tremor; motor incoordination; blindness ; time to onset was 177-395 d	Burbacher et al. 1988
Monkey/ *Macaca fascicularis*/4 M, 1 F exposed, 1 M, 2 F controls	6.5-7 yr 7 d/wk 1 x/d	LOAEL = 0.05	Six years after end of dosing (follow-up study to Rice and Gilbert 1982): decreased fine motor performance; diminished touch and pinprick sensitivity; impaired high frequency hearing ($p<0.05$)	Rice 1989b; Rice and Gilbert 1992

Table 6-14
NOAELs and LOAELs for Developmental Toxicity of Methylmercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	NOAEL, LOAEL (mg/kg-day)	Effects	Reference
Rat/Holtzman /5 F	during gestation, during lactation, or postnatal days 21-30	LOAEL=2.5	Decreased visual evoked potential latencies for peaks in 30-day old pups exposed during gestation, during lactation, or during postnatal days 21-30.	Zenick 1976
Rat/Charles River CD/ 20 F	47 d prior to and during gestation	NOAEL=0.7, LOAEL=1.4	Ultrastructural changes, dose-related decrease in biochemical activity in mitochondria of fetal hepatocytes	Fowler and Woods 1977
Rat/Long-Evans/4 exposed, 6 control	Once Gd 7	LOAEL=4	Increased P1-N1 amplitudes and decreased P2 and N2 latencies of cortically visual evoked potential	Dyer et al. 1978
Rat/Wistar-Neuherberg/ No. F. NS	4 d Gd 6-9	LOAEL=0.04	Impaired ability to perform operant conditioning procedures (number of responses on lever required in specified period of time) (	Musch et al. 1978
Rat/Wistar/10 F	4 d 1 x/d Gd 6-9	NOAEL=0.004, LOAEL=0.008	Reduction in behavioral performance in offspring of treated mice following operant conditioning	Bornhausen et al. 1980
Rat/Sprague-Dawley/ 15-19 F	4 d 1 x/d Gd 6-9	NOAEL=1.6, LOAEL=4.8	Delayed vaginal patency, delayed surface righting, retarded swimming development, lower activity, impaired complex water maze performance. Increased mortality of pups at 1-21 days of age	Vorhees 1985

Table 6-14 (continued)
NOAELs and LOAELs from Developmental Toxicity of Methylmercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	NOAEL, LOAEL (mg/kg-day)	Effects	Reference
Rat/Wistar/ 38 M, 38 F	during gestation and lactation	NOAEL=0.2, LOAEL=0.6	Increase in response latency in male ($p<0.05$) and female pups ($p<0.01$) and in passiveness ($p<0.05$) in visual discrimination reversal task	Schreiner et al. 1986
Rat/HAN-Wistar/10 F	13 days prior to mating until post-natal day 21	LOAEL=0.2	Delayed sexual maturity (vaginal opening and testes descent)	Suter and Schön 1986
Rat/Wistar/16 F	2 wk prior to mating through weaning	LOAEL=0.08	Impaired tactile-kinesthetic function ($p \leq 0.05$)	Elsner 1991
Mouse/SvSl/ No. F NS	Once Gd 7 or 9 (i.p.)	LOAEL=0.16	Impaired swimming ability and open-field behavior ($p<0.05$) in 30-day old pups.	Spyker et al. 1972
Monkey/ *Macaca fascicularis*/9 F exposed, 8 F control	approx. 1-3 yr 1 x/d prior to mating through gestation	LOAEL=0.04	Impaired visual recognition memory (data pooled from both groups of infants of exposed mothers) compared to unexposed controls; test performed at 50-60 days of age.	Gunderson et al. 1988
Monkey/ *Macaca fascicularis*/ 12 F exposed, 13 F control	approx. 4 mo to 2 yr 1 x/d prior to mating through gestation	LOAEL=0.04	Decrease in social play behavior and concomitant increase in nonsocial passive behavior compared to unexposed controls; tests performed at 2 weeks to 8 months of age.	Gunderson et al. 1988

Table 6-14 (continued)
NOAELs and LOAELs from Developmental Toxicity of Methylmercury in Animals

Species/ Strain/ No. per Sex per Group	Exposure Duration	NOAEL, LOAEL (mg/kg-day)	Effects	Reference
Monkey/ *Macaca fascicularis*/5 mothers	4-4.5 yr 1 x/d in utero and postnatally	LOAEL=0.01	Spatial visual impairment	Rice and Gilbert 1990
Monkey/ *Macaca fascicularis*/4 M, 1 F exposed, 1 M, 2 F controls	6.5-7 yr 7 d/wk 1 x/d	LOAEL=0.05	Six years after end of dosing (follow-up study to Rice and Gilbert 1982); decreased fine motor performance; diminished touch and pinprick sensitivity; impaired high frequency hearing ($p<0.05$).	Rice 1989b; Rice and Gilbert 1992
Monkey/ *Macaca fascicularis*/ 23 F	unspecified period prior to mating through gestation	NOAEL=0.08	No effect on spatial memory of adult offspring of animals treated with methylmercury hydroxide (data pooled from 24 animals, all treated groups).	Gilbert et al. 1993
Monkey/*Saimiri sciureus*/3 F	week 11 or 14.5 until parturition	LOAEL=0.7 to 0.9 ppm methylmercury in maternal blood	Monkeys exposed *in utero* tested (on learned lever pulling activity) at ages 5-6 yr. decreased sensitivity to degrees in reinforcement; change in reinforcement degree resulted in either no behavior change or slow change by comparison to controls.	Newland et al. 1994

At least three long term studies of non-human primates exposed *in utero* or as infants have been undertaken. Description of the study populations (adapted from Rice 1996) is given in Table 6-15.

Postnatal, pre-pubescent exposure of the primates approximates the exposures experienced by human children in both the short term (e.g. Iraq) and long term, steady state (e.g. Seychelles) scenarios used in risk assessment. According to Guidelines for Risk Assessment established by the U.S. EPA (1991), exposures of organisms until the time of sexual maturation should be considered in the assessment of developmental toxicants.

Exposure of monkeys to methylmercury during development has produced effects on sensory systems; visual, auditory and somatosensory changes have been observed. These changes were

apparently permanent. There were inter-individual variations in the degree and type of impairment. These observations have implications for human studies in that they emphasize the necessity of assessing the function integrity of multiple sensory systems.

Rice (1996) noted that delayed neurotoxic effects were observed in monkeys six years after exposure was stopped. This was expressed as clumsiness and decreased ability to grasp a variety of objects when the animals were 13 years old. The author pointed out the relevance of this finding to the potential for neurodegenerative disease development in aging humans exposed to methylmercury earlier in life. There is increasing evidence, from Minamata and other exposures, that degenerative processes associated with aging are exacerbated by mercury exposure (Igata 1993; Schantz et al. 1996).

Monkeys exposed to methylmercury *in utero* showed variable effects on cognitive processes (Rice 1996). These animals were delayed in development of object permanence (ability to conceptualize the existence of a hidden object) and recognition memory (recognition of faces). These abilities, however, did not appear to be impaired in adult animals. The methylmercury effect appears to have been a developmental delay rather than a permanent impairment. The emotional and social sequelae of a similar reversible developmental delay in humans is not known. Newland et al. (1994) demonstrated a persistent learning impairment in a group of three squirrel monkeys (*Saimiri sciureus*) exposed to methylmercury *in utero*. They concluded that the toxic effect led to insensitivity to changes in the consequences of behaviors.

Table 6-15
Studies in Non-Human Primates

Investigators /Exposure schedule	dose µg/kg/d	n	Total blood mercury in ppm		
Rice and co-workers birth- 7 yrs	0	5	peak at 200 days <0.1		Steady state <0.1
	50	5	1.2		0.7
in utero - 4yrs	0	5	mothers <0.1	birth <0.1	Steady state <0.1
	10	1	0.3	0.5	0.2
	25	2	0.7	0.9	0.4
	50	5	1.4	2.7	0.7
Gunderson, Burbacher et al. *in utero*	0	12	mothers	birth	
	50	12	1.1	1.6	
Newland et al *in utero*	0	3	mothers	birth	
		3	0.7 - 0.9		

The study of visual psychophysics provides information about function and dysfunction in the visual system, which can be applied to studies in humans. For example data on contrast sensitivity functions in non-human primates provide links between important features of visual function as expressed in behavior and the neural mechanisms underlying vision. The learning impairments observed in behavior under concurrent schedules not only raise concerns about cognitive effects of methylmercury exposure but also point to behavioral mechanisms by which these effects occur. Results suggesting methylmercury-related deficits in the visual recognition of faces are congruent with well-established areas of neuroscience that show how higher-order functioning is accomplished in the primate sensory (including visual) cortex. These results point to links between the integration of complex visual information and higher order cognitive abilities.

The sensory and motor deficits observed in animals exposed to methylmercury indicate that the exposed individual is missing the full complement of important capabilities. There are implications for the adversity and long-term impact of some of the subtle changes in cognitive ability noted in children exposed to mercury *in utero*. This research is leading to the recognition that forms of learning and reading dysfunction in people can be traced to subtle alterations in sensory systems; these findings raise concerns about deficits in functional domains not traditionally linked directly to sensory function. The motor deficits are consistent with neural systems that are affected by methylmercury and, therefore, indicate non-trivial impairment of the individual. Whether the cognitive endpoints are traceable to this sensory loss remains to be determined, but some, such as the learning deficits under concurrent schedules or alterations in fixed-interval schedule performance may be independent of such loss.

The rodent studies support conclusions drawn from non-human primates, although the results are not always as consistent. Rats have most often been used, and the endpoints identified to date have usually been less specific than those examined in the primate literature. Deficits in schedule-controlled operant behavior (Bornhausen et al. 1980; Schreiner et al. 1986) and subtle characteristics of motor function (Elsner 1991) have, however, been reported. The rodent studies generally find effects at doses predictable from a consideration of the kinetics of methylmercury in these species and the sensitivity of the procedures used. Data on cognitive function generally show only weak effects and at high dose. Most often observed have been deficiencies in motor function. Sensory system function has not been as extensively tested, although there have been reports of effects for *in utero* exposure on visual evoked potentials (Zenick 1976; Dyer et al. 1978).

Rice (1996) used data from animal studies described above to derive RfDs for methylmercury. She identified LOAELs (in the absence of a NOAEL) of 0.01 to 0.05 mg/kg/day from the studies of effects in monkeys exposed *in utero* and/or post partum. Standard U.S.EPA methodology was followed in the application of 10-fold uncertainty factors for the following: variability in human populations, extrapolation from animal data and use of a LOAEL in the absence of a NOAEL. Dividing by the uncertainty factor of 1000 results in a range of RfDs of 0.01 to 0.05 µg/kg/day. If one uses the rat data, a NOAEL of 0.005 mg/kg/day is identified and two 10 fold uncertainty factors are most appropriate (for human variability and extrapolation from animal data). The resulting RfD would be 0.05 µg/kg/day. RfDs based on use of sensitive, but relevant, endpoints measured in animals are 5- to 10-fold lower than those calculated from data in humans. This leads to a conclusion that the RfD based on observation of clinical and other effects in Iraqi children is not unduly conservative.

Uncertainty in the dose conversion (maternal hair mercury to dietary intake)

It was assumed in the derivation of the RfD that there was no substantial difference in pharmacokinetics of methylmercury as a consequence of the food medium in which it is presented to humans. The recent SAB report (U.S.EPA 1997) makes the following statement.

There is no compelling evidence to suggest that the toxicokinetics of methylmercury (MeHg) ingested in grain (as in the Iraqi poisoning episode) is different from that resulting form ingestion of fish (the typical exposure route of humans). The best evidence for this is a study in which cats were fed contaminated fish, control fish, or MeHg in a non-fish diet (Charbonneau *et al*., 1974). No differences were observed in degree of MeHg neurotoxicity, latency to toxicity (ataxia), tissue levels or distribution of Hg.

Gearhart et al. (1995) applied a PBPK model to their benchmark dose calculated in ppm maternal hair mercury from the Kjellstrom et al. (1986a, b, 1989) data. Details of the application of this model were not provided in the publication. A dose-conversion factor of about 0.05 [ppm mercury in hair/(µg/kg-day)] can be estimated from Figure 5c in Gearhart et al. (1995). Applying this factor to the benchmarks of 17 to 50 ppm mercury in hair yields the reported intake range of 0.85 to 25 µg/kg-day. Although this dose-conversion factor is about half of that estimated by the U.S. EPA, the two estimates are consistent when duration of exposure is considered. The Gearhart et al. (1995) pharmacokinetic model predicts that equilibrium is not reached until about 400 days. The dose conversion of 0.05 corresponds to hair mercury levels at equilibrium and is the appropriate factor to apply to the New Zealand hair concentrations, which arose from longer-term exposure. In contrast, the Iraqi exposure was only for a few months (Marsh et al. 1978). A dose conversion of about 0.1, which is virtually the same as that used by the U.S. EPA, can be estimated for a 3-month exposure from Figure 4 in Gearhart et al. (1995) and from Sherlock et al. (1984) assuming a hair:blood mercury concentration ratio of 250:1.

In their 1994 Toxicological Profile, ATSDR used the analysis reported by Cox et al. (1989), (see discussion below) of the Iraqi developmental data in the derivation of an intermediate MRL (minimal

risk level). Using delayed onset of walking as the critical effect, a LOAEL of 14 ppm mercury in hair was determined. A dose conversion from ppm hair to daily intake to maintain blood mercury levels in pregnant women was done in a very similar manner to that employed by U.S. EPA. Values for parameters in the equation on page 6-18 were consistent between the two Agencies with one exception; namely the use of a blood volume of 4.1L by ATSDR compared to 5L by U.S. EPA. The methylmercury intake level calculated by ATSDR to maintain a hair level of 14 ppm is 1.2 μg/kg-day compared to 1.1 μg/kg-day to maintain a hair level of 11 ppm (used by U.S. EPA).

ATSDR (1997) has recently released for public comment an updated Toxicological Profile with a revised Minimal Risk Level (MRL) for methylmercury. In their dose conversion, they used the following values: hair:blood ratio = 250; body weight = 60 kg; blood volume = 4.2 L; elimination constant = 0.014; absorbed dose found in the blood = 0.05; absorption factor = 95%.

The state of New Jersey currently uses an RfD of 0.7×10^{-4} mg/kg-day (described in Stern 1993) compared to the U.S. EPA's RfD of 1×10^{-4} mg/kg-day. The critical effect chosen was developmental endpoints in the Iraqi children exposed *in utero* including delayed onset of walking. A recent discussion of this RfD was presented in the context of the external peer review of the Mercury Study Report to Congress. Stern described the LOAEL as the mercury hair level equivalent to a mercury blood level of 44 μg/L. To determine the intake level, the equation on page 6-18 was used but with different values for two parameters; namely, b and f.

$$= 0.70 \ \mu g/kg\text{-}day$$

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

$$= \frac{44 \ \mu g/L \times 0.013 \ days^{-1} \times 5L}{0.95 \times 0.077 \times 60 \ kg}$$

Choice of the value of 0.077 for f, fraction of daily intake taken up by blood was based on a paper by Smith et al. (1994) which was not published at the time of the RfD/RfC work group discussions and was not considered by them in choosing the parameter values. Smith et al. (1994) (described briefly in chapter 2 of this volume) presents a study of methylmercury excretion kinetics based on measurement of i.v. administered methylmercury (1.7-7.4 µg) in blood, urine and feces of 7 male volunteers. The authors claim that data from this study are superior to those from previous studies in accounting for the portion of the labeled mercury which is metabolized to inorganic mercury. Based on the linear extrapolation of the plot of blood concentration of methylmercury versus time, the authors calculated that approximately 7.5% of the methylmercury remained in the blood following rapid equilibration among tissue compartments. Based on fitting the experimental data to a five compartment pharmacokinetic model, they calculate that 7.7% (geometric mean) of the methylmercury is found in the blood. It should be noted that the values for this parameter among the seven subjects ranged from 6.5-9.5%.

Smith et al. (1994) taking conversion to inorganic mercury into account, reported an overall estimate (geometric mean) of the half-life in blood (methylmercury-specific as per discussion in previous paragraph) of 45 days ($0.015 \ days^{-1}$). The half-lives (elimination constants) for the 7 subjects ranged from 35 days ($0.020 \ days^{-1}$) to 53 days ($0.013 \ days^{-1}$). Stern (1993) notes the half-life reported by Cox et al. (1989) was 48 days with a range of about 18-37. This corresponds to a value for b of $0.0144 \ day^{-1}$. The mean value is not reported by Cox, but a Monte Carlo simulation of the data estimated a mean of about 47 days. The most frequently reported value (mode), however, was 55 days corresponding to a

value for b of 0.013 day^{-1}. Ultimately the value of b = 0.013 day^{-1} was chosen by Stern as the most "typical" value.

Uncertainty factors

The current RfD was derived by application of an uncertainty factor of 10. This was intended to cover three areas of uncertainty: lack of data from a two-generation reproductive assay; variability in the human population, in particular the wide variation in biological half-life of methylmercury and the variation that occurs in the hair to blood ratio for mercury; and lack of data on long term sequelae of developmental effects. There was no factor applied for sensitive subpopulations as data were obtained from exposure to a sensitive human group; namely, the developing fetus.

The interpretation by some risk assessors is that the effects noted in the Iraqi population exposed to contaminated grain are not being seen at similar doses of methylmercury delivered *in utero* via contaminated seafood. One assessment by a scientist at FDA is that the U.S. EPA RfD of $1.0x10^{-4}$ mg/kg-day for methylmercury is somewhat conservative and is certainly protective; a suggestion was made that the uncertainty factor could be decreased to 3, resulting in a RfD of $3.0x10^{-4}$ mg/kg-day.

Stern (1997) did an analysis of the degree of uncertainty associated with interindividual variability applied to the dose conversion from maternal hair mercury to ingested daily dose of methylmercury. His conclusion was that a calculated ingested dose intended to be inclusive of 95-99% of women 18-40 years old would be 0.1 to 0.3 µg/kg/day (by contrast to EPA's calculated 1 µg/kg/day). His recommended uncertainty factor of three (for lack of reproductive data and data on long-term sequelae) would result in a RfD of 0.01 to 0.03 µg/kg/day.

ATSDR has recently released for public comment an updated Toxicological Profile with a revised Minimal Risk Level (MRL) for methylmercury. This report chooses as a NOAEL the median maternal hair mercury reported by Davidson et al. (1995) for the 29 month old Seychellois children tested with the BSID and Bayley Infant Behavior record. The Toxicological Profile characterizes the reported decrease in the male children's activity level as not adverse and chooses use of a midpoint of all measured maternal hair levels rather than the highest measure or median of the top quartile. An uncertainty factor was not used to account for human variability.

The recent review of the Mercury Study Report to Congress by the SAB recommended that EPA consider information suggesting that the uncertainty factor applied to the Iraqi data be increased. Their arguments are as follows.

For example, the Faeroe Islands data (also animal data) indicates that the fetal exposure may be greater than maternal exposure. In this study fetal cord blood mercury levels averaged 80.2 ppb while maternal blood levels were only 38.1 ppb. Animal data supports that the fetus may act as a sink for mercury. The report extensively reviews blood mercury kinetics but has little to say about fetal brain mercury levels. Although the data are slight there are indications from recent monkey studies that the brain mercury half-life is very long (Vahter *et al*. 1995). The RfD for MeHg is based on results from an acute exposure study while most MeHg exposure is thought to be long term. This may be an additional reason to increase the uncertainty factor. There are also indications of age related changes where MeHg may accelerate neurodegeneration associated with aging from human data (Igata, 1993) and animal data (Rice 1989a; 1989b)). In evaluating neurotoxic effects from low exposures such as with methylmercury, it must be remembered that few individuals may actually demonstrate clinical signs of disease but many individuals may suffer subtle changes which can produce total population effects.

Risks among subpopulations

The recent review by the SAB of the Mercury Study Report to Congress discusses at some length the likelihood that there are differential responses among human subgroups. That discussion is excerpted below.

Effect modification occurs when, at equal doses of a toxicant, adverse outcomes are observed in some members of a population but not others. The affected individuals may possess one or more distinctive characteristics such as age (stage of development), gender, social class, or certain premorbid health factors (e.g., diabetes, liver disease, pulmonary dysfunction) or genetic predisposing factors that are not well represented in unaffected members of the population.

Most studies of effects of environmental agents on child development have treated potential effect modifiers as covariates or confounders in multiple linear regression models without interaction terms models, or as matching variables in comparing so-called "exposed" and "unexposed" groups. Interactions are infrequently explored or are dismissed.

The animal literature, however, presents numerous examples of neurotoxicity enhancement or buffering as a result of species, strain, drug, and physical and social environmental interactions. An example from the methylmercury literature is the phenomenon of male susceptibility. Studies have shown that the relative risk of perinatal morbidity and mortality is higher in human males (Abramowizc and Barnett 1970; Naeye et al, 1971), including the risk of poor reproductive outcomes and postnatal development due to fetal exposure to industrial pollutants (Scragg et al. 1977; McKeown-Eyssen et al. 1983). Males also have a higher rate of cognitive developmental disability in the general population (Gross and Wilson 1974) and display more profound intellectual deficits as a result of cortical lesions (Bornstein and Matazarro 1984; Inglis and Lawson 1981). The McKeown-Eyssen et al. (1983) study reported gender-related differences including dose-related deficits in sensorimotor behaviors assessed in the BSID and increased prevalence of abnormal muscle tone and deep tendon. The latter effect was not associated with methylmercury dose in females. In the Iraqi poisoning more severe neurological effects were observed in male children (Marsh et al. 1987). Animal experiments have also observed sex differences in neurodevelopmental vulnerability. For example, in Sager et al. (1984) a single low dose of methylmercury administered to neonatal mice resulted in mitotic arrest in cells of the granule layer of the cerebellum only in males.

There is evidence that lifestyle factors such as the quality of the home environment and nutrition play a role in the expression of developmental neurotoxicity. The literature on connections between social factors and methylmercury toxicity is sparse. Studies on lead, however, have found greater neurocognitive deficits in exposed individuals from the lowest socio-economic groups (e.g., Bellinger et al. 1989; Dietrich et al. 1987; Harvey et al. 1984; Lansdown et al. 1986).

The positive nutritional factors of a seafood diet may be a factor in the greater delay in the onset of the Minamata as compared to the Iraqi outbreaks. Early results from the Faroe Islands studies have shown a positive association between cord blood methylmercury concentrations and birth weight (Grandjean et al. 1992; Grandjean et al. 1995). The authors attribute the finding to the benefits of n-3 polyunsaturated fatty acids in a high seafood diet and to the benefits of breast-feeding which, in itself, can lead to higher methylmercury intake by the infant.

The protective effects of genetic and environmental factors may be expressed in the studies of Seychellois children (Davidson et al. 1995). At 19 and 29, months scores on the Psychomotor Development Index (PDI) were very negatively skewed. The mean PDI scores at 19 and 29 months were 1.7 and 1.3 standard deviations above the United States means of 100 +/- 16 points respectively. That is, means for the PDI recorded for Seychelles children are above what would be classified as "accelerated performance" on these scales. The "developmental health" of this population is also reflected in the small number of subjects attaining abnormal scores on the DDST-R by comparison to samples in the United States. Only 3 out of 737 individual examinations or 0.4% were rated as abnormal (i.e., below

the 10th percentile for U.S. norms). Accelerated motor development has been noted in previous studies of African cultures and is also observed in African-American infants under two years of age.

The conclusion of the SAB, however, was that "the data regarding effect modification in human epidemiologic studies of mercury poisoning are currently too meager to base separate estimates of human health risks or establish different RFD's for various subpopulations."

Other areas of uncertainty

Birth date uncertainty would have an impact on exposure uncertainty if correspondence of exposure and gestation was estimated (Marsh et al. 1978) from birth date to any great extent. That is, exposure may have occurred to a lesser extent (or not at all) than assumed during the critical period of gestation. The result would be a lower exposure associated with the observation, depending on the width of the critical time window during gestation and on the importance of duration of exposure in the elicitation of the particular effect. If the exposure occurred after the critical period, any observation of an effect would be attributed to causes other than methylmercury and be included in the background.

Several scientists have suggested that a developmental toxicity RfD is needed for methylmercury. This may not be necessary, however, if the critical effect is developmental toxicity and the uncertainty factors used to estimate the lifetime RfD do not involve an adjustment for less than lifetime exposure nor lack of complete data base.

6.3.1.2 Inhalation Reference Concentrations (RfCs)

Elemental mercury

The U.S. EPA has determined an RfC of $3x10^{-4}$ mg/m^3 for elemental mercury (U.S. EPA 1994). The inhalation RfC is based on neurologic toxicity observed in several human occupational studies. The observed neurologic changes included hand tremor, increases in memory disturbances and slight subjective and objective evidence of autonomic dysfunction. Fawer et al. (1983) measured intention tremor (tremors that occur at the initiation of voluntary movements) in workers exposed to a TWA concentration of 0.026 mg/m^3 over an average of 15.3 years. It was noted, however, that the tremors may have resulted from intermittent exposures to concentrations higher than the TWA.

Piikivi and colleagues conducted several studies in chloralkali workers on electroencephalogram (EEG) abnormalities (Piikivi and Toulonen 1989); subjective measures of memory disturbance and sleep disorders and objective disturbances in psychological performance (Piikivi and Hanninen 1989); and subjective and objective symptoms of autonomic dysfunction such as induced pulse rate variations and blood pressure responses (Piikivi 1989). U.S. EPA extrapolated an occupational exposure level associated with these neurological changes of 0.025–0.030 mg/m^3 from blood levels, based on a conversion factor calculated by Roels et al. (1987). The LOAEL (0.025 mg/m^3 adjusted to 0.009 mg/m^3 for continuous exposure of the general population) was divided by an uncertainty factor of 30 (10 to protect sensitive individuals and for use of a LOAEL, and 3 for the lack of reproductive studies in the database) to yield the RfC of $3x10^{-4}$ mg/m^3.

The RfC was, thus, calculated in the following way.

$$RfC = \frac{LOAEL\ mg\ Hg/m^3}{UF}$$
$$= \frac{0.009\ mg/m^3}{30}$$
$$= 0.0003\ mg/m^3$$

This reference concentration was reviewed and verified by the RfD/RfC Work Group and was verified on April 19, 1990. It was released under a special action by U.S. EPA (Jarabek 1992, personal communication).

Confidence in the critical study, the data base, and, thus in the RfC were rated "medium" by the Work Group. Factors which were positive for confidence in the critical study were the use of a sufficient number of human subjects, inclusion of appropriate controls, sufficient exposure duration and that the LOAEL can be corroborated in other studies. It was noted, however, that for all but one of the studies, exposure had to be extrapolated from blood mercury levels. The lack of human or multispecies reproductive or developmental studies precluded higher confidence in the data base.

Inorganic (mercuric) mercury

Developmental toxicity (skeletal abnormalities and retarded growth) in mice (Selypes et al. 1984) and autoimmune disease in Brown-Norway rats (Bernaudin et al. 1981) have also been observed following inhalation exposures. Due to the limitations of these inhalation studies and the inadequacy of the remaining toxicologic and pharmacokinetic data bases, the RfD/RfC Work Group determined the derivation of an RfC is not possible. The posting of this determination on IRIS is proceeding concurrent with the finalization of this Report to Congress.

Methylmercury

No estimate of risk from inhalation of methylmercury has been done by U.S. EPA.

6.3.1.3 Estimation of Risk from Dermal Exposure

The dermal contribution of the different mercury species to the total systemic exposure of each of these mercury species may be important for a full characterization of risk to the potentially exposed human populations. Many of the necessary data needed for conducting a dermal risk assessment are currently lacking or not well enough understood to assess systemic exposure and risks from dermal exposure to mercury species.

For any of these mercury species to be a dermal health hazard they must be absorbed across the skin (epidermis and dermis) and be systemically distributed to the affected critical organ systems (kidneys or CNS) via the circulatory system. The percutaneous absorption for each mercury species is dependent on skin-specific factors (e.g., skin thickness, hydration, age, condition, circulation, and temperature) and compound-specific factors (e.g., lipophilicity, polarity, chemical structure, volatility, and solubility), which are involved in determining the rate and amount of absorption by the cutaneous route. Currently there are few known or agreed upon percutaneous absorption rates available for any of the mercury species of interest. Some data on percutaneous absorption (Kp = Permeability coefficient) for the mercuric forms of mercury in aqueous media have been reported in U.S. EPA (1992).

The media (aqueous, vapors or soil) where the mercury species are found must also be considered in dermal risk assessments. Each medium has its own set of factors that impact the specific percutaneous absorption rates for each of the mercury species. For example, mercury compounds found in aqueous media are dependent on factors such as solubility in water and increased hydration of the skin. Mercury compounds associated with soil must be assessed for binding to the particular soil type of concern, the adhesion of the soil of concern to skin, the desorption of the mercury compound from the soil, and the absorption of the mercury compound across the skin and into the circulatory system. Other aspects that must be considered with a dermal assessment are binding or sequestration of the mercury species at the site of exposure or closely nearby, and the metabolism of the mercury species in the skin that may result in oxidation/reduction of the mercury species to other valence states (thereby, potentially resulting in different critical effects than from the originally absorbed compound).

At present, many of the necessary mercury species/media factors have not been fully ascertained and as a result credible dermal risk assessments cannot be accomplished at this time. A more extensive discussion of dermal exposure assessment for risk assessment can be found in *Dermal Exposure Assessment: Principles and Applications* (EPA/600/8-91/011B, January 1992).

6.3.2 Developmental Effects

6.3.2.1 Elemental Mercury

Elemental mercury was judged to have sufficient animal data for developmental toxicity. The two studies which contribute most to the level of concern are Danielsson et al. (1993) and Fredriksson et al. (1992). Both of these studies are limited as a basis for an RfD_{DT} by the small numbers of animals tested and by the very few dose groups. A further limitation is lack of data on gender differences. No RfD_{DT} for elemental mercury is available at this time.

6.3.2.2 Inorganic Mercury

There are no data from human studies which are suitable for derivation of an RfD_{DT}. Inspection of available animal studies indicates that there are five reports of developmental effects of inorganic mercury given orally. In all of these, exposure was by gavage to pregnant animals, and effects were monitored in progeny. Three papers were reported as abstracts giving few experimental details.

Rizzo and Furst (1972) treated Long Evans rats (5/group) with a single gavage dose of 2 mg Hg/kg as mercuric oxide on either day 5, 12, or 19 of gestation. Animals were sacrificed on day 20 or 21 of gestation. No effects of treatment on gestation day 12 or 19 were noted. According to the authors treatment on day 5 resulted in a higher percentage of growth retardation and inhibition of eye formation, but no statistical analyses were done.

In Gale (1974), pregnant Golden hamsters were administered 0, 2.5, 5, 16, 22, 32, 47, or 63 mg Hg/kg-day mercuric acetate via gavage on gestation day 8. When the pregnant animals were sacrificed on day 12 or 14, there was a significant increase in the incidence of abnormal fetuses including small, retarded, or edematous (combined), and/or malformed fetuses. The NOAEL for developmental toxicity was 5 mg mercuric chloride/kg or 2 mg Hg/kg. Maternal toxicity included dose-related weight loss, diarrhea, slight tremor, somnolence, tubular necrosis and hepatocellular vacuolization, but insufficient data were provided to allow determination of a LOAEL or NOAEL for maternal toxicity.

The advantage of the Gale (1974) study as a basis for quantitation of potential risk is that several doses of inorganic mercury were tested; the spacing of the doses was adequate for identification of both a LOAEL and NOAEL. It is not recommended, however, that the NOAEL in Gale (1974) serve as the basis for an RfD_{DT}. There were relatively few animals tested (decreasing the overall sensitivity of the assay) and not all endpoints were thoroughly evaluated. The test compound was administered on only one day of gestation, and there is some question as to the suitability of the golden hamster for developmental assays. The data base for developmental effects, while generally supportive of the LOAEL is not adequate to determine if the measured endpoints were the most sensitive for developmental effects of inorganic mercury.

6.3.2.3 Methylmercury

Weight of evidence for developmental toxicity indicates that a developmental toxicity RfD is appropriate for methylmercury. A separate RfD_{DT} may not be necessary as the critical effect for the lifetime RfD is developmental toxicity. The current RfD ($1x10^{-4}$ mg/kg-day) was based on developmental endpoints in offspring of women exposed during pregnancy; it may be taken as protective against developmental toxicity. For less than chronic exposures it should be noted that the RfD_{DT} is not intended as a lifetime exposure value.

6.3.3 Germ Cell Mutagenicity

Data do not support the generation of quantitative estimates for germ cell mutagenicity for any form of mercury.

6.3.4 Carcinogenic Effects

6.3.4.1 Elemental Mercury

Elemental mercury is categorized as D, unable to classify as to human carcinogenicity. A quantitative estimate for carcinogenic effect is, thus, inappropriate.

6.3.4.2 Inorganic Mercury

Quantification of the potential carcinogenic effects of mercuric chloride (classified as C, possible human carcinogen) was not done. No increase in tumor incidence was observed in a carcinogenicity study in which white Swiss mice were given 0.95 mg Hg/kg-day as mercuric chloride in drinking water (Schroeder and Mitchener 1975). No statement regarding carcinogenicity was reported in a 2-year feeding study in which rats were administered mercuric acetate in the diet at doses of 0, 0.02, 0.1, 0.4, 1.7 and 6.9 mg Hg/kg-day (Fitzhugh et al. 1950).

The incidence of squamous cell papillomas of the forestomach and thyroid follicular cell carcinomas from NTP (1993) was evaluated. No slope factor was based on the forestomach tumors because this type of tumor is probably the result of irritation of the forestomach, cell death and epithelial proliferation. The carcinogenic mechanism may be specific to irritation at the high doses used in the bioassay; use of these tumors as a basis for human health assessment of low doses of inorganic mercury is inappropriate.

Regarding the thyroid carcinomas, a variety of drugs, chemicals, and physiological perturbations result in the development of thyroid follicular tumors in rodents. For a number of chemicals, the mechanism of tumor development appears to be a secondary effect of longstanding hypersecretion of thyroid-stimulating hormone by the pituitary (Capen and Martin 1989; McClain 1989). In the absence of such long-term stimulatory effects, induction of thyroid follicular cell cancer by such chemicals usually does not occur (Hill 1989). Use of the incidence of thyroid tumors from NTP (1993) in low dose extrapolation is, thus, questionable.

6.3.4.3 Methylmercury

Quantification of the potential carcinogenic effects of methylmercury (classified as C, possible human carcinogen) was not done. No increased incidence of tumors was seen in rats exposed to doses of up to 0.34 mg Hg/kg-day for 130 weeks (Mitsumori et al. 1983, 1984) or in cats exposed to a diet containing up to 0.176 mg Hg/kg-day for 2 years (Charbonneau et al. 1976).

No slope factor was calculated for methylmercury based on the incidence of renal epithelial tumors in male mice. The two studies by Mitsumori et al. (1981, 1990) were limited by high mortality in the high-dose males, the only group to exhibit a statistically significant increase in tumor incidence. The study by Hirano et al. (1986) was not limited by survival problems, but the tumors were observed in conjunction with nephrotoxicity and appear to be a high-dose phenomenon that may not be linear at low doses. The tumors appeared to originate from focal hyperplasia of the tubular epithelium induced as a reparative change. The hyperplasia was not observed in tubular epithelium that was undergoing early degenerative changes; thus, the tumors may not occur where degenerative changes do not occur. The appropriateness of deriving a quantitative risk estimate using the assumption of linearity at low doses based on data for which a threshold may exist is questionable.

6.4 Risk Assessments Done By Other Groups

Quantitative estimates of hazards of oral exposure to methylmercury exposure have been considered by the Food and Drug Administration, Agency for Toxic Substances and Disease Registry (ATSDR), the Department of Energy and several State agencies. Several inhalation workplace exposure limits are available in the United States and other countries.

6.4.1 Food and Drug Administration

In 1969, in response to the poisonings in Minamata Bay and Niigata, Japan, the U.S. FDA proposed an administrative guideline of 0.5 ppm for mercury in fish and shellfish moving in interstate commerce. This limit was converted to an action level in 1974 (Federal Register 39, 42738, December 6, 1974) and increased to 1.0 ppm in 1979 (Federal Register 44; 3990, January 19, 1979) in recognition that exposure to mercury was less than originally considered. In 1984, the 1.0 ppm action level was converted from a mercury standard to one based on methylmercury (Federal Register 49, November 19, 1984).

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

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

6.4.2 ATSDR

ATSDR has established Minimal Risk Levels (MRLs) for elemental, inorganic and methylmercury (ATSDR 1994). Recently a revised Toxicological Profile has been released for public comment (ATSDR 1997).

An acute inhalation MRL of 0.00002 mg/m^3 has been derived for elemental mercury vapor based on neurodevelopmental changes in rats. Specifically, the effects were changes in locomotor activity at 4 months of age and an increased time to complete a radial arm maze at 6 months of age following exposure to 0.05 mg Hg/m^3 for 1 hour during post-partum days 11–17 (Fredriksson et al. 1992). A chronic inhalation MRL of 0.000014 mg/m^3 was derived for elemental mercury vapor based on a significant increased in the average velocity of naturally occurring tremors in occupational workers (Fawer et al. 1983). The revised chronic MRL is calculated to be 0.0002 mg/m^3 by application of an uncertainty factor of 30 to a LOAEL of 0.026 mg/m^3 for increased frequency of tremors in occupationally exposed workers (Fawer et al. 1983).

Acute and intermediate oral MRLs were derived for inorganic mercury based on kidney effects reported in the 1993 NTP study of mercuric chloride. The acute oral MRL was 0.007 mg Hg/kg-day based on a 2-week study reporting a NOAEL of 0.93 mg Hg/kg-day for renal effects in rats (NTP 1993). At higher doses, an increased incidence of tubular necrosis was observed. The intermediate oral MRL of 0.002 mg Hg/kg-day was established, based on a 6-month study reporting a NOAEL of 0.23 mg Hg/kg-day for renal effects (increased absolute and relative kidney weights) (NTP 1993). There is no indication that these values have been revised in the 1997 document.

An acute-intermediate oral MRL of 0.00012 mg Hg/kg-day was established in 1994 for methylmercury. ATSDR derived their assessment from the Marsh et al. (1981) and Cox et al. (1989) data; the MRL is based on the lowest observed peak of total mercury concentration in maternal hair (0.0012 mg/kg-day equivalent to a LOAEL of 14 ppm mercury in maternal hair) during pregnancy associated with a delayed onset of walking in offspring in Iraqi children. This assessment is discussed in section 6.3.1.1 of this volume.

The 1997 Toxicological Profile calculates a chronic MRL for methylmercury of 0.5 µg/kg/day. This report chooses as a NOAEL the median maternal hair mercury of 5.9 ppm reported by Davidson et al. (1995) for the 29 month old Seychellois children tested with the BSID and Bayley Infant Behavior record. The Toxicological Profile characterizes the reported decrease in the male children's activity level as not adverse and chooses use of a midpoint of all measured maternal hair levels rather than the highest measure or median of the top quartile. Dose conversion was done as in the 1994 document to give an estimated ingested dose of 0.5 µg/kg/day. An uncertainty factor was not used to account for human variability.

6.4.3 Department of Energy

Brookhaven Laboratories has prepared a report for Office of Clean Coal Technology, DOE. This report describes a probabilistic-based assessment which considered the potential increased health risk for paresthesia in adults. Their estimate is based upon a yearly emission rate of 180 kg/year from all fossil fuel power plants in the United States. This estimate represents less than 1% of the existing global pool of mercury that is introduced into the environment. Based upon the most sensitive adult sign of paresthesia, the mercury emissions from power plants would result in an increased risk for paresthesia of 0.004–0.007% with an upper 95th percentile risk of 0.013–0.017% (Lipfert et al. 1994).

6.4.4 National Institute of Environmental Health Sciences (NIEHS)

NIEHS, part of the National Institutes of Health, was required under section 301 of the CAA "to conduct, and transmit to the Congress by November 15, 1993, a study to determine the threshold level of mercury exposure below which adverse human health effects are not expected to occur." In section 112 (n)(1)(C), NIEHS was encouraged to evaluate the health effects threshold for mercury in the absence of specifics as to species of mercury but to consider mercury in fish. As mercury in fish is primarily in the form of methylmercury, the NIEHS limited their consideration to this species.

The report was completed in 1993 and delivered to Office of Management and Budget for clearance. It describes dose- response assessments for methylmercury done by WHO, FDA and U.S. EPA and presents all three estimates as recommended for tolerable mercury concentrations. The NIEHS report also describes estimates of fish consumption by the U.S. population.

6.4.5 Department of Labor

OSHA established a Permissible Exposure Limit (PEL), time-weighted average of 0.05 mg Hg/m^3 for mercury vapor, with a notation for skin exposure (U.S. Department of Labor 1989). A PEL as a ceiling value of 0.1 mg Hg/m^3, also with a notation for dermal exposure was set for aryl mercury and inorganic mercury compounds.

NIOSH determined a Recommended Exposure Limit (REL), time-weighted average, of 0.05 mg Hg/m^3 for mercury and 0.1 mg Hg/m^3 for aryl and inorganic mercury compounds (NIOSH 1973, 1988).

6.4.6 Various States

A number of states have released fish consumption advisories based upon their independent analysis of the available scientific literature for methylmercury. Most active among these states are Michigan, New Jersey, Maine, Idaho, and Oregon. Generally, there is a trend to move to more conservative values based upon developmental neurotoxicity defined in the Marsh et al. (1981) and Cox et al. (1989) papers. The methylmercury RfD of $0.7x10^{-4}$ mg/kg-day used by the state of New Jersey is discussed in section 6.3.1.1. Some states are waiting for more specific guidance from U.S. EPA.

6.4.7 World Health Organization

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

6.4.8 ACGIH

The ACGIH has established Threshold Limit values (TLV) as eight-hour time-weighted averages. They include the following:

Aryl mercury compounds	0.1 mg Hg/m^3
Mercury vapor	0.05 mg Hg/m^3
Inorganic mercury	0.1 mg Hg/m^3

No STEL is recommended at this time. The Biological Exposure Indices Committee has recommended values for inorganic mercury in urine and blood of 35 µg/g creatinine and 15 µg/L respectively.

The ACGIH classified inorganic mercury including elemental mercury as follows: A4- Not classifiable as a Human Carcinogen: There are inadequate data on which to classify the agent in terms of its carcinogenicity in humans and/or animals.

7. ONGOING RESEARCH AND RESEARCH NEEDS

7.1 Ongoing Research

Table 7-1 lists ongoing research projects abstracted from the Federal Research in Progress Data Base (FEDRIP, 1994).

Table 7-1
Ongoing Research

Investigator	Affiliation	Research Description	Sponsor
		Human	
T. Clarkson	University of Rochester, Rochester, NY	Dose-response relationships in humans exposed to methylmercury and prenatal and early postnatal body burdens of methylmercury.	National Institute of Environmental Health Sciences (NIEHS)
P. Grandjean	Odense University, Odense, Denmark	Neurotoxicity risk from exposure to methylmercury from seafood	NIEHS
W. Markesbery	University of Kentucky, Lexington, KY	Role of mercury and dental amalgams in Alzheimer's disease	National Institute on Aging
M. Martin	University of Washington, Seattle, WA	Epidemiology of mercury in dentists	National Institute of Dental Research
R. Mitchell	University of Kentucky, Lexington, KY	Amalgam restorations and the relative risk of adverse pregnancy outcome	National Institute of Dental Research
G. Myers	University of Rochester, Rochester, NY	Child development following prenatal methylmercury exposure via fish	NIEHS
T. Okabe	Baylor College of Dentistry, Dallas, TX	Establish maximum levels of exposure from amalgams for dental patients and personnel	National Institute of Dental Research
M. Owens	Science Applications International Corp, Falls Church, VA	Potential and adverse effects associated with dental amalgam	National Institute of Dental Research
M. Rosenman	Morehouse College, Atlanta, GA	Effect of mercury in amalgam and urine to cognitive functioning in children	National Institute of General Medical Sciences
D. Savitz	University of North Carolina Chapel Hill, Chapel Hill, NC	Mercury and reproductive health in women dentists	National Institute of Dental Research
		Animal	
P. Bigazzi	University of Connecticut, Farmington, CT	Mercury induced auto-immune disease in rats	NIEHS
T. Burbacher	University of Washington, Seattle, WA	Developmental effects of methylmercury in monkeys and rats	NIEHS
K. Mottet	University of Washington, Seattle, WA	Long-term toxicity associated with inorganic mercury and methylmercury	NIEHS
K. Pollard	University of California, San Diego, CA	Animal model of systemic autoimmunity induced by mercury	National Institute of Arthritis and Musculoskeletal and Skin Diseases

Table 7-1
Ongoing Research (continued)

Investigator	Affiliation	Research Description	Sponsor
B. Weiss	University of Rochester, Rochester, NY	Neurotoxicity throughout the lifespan of mice exposed prenatally to methylmercury	NIEHS
Mechanistic			
W. Atchison	Michigan State University, East Lansing, MI	Neurotoxic mechanism of chronic methylmercury poisoning	NIEHS
D. Barfuss	Georgia State University, Atlanta, GA	Transport and toxicity of inorganic mercury in the nephron	NIEHS
T. Jensen	Herbert H. Lehman College, New York, NY	Effect on membrane structure and organelle distribution	National Institute of General Medical Sciences
D. Lawrence	Albany Medical College, Albany, NY	Effects of metals on the structure and function of murine and human lymphocytes	NIEHS
R. Noelle	Dartmouth Medical School, Hanover, NH	Effect of mercury on β-lymphocyte function	NIEHS
K. Pollard	Scripps Research Institute, San Diego, CA	Mechanisms of autoantibody response induced by mercury which target the nucleolus	National Institute of Allergy and Infectious Diseases
B. Rajanna	Selma University, Selma, AL	Biomechanisms of heavy metal toxicity in rats	National Institute of General Medical Sciences
K. Ruehl	Rutgers University, New Brunswick, NJ	Mechanism of methylmercury neurotoxicity during development in mice	NIEHS
T. Sarafian	University of California, Los Angeles, CA	Effect of methylmercury on protein phosphorylation in cerebellar granule cells in brain	NIEHS
J. Stokes	Mount Desert Island Biological Lab, Salsbury Cove, ME	Effects of mercurials on transport properties of the bladder	NIEHS
R. Zalups	Mercer University School of Medicine	Cytotoxicity of mercuric chloride to isolated rat proximal tubular cells	NIEHS

Two of these ongoing studies deserve further discussion because they may fill critical data needs for the development of a reference dose for methylmercury. The first is the Seychelles Islands Study led by Dr. T.W. Clarkson from the University of Rochester. The objective of this study is to define the extent of human health risks from prenatal exposure to methylmercury. Dose-response relationships in a human population with dietary exposure to methylmercury at levels believed to be in the range of the threshold for developmental toxicity are being studied. Both prenatal and early postnatal body burdens of methylmercury will be examined as well as transport to the brain.

This study is testing the hypothesis, developed in previous studies of prenatal exposure in the Iraq population, that subtle psychological and behavioral changes in prenatally exposed children can be quantitatively related using dose-response models to the mother's methylmercury exposure during pregnancy. In the Seychelles, a group of islands off the coast of Africa near Madagascar, a group of 779 infants who were prenatally exposed to methylmercury through maternal fish consumption is being studied with annual administration of neurodevelopmental, psychological and educational testing of the

children through 5.5 years of age. This population consumes a relatively large amount of marine fish and marine mammals, both of which are likely to contain methylmercury. The study is testing the hypothesis that methylmercury concentration in hair correlates with methylmercury in the brain by using human autopsy data. Mechanisms of transport of methylmercury across the blood brain barrier also are being studied to understand better the factors that limit the accuracy of hair mercury as a biological marker for target tissue levels. Findings reported in recent publications are summarized in section 3.3.1.1.

The second study is the Faroe Islands Study led by Dr. P.A. Grandjean from Odense University in Denmark. The purpose of this study is to determine whether a neurotoxic risk is present from methylmercury exposure from seafood and, if so, the threshold for such effects. This study is examining a cohort of 1,000 children in the Faroe Islands, located in the North Atlantic between Scotland and Iceland. As is the case in the Seychelles, this population consumes a relatively large amount of seafood; consumption includes marine fish and marine mammals. Intrauterine exposures were determined by mercury analysis of umbilical cord blood and maternal hair collected at consecutive births during 21 months in 1986 and 1987. In 13 percent of the births, mercury levels were greater than 10 ppm in maternal hair, and 25 percent of the cord blood samples had a mercury concentration above the corresponding level of 40 µg/L. No cases of gross methylmercury poisoning have been observed. The persistence of mercury in the body is being assessed from mercury hair concentrations in the children at one and six years of age, and dietary information is being collected. A detailed pediatric examination and a test battery to identify possible subtle signs of neurobehavioral dysfunction are being conducted. The test battery includes psychological tests and neurophysiological measurement of evoked potentials; these methods are known from previous research to be particularly sensitive to the types of neurotoxicity expected.

The Faroese population was chosen for this study because of the homogeneity and stability of the population and the efficient coverage of the Danish health care system. The cohort includes 75% of all births occurring during the sampling period. A high participation rate (about 80%) is expected at the 6-year examination period. Alcohol use is minimal in Faroese women (75% were abstainers during pregnancy), and 60% are nonsmokers. The lead exposure is low (median lead concentration in cord blood was 1.7 µg/100 mL). Exposure to polychlorinated biphenyls (PCB), however, may be a confounder, and alcohol intake of the fathers may have been high. Due to the high seafood intake, selenium exposure is increased, and its possible protective action against mercury toxicity is being examined. Findings reported at recent scientific meetings are summarized in section 3.3.1.1.

7.2 Research Needs

In addition to the ongoing studies described above, further research is necessary for refinement of the U.S. EPA's risk assessments for mercury and mercury compounds. In order to reduce uncertainties in the current estimates of the oral reference doses (RfDs) and inhalation reference concentrations (RfCs), longer-term studies with low-dose exposures are necessary. In particular, epidemiological studies should emphasize comprehensive exposure data with respect to both dose and duration of exposure. The current RfD and RfC values have been determined for the most sensitive toxicity endpoint for each compound; that is, the neurological effects observed following exposure to elemental or methylmercury, and the renal autoimmune glomerulonephritis following exposure to inorganic mercury. For each of these compounds, experiments conducted at increasingly lower doses with more sensitive measures of effect will improve understanding of the respective dose-response relationships at lower exposure levels and the anticipated thresholds for the respective effects in humans. Similar information from developmental toxicity studies would allow determination of RfDs for developmental toxicity (RfD_{dt}) for elemental and inorganic mercury. For inorganic mercury, furthermore, the many ongoing studies in which mechanisms of action are being investigated will greatly assist in quantifying the risks posed by these compounds.

Well-conducted studies are also needed to clarify exposure levels at which toxic effects other than those defined as “critical” could occur in humans. For all three forms of mercury, data are inadequate, conflicting, or absent for the following: adverse reproductive effects (effects on function or outcome, including multigeneration exposure); impairment of immune function; and genotoxic effects on human somatic or germinal cells (elemental and inorganic mercury). Investigations that relate the toxic effects to biomonitoring data will be invaluable in quantifying the risks posed by these mercury compounds. In addition, work should focus on subpopulations that have elevated risk because they are exposed to higher levels of mercury at home or in the workplace, because they are also simultaneously exposed to other hazardous chemicals, or because they have an increased sensitivity to mercury toxicity. Information on postnatal exposure without prenatal exposure is limited; therefore, analyzing the potential risks associated with mercury exposure of young children is difficult.

There are data gaps in the carcinogenicity assessments for each of the mercury compounds. The U.S. EPA's weight-of-evidence classification of elemental mercury (Group D) is based on studies in workers who were also potentially exposed to other hazardous compounds including radioactive isotopes, asbestos, or arsenic. There were no appropriate animal studies available for this compound.

Studies providing information on the mode of action of inorganic mercury and methylmercury in producing tumors will be of particular use in defining the nature of the dose response relationship.

The assessment of both noncarcinogenic effects and carcinogenic effects will be improved by an increased understanding of the toxicokinetics of these mercury compounds. In particular, quantitative studies that compare the three forms of mercury across species and/or across routes of exposure are vital for the extrapolation of animal data when assessing human risk. For elemental mercury there is a need for quantitative assessment of the relationship between inhaled concentration and delivery to the brain or fetus; in particular the rate of elemental to mercuric conversion mediated by catalase and the effect of blood flow. Such assessment is needed for evaluation of the impact of mercury exposure from dental amalgam.

Work has been done on development of physiologically-based pharmacokinetic models. While one of these has developed a fetal submodel, data on fetal pharmacokinetics are generally lacking. The toxicokinetics of mercury as a function of various developmental stages should be explored. Elemental mercury and methylmercury appear to have the same site of action in adults; research is, therefore, needed on the potential for neurotoxicity in newborns when the mother is exposed. This work should be accompanied by pharmacokinetic studies and model development.

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APPENDIX A

DOSE CONVERSIONS

APPENDIX A

DOSE CONVERSIONS

All doses in the tables in Section 4 were adjusted for the amount of mercury in the compound.

For example, for animals administered 1 mg/kg/day mercuric chloride:

Molecular weight of mercuric chloride = 271.5
Molecular weight of mercury = 200.6
Dose of mercury = 1 mg Hg/kg/day x 200.6/271.5 = 0.74 mg Hg/kg/day

(1) To convert from ppm in feed to mg/kg body weight/day, the following equation was used:

mg toxicant (T)/kg body weight/day = mg T/kg food x food factor

where food factor = kg food per day intake/kg body weight

(2) To convert from ppm in water to mg/kg body weight/day, the following equation was used:

mg T/kg body weight/day = mg T/L water x L water per day intake/kg body weight

where L is liters of water intake per day

Species	***Sample Values Used***		
	Water intake/day (Liter/day)	***Body weight (kg)***	***Food factor (kg food/kg body weight)***
Mouse	0.0057	0.03	0.13
Rat	0.049	0.35	0.05
Rabbit	0.41	3.8	0.049

(3) To convert from ppm in air to mg/m^3 for a vapor, the following equation was used:

$1\ mg/m^3 = 1\ ppm \times molecular\ weight/24.45$

APPENDIX B

SUMMARIES FOR THE INTEGRATED RISK INFORMATION SYSTEM (IRIS)

I.B. REFERENCE CONCENTRATION FOR CHRONIC INHALATION EXPOSURE (RfC)

Substance Name -- Elemental mercury (Hg)
CASRN -- 7439-97-6
Preparation date -- 3/12/90

I.B.1. INHALATION RfC SUMMARY

Critical Effect	Exposures*	UF	MF	RfC
Hand tremor; increases in memory disturbances; slight subjective and objective evidence of autonomic dysfunction Human occupational inhalation studies Fawer et al., 1983; Piikivi and Tolonen, 1989; Piikivi and Hanninen, 1989; Piikivi, 1989; Ngim et al., 1992; Liang et al., 1993	NOAEL: None LOAEL: 0.025 mg/cu.m (converted to LOAEL [ADJ] of 0.009 mg/cu.m	30	1	3E-4 mg/cu.m

* Conversion Factors and Assumptions: This is an extrarespiratory effect of a vapor (gas). The LOAEL is based on an 8-hour TWA occupational exposure. MVho = 10 cu.m/day, MVh = 20 cu.m/day. LOAEL(HEC) = LOAEL(ADJ) = 0.025 mg/cu.m x MVho/MVh x 5 days/7 days = 0.009 mg/cu.m. Air concentrations (TWA) were measured in the Fawer et al. (1983), Ngim et al. (1992), and Liang et al. (1993) studies. Air concentrations were extrapolated from blood levels based on the conversion factor of Roels et al. (1987) as described in the Additional Comments section for the studies of Piikivi and Tolonen (1989), Piikivi and Hanninen (1989), and Piikivi (1989).

I.B.2. PRINCIPAL AND SUPPORTING STUDIES (INHALATION RfC)

Fawer, R.F., U. DeRibaupierre, M.P. Guillemin, M. Berode and M. Lobe. 1983. Measurement of hand tremor induced by industrial exposure to metallic mercury. J. Ind. Med. 40: 204-208.

Piikivi, L. and U. Tolonen. 1989. EEG findings in chlor-alkali workers subjected to low long term exposure to mercury vapor. Br. J. Ind. Med. 46: 370-375.

Piikivi, L. and H. Hanninen. 1989. Subjective symptoms and psychological performance of chlorine-alkali workers. Scand. J. Work Environ. Health. 15: 69-74.

Piikivi, L. 1989. Cardiovascular reflexes and low long-term exposure to mercury vapor. Int. Arch. Occup. Environ. Health. 61: 391-395.

Ngim, C.H., S.C. Foo, K.W. Boey and J. Jeyaratnam. 1992. Chronic neurobehavioral effects of elemental mercury in dentists. Br. J. Ind. Med. 49: 782-790.

Liang, Y-X., R-K. Sun, Y. Sun, Z-Q. Chen and L-H. Li. 1993. Psychological effects of low exposure to mercury vapor: Application of a computer-administered neurobehavioral evaluation system. Environ. Res. 60: 320-327.

Fawer et al. (1983) used a sensitive objective electronic measure of intention tremor (tremors that occur at the initiation of voluntary movements) in 26 male workers (mean age of 44 years) exposed to low levels of mercury vapor in various occupations: fluorescent tube manufacture (n=7), chloralkali plants (n=12), and acetaldehyde production (n=7). Controls (n=25; mean age of 44.6 years) came from the same factories but were not exposed occupationally. Personal air samples (two per subject) were used to characterize an average exposure concentration of 0.026 mg/cu.m. It should be noted that it is likely that the levels of mercury in the air varied during the period of exposure and historical data indicate that previous exposures may have been higher. Exposure measurements for the control cohort were not performed. The average duration of exposure was 15.3 years. The measures of tremor were significantly increased in the exposed compared to control cohorts, and were shown to correspond to exposure and not to chronologic age. These findings are consistent with neurophysiological impairments that might result from accumulation of mercury in the cerebellum and basal ganglia. Thus, the TWA of 0.026 mg/cu.m was designated a LOAEL. Using the TWA and adjusting for occupational ventilation rates and workweek, the resultant LOAEL(HEC) is 0.009 mg/cu.m.

Piikivi and Tolonen (1989) used EEGs to study the effects of long-term exposure to mercury vapor in 41 chloralkali workers exposed for a mean of 15.6 +/- 8.9 years as compared with matched referent controls. They found that the exposed workers, who had mean blood Hg levels of 12 ug/L and mean urine Hg levels of 20 ug/L, tended to have an increased number of EEG abnormalities when analyzed by visual inspection only. When the EEGs were analyzed by computer, however, the exposed workers were found to have significantly slower and attenuated brain activity as compared with the referents. These changes were observed in 15% of the exposed workers. The frequency of these changes correlated with cortical Hg content (measured in other studies); the changes were most prominent in the occipital cortex less prominent in the parietal cortex, and almost absent in the frontal cortex. The authors extrapolated an exposure level associated with these EEG changes of 0.025 mg/cu.m from blood levels based on the conversion factor calculated by Roels et al. (1987).

Piikivi and Hanninen (1989) studied the subjective symptoms and psychological performances on a computer-administered test battery in 60 chloralkali workers exposed to mercury vapor for a mean of 13.7 +/- 5.5 years as compared with matched referent controls. The exposed workers had mean blood Hg levels of 10 ug/L and mean urine Hg levels of 17 ug/L. A statistically significant increase in subjective measures of memory disturbance and sleep disorders was found in the exposed workers. The exposed workers also reported more anger, fatigue and confusion. No objective disturbances in perceptual motor, memory or learning abilities were found in the exposed workers. The authors extrapolated an exposure level associated with these subjective measures of memory disturbance of 0.025 mg/cu.m from blood levels based on the conversion factor calculated by Roels et al. (1987).

Both subjective and objective symptoms of autonomic dysfunction were investigated in 41 chloralkali workers exposed to mercury vapor for a mean of 15.6 +/- 8.9 years as compared with matched referent controls (Piikivi, 1989). The quantitative non-invasive test battery consisted of measurements of pulse rate variation in normal and deep breathing, in the Valsalva maneuver and in vertical tilt, as well as blood pressure responses during standing and isometric work. The exposed workers had mean blood levels of 11.6 ug/L and mean urine levels of 19.3 ug/L. The exposed workers complained of more subjective symptoms of autonomic dysfunction than the controls, but the only statistically significant difference was an increased reporting of palpitations in the exposed workers. The quantitative tests revealed a slight decrease in pulse rate variations, indicative of autonomic reflex dysfunction, in the exposed workers. The authors extrapolated an exposure level associated with these subjective and objective measures of autonomic dysfunction of 0.030 mg/cu.m from blood levels based on the conversion factor calculated by Roels et al. (1987).

Two more recent studies in other working populations corroborate the neurobehavioral toxicity of low-level mercury exposures observed in the Fawer et al. (1983), Piikivi and Tolonen (1989), Piikivi and Hanninen (1989), and Piikivi (1989) studies.

Ngim et al. (1992) assessed neurobehavioral performance in a cross-sectional study of 98 dentists (38 female, 60 male; mean age 32, range 24-49 years) exposed to TWA concentrations of 0.014 mg/cu.m (range 0.0007 to 0.042 mg/cu.m) versus 54 controls (27 female, 27 male; mean age 34, range 23-50 years) with no history of occupational exposure to mercury. Air concentrations were measured with personal sampling badges over typical working hours (8-10 hours) and converted to an 8-hour TWA. No details on the number of exposure samples or exposure histories were provided. Blood samples from the exposed cohort were also taken and the data supported the correspondence calculated by Roels et al. (1987). Based on extrapolation of the average blood mercury concentration (9.8 ug/L), the average exposure concentration would be estimated at 0.023 mg/cu.m. The average duration of practice of the exposed dentists was 5.5 years. Exposure measurements of the control cohort were not performed. The exposed and control groups were adequately matched for age, amount of fish consumption, and number of amalgam dental fillings. The performance of the dentists was significantly worse than controls on a number of neurobehavioural tests measuring motor speed (finger tapping), visual scaning, visumotor coordination and concentration, visual memmory, and visuomotor coordination speed. These neurobehavioral effects are consistent with central and peripheral neurotoxicity and the TWA is considered a LOAEL. Using the TWA and adjusting for occupational ventilation rates and the reported 6-day workweek, the resultant LOAEL(HEC) is 0.006 mg/cu.m.

Liang et al. (1993) investigated workers in a fluorescent lamp factory with a computer-adminstered neurobehavioral evaluation system and a mood inventory profile. The exposed cohort (mean age 34.2 years) consisted of 19 females and 69 males exposed to ninterruptedly for at least 2 years prior to the study. Exposure was monitored with area samplers and ranged from 0.008 to 0.085 mg/cu.m across worksites. No details on how the exposure profiles to account for time spent in different worksites were constructed. The average exposure was estimated at 0.033 mg/cu.m. (range 0.005 to 0.19 mg/cu.m). The average duration was of working was 15.8 years for the exposed cohort. Urinary excretion was also monitored and reported to average 0.025 mg/L. The control cohort (mean age 35.1 years) consisted of 24 females and 46 males recruited from an embroidery factory. The controls were matched for age, education, smoking and drinking habits. Exposure measurements for the control cohort were not performed. The exposed cohort performed significantly worse than the control on tests of finger tapping, mental arithmetic, two-digit searches, switiching attention, and visual reaction time. The effect on performance persisted after the confounding factor of chronological age was controlled. Based

on these neurobehavioral effects, the TWA of 0.033 mg/cu.m is designated as LOAEL. Using the TWA and adjusting for occupational ventilation rates and workweek, the resultant LOAEL(HEC) is 0.012 mg/cu.m.

The above studies were taken together as evidence for a LOAEL based on neurobehavioral effects of low-level mercury exposures. The LOAEL(HEC) levels calculated on measured air concentration levels of the Ngim et al. (1992) and the Liang et al. (1993) studies bracket that calculated based on the air concentrations measured by Fawer et al. (1983) as a median HEC level. Extrapolations of blood levels, used as biological monitoring that accounts for variability in exposure levels, also converge at 0.025 mg/cu.m as a TWA which results in the same HEC level. Thus, the TWA level of 0.025 mg/cu.m was used to represent the exposure for the synthesis of the studies described above. Using this TWA and taking occupational ventilation rates and workweek into account results in a LOAEL(HEC) of 0.009 mg/cu.m.

I.B.3. UNCERTAINTY AND MODIFYING FACTORS (INHALATION RfC)

UF -- An uncertainty factor of 10 was used for the protection of sensitive human subpopulations (including concern for acrodynia - see Additional Comments section) together with the use of a LOAEL. An uncertainty factor of 3 was used for lack of data base, particularly developmental and reproductive studies.

MF -- None

I.B.4. ADDITIONAL COMMENTS (INHALATION RfC)

Probably the most widely recognized form of hypersensitivity to mercury poisoning is the uncommon syndrome known as acrodynia, also called erythredema polyneuropathy or pink disease (Warkany and Hubbard, 1953). Infantile acrodynia was first described in 1828, but adult cases have also since been reported. While acrodynia has generally been associated with short-term exposures and with urine levels of 50 ug/L or more, there are some cases in the literature in which mercury exposure was known to have occurred, but no significant (above background) levels in urine were reported. There could be many reasons for this, but the most likely is that urine levels are not a simple measure of body burden or of target tissue (i.e., brain levels); however, they are the best means available for assessing the extent of exposure. It was felt that the RfC level estimated for mercury vapor based on neurotoxicity of chronic exposure in workers is adequate to protect children from risk of acrodynia because such exposures of long duration would be expected to raise urine levels by only 0.12 ug/L against a background level of up to 20 ug/L (i.e., such exposures would not add significantly to the background level of mercury in those exposed).

Roels et al. (1987) investigated the relationships between the concentrations of metallic mercury in air and levels monitored in blood or urine in workers exposed during manufacturing of dry alkaline batteries. Breathing zone personal samples were used to characterize airborne mercury vapors. Total mercury in blood and urine samples were analyzed using atomic absorption. The investigation controlled

for several key factors including the use of reliable personal air monitoring, quality control for blood and urine analyses, standardization of the urinary mercury concentration for creatinine concentration, and stability of exposure conditions (examined subjects were exposed to mercury vapor for at least 1 year). Strong correlations were found between the daily intensity of exposure to mercury vapor and the end of workshift levels in blood (r=0.86; n=40) or urine (r=0.81; n=34). These relationships indicated a conversion factor of 1:4.5 (air:blood) and 1:1.22 (air:urine as ug/g creatinine). These factors were used to extrapolate blood or urine levels associated with effects in the reported studies to airborne mercury levels.

Sensory and motor nerve conduction velocities were studied in 18 workers from a mercury cell chlorine plant (Levine et al., 1982). Time-integrated urine Hg levels were used as an indicator of mercury exposure. Using linearized regression analysis, the authors found that motor and sensory nerve conduction velocity changes (i.e., prolonged distal latencies correlated with the time-integrated urinary Hg levels in asymptomatic exposed workers) occurred when urinary Hg levels exceeded 25 ug/L. This study demonstrates that mercury exposure can be associated with preclinical evidence of peripheral neurotoxicity.

Singer et al. (1987) studied nerve conduction velocity of the median motor, median sensor and sural nerves in 16 workers exposed to various inorganic mercury compounds (e.g., mercuric oxides, mercurial chlorides, and phenyl mercuric acid) for an average of 7.3 +/- 7.1 years as compared with an unexposed control group using t-tests. They found a slowing of nerve conduction velocity in motor, but not sensory, nerves that correlated with increased blood and urine Hg levels and an increased number of neurologic symptoms. The mean mercury levels in the exposed workers were 1.4 and 10 ug/L for blood and urine, respectively. These urine levels are 2-fold less than those associated with peripheral neurotoxicity in other studies (e.g., Levine et al., 1982). There was considerable variability in the data presented by Singer et al. (1987), however, and the statistical analyses (t-test) were not as rigorous as those employed by Levine et al. (1982) (linearized regression analysis). Furthermore, the subjects in the Levine et al. (1982) study were asymptomatic at higher urinary levels than those reported to be associated with subjective neurological complaints in the workers studied by Singer et al. (1987). Therefore, these results are not considered to be as reliable as those reported by Levine et al. (1982).

Miller et al. (1975) investigated several subclinical parameters of neurological dysfunction in 142 workers exposed to inorganic mercury in either the chloralkali industry or a factory for the manufacture of magnetic materials. They reported a significant increase in average forearm tremor frequency in workers whose urinary Hg concentrations exceeded 50 ug/L as compared with unexposed controls. Also observed were eyelid fasciculation, hyperactive deep-tendon reflexes and dermatographia, but there was no correlation between the incidence of these findings and urinary Hg levels.

Roels et al. (1985) examined 131 male and 54 female workers occupationally exposed to mercury vapor for an average duration of 4.8 years. Urinary mercury (52 and 37 ug/g creatinine for males and females, respectively) and blood mercury levels (14 and 9 ug/L for males and females, respectively) were recorded, but atmospheric mercury concentration was not provided. Symptoms indicative of CNS disorders were reported but not related to mercury exposure. Minor renal tubular effects were detected in mercury-exposed males and females and attributed to current exposure intensity rather (urinary Hg >50 ug/g creatinine) than exposure duration. Male subjects with urinary mercury levels of >50 ug/g creatinine exhibited preclinical signs of hand tremor. It was noted that females did not exhibit this effect and that their urinary mercury never reached the level of 50 ug/g creatinine. A companion study (Roels et al., 1987) related air mercury (Hg-air)levels to blood mercury (Hg-blood) and

urinary mercury (Hg-U) values in 10 workers in a chloralkali battery plant. Duration of exposure was not specified. A high correlation was reported for Hg-air and Hg-U for preshift exposure ($r=0.70$, $p<0.001$) and post-shift ($r=0.81$, $p<0.001$) measurements. Based on these data and the results of their earlier (1985) study, the investigators suggested that some mercury-induced effects may occur when Hg-U levels exceed 50 ug/g creatinine, and that this value corresponds to a mercury TWA of about 40 ug/cu.m.

A survey of 567 workers at 21 chloralkali plants was conducted to ascertain the effects of mercury vapor inhalation (Smith et al., 1970). Mercury levels ranged from <0.01 to 0.27 mg/cu.m and chlorine concentrations ranged from 0.1 to 0.3 ppm at most of the working stations of these plants. Worker exposure to mercury levels (TWA) varied, with 10.2% of the workers being exposed to <0.01 mg/cu.m, 48.7% exposed to 0.01 to 0.05 mg/cu.m, 25.6% exposed to 0.06 to 0.10 mg/cu.m and 4.8% exposed to 0.24 to 0.27 mg/cu.m (approximately 85% were exposed to Hg levels less than or equal to 0.1 mg/cu.m). The duration of employment for the examined workers ranged from one year (13.3%) to >10 years (31%), with 55.7% of the workers being employed for 2 or 9 years. A group of 600 workers not exposed to chlorine served as a control group for assessment of chlorine effects, and a group of 382 workers not exposed to either chlorine or mercury vapor served as the reference control group. A strong positive correlation ($p<0.001$) was found between the mercury TWAs and the reporting of subjective neuropsychiatric symptoms (nervousness, insomnia), occurrence of objective tremors, and weight and appetite loss. A positive correlation ($p<0.001$) was also found between mercury exposure levels and urinary and blood mercury levels of test subjects. No adverse alterations in cardiorespiratory, gastrointestinal, renal or hepatic functions were attributed to the mercury vapor exposure. Additionally, biochemical (hematologic data, enzyme activities) and clinical measurements (EKG, chest X-rays) were no different between the mercury-exposed and non-exposed workers. No significant signs or symptoms were noted for individuals exposed to mercury vapor concentrations less than or equal to 0.1 mg/cu.m. This study provides data indicative of a NOAEL of 0.1 mg Hg/cu.m and a LOAEL of 0.18 mg Hg/cu.m. In a followup study conducted by Bunn et al. (1986), however, no significant differences in the frequency of objective or subjective findings such as weight loss and appetite loss were observed in workers exposed to mercury at levels that ranged between 50 and 100 ug/L. The study by Bunn et al. (1986) was limited, however, by the lack of information provided regarding several methodological questions such as quality assurance measures and control of possible confounding variables.

The mercury levels reported to be associated with preclinical and symptomatic neurological dysfunction are generally lower than those found to affect kidney function, as discussed below.

Piikivi and Ruokonen (1989) found no evidence of glomerular or tubular damage in 60 chloralkali workers exposed to mercury vapor for an average of 13.7 +/- 5.5 years as compared with their matched referent controls. Renal function was assessed by measuring urinary albumin and N-acetyl-beta-glucosaminidase (NAG) activity. The mean blood Hg level in the exposed workers was 14 ug/L and the mean urinary level was 17 ug/L. The authors extrapolated the NOAEL for kidney effects based on these results of 0.025 mg/cu.m from blood levels using the conversion factor calculated by Roels et al. (1987).

Stewart et al. (1977) studied urinary protein excretion in 21 laboratory workers exposed to 10-50 ug/cu.m of mercury. Their urinary level of mercury was about 35 ug/L. Increased proteinuria was found in the exposed workers as compared with unexposed controls. When preventive measure were instituted to limit exposure to mercury, proteinuria was no longer observed in the exposed technicians.

Lauwerys et al. (1983) found no change in several indices of renal function (e.g., proteinuria, albuminuria, urinary excretion of retinol-binding protein, aminoaciduria, creatinine in serum, beta-2-microglobulin in serum) in 62 workers exposed to mercury vapor for an average of 5.5 years. The mean urinary Hg excretion in the exposed workers was 56 ug/g creatinine, which corresponds to an exposure level of about 46 ug/cu.m according to a conversion factor of 1:1.22 (air:urine [ug/g creatinine]) (Roels et al., 1987). Despite the lack of observed renal effects, 8 workers were found to have an increased in serum anti-laminin antibodies, which can be indicative of immunological effects. In a followup study conducted by Bernard et al. (1987), however, there was no evidence of increased serum anti-laminin antibodies in 58 workers exposed to mercury vapor for an average of 7.9 years. These workers had a mean urinary Hg excretion of 72 ug/g creatinine, which corresponds to an exposure levels of about 0.059 mg/cu.m.

Stonard et al. (1983) studied renal function in 100 chloralkali workers exposed to inorganic mercury vapor for an average of 8 years. No changes in the following urinary parameters of renal function were observed at mean urinary Hg excretion rates of 67 ug/g creatinine: total protein, albumin, alpha-1-acid glycoprotein, beta-2-microglobulin, NAG, and gamma-glutamyl transferase. When urinary Hg excretion exceeded 100 ug/g creatinine, a small increase in the prevalence of higher activities of NAG and gamma-glutamyl transferase was observed.

The mercury levels reported to be associated with preclinical and symptomatic neurological dysfunction and kidney effects are lower than those found to pulmonary function, as discussed below.

McFarland and Reigel (1978) described the cases of 6 workers who were acutely exposed (4-8 hours) to calculated metallic mercury vapor levels of 1.1 to 44 mg/cu.m. These men exhibited a combination of chest pains, dyspnea, cough, hemoptysis, impairment of pulmonary function (reduced vital capacity), diffuse pulmonary infiltrates and evidence of interstitial pneumonitis. Although the respiratory symptoms resolved, all six cases exhibited chronic neurological dysfunction, presumably as a result of the acute, high-level exposure to mercury vapor.

Lilis et al. (1985) described the case of a 31-year-old male who was acutely exposed to high levels of mercury vapor in a gold-extracting facility. Upon admission to the hospital, the patient exhibited dyspnea, chest pain with deep inspiration, irregular infiltrates in the lungs and reduced pulmonary function (forced vital capacity [FVC]). The level of mercury to which he was exposed is not known, but a 24-hour urine collection contained 1900 ug Hg/L. Although the patient improved gradually over the next several days, 11 months after exposure he still showed signs of pulmonary function abnormalities (e.g., restriction and diffusion impairment).

Levin et al. (1988) described four cases of acute high-level mercury exposure during gold ore purification. The respiratory symptoms observed in these four cases ranged from minimal shortness of breath and cough to severe hypoxemia. The most severely affected patient exhibited mild interstitial lung disease both radiographically and on pulmonary function testing. One patient had a urinary Hg level of 245 ug/L upon hospital admission. The occurrence of long-term respiratory effects in these patients could not be evaluated since all but one refused follow-up treatment.

Ashe et al. (1953) reported that there was no histopathological evidence of respiratory damage in 24 rats exposed to 0.1 mg Hg/cu.m 7 hr/day, 5 days/week for 72 weeks. This is equivalent to a NOAEL[HEC] of 0.07 mg/cu.m.

Kishi et al. (1978) observed no histopathological changes in the lungs of rats exposed to 3 mg/cu.m of mercury vapor 3 hours/day, 5 days/week for 12-42 weeks.

Beliles et al. (1967) observed no histopathological changes in the lungs of pigeons exposed to 0.1 mg/cu.m of mercury vapor 6 hours/day, 5 days/week for 20 weeks.

Neurological signs and symptoms (i.e., tremors) were observed in 79 workers exposed to metallic mercury vapor whose urinary mercury levels exceeded 500 ug/L. Short-term memory deficits were reported in workers whose urine levels were less than 500 ug/L (Langolf et al., 1978).

Impaired performance in mechanical and visual memory tasks and psychomotor ability tests was reported by Forzi et al. (1978) in exposed workers whose urinary Hg levels exceeded 100 ug/L.

Decreased strength, decreased coordination, increased tremor, decreased sensation and increased prevalence of Babinski and snout reflexes were exhibited by 247 exposed workers whose urinary Hg levels exceeded 600 ug/L. Evidence of clinical neuropathy was observed at urinary Hg levels that exceeded 850 ug/L (Albers et al., 1988).

Preclinical psychomotor dysfunction was reported to occur at a higher incidence in 43 exposed workers (mean exposure duration of 5 years) whose mean urinary excretion of Hg was 50 ug/L. Workers in the same study whose mean urinary Hg excretion was 71 ug/L had a higher incidence of total proteinuria and albuminuria (Roels et al., 1982).

Postural and intention tremor was observed in 54 exposed workers (mean exposure duration of 7.7 years) whose mean urinary excretion of Hg was 63 ug/L (Roels et al., 1989).

Verbeck et al. (1986) observed an increase in tremor parameters with increasing urinary excretion of mercury in 21 workers exposed to mercury vapor for 0.5-19 years. The LOAEL for this effect was a mean urinary excretion of 35 ug/g creatinine.

Rosenman et al. (1986) evaluated routine clinical parameters (physical exams, blood chemistry, urinalysis), neuropsychological disorders, urinary NAG, motor nerve conduction velocities and occurrence of lenticular opacities in 42 workers of a chemical plant producing mercury compounds. A positive correlation ($p<0.05$ to $p<0.001$) was noted between urinary mercury (levels ranged from 100-250 ug/L) and the number of neuropsychological symptoms, and NAG excretions and the decrease in motor nerve conduction velocities.

Evidence of renal dysfunction (e.g., increased plasma and urinary concentrations of beta-galactosidase, increased urinary excretion of high-molecular weight proteins and a slightly increased plasma beta-2-microglobulin concentration) was observed in 63 chloralkali workers. The incidence of these effects increased in workers whose urinary Hg excretion exceeded 50 ug/g creatinine (Buchet et al., 1980).

Increased urinary NAG levels were found in workers whose urinary Hg levels exceeded 50 ug/L (Langworth et al., 1992).

An increase in the concentration of urinary brush border proteins (BB-50) was observed in 20 workers whose mean urinary Hg excretion exceeded 50 ug/g creatinine (Mutti et al., 1985).

Foa et al. (1976) found that 15 out of 81 chloralkali workers exposed to 60-300 ug/cu.m mercury exhibited proteinuria.

An increased excretion of beta-glutamyl transpeptidase, indicative of renal dysfunction, was found in 509 infants dermally exposed to phenylmercury via contaminated diapers (Gotelli et al., 1985).

Berlin et al. (1969) exposed rats, rabbits and monkeys to 1 mg/cu.m of mercury vapor for 4 hours and measured the uptake and distribution of mercury in the brain as compared with animals injected intravenously with the same doses of mercury as mercuric salts. Mercury accumulated in the brain following inhalation exposure to metallic mercury vapor at levels that were 10 times higher than those observed following intravenous injection of the same dose of mercury as mercuric salts. These results demonstrate that mercury is taken up by the brain following inhalation of the vapor at higher levels than other forms of mercury and that this occurs in all species studied.

Limited animal studies concerning inhalation exposure to inorganic mercury are available. The results of a study conducted by Baranski and Szymczyk (1973) were reported in an English abstract. Adult female rats were exposed to metallic mercury vapor at 2.5 mg/cu.m for 3 weeks prior to fertilization and during gestation days 7-20. A decrease in the number of living fetuses was observed in the dams compared with unexposed controls, and all pups born to the exposed dams died by the sixth day after birth. However, no difference in the occurrence of developmental abnormalities was observed between exposed and control groups. The cause of death of the pups in the mercury-exposed group was unknown, although an unspecified percentage of the deaths was attributed by the authors to a failure of lactation in the dams. Death of pups was also observed in another experiment where dams were only exposed prior to fertilization (to 2.5 mg/cu.m), which supports the conclusion that the high mortality in the first experiment was due at least in part to poor health of the mothers. Without further information, this study must be considered inconclusive regarding developmental effects.

The only other study addressing the developmental toxicology of mercury is the one reported in abstract form by Steffek et al. (1987) and, as such, is included as a supporting study. Sprague-Dawley rats (number not specified) were exposed by inhalation to mercury vapor at concentrations of 0.1, 0.5 or 1.0 mg/cu.m throughout the period of gestation (days 1-20) or during the period of organogenesis (days 10-15). The authors indicated the exposure protocols to be chronic and acute exposure, respectively. At either exposure protocol, the lowest mercury level produced no detectable adverse effect. At 0.5 mg/cu.m, an increase in the number of resorptions (5/41) was noted for the acute group, and two of 115 fetuses exhibited gross cranial defects in the chronic group. At 1.0 mg/cu.m, the number of resorptions was increased in acute (7/71) and chronic (19/38) groups and a decrease in maternal and fetal weights also was detected in the chronic exposure group. No statistical analysis for these data was provided. A LOAEL of 0.5 mg/cu.m is provided based on these data.

Mishinova et al. (1980) investigated the course of pregnancy and parturition in 349 women exposed via inhalation to metallic mercury vapors (unspecified concentrations) in the workplace as compared to 215 unexposed women. The authors concluded that the rates of pregnancy and labor complication were high among women exposed to mercury and that the effects depended on "the length of service and concentration of mercury vapors." Lack of sufficient details preclude the evaluation of dose-response relationships.

In a questionnaire that assessed the fertility of male workers exposed to mercury vapor, Lauwerys et al. (1985) found no statistically significant change in the observed number of children born

to the exposed group compared with a matched control group. The urinary excretion of mercury in the exposed workers ranged from 5.1 to 272.1 ug/g creatinine.

Another study found that exposure to metallic mercury vapor caused prolongation of estrus cycles in animals. Baranski and Szymczyk (1973) reported that female rats exposed via inhalation to mercury vapor at an average of 2.5 mg/cu.m, 6 hours/day, 5 days/week for 21 days experienced longer estrus cycles than unexposed animals. In addition, estrus cycles during mercury exposure were longer than normal estrus cycles in the same animals prior to exposure. Although the initial phase of the cycle was protracted, complete inhibition of the cycle did not occur. During the second and third weeks of exposure, these rats developed signs of mercury poisoning including restlessness, seizures and trembling of the entire body. The authors speculated that the effects on the estrus cycle were caused by the action of mercury on the CNS (i.e., damage to the hypothalamic regions involved in the control of estrus cycling).

Renal toxicity has been reported following oral exposure to inorganic mercury salts in animals, with the Brown-Norway rat appearing to be uniquely sensitive to this effect. These mercury-induced renal effects in the Brown-Norway rat are the basis for the oral RfD for mercurial mercury. Several investigators have produced autoimmune glomerulonephritis by administering HgCl2 to Brown-Norway rats (Druet et al., 1978).

The current OSHA standard for mercury vapor is 0.05 mg/cu.m. NIOSH recommends a TWA Threshold Limit Value of 0.05 mg/cu.m for mercury vapor.

I.B.5. CONFIDENCE IN THE INHALATION RfC

Study -- Medium
Data Base -- Medium
RfC -- Medium

Due to the use of a sufficient number of human subjects, the inclusion of appropriate control groups, the exposure duration, the significance level of the reported results and the fact that exposure levels in a number of the studies had to be extrapolated from blood mercury levels, confidence in the key studies is medium. The LOAEL values derived from these studies can be corroborated by other human epidemiologic studies. The adverse effects reported in these studies are in accord with the well-documented effects of mercury poisoning. The lack of human or multispecies reproductive/developmental studies precludes assigning a high confidence rating to the data base and inadequate quantification of exposure levels. Based on these considerations, the RfC for mercury is assigned a confidence rating of medium.

I.B.6. EPA DOCUMENTATION AND REVIEW OF THE INHALATION RfC

Source Document -- U.S. EPA, 1995

This IRIS summary is included in The Mercury Study Report to Congress which was reviewed by OHEA and EPA's Mercury Work Group in November 1994. An interagency review by scientists from

other federal agencies took place in January 1995. The report was also reviewed by a panel of non-federal external scientists in January 1995 who met in a public meeting on January 25-26. All reviewers comments have been carefully evaluated and considered in the revision and finalization of this IRIS summary. A record of these comments is summarized in the IRIS documentation files.

Other EPA Documentation -- None

Agency Work Group Review -- 11/16/89, 03/22/90, 04/19/90

Verification Date -- 04/19/90

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_II. CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Substance Name -- Mercury, elemental
CASRN -- 7439-97-6
Preparation Date -- 5/24/94

_II.A. EVIDENCE FOR CLASSIFICATION AS TO HUMAN CARCINOGENICITY

_II.A.1 WEIGHT-OF-EVIDENCE CLASSIFICATION

Classification -- D; not classifiable as to human carcinogenicity

Basis -- Based on inadequate human and animal data. Epidemiologic studies failed to show a correlation between exposure to elemental mercury vapor and carcinogenicity; the findings in these studies were confounded by possible or known concurrent exposures to other chemicals, including human carcinogens, as well as lifestyle factors (e.g., smoking). Findings from genotoxicity tests are severely limited and provide equivocal evidence that mercury adversely affects the number or structure of chromosomes in human somatic cells.

_II.A.2 HUMAN CARCINOGENICITY DATA

Inadequate. A number of epidemiological studies were conducted that examined mortality among elemental mercury vapor-exposed workers. Conflicting data regarding a correlation between mercury exposure and an increased incidence of cancer mortalities have been obtained. All of the studies have limitations that complicate interpretation of their results for associations between mercury exposure and induction of cancer; increased cancer rates were attributable to other concurrent exposures or lifestyle factors.

A retrospective cohort study examined mortality among 5663 white males who worked between 1953 and 1963 at a plant in Oak Ridge, Tennessee, where elemental mercury was used for lithium isotope separation (Cragle et al., 1984). The workers were divided into three cohorts: exposed workers who had been monitored on a quarterly basis for mercury levels in urine (n=2,133); workers exposed in the mercury process section for whom urinalysis monitoring data were not collected (n=270); and unexposed workers from other sections of the nuclear weapons production facility (n=3260). The study subjects worked at least 4 months during 1953-1958 (a period when mercury exposures were likely to be high); mortality data from death certificates were followed through the end of 1978. The mean age of the men at first employment at the
facility was 33 years, and the average length of their employment was >16 years with a mean of 3.73 years of estimated mercury exposure. Air mercury levels were monitored beginning in 1955; during 1955 through the third quarter of 1956, air mercury levels were reportedly above 100 ug/cu.m in 30-80% of the samples. Thereafter, air mercury levels decreased to concentrations below 100 ug/cu.m. The mortality experience (i.e., the SMR) of each group was compared with the age-adjusted mortality experience of the U.S. white male population. Among exposed and monitored workers, no significant increases in mortality from cancer at any site were reported, even after the level or length of exposure

was considered. A significantly lower mortality from all causes was observed. An excessive number of deaths was reportedly due to lung cancer in the exposed and monitored workers (42 observed, 31.36 expected), but also in the unexposed workers (71 observed, 52.93 expected). The SMR for each group was 1.34; the elevated incidence of lung cancer deaths was, therefore, attributed to some other factor at the plant and/or to lifestyle factors (e.g., smoking) common to both the exposed and unexposed groups. Study limitations include small cohort sizes for cancer mortality, which limited the statistical stability of many comparisons.

Barregard et al. (1990) studied mortality and cancer morbidity between 1958 and 1984 in 1190 workers from eight Swedish chloralkali plants that used the mercury cell process in the production of chlorine. The men included in the study had been monitored for urinary or blood mercury for more than one year between 1946 and 1984. Vital status and cause of death were ascertained from the National Population Register and the National Bureau of Statistics. The cancer incidence of the cohort was obtained from the Swedish Cancer Register. The observed total mortality and cancer incidences were compared with those of the general Swedish male population. Comparisons were not made between exposed and unexposed workers. Mean urinary mercury levels indicated a decrease in exposure between the 1950s and 1970s; the mean urinary mercury level was 200 ug/L during the 1950s, 150 ug/L during the 1960s and 50 ug/L in the 1970s. Mortality from all causes was not significantly increased in exposed workers. A significant increase in deaths from lung tumors was observed in exposed workers 10 years or more after first exposure (rate ratio, 2.0; 95% CI, 1.0-3.8). Nine of the 10 observed cases of lung cancer occurred among workers (457 of the 1190) possibly exposed to asbestos as well as to mercury. No dose response was observed with respect to mercury exposure and lung tumors. This study is limited because no quantitation was provided on smoking status, and results were confounded by exposure to asbestos.

Ahlbom et al. (1986) examined the cancer mortality during 1961-1979 of cohorts of Swedish dentists and dental nurses aged 20-64 and employed in 1960 (3454 male dentists, 1125 female dentists, 4662 female dental nurses). Observed incidences were compared with those expected based on cancer incidence during 1961-1979 among all Swedes employed during 1960 and the proportion of all Swedes employed as dentists and dental nurses. Data were stratified by sex, age (5-year age groups) and county. The incidence of glioblastomas among the dentists and dental nurses combined was significantly increased compared to survival rates (SMR, 2.1; 95% CI, 1.3-3.4); the individual groups had apparently elevated SMRs (2.0-2.5), but the 95%
confidence intervals of these groups included unity. By contrast, physicians and nurses had SMRs of only 1.3 and 1.2, respectively. Exposure to mercury could not be established as the causative factor because exposure to other chemicals and X-rays was not ruled out.

Amandus and Costello (1991) examined the association between silicosis and lung cancer mortality between 1959 and 1975 in 9912 white male metal miners employed in the United States between 1959 and 1961. Mercury exposures were not monitored. Exposures to specific metals among the silicotic and nonsilicotic groups were analyzed separately. Lung cancer mortality in both silicotic and nonsilicotic groups was compared with rates in white males in the U.S. population. Both silicotic (n=11) and nonsilicotic mercury miners (n=263) had significantly increased lung cancer mortality (SMR, 14.03; 95% CI, 2.89-40.99 for silicotics. SMR, 2.66; 95% CI, 1.15-5.24 for nonsilicotics). The analysis did not focus on mercury miners, and confounders such as smoking and radon exposure were not analyzed with respect to mercury exposure. This study is also limited by the small sample size for non-silicotic mercury miners.

A case-control study of persons admitted to a hospital in Florence, Italy, with lung cancer between 1981-1983 was performed to evaluate occupational risk factors (Buiatti et al., 1985). Cases were matched with one or two controls (persons admitted to the hospital with diagnoses other than lung cancer or suicide) with respect to sex, age, date of admission and smoking status. Women who had "ever worked" as hat makers had a significantly increased risk of lung cancer. The duration of employment as a hat maker averaged 22.2 years, and latency averaged 47.8 years. Workers in the Italian hat industry were known to be occupationally exposed to mercury; however, the design of this study did not allow evaluation of the relationship between cumulative exposure and cancer incidence. In addition, interpretation of the results of this study is limited by the small sample size (only 6/376 cases reported this occupation) and by exposure of hat makers to other pollutants including arsenic, a known lung carcinogen.

Ellingsen et al. (1992) examined the total mortality and cancer incidence among 799 workers employed for more than 1 year in two Norwegian chloralkali plants. Mortality incidence between 1953 and 1988 and cancer incidence between 1953 and 1989 were examined. Mortality and cancer incidence were compared with that of the age-adjusted general male Norwegian population. No increase in total cancer incidence was reported, but lung cancer was significantly elevated in the workers (rate ratio, 1.66; 95% CI, 1.0-2.6). No causal relationship can be drawn from the study between mercury exposure and lung cancer because no correlation existed between cumulative mercury dose, years of employment or latency time. Also, the prevalence of smoking was 10 20% higher in the exposed workers, and many workers were also exposed to asbestos.

_II.A.3 ANIMAL CARCINOGENICITY DATA

Inadequate. Druckrey et al. (1957) administered 0.1 mL of metallic mercury to 39 male and female rats (BD III and BD IV strains) via intraperitoneal injection. Among the rats surviving longer than 22 months, 5/12 developed peritoneal sarcomas. The increase in the incidence of sarcomas was observed only in those tissues that had been in direct contact with the mercury. Although severe kidney damage was reported in all treated animals, no renal tumors or tumors at any site other than the peritoneal cavity were observed.

_II.A.4 SUPPORTING DATA FOR CARCINOGENICITY

Cytogenetic monitoring studies of workers occupationally exposed to mercury by inhalation provide very limited evidence that mercury adversely affects the number or structure of chromosomes in human somatic cells. Popescu et al. (1979) compared four men exposed to elemental mercury vapor with an unexposed group and found a statistically significant increase in the incidence of chromosome aberrations in the WBCs from whole blood. Verschaeve et al. (1976) found an increase in aneuploidy after exposure to low concentrations of vapor, but results could not be repeated in later studies (Verschaeve et al., 1979). Mabille et al. (1984) did not find increases in structural chromosomal aberrations of lymphocytes of exposed workers. Similarly, Barregard et al. (1991) found no increase in the incidence or size of micronuclei and no correlation between micronuclei and blood or urinary mercury levels of chloralkali workers. A statistically significant correlation was observed between

cumulative exposure to mercury and micronuclei induction in T lymphocytes in exposed workers, suggesting a genotoxic effect.

_II.B QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

None.

_II.C QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM INHALATION EXPOSURE

None.

_II.D EPA DOCUMENTATION, REVIEW, AND CONTACTS (CARCINOGENICITY ASSESSMENT)

_II.D.1 EPA DOCUMENTATION

Source document -- U.S. EPA, 1995

This IRIS summary is included in The Mercury Study Report to Congress which was reviewed by OHEA and EPA's Mercury Work Group in November 1994. An interagency review by scientists from other federal agencies took place in January 1995. The report was also reviewed by a panel of non-federal external scientists in January 1995 who met in a public meeting on January 25-26. All reviewers comments have been carefully evaluated and considered in the revision and finalization of this IRIS summary. A record of these comments is summarized in the IRIS documentation files.

_II.D.2 REVIEW (CARCINOGENICITY ASSESSMENT)

Agency Work Group Review -- 01/13/88, 03/03/94

Verification Date -- 03/03/94

_II.D.3 U.S. EPA CONTACTS (CARCINOGENICITY ASSESSMENT)

Rita Schoeny / NCEA -- (513)569-7544

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I.A REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Substance Name -- Mercuric chloride (HgCl2)
CASRN -- 7487-94-7
Preparation Date -- 11/01/88

I.A.1 ORAL RfD SUMMARY

Critical Effect	Experimental Doses*	UF	MF	RfD
Autoimmune effects	NOAEL: None	1000	1	3E-4 mg/kg-day
Rat Subchronic Feeding and Subcutaneous Studies	LOAEL: 0.226 mg/kg-day			
	LOAEL: 0.317 mg/kg-day			
	LOAEL: 0.633 mg/kg-day			
U.S. EPA, 1987				

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* Conversion Factors and Assumptions -- Dose conversions in the three studies employed a 0.739 factor for HgCl2 to Hg2+, a 100% factor for subcutaneous (s.c.) to oral route of exposure, and a time-weighted average for days/week of dosing. This RfD is based on the back calculations from a Drinking Water Equivalent Level (DWEL), recommended to and subsequently adopted by the Agency, of 0.010 mg/L: (RfD = 0.010 mg/L x 2 L/day/70 kg bw = 0.0003 mg/kg bw/day). The LOAEL exposure levels, utilized in the three studies selected as the basis of the recommended DWEL, are from Druet et al. (1978), Bernaudin et al. (1981) and Andres (1984), respectively.

I.A.2 PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

U.S. EPA. 1987. Peer Review Workshop on Mercury Issues. Summary Report. Environmental Criteria and Assessment Office, Cincinnati, OH. October 26-27.

On October 26-27, 1987, a panel of mercury experts met at a Peer Review Workshop on Mercury Issues in Cincinnati, Ohio, and reviewed outstanding issues concerning the health effects and risk assessment of inorganic mercury (U.S. EPA, 1987). The following five consensus conclusions and recommendations were agreed to as a result of this workshop:

1) The most sensitive adverse effect for mercury risk assessment is formation of mercuric-mercury-induced autoimmune glomerulonephritis. The production and deposition of IgG antibodies to the glomerular basement membrane can be considered the first step in the formation of this mercuric-mercury-induced autoimmune glomerulonephritis.

2) The Brown Norway rat should be used for mercury risk assessment. The Brown Norway rat is a good test species for the study of Hg2+-induced autoimmune glomerulonephritis. The Brown Norway rat is not unique in this regard (this effect has also been observed in rabbits).

3) The Brown Norway rat is a good surrogate for the study of mercury-induced kidney damage in sensitive humans. For this reason, the uncertainty factor used to calculate criteria and health advisories (based on risk assessments using the Brown Norway rat) should be reduced by 10-fold.

4) Hg2+ absorption values of 7% from the oral route and 100% from the s.c. route should be used to calculate criteria and health advisories.

5) A DWEL of 0.010 mg/L was recommended based on the weight of evidence from the studies using Brown Norway rats and limited human tissue data.

Three studies using the Brown Norway rat as the test strain were chosen from a larger selection of studies as the basis for the panel's recommendation of 0.010 mg/L as the DWEL for inorganic mercury. The three studies are presented below for the sake of completeness. It must be kept in mind, however, that the recommended DWEL of 0.010 mg/L and back calculated oral RfD of 0.0003 mg/kg-day were arrived at from an intensive review and workshop discussions of the entire inorganic mercury data base, not just from one study.

In the Druet et al. (1978) study, the duration of exposure was 8-12 weeks; s.c. injection was used instead of oral exposure. In this study the development of kidney disease was evaluated. In the first phase the rats developed anti-GBM antibodies. During the second phase, which is observed after 2-3 months, the patterns of fixation of antisera changed from linear to granular as the disease progressed. The immune response was accompanied by proteinuria and in some cases by a nephrotic syndrome.

Both male and female Brown Norway rats 7-9 weeks of age were divided into groups of 6-20 animals each. The numbers of each sex were not stated. The animals received s.c. injections of mercuric chloride (HgCl2) 3 times weekly for 8 weeks, with doses of 0, 100, 250, 500, 1000 and 2000 ug/kg. An additional group was injected with a 50 ug/kg dose for 12 weeks. Antibody formation was measured by the use of kidney cryostat sections stained with a fluoresceinated sheep anti-rat IgG antiserum. Urinary protein was assessed by the biuret method (Druet et al., 1978).

Tubular lesions were observed at the higher dose levels. Proteinuria was reported at doses of 100 ug/kg and above, but not at 50 ug/kg. Proteinuria was considered a highly deleterious effect, given that affected animals developed hypoalbuminemia and many died. Fixation of IgG antiserum was detected in all groups except controls (Druet et al., 1978).

Bernaudin et al. (1981) reported that mercurials administered by inhalation or ingestion to Brown Norway rats developed a systemic autoimmune disease. The HgCl2 ingestion portion of the study involved the forcible feeding of either 0 or 3000 ug/kg-week of HgCl2 to male and female Brown Norway rats for up to 60 days. No abnormalities were reported using standard histological techniques in either experimental or control rats. Immunofluorescence histology revealed that 80% (4/5) of the mercuric-exposed rats were observed with a linear IgG deposition in the glomeruli after 15 days of exposure. After 60 days of HgCl2 exposure, 100% (5/5) of the rats were seen with a mixed linear and

granular pattern of IgG deposition in the glomeruli and granular IgG deposition in the arteries. Weak proteinuria was observed in 60% (3/5) of the rats fed $HgCl_2$ for 60 days. The control rats were observed to have no deposition of IgG in the glomeruli or arteries as well as normal urine protein concentrations.

Andres (1984) administered $HgCl_2$ (3 mg/kg in 1 mL of water) by gavage to five Brown Norway rats and two Lewis rats twice a week for 60 days. A sixth Brown Norway rat was given only 1 mL of water by gavage twice a week for 60 days. All rats had free access to tap water and pellet food. After 2-3 weeks of exposure, the Brown Norway $HgCl_2$-treated rats started to lose weight and hair. Two of the $HgCl_2$-treated Brown Norway rats died 30-40 days after beginning the study. No rats were observed to develop detectable proteinuria during the 60-day study. The kidneys appeared normal in all animals when evaluated using standard histological techniques, but examination by immunofluorescence showed deposits of IgG present in the renal glomeruli of only the mercuric-treated Brown Norway rats. The Brown Norway treated rats were also observed with mercury-induced morphological lesions of the ileum and colon with abnormal deposits of IgA in the basement membranes of the intestinal glands and of IgG in the basement membranes of the lomina propria. All observations in the Lewis rats and the control Brown Norway rat appeared normal.

I.A.3 UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

UF -- An uncertainty factor of 1000 was applied to the animal studies using Brown Norway rats as recommended in U.S. EPA (1987). An uncertainty factor was applied for LOAEL to NOAEL conversion: 10 for use of subchronic studies and a combined 10 for both animal to human and sensitive human populations.

MF -- None

I.A.4 ADDITIONAL STUDIES / COMMENTS (ORAL RfD)

Kazantzis et al. (1962) performed renal biopsies in 2 (out of 4) workers with nephrotic syndrome who had been occupationally exposed to mercuric oxide, mercuric acetate and probably mercury vapors. Investigators reported that the nephrotic syndrome observed in 3 of the 4 workers may have been an idiosyncratic reaction since many other workers in a factory survey had similarly high levels of urine mercury without developing proteinuria. This conclusion was strengthened by work in Brown Norway rats indicating a genetic (strain) susceptibility and that similar mercury-induced immune system responses have been seen in affected humans and the susceptible Brown Norway rats (U.S. EPA, 1987).

The only chronic ingestion study designed to evaluate the toxicity of mercury salts was reported by Fitzhugh et al. (1950). In this study, rats of both sexes (20-24/group) were given 0.5, 2.5, 10, 40 or 160 ppm mercury as mercuric acetate in their food for up to 2 years. Assuming food consumption was equal to 5% bw/day, the daily intake would have been 0.025, 0.125, 0.50, 2.0 and 8.0 mg/kg for the five groups, respectively. At the highest dose level, a slight depression of body weight was detected in male rats only. The statistical significance of this body-weight depression was not stated. Kidney weights were significantly ($p<0.05$) increased at the 2.0 and 8.0 mg/kg dose levels. Pathological changes originating in the proximal convoluted tubules of the kidneys were also noted, with more severe effects

in females than males. The primary weaknesses of this study were (1) the lack of reporting on which adverse effects were observed with which dosing groups and (2) that the most sensitive strain, the Brown Norway rat, was not used for evaluating the mercury-induced adverse health effects.

NTP (1993) conducted subchronic and chronic gavage toxicity studies on Fischer 344 rats and B6C3F1 mice to evaluate the effects of HgCl2, and the kidney appeared to be the major organ affected. In the 6-month study, Fischer 344 rats (10/sex /group) were administered 0, 0.312, 0.625, 1.25, 2.5 or 5 mg/kg-day of HgCl2 (0.23, 0.46, 0.92, 1.9 and 3.7 mg/kg-day) 5 days/week by gavage. Survival was not affected, although body-weight gains were decreased in males at high dose and in females at or above the 0.46 mg/kg-day dose. Absolute and relative kidney weights were significantly increased in both sexes with exposure to at least 0.46 mg/kg-day. In males, the incidence of nephropathy was 80% in the controls and 100% for all treated groups; however, severity was minimal in the controls and two low-dose groups and minimal to mild in the 0.92 mg/kg-day group and higher. In females, there was a significant increased incidence of nephropathy only in the high-dose group (4/10 with minimal severity). Nephropathy was characterized by foci of tubular regeneration, thickened tubular basement membrane and scattered dilated tubules containing hyaline casts. No treatment-related effects were observed in the other organs; however, histopathology on the other organs was performed only on control and high-dose rats.

B6C3F1 mice (10/sex/group) were administered 0, 1.25, 2.5, 5, 10 or 20 mg/kg-day HgCl2 (0, 0.92, 1.9,3.7, 7.4 or 14.8 mg/kg-day) 15 days/week by gavage for 6 months (NTP 1993). A decrease in body-weight gain was reported in only the males at the highest dose tested. Significant increases occurred in absolute kidney weights of male mice at 3.7 mg/kg-day or greater and relative kidney weights of male mice at 7.4 and 14.8 mg/kg-day doses. The kidney weight changes corresponded to an increased incidence of cytoplasmic vacuolation of renal tubule epithelium in males exposed to at least 3.7 mg/kg-day. The exposed female mice did not exhibit any histopathologic changes in the kidneys.

In the 2-year NTP study, Fischer 344 rats (60/sex/group) were administered 0, 2.5 and 5 mg/kg-day HgCl2 (1.9 and 3.7 mg/kg-day) 5 days week by gavage (NTP, 1993). After 2 years, survival was reduced in only the treated male rat groups compared with the control. Mean body weights were decreased in both male and female treated groups. After 2 years, an increased incidence of nephropathy of moderate-to-marked severity and increased incidence of tubule hyperplasia was observed in the kidneys of exposed males compared with the controls. The control males exhibited nephropathy, primarily of mild-to-moderate severity. Hyperparathyroidism, mineralization of various tissues and fibrous osteodystrophy were observed and considered secondary to the renal impairment. No significant differences were found in renal effects between exposed and control females. Other nonneoplastic effects included an increased incidence of forestomach hyperplasia in the exposed males and high-dose females.

NTP (1993) also administered to B6C3F1 mice (60/sex/group) daily oral gavage doses of 0, 5 or 10 mg/kg-day HgCl2 (0, 3.7 and 7.4 mg/kg-day) 5 days/week by gavage for 2 years. Survival and body weights of mice were slightly lower in HgCl2-treated mice compared with controls. Absolute kidney weights were significantly increased in the treated males, while relative kidney weights were significantly increased in high-dose males and both low- and high-dose females. Histopathology revealed an increase in the incidence and severity of nephropathy in exposed males and an increase in the incidence of nephropathy in exposed females. Nephropathy was defined as foci of proximal convoluted tubules with thickened basement membrane and basophilic cells with scant cytoplasm. Some affected

convoluted tubules contained syaline casts. Also, an increase in nasal cavity inflammation (primarily infiltration of granulocytes in nasal mucosa) was observed in the exposed animals.

Gale and Ferm (1971) studied the teratogenic effects of mercuric acetate on Syrian golden hamsters. Single doses of 2, 3 or 4 mg/kg were injected by the i.v. route on day 8 of gestation. Growth retardation, increased resorption rates and edema of the fetuses were found at all three dose levels, while an increase in the number of abnormalities was detected at the two higher doses. In a more recent study, Gale (1981) compared the embryotoxic effects of a single s.c. dose of 15 mg/kg mercuric acetate on the eighth day of gestation in five inbred strains and one noninbred strain of Syrian hamsters. While strain differences were apparent, a variety of abnormalities were reported in all the strains. Gale (1974) also compared the relative effectiveness of different exposure routes in Syrian hamsters. The following sequence of decreasing efficacy was noted for mercuric acetate; i.p. > i.v. > s.c. > oral. The lowest doses used, 2 mg/kg for i.p. and 4 mg/kg for the other three routes, were all effective in causing increased resorption and percent abnormalities.

In male mice administered a single i.p. dose of 1 mg/kg HgCl2, fertility decreased between days 28 and 49 post treatment with no obvious histological effects noted in the sperm (Lee and Dixon, 1975). The period of decreased fertility indicated that spermatogonia and premeiotic spermatocytes were affected. The effects were less severe than following a similar dose of methyl mercury. A single i.p. dose of 2 mg/kg HgCl2 in female mice resulted in a significant decrease in the total number of implants and number of living embryos and a significant increase in the percentage of dead implants (Suter, 1975). These effects suggest that mercury may be a weak inducer of dominant lethal mutations.

I.A.5 CONFIDENCE IN THE ORAL RfD

Study -- N/A
Data Base -- High
RfD -- High

No one study was found adequate for deriving an oral RfD; however, based on the weight of evidence from the studies using Brown Norway rats and the entirety of the mercuric mercury data base, an oral RfD of high confidence results.

I.A.6 EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD

Source Document -- U.S. EPA, 1988

This IRIS summary is included in The Mercury Study Report to Congress, which was reviewed by OHEA and EPA's Mercury Work Group in November 1994. An interagency review by scientists from other federal agencies took place in January 1995. The report was also reviewed by a panel of non-federal external scientists in January 1995 who met in a public meeting on January 25-26. All reviewers comments have been carefully evaluated and considered in the revision and finalization of this IRIS summary. A record of these comments is summarized in the IRIS documentation files.

Other Docmentation -- U.S. EPA, 1987

Agency Work Group Review -- 08/05/85, 02/05/86, 08/19/86, 11/16/88

Verification Date -- 11/16/88

_I.A.7 EPA CONTACTS (ORAL RfD)

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II. CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Substance Name -- Mercuric Chloride
CASRN -- 7487-94-7
Preparation Date -- 5/24/94

II.A EVIDENCE FOR CLASSIFICATION AS TO HUMAN CARCINOGENICITY

II.A.1 WEIGHT-OF-EVIDENCE CLASSIFICATION

Classification -- C; possible human carcinogen

Basis -- Based on the absence of data in humans and limited evidence of carcinogenicity in rats and mice. Focal papillary hyperplasia and squamous cell papillomas in the forestomach as well as thyroid follicular cell adenomas and carcinomas were observed in male rats gavaged with mercuric chloride for 2 years. The relevance of the forestomach papillomas to assessment of cancer in humans is questionable because no evidence indicated that the papillomas progressed to malignancy. The relevance of the increase in thyroid tumors has also been questioned because these tumors are generally considered to be secondary to hyperplasia; this effect was not observed in the high-dose males. It should also be noted that the authors considered the doses used in the study to exceed the MTD for male rats. In the same study, evidence for increases in squamous cell papillomas in the forestomach of female rats was equivocal. In a second study, equivocal evidence for renal adenomas and adenocarcinomas was observed in male mice; there was a significant positive trend. This tumor type is rare in mice, and the increase in incidence was statistically significant when compared with historic controls. Two other nonpositive lifetime rodent studies were considered inadequate. Mercuric chloride showed mixed results in a number of genotoxicity assays.

II.A.2 HUMAN CARCINOGENICITY DATA

None. No data are available on the carcinogenic effects of mercuric chloride in humans.

II.A.3 ANIMAL CARCINOGENICITY DATA

Limited. The results from a dietary study in rats and mice show equivocal evidence for carcinogenic activity in male mice and female rats and some evidence for carcinogenic activity in male rats. Two other dietary studies did not show any evidence for carcinogenicity, but these studies are limited by inadequacies in the data and experimental design, including the small number of animals/dose and/or a lack of complete histopathological examinations.

Mercuric chloride (purity >99%) was administered by gavage in water at doses of 0, 2.5 or 5 (mg/kg)/day (0, 1.9 and 3.7 (mg/kg)/day) to 60 F344 rats/sex/group, 5 days/week for 104 weeks (NTP,

1993). An interim sacrifice (10/sex/dose) was conducted after 15 months of exposure. Complete histopathological examinations were performed on all animals found dead, killed in extremis, or killed by design. Survival after 24 months was lower in low- and high-dose males at a statistically significant rate; survival was 43, 17 and 8% in control, low-, and high-dose males, respectively, and 58, 47 and 50% in control, low-, and high-dose females, respectively. During the second year of the study, body weight gains of low- and high-dose males were 91 and 85% of controls, respectively, and body weight gains of low- and high-dose females were 90 and 86% of controls, respectively. At study termination, nephropathy was evident in almost all male and female rats including controls, but the severity was much greater in treated males. The incidence of "marked" nephropathy was 6/50, 29/50 and 29/50 in control, low- and high-dose males, respectively. Squamous cell papillomas of the forestomach showed a statistically significant positive trend with dose by life table adjusted analysis; the incidences were 0/50, 3/50 and 12/50 in control, low- and high-dose males, respectively. For females, the incidence was 0/50, 0/49 and 2/50 in control, low- and high-dose groups, respectively. These neoplasms are rare in male rats and occurred in only 1/264 historical controls. The incidence of papillary hyperplasia of the stratified squamous epithelium lining of the forestomach was elevated at a statistically significant rate in all dosed males (3/49, 16/50 and 35/50 in control, low- and high-dose males, respectively) and in high-dose females (5/50, 5/49 and 20/50 in control, low-and high-dose females, respectively). The incidence of thyroid follicular cell carcinomas, adjusted for survival, showed a significant positive trend in males; the incidence was 1/50, 2/50 and 6/50 in control, low- and high-dose groups, respectively. The combined incidence of thyroid follicular cell neoplasms (adenoma and/or carcinoma) was not significantly increased (2/50, 6/50 and 6/50 in control, low- and high-dose males, respectively). In female rats a significant decrease in the incidence of mammary gland fibroadenomas was observed (15/50, 5/48 and 2/50 in control, low- and high-dose females, respectively). The high mortality in both groups of treated males indicates that the MTD was exceeded in these groups and limits the value of the study for assessment of carcinogenic risk. NTP (1993) considered the forestomach tumors to be of limited relevance to humans because the tumors did not appear to progress to malignancy. NTP (1993) also questioned the relevance of the thyroid carcinomas because these neoplasms are usually seen in conjunction with increased incidences of hyperplasia and adenomas. In this study, however, no increases in hyperplasia or adenomas were observed. Hyperplasia incidence was 2/50, 4/50 and 2/50 in control, low- and high-dose males, respectively; adenoma incidence was 1/50, 4/50 and 0/50 in control, low- and high-dose males, respectively.

In the same study, mercuric chloride was administered by gavage in water at doses of 0, 5 or 10 (mg/kg)/day (0, 3.7 and 7.4 (mg/kg)/day) to 60 B6C3F1 mice/sex/group 5 days/week for 104 weeks (NTP, 1993). An interim sacrifice (10/sex/dose) was conducted after 15 months of exposure. Terminal survival and body weight gain were not affected in either sex by the administration of mercuric chloride. It should be noted that survival of high-dose females was lower than controls; female survival rates were 82, 70 and 62% in control, low- and high-dose females, respectively. Female mice exhibited a significant increase in the incidence of nephropathy (21/49, 43/50 and 42/50 in control, low- and high-dose females, respectively). Nephropathy was observed in 80-90% of the males in all groups. The severity of nephropathy increased with increasing dose. The incidence of renal tubular hyperplasia was 1/50, 0/50 and 2/49 in control, low- and high-dose males. The combined incidence of renal tubular adenomas and adenocarcinomas was 0/50, 0/50 and 3/49 in control, low- and high-dose males, respectively. Although no tumors were seen in the low-dose males, a statistically significant positive trend for increased incidence with increased dose was observed. These observations were considered important because renal tubular hyperplasia and tumors in mice are rare. The 2-year historical incidence of renal tubular adenomas or adenocarcinomas in males dosed by gavage with water was 0/205, and only 4 of the nearly 400 completed NTP studies have shown increased renal tubular neoplasms in mice. Data from this study

were not statistically compared with historical control data by NTP. EPA's analysis of the reported data with Fisher's Exact test showed that the incidence of renal tubular adenomas or adenocarcinomas in the high-dose males was significantly elevated when compared with historical controls (Rice and Knauf, 1994).

A 2-year feeding study in rats (20 or 24/sex/group; strain not specified) was conducted in which mercuric acetate was administered in the diet at doses of 0, 0.5, 2.5, 10, 40 and 160 ppm (0, 0.02, 0.1, 0.4, 1.7 and 6.9 (mg Hg/kg)/day (Fitzhugh et al., 1950). Survival was not adversely affected in the study. Increases in kidney weight and renal tubular lesions were observed at the two highest doses. No statement was made in the study regarding carcinogenicity. This study was not intended to be a carcinogenicity assay, and the number of animals/dose was rather small. Histopathological analyses were conducted on only 50% of the animals (complete histopathology conducted on only 31% of the animals examined), and no quantitation of results or statistical analyses were performed.

No increase in tumor incidence was observed in a carcinogenicity study using white Swiss mice (Schroeder and Mitchener, 1975). Groups of mice (54/sex/group) were exposed until death to mercuric chloride in drinking water at 5 ppm Hg (0.95 (mg/kg)/day). No effects on survival or body weights were observed. After dying, mice were weighed and dissected. The animals were examined for gross tumors, and some sections were made of the heart, lung, liver, kidney and spleen for microscopic examination. No toxic effects of mercuric chloride were reported in the study. No statistically significant differences were observed in tumor incidences for treated animals and controls. This study is of limited use for evaluation of carcinogenicity because complete histological examinations were not performed, only a single dose was tested, and the MTD was not achieved.

II.A.4 SUPPORTING DATA FOR CARCINOGENICITY

The increasing trend for renal tubular cell tumors in mice observed in the NTP (1993) study receives some support from similar findings in mice after chronic dietary exposure to methylmercury (Hirano et al., 1986; Mitsumori et al., 1981, 1990). In these studies, dietary exposure to methylmercuric chloride resulted in increases in renal tubular tumors at doses wherein substantial nephrotoxicity was observed (see methylmercury file on IRIS).

As summarized in NTP (1993) and U.S. EPA (1985), mercuric chloride has produced some positive results for clastogenicity in a variety of in vitro and in vivo genotoxicity assays; mixed results regarding its mutagenic activity have been reported. Mercuric chloride was negative in gene mutation tests with Salmonella typhimurium (NTP, 1993; Wong, 1988) but produced DNA damage as measured in the Bacillus subtilis rec assay (Kanematsu et al., 1980). A weakly positive response for gene mutations was observed in mouse lymphoma (L5178Y) cells in the presence of microsomal activation (Oberly et al., 1982). DNA damage has also been observed in assays using rat and mouse embryo fibroblasts (Zasukhina et al., 1983), CHO cells and human KB cells (Cantoni and Costa, 1983; Cantoni et al., 1982, 1984a,b; Christie et al., 1984, 1986; NTP, 1993; Williams et al., 1987). Mercuric chloride also produced chromosome aberrations and SCEs in CHO cells (Howard et al., 1991) and chromosome aberrations in human lymphocytes (Morimoto et al., 1982). Sex-linked recessive lethal mutations were not observed in male Drosophila melanogaster (NTP, 1993).

Although mice given intraperitoneal doses of mercuric chloride have shown no increase in chromosomal aberrations in bone marrow cells (Poma et al., 1981) and no increase in aneuploidy in spermatogonia (Jagiello and Lin, 1973), mercuric chloride administered to mice by gavage induced a dose-related increase in chromosome aberrations and aberrant cells in the bone marrow (Ghosh et al., 1991). Similarly, an increased incidence of chromosomal aberrations (primarily deletion and numeric aberrations) was observed in livers of fetal mice exposed to mercury in utero as the result of maternal inhalation of aerosols of mercuric chloride (Selypes et al., 1984). Positive dominant lethal results (increased resorptions and post-implantation deaths in untreated females) have been obtained in studies in which male rats were administered mercuric chloride orally (Zasukhina et al., 1983). A slight increase in post-implantation deaths and a decrease in living embryos were also reported in treated female mice mated to untreated males (Suter, 1975); however, it was not clear whether these effects were the result of germ cell mutations or were secondary to maternal toxicity.

The effects of mercuric chloride on genetic material has been suggested to be due to the ability of mercury to inhibit the formation of the mitotic spindle, an event known as c-mitosis (U.S. EPA, 1985).

_II.B QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

None. The incidences of squamous cell papillomas of the forestomach and thyroid follicular cell carcinomas were evaluated. No slope factor was derived using the forestomach tumors because these tumors are probably the result of doses of mercuric chloride above-MTD resulting in irritation of the forestomach and subsequent cell death and epithelial proliferation. The carcinogenic mechanism for mercuric chloride at the high doses observed may be specific to effects of irritation of the forestomach.

Regarding the thyroid carcinomas, a variety of drugs, chemicals and physiological perturbations result in the development of thyroid follicular tumors in rodents. For a number of chemicals, the mechanism of tumor development appears to be a secondary effect of long-standing hypersecretion of thyroid-stimulating hormone by the pituitary (Capen and Martin, 1989; McClain, 1989). In the absence of such long-term stimulatory effects, induction of thyroid follicular cell cancer by such chemicals usually does not occur (Hill, 1989). The mechanism whereby thyroid tumors developed in the NTP (1993) assay is very unclear given that hyperplasia was not observed. The study reviewers concluded that it was difficult to associate the increase in thyroid tumors with mercuric chloride administration. Thus, it would be of questionable value to use the thyroid tumors in rats as the basis for a quantitative cancer risk estimate for humans.

All tumors in rats were observed at doses equalling or exceeding the MTD. Kidney tumors in mice were observed in only the high-dose males. The increased incidence was not statistically significant in comparison to the concurrent controls, but was significant when compared with historical controls. A linear low-dose extrapolation based on the male mouse kidney tumor data (three tumors in the high-dose group only) is not appropriate.

_II.C QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM INHALATION EXPOSURE

None.

_II.D EPA DOCUMENTATION, REVIEW AND CONTACTS (CARCINOGENICITY ASSESSMENT)

_II.D.1 EPA DOCUMENTATION

Source Document -- U.S. EPA, 1995

This IRIS summary is included in The Mercury Study Report to Congress which was reviewed by OHEA and EPA's Mercury Work Group in November 1994. An interagency review by scientists from other federal agencies took place in January 1995. The report was also reviewed by a panel of non-federal external scientists in January 1995 who met in a public meeting on January 25-26. All reviewers comments have been carefully evaluated and considered in the revision and finalization of this IRIS summary. A record of these comments is summarized in the IRIS documentation files.

_II.D.2 REVIEW (CARCINOGENICITY ASSESSMENT)

Agency Work Group Review -- 03/03/94

Verification Date -- 03/03/94

_II.D.3 U.S. EPA CONTACTS (CARCINOGENICITY ASSESSMENT)

Rita Schoeny / NCEA -- (513)569-7544

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I.A. REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Chemical -- Methylmercury (MeHg)
CASRN -- 22967-92-6
Preparation Date -- 2/10/95

I.A.1 ORAL RfD SUMMARY

Critical Effect	Experimental Doses*	UF	MF	RfD
Developmental neurologic abnormalities in human infants Human epidemiologic studies	Benchmark Dose: 11 ppm in hair; equivalent to maternal blood levels 44 ug/L and body burdens of 69 ug or daily intake of 1.1 ug/kg-day	10	1	1E-4 mg/kg-day

Marsh et al., 1987; Seafood Safety, 1991

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* Conversion Factors and Assumptions -- Maternal daily dietary intake levels were used as the dose surrogate for the observed developmental effects in the infants. The daily dietary intake levels were calculated from hair concentrations measured in the mothers. This conversion is explained in the text below. A benchmark dose approach was used rather than a NOAEL/LOAEL approach to analyze the neurological effects in infants as the response variable. This analysis is also explained in the text below.

I.A.2 PRINCIPAL STUDIES (ORAL RfD)

Marsh, D.O., T.W. Clarkson, C. Cox, L. Amin-Zaki and S. Al-Trkiriti. 1987. Fetal methylmercury poisoning: Relationship between concentration in a single strand of maternal hair and child effects. Arch. Neurol. 44: 1017-1022.

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In 1971-1972 many citizens in rural Iraq were exposed to MeHg-treated seed grain that was mistakenly used in home-baked bread. Latent toxicity was observed in many adults and children who had consumed bread over a 2- to 3-month period. Infants born to mothers who ate contaminated bread during gestation were the most sensitive group. Often infants exhibited neurologic abnormalities while their mothers showed no signs of toxicity. Some information indicates that male infants are more sensitive than females. Among the signs noted in the infants exposed during fetal development were cerebral palsy, altered muscle tone and deep tendon reflexes as well as delayed developmental milestones, i.e., walking by 18 months and talking by 24 months. The neurologic signs noted in adults

included paresthesia, ataxia, reduced visual fields and hearing impairment. Some mothers experienced paresthesia and other sensory disturbances but these symptoms were not necessarily correlated with neurologic effects in their children. Unique analytic features of mercury (Hg), that is, analysis of segments of hair correlated to specific time periods in the past permitted approximation of maternal blood levels that the fetuses were exposed to in utero. The data collected by Marsh et al. (1987) summarizes clinical neurologic signs of 81 mother and child pairs. From x-ray fluorescent spectrometric analysis of selected regions of maternal scalp hair, concentrations ranging from 1 to 674 ppm were determined and correlated with clinical signs observed in the affected members of the mother-child pairs. Among the exposed population were affected and unaffected individuals throughout the dose-exposure range.

While the purpose of the Seafood Safety publication was to critique the quantitative risk assessment that FDA had performed for MeHg, this material is included in the EPA risk assessment because the Tables of Incidence of various clinical effects in children that were provided in the FDA assessment readily lend themselves to a benchmark dose approach. Specifically the continuous data for the Iraqi population that was reported in Marsh et al. (1987) are placed in five dose groups and incidence rates are provided for delayed onset of walking, delayed onset of talking, mental symptoms, seizures, neurological scores above 3 and neurological scores above 4 for affected children. Neurologic scores were determined by clinical evaluation for cranial nerve signs, speech, involuntary movement, limb tone strength, deep tendon reflexes, plantar responses, coordination, dexterity, primitive reflexes, sensation, posture, and ability to sit, stand and run. This paper provided groupings of the 81 mother-infant pairs for various effects, and the authors present the data in Tables 6-11 through 6-16B.

EQUATION USED FOR CALCULATION OF DAILY DOSE: From the concentration of Hg present in maternal hair, a corresponding blood concentration value is determined. A hair concentration of 11 ppm converts to a blood concentration of 44 ug/L; the following equation can then be used to determine the daily dose that corresponds to that blood concentration of Hg. Use of this equation is based on the assumption that steady-state conditions exist and that first-order kinetics for Hg are being followed.

$$d = (C \times b \times V)/(A \times f)$$

d (ug/day) = 44 ug/L multiplied by 0.014 multiplied by 5 liters divided by 0.95 then divided by 0.05 yields 65 ug/day

where:

d = daily dietary intake (expressed as ug of MeHg)

C = concentration in blood (expressed as ug/L)

b = elimination constant (expressed as days-1)

V = volume of blood in the body (expressed as liters)

A = absorption factor (unitless)

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

The following sections provide the data and rationale supporting the choice of parameter values used in the conversion equation. It should be noted that even if the upper or lower ranges of the parameter values were used, the conversion factor precision remains the same due to rounding error. The Agency realizes that new pharmacokinetic data may become available that warrant a change to some of these parameters.

HAIR TO BLOOD CONCENTRATION RATIO: The hair:blood concentration ratio for total Hg is frequently cited as 250. The following description provides a justification of why we have chosen to use the ratio of 250:1. Ratios reported in the literature range from 140 to 370, a difference of more than a factor of 2.5. Differences in the location of hair sampled (head vs chest and distance from scalp) may contribute to the differences observed. As much as a 3-fold seasonal variation in Hg levels was observed in average hair levels for a group of individuals with moderate to high fish consumption rates, with yearly highs occurring in the fall and early winter (Phelps et al., 1980; Suzuki et al., 1993). The high slope reported by Tsubaki and Irukayama (1977) may have reflected the fact that Hg levels were declining at the time of sampling so that the hair levels reflect earlier, higher blood levels. Phelps et al. (1980) obtained multiple blood samples and sequentially analyzed lengths of hair from individuals. Both hair and blood samples were taken for 339 individuals in Northwestern Ontario. After reviewing the various reports for converting hair concentrations to blood concentrations, the Phelps paper was selected because of the large sample size and the attention to sampling and analysis. The ratio Phelps observed between the total Hg concentration in hair taken close to the scalp and simultaneous blood sampling for this group was 296. To estimate the actual ratio, the authors assumed that blood and hair samples were taken following complete cessation of MeHg intake. They also assumed a half-life of MeHg in blood of 52 days and a lag of 4 weeks for appearance of the relevant level in hair at the scalp. Phelps also determined that 94% of the Hg in hair was MeHg. Based on these assumptions, they calculated that if the actual hair:blood ratio was 200, they would have observed a ratio of 290. Based on these and other considerations, Phelps states that the actual ratio is "probably higher than 200, but less than the observed value of 296." As the authors point out, 2/3 of the study population were sampled during the falling phase of the seasonal variation (and 1/3 or less in the rising phase). This methodology would tend to result in a lower observed ratio; therefore, the actual average is likely to be greater than 200.

In view of these limitations a value of 250 was considered acceptable for the purpose of estimating average blood levels in the Iraqi population.

CALCULATION OF DIETARY INTAKE FROM BLOOD CONCENTRATION: The first step in this process is to determine the fraction of Hg in diet that is absorbed. Radio-labeled methyl-mercuric nitrate ($MeHgNO_3$) was administered in water to three healthy volunteers (Aberg et al., 1969). The uptake was >95%. Miettinen et al. (1971) incubated fish liver homogenate with radio-labeled $MeHgNO_3$ to produce methylmercury proteinate. The proteinate was then fed to fish that were killed after a week and then cooked and fed to volunteers after confirmation of MeHg in the fish. Mean uptake exceeded 94%. Based on these experimental results, this derivation used an absorption factor of 0.95.

The next step involves determining the fraction of the absorbed dose that is found in the blood. There are three reports on the fraction of absorbed MeHg dose distributed to blood volume in humans. Kershaw et al. (1980) report an average fraction of 0.059 of absorbed dose in total blood volume, based on a study of five adult male subjects who ingested MeHg-contaminated tuna. In a group of nine male and six female volunteers who had received 203 Hg-methylmercury in fish approximately 10% of the total body burden was present in 1 liter of blood in the first few days after exposure, dropping to approximately 5% over the first 100 days (Miettinen et al., 1971). In another study, an average value of

1.14% for the percentage of absorbed dose in 1 kg of blood was derived from subjects who consumed a known amount of MeHg in fish over a 3-month period (Sherlock et al., 1982). Average daily intake in the study ranged from 43-233 ug/day and a dose-related effect on percentage of absorbed dose was reported that ranged from 1.03-1.26% in 1 liter of blood (each of these values should be multiplied by 5 [since there are approximately 5 liters of blood in an adult human body] to yield the total amount in the blood compartment). The value 0.05 has been used for this parameter in the past (WHO, 1990).

ELIMINATION CONSTANT: Based on data taken from four studies, reported clearance half-times from blood or hair ranged from 48-65 days. Two of these studies included the Iraqi population exposed during the 1971-1972 outbreak. The value from the Cox study (Cox et al., 1989) is derived from the study group that included the mothers of the infants upon which this risk assessment is based. The average elimination constant of the four studies is 0.014; the average of individual values reported for 20 volunteers ingesting from 42-233 ug Hg/day in fish for 3 months (Sherlock et al., 1982) is also 0.014.

VOLUME OF BLOOD IN THE BODY AND BODY WEIGHT: Blood volume is 7% of body weight as has been determined by various experimental methods and there is an increase of 20 to 30% (to about 8.5 to 9%) during pregnancy (Best and Taylor, 1961). Specific data for the body weight of Iraqi women were not found. Assuming an average body weight of 60 kg. (Snyder et al., 1981) and a blood volume of 9% of body weight during pregnancy, a blood volume of 5.4 liters is derived.

DERIVATION OF A BENCHMARK DOSE: Benchmark dose estimates were made for excess risk above background based on a combination of all childhood neurologic end points. This method was chosen since the Agency felt that any childhood neurologic abnormality is considered an adverse effect and likely to have serious sequelae lasting throughout lifetime. In addition, grouping of all neurologic endpoints provided a much better goodness of fit of the data than when any endpoint was used individually. The endpoints that were grouped delayed the onset of walking and talking, neurologic scores <3, mental symptoms, and seizures. Using these data sets taken from the Seafood Safety paper, benchmark doses at the 1, 5 and 10% incidence levels were constructed using both Weibull and polynomial models. The Weibull model places the maximum likelihood estimate with corresponding 95% confidence level at 11 ppm of MeHg in maternal hair. The Agency decided to use the lower 95% confidence level for the 10% incidence rate. Recent research by Faustman et al. (1994) and Allen et al. (1994a,b) suggests that the 10% level for the benchmark dose roughly correlates with a NOAEL for quantal developmental toxicity data. The 95% lower confidence limits on doses corresponding to the 1, 5, and 10% levels were calculated using both models and the values determined using the polynomial model always fell within 3% of the Weibull values. For final quantitative analysis the Weibull model was chosen because of goodness of fit of the data and because this model has been used in the past by the Agency for developmental effects. The experience of the Agency indicates that this model performs well when modeling for developmental effects.

I.A.3 UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

UF -- An uncertainty factor of 3 is applied for variability in the human population, in particular the variation in the biological half-life of MeHg and the variation that occurs in the hair:blood ratio for Hg. In addition, a factor of 3 is applied for lack of a two-generation reproductive study and lack of data for the effect of exposure duration on sequelae of the developmental neurotoxicity effects and on adult paresthesia. The total UF is 10.

MF -- None

I.A.4 ADDITIONAL STUDIES/COMMENTS (ORAL RfD)

McKeown-Eyssen et al. (1983) have provided a report of neurologic abnormalities in four communities of Cree Indians in northern Quebec. A group of 247 children first exhibited clinical signs consistent with MeHg exposure between 12 and 30 months of age. An attempt was made to account for possible confounding factors; the interviewers determined alcohol and tobacco consumption patterns among the mothers of affected children. Age of the mothers and multiparity was also taken into account in analysis of the data. The average indices of exposure were the same for boys and girls at 6 ug/g; only 6% had exposure above 20 ug/g. The prevalence of multiple abnormal neurologic findings was about 4% for children of both sexes. The most frequently observed abnormality was delayed deep tendon reflexes; this was seen in 11.4% of the boys and 12.2% of the girls. These investigators found that when there was a positive association between maternal Hg exposure and abnormal neurologic signs in boys, the incidence rate was 7.2%. The incidence rate for neurologic disorders in daughters was less and was found to be not statistically significant. Disorders of muscle tone were usually confined to the legs. Persistence of the Babinski reflex and incoordination due to delayed motor development were seen with equal frequency for both sexes. The discriminant analysis conducted for the boys to distinguish the 15 cases with abnormal muscle tone or reflexes from the 82 normal controls was unable to separate differences between these groups based on confounding variables. The prevalence of abnormality of muscle tone or reflexes was found to increase 7 times with each increase of 10 ug/g of the prenatal exposure index. Although this study provides supportive data for the RfD, it is not included with the principal studies because it was confounded by alcoholism and smoking among mothers.

Studies performed in New Zealand investigated the mental development of children who had prenatal exposure to MeHg (Kjellstrom et al., 1986, 1989). A group of 11,000 mothers who regularly ate fish were initially screened by survey and of these about 1000 had consumed fish in three meals per week during pregnancy. Working from this large population base, 31 matched pairs were established. For proper comparison a reference child matched for ethnic group and age of mother, child's birthplace and birth date was identified for each high Hg child. Retrospective Hg concentrations were determined from the scalp hair of the mothers to match the period of gestation. The average hair concentration for high-exposure mothers was 8.8 mg/kg and for the reference group it was 1.9 mg/kg.

The children of exposed mothers were tested at 4 and 6 years of age. At 4 years of age the children were tested using the Denver Developmental Screen Test (DDST) to assess the effects of Hg. This is a standardized test of a child's mental development that can be administered in the child's home. It consists of four major function sectors: gross motor, fine motor, language, and personal-social. A developmental delay in an individual item is scored as abnormal, questionable when the child has failed in their response and at least 90% of the children can pass this item at a younger age. The results of the DDST demonstrated 2 abnormal scores and 14 questionable scores in the high Hg-exposed group and 1 abnormal and 4 questionable scores in the control group. Analysis of the DDST results by sector showed that developmental delays were most commonly noted in the fine motor and language sectors but the differences for the experimental and control groups were not significant. The investigators noted that differences in performance of the DDST between high Hg-exposed and referent children could be due to

confounding variables and that DDST results are highly dependent upon the age of the child. Standardized vision tests and sensory tests were also performed to measure development of these components of the nervous system. The prevalence for developmental delay in children was 52% from high Hg mothers and 17% from mothers of the reference group. In comparison to other studied populations, the hair Hg concentration of the mothers in this study were lower than those associated with CNS effects in children exposed in Japan and Iraq. Results of the DDST demonstrated 2 abnormal scores and 14 questionable scores in the high Hg-exposed group and 1 abnormal and 4 questionable scores in the control group. Analysis of the DDST results by sector showed that developmental delays were most commonly noted in the fine motor and language sectors but the differences for the experimental and control groups were not significant. The data obtained from this study is too limited for detailed dose-response analysis. The differences in performance of the DDST between high Hg-exposed and referent children could be due to confounding variables. DDST results are highly dependent upon the age of the child. Infants of the Hg-exposed group more frequently had low birth weights and were more likely to be born prematurely. Use of this study is also limited by the fact that there was only a 44% participation rate.

A second stage follow-up of the original Kjellstrom study was carried out when the children were 6 years old to confirm or refute the developmental findings observed at age 4 (Kjellstrom et al., 1989). In this later study the high exposure children were compared with three control groups with lower prenatal Hg exposure. The mothers of children in two of these control groups had high fish consumption and average hair Hg concentrations during pregnancy of 3-6 mg/kg and 0-3 mg/kg, respectively. For this study the high exposure group was matched for maternal ethnic group, age, smoking habits, residence, and sex of the child. For this second study, 61 of 74 high-exposure children were available for study. Each child was tested at age 6 with an array of scholastic, psychological, and behavioral tests which included the Test of Language Development (TOLD), the Wechsler Intelligence Scale for Children, and the McCarthy Scale of Children's Abilities. The results of the tests were compared between groups. Confounding was controlled for by using linear multiple regression analysis. A principal finding was that normal results of the psychological test variables were influenced by ethnic background and social class. The high prenatal MeHg exposure did decrease performance in the tests, but it contributed only a small part of the variation in test results. The investigation found that an average hair Hg level of 13-15 mg/kg during pregnancy was consistently associated with decreased test performance. Due to the small size of the actual study groups it was not possible to determine if even lower exposure levels might have had a significant effect on test results. The Kjellstrom studies are limited for assessing MeHg toxicity because the developmental and intelligence tests used are not the most appropriate tests for defining the effects of MeHg. Also, greater significance was seen in differences of cultural origins of the children than the differences in maternal hair MeHg concentrations.

The initial epidemiologic report of MeHg poisoning involved 628 human cases that occurred in Minamata Japan between 1953 and 1960 (Tsubaki and Irukayama, 1977). The overall prevalence rate for the Minamata region for neurologic and mental disorders was 59%. Among this group 78 deaths occurred and hair concentrations of Hg ranged from 50-700 ug/g. Hair Hg concentrations were determined through the use of less precise analytic methods than were available for later studies. The specific values derived from these studies do not contribute directly to quantitative risk assessment for MeHg. The most common clinical signs observed in adults were paresthesia, ataxia, sensory disturbances, tremors, impairment of hearing and difficulty in walking. This particular group of neurologic signs has become known as "Minimata disease." Examination of the brains of severely affected patients that died revealed marked atrophy of the brain (55% normal volume and weight) with

cystic cavities and spongy foci. Microscopically, entire regions were devoid of neurons, granular cells in the cerebellum, golgi cells and Purkinje cells. Extensive investigations of congenital Minamata disease were undertaken and 20 cases that occurred over a 4-year period were documented. In all instances the congenital cases showed a higher incidence of symptoms than did their mothers. Severe disturbances of nervous function were described and the affected offspring were very late in reaching developmental milestones. Hair concentrations of Hg in affected infants ranged from 10 to 100 ug/g. Data on hair Hg levels for the mothers during gestation were not available.

Rice (1989) dosed five cynomolgus monkeys (Macaca fascicularis) from birth to 7 years of age with 50 ug/kg-day and performed clinical and neurologic examinations during the dosing period and for an additional 6 years. As an indicator of the latent effects of MeHg, objective neurologic examinations performed at the end of the observation period revealed insensitivity to touch and loss of tactile response. In addition, monkeys dosed with MeHg were clumsier and slower to react when initially placed in an exercise cage as well as in the later stages of the observation period.

Gunderson et al. (1986) administered daily doses of 50-70 ug/kg of MeHg to 11 crab-eating macaques (Macaca fascicularis) throughout pregnancy which resulted in maternal blood levels of 1080-1330 ug/L in mothers and 1410-1840 ug/L in the offspring. When tested 35 days after birth the infants exhibited visual recognition deficits.

In another study, groups of 7 or 8 female crab-eating macaques (Macaca fascicularis) were dosed with 0.50 and 90 ug/kg-day of MeHg through four menstrual cycles (Burbacher et al., 1984). They were mated with untreated males and clinical observations were made for an additional 4 months. Two of seven high-dose females aborted and three did not conceive during the 4-month mating period; the other two females delivered live infants. Two of seven females of the 50 ug/kg-day dose group aborted; the remaining females delivered live infants. All 8 females of the control group conceived and 6 delivered live infants. These reproductive results approached but did not reach statistical significance. Reproductive failure within dose groups could be predicted by blood Hg levels. The dams did not show clinical signs of MeHg poisoning during the breeding period or gestation but when females were dosed with 90 ug/kg-day for 1 year 4/7 did show adverse neurologic signs.

Bornhausen et al. (1980) reported a decrease in operant behavior performance in 4-month-old rats whose dams had received 0.005 and 0.05 mg/kg-day of MeHg on days 6 through 9 of gestation. A statistically significant effect (<0.05) was observed in offspring whose dams had received 0.01 and 0.05 mg/kg during gestation. The authors postulated that more severe effects of in utero exposure would be seen in humans since the biological half-time of Hg in the brain of humans is 5 times longer than the rat. In addition, much longer in utero exposure to Hg would occur in humans since gestation is much longer in chronologic time.

In another investigation groups of Wistar rats (50/sex/dose) were administered daily doses of 2, 10, 50 and 250 ug/kg-day of MeHg for 26 months (Munro et al., 1980). Female rats that received 25 ug/kg-day had reduced body weight gains and showed only minimal clinical signs of neurotoxicity; however, male rats that received this dose did show overt clinical signs of neurotoxicity, had decreased hemoglobin and hematocrit values, had reduced weight gains, and showed increased mortality. Histopathologic examination of rats of both sexes receiving 25 ug/kg-day revealed demyelination of dorsal nerve roots and peripheral nerves. Males showed severe kidney damage and females had minimal renal damage. This study showed a NOAEL of 5 ug/kg-day and a LOAEL of 25 ug/kg-day.

A 2-year feeding study of MeHg chloride was conducted in B6C3F1 mice (60 mice/sex/group) at doses of 0, 0.4, 2 and 10 ppm (0, 0.04, 0.17, and 0.83 mg/kg-day) to determine chronic toxicity and possible carcinogenic effects (Mitsumori et al., 1990). The mice were examined clinically during the study and neurotoxic signs characterized by posterior paralysis were observed in 33 males after 59 weeks and 3 females after 80 weeks in the 10 ppm group. A marked increase in mortality and a significant decrease in body weight gain were also observed in the 10 ppm male dose group, beginning at 60 weeks. Post mortem examination revealed toxic encephalopathy consisting of neuronal necrosis of the brain and toxic peripheral sensory neuropathy in both sexes of the 10 ppm group. An increased incidence of chronic nephropathy was observed in the 2 and 10 ppm males. Based upon this study a NOAEL of 0.04 mg/kg-day and a LOAEL of 0.17 mg/kg-day was determined. These results indicated that B6C3F1 mice are more sensitive to the neurotoxic effects of MeHg than ICR mice.

KINETICS: MeHg in the diet is almost completely absorbed into the bloodstream. Animal studies indicate (Walsh, 1982) that age has no effect on the efficiency of the gastrointestinal absorption, which is usually in excess of 90%. From the bloodstream MeHg is distributed to all tissues, and distribution is complete within 4 days in humans. The time necessary to reach peak brain levels from a single oral dose is 1 or 2 days longer than other tissues and at this time the brain contains 6% of the total dose. Also at this time the brain concentration is six times that of the blood.

Methylmercury is converted to inorganic Hg in various tissues at different rates in mammals. The fraction of total Hg present as Hg++ depends on the duration of exposure and the time after cessation of exposure. The percentages of total Hg present as inorganic Hg++ in tissues of the Iraqi population exposed for 2 months were: whole blood 7%, plasma 22%, breast milk 39% and urine 73%. Measurements in the hepatic tissue of patients that had died was 16-40% of Hg++.

The fecal pathway accounts for 90% of the total elimination of Hg in mammals after exposure to MeHg. Essentially all Hg in feces is in the inorganic form. The process of fecal elimination begins with biliary excretion with extensive recycling of both MeHg and Hg++ complexed with glutathione. Inorganic Hg is poorly absorbed across the intestinal wall, but MeHg is readily reabsorbed such that a secretion-resorption cycle is established. The intestinal microflora convert MeHg to inorganic Hg.

Whole body half-times determined in human volunteers averaged 70 days with a range of 52-93 days. Observations of blood half-times is 50 days with a range of 39-70 days. Lactating women have a significantly shorter whole body half-time of 42 days compared with 79 days in nonlactating women.

Selenium is known to bioconcentrate in fish and it is thought that simultaneous ingestion of selenium may offer a protective effect for the toxicity of MeHg based upon its antioxidant properties. Selenium has been observed to correlate with Hg levels in blood (Granjean and Weihe, 1992).

I.A.5 CONFIDENCE IN THE ORAL RfD

Study -- Medium
Data Base -- Medium
RfD -- Medium

The benchmark dose approach allowed use of the entire dose-response assessment and the calculation of a value that was consistent with the traditional NOAEL/LOAEL approach. In addition, the results of laboratory studies with nonhuman primates support the quantitative estimate of the NOAEL/LOAEL range of the benchmark dose that was indicated by the human studies. The reported literature covers detailed studies of human exposures with quantitation of MeHg by analysis of specimens from affected mother-fetus pairs. A strength of the Marsh study is the fact that the quantitative data came directly from the affected population and quantitation is based on biological specimens obtained from affected individuals. Unfortunately, a threshold was not easily defined and extended application of modeling techniques were needed to define the lower end of the dose-response curve. This may indicate high variability of response to MeHg in the human mother-fetal pairs or misclassification in assigning pairs to the cohort. Recent concerns expressed in the research community relate to the applicability of a dose-response estimate based on a grain-consuming population when the actual application is likely to help characterize risk for fish-consuming segments of the population. Confidence in the supporting data base is medium. Confidence in the RfD is medium.

I.A.6 EPA DOCUMENTATION

Source Document -- U.S. EPA, 1995

This IRIS summary is included in The Mercury Study Report to Congress, which was reviewed by OHEA and EPA's Mercury Work Group in November 1994. An interagency review by scientists from other federal agencies took place in January 1995. The report was also reviewed by a panel of non-federal external scientists in January 1995 who met in a public meeting on January 25-26. All reviewers comments have been carefully evaluated and considered in the revision and finalization of this IRIS summary. A record of these comments is summarized in the IRIS documentation files.

Other EPA Documentation -- U.S. EPA, 1980, 1984, 1987, 1988

Agency Work Group Review -- 12/02/85, 03/25/92, 02/17/94, 08/04/94, 09/08/94, 09/22/94, 10/13/94, 11/23/94

Verification Date -- 11/23/94

I.A.7 EPA CONTACTS (ORAL RfD)

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II. CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Substance Name -- Methylmercury
CASRN -- 22967-92-6
Preparation Date -- 5/24/94

II.A EVIDENCE FOR CLASSIFICATION AS TO HUMAN CARCINOGENICITY

II.A.1 WEIGHT-OF-EVIDENCE CLASSIFICATION

Classification -- C; possible human carcinogen

Basis -- Based on inadequate data in humans and limited evidence of carcinogenicity in animals. Male ICR and B6C3F1 mice exposed to methylmercuric chloride in the diet had an increased incidence of renal adenomas, adenocarcinomas and carcinomas. The tumors were observed at a single site and in a single species and single sex. The renal epithelial cell hyperplasia and tumors were observed only in the presence of profound nephrotoxicity and were suggested to be a consequence of reparative changes in the cells. Several nonpositive cancer bioassays were also reported. Although genotoxicity test data suggest that methylmercury is capable of producing chromosomal and nuclear damage, there are also nonpositive genotoxicity data.

II.A.2 HUMAN CARCINOGENICITY DATA

Inadequate. Three studies were identified that examined the relationship between methylmercury exposure and cancer. No persuasive evidence of increased carcinogenicity attributable to methylmercury exposure was observed in any of the studies. Interpretation of these studies, however, was limited by poor study design and incomplete descriptions of methodology and/or results.

Tamashiro et al. (1984) evaluated the causes of death in 334 subjects from the Kumamoto Prefecture who had been diagnosed with Minamata disease (methylmercury poisoning) and died between 1970 and 1980. The subjects involved fishermen and their families who had been diagnosed with the disease; thus, Minamata disease was used as a surrogate for methylmercury exposure. The controls were selected from all deaths that had occurred in the same city or town as the cases and were matched on the basis of sex, age at death (within 3 years) and year of death; two controls were matched to each subject. Malignant neoplasms were designated as the underlying cause of death in 14.7% (49/334) of the subjects and 20.1% (134/668) of the controls. For 47 subjects in which Minamata disease was listed as the underlying cause of death, the investigators reanalyzed the mortality data and selected one of the secondary causes to be the underlying cause of death in order to allow examination of the subjects and controls under similar conditions and parameters. The three subjects for which Minamata disease was listed as the only cause of death were excluded from further analysis. Using the Mantel-Haenzel method to estimate odds ratios, no significant differences were observed between the subjects and controls with respect to the proportion of deaths due to malignant neoplasms among males, females or both sexes combined. The estimated odds ratios and 95% confidence intervals were 0.84 (0.49-1.43), 0.58 (0.28-1.21) and 0.75 (0.50-1.11) for males, females and both sexes combined. Similarly, no increases in odds ratio were observed among the subjects relative to the controls when malignant neoplasms were

identified as a secondary cause of death or were listed on death certificates as one of the multiple causes of death. These data suggest that cancer incidence was not increased in persons with overt signs of methylmercury poisoning when compared with persons for whom no diagnosis of methylmercury poisoning had been made. Interpretation is limited by potential bias in designating the cause of death among patients with known Minamata disease and by the uncertainty regarding the extent of methylmercury exposure and undiagnosed Minamata disease among the controls. In a subsequent study, Tamashiro et al. (1986) compared the mortality patterns (between 1970 and 1981) among residents of the Fukuro and Tsukinoura districts in the Kumamoto Prefecture (inhabited mainly by fishermen and their families) with that of age-matched residents of Minamata City (also in the Kumamoto Prefecture) who died between 1972 and 1978. In this study, high exposure to methylmercury was inferred from residence in a district believed to have higher intake of local seafood. By contrast, in the 1984 study described above, high methylmercury exposure was inferred from a diagnosis of Minamata disease. A total of 416 deaths were recorded in the Fukuro and Tsukinoura districts in 1970-1981, and 2325 deaths were recorded in Minamata City in 1972-1978. No statistically significant increase in the overall cancer mortality rate was observed; however, an increase in the mortality rate due to liver cancer was observed (SMR, 207.3; 95% CI, 116.0-341.9). Analysis of mortality by sex showed a statistically significant increase in the rate of liver cancer only among males (SMR, 250.5; 95% CI, 133.4-428.4). Males also had statistically significant higher mortality due to chronic liver disease and cirrhosis. The authors note that these results should be interpreted with caution because the population of the Fukuro and Tsukinoura districts had higher alcohol consumption and a higher prevalence of hepatitis B (a predisposing factor for hepatocellular cancer). Interpretation of these results is also limited by an incomplete description of the methodology used to calculate the SMRs; it is unclear whether the study authors used appropriate methods to compare mortality data collected over disparate time frames (12 years for exposed and 7 years for controls).

In a study from Poland, Janicki et al. (1987) reported a statistically significant increase in mercury content of hair in leukemia patients (0.92 +/-1.44 ppm [sic]; n=47) relative to that in healthy unrelated patients (0.49 +/-0.41 ppm; n=79). Similarly, the mercury content in the hair of a subgroup of leukemia patients (0.69 +/- 0.75; n=19) was significantly greater than that in healthy relatives who had shared the same residence for at least 3 years preceding the onset of the disease (0.43 +/- 0.24 ppm; n=52). When patients with specific types of leukemia were compared with the healthy unrelated subjects (0.49 +/- 0.41 ppm; n=79), only those with acute leukemia (type not specified; 1.24 +/- 1.93 ppm; n=23) had a significantly increased hair mercury content. No significant differences in hair mercury content were observed in 9 patients with chronic granulocytic leukemia or 15 patients with chronic lymphocytic leukemia when compared with the unrelated, healthy controls. The authors inferred that acute leukemia was associated with increased level of mercury in hair. This study is of limited use for cancer risk assessment because of the following: uncertainty regarding the correlation between the chronology of incorporation of mercury in the hair and onset of the disease; the small population studied; the failure to describe adequately the characteristics of the leukemia patients or healthy controls (age distribution, length of residence in the region, criteria for inclusion in the study); uncertainty regarding the source of mercury exposure (the authors presumed that exposure was the result of use of methylmercury-containing fungicides); and the failure to address exposure to other chemicals or adjust for other leukemia risk factors. Furthermore, the variability of hair mercury content was large, and the mean hair mercury levels were within normal limits for all groups. Thus, the statistical significance may have been due to chance.

The carcinogenic effects of organomercury seed dressing exposure were investigated in a series of case-control studies for incidence of soft-tissue sarcomas (Eriksson et al., 1981, 1990; Hardell and

Eriksson, 1988) or malignant lymphomas (Hardell et al., 1981). These studies were conducted in Swedish populations exposed to phenoxyacetic acid herbicides or chlorophenols (the exposures of primary interest in the studies), organomercury seed dressings, or other pesticides. Exposure frequencies were derived from questionnaires and/or interviews. Control groups from the same region of the country were matched to cases based on vital status. A total of 402 cases of soft-tissue sarcoma and (among persons not exposed to phenoxyacetic acid herbicides) 128 cases of malignant lymphoma were reported. In each study, the odds ratio for exposure to organomercury in seed dressings and the incidence of sarcoma or lymphoma was either <1.0 or the range of the 95% confidence interval for the odds ratio included 1.0; therefore, no association was indicated for organomercury exposure and soft-tissue sarcoma or malignant lymphoma. The study subjects were likely to have experienced exposures to the other pesticides and chemicals.

II.A.3 ANIMAL CARCINOGENICITY DATA

Limited. Three dietary studies in two strains of mice indicate that methylmercury is carcinogenic. Interpretation of two of the positive studies was complicated by observation of tumors only at doses that exceeded the MTD. A fourth dietary study in mice and four dietary studies in rats failed to indicate carcinogenicity associated with methylmercury exposure. Interpretation of four of the nonpositive studies was limited because of deficiencies in study design or failure to achieve an MTD.

Methylmercuric chloride (>99% pure) was administered in the diet at levels of 0, 0.4, 2 or 10 ppm (0, 0.03, 0.15 and 0.73 (mg/kg)/day in males and 0, 0.02, 0.11 and 0.6 (mg/kg)/day in females) to 60 ICR mice/sex/group for 104 weeks (Hirano et al., 1986). Interim sacrifices (6/sex/group) were conducted at 26, 52 and 78 weeks. Complete histopathological examinations were performed on all animals found dead, killed in extremis or killed by design. Mortality, group mean body weights and food consumption were comparable to controls. The first renal tumor was observed at 58 weeks in a high-dose male, and the incidence of renal epithelial tumors (adenomas or adenocarcinomas) was significantly increased in high-dose males (1/32, 0/25, 0/29 and 13/26 in the control, low-, mid- and high-dose groups, respectively). Ten of the 13 tumors in high-dose males were adenocarcinomas. These tumors were described as solid type or cystic papillary types of adenocarcinomas. No invading proliferation into the surrounding tissues was observed. The incidence of renal epithelial adenomas was not significantly increased in males, and no renal adenomas or adenocarcinomas were observed in any females studied. Focal hyperplasia of the tubular epithelium was reported to be increased in high-dose males (13/59; other incidences not reported). Increases in non-neoplastic lesions in high-dose animals provided evidence that an MTD was exceeded. Non-neoplastic lesions reported as increased in treated males included the following: epithelial degeneration of the renal proximal tubules; cystic kidney; urinary cast and pelvic dilatation; and decreased spermatogenesis. Epithelial degeneration of the renal proximal tubules and degeneration or fibrosis of the sciatic nerve was reported in high-dose females.

Methylmercuric chloride (>99% pure) was administered in the diet at levels of 0, 0.4, 2 or 10 ppm (0, 0.3, 0.14 and 0.69 (mg/kg)/day in males and 0, 0.03, 0.13 and 0.60 (mg/kg)/day in females) to 60 B6C3F1 mice/sex/group for 104 weeks (Mitsumori et al., 1990). In high-dose males, a marked increase in mortality was observed after week 60 (data presented graphically; statistical analyses not performed by authors). Survival at study termination was approximately 50, 60, 60 and 20% in control, low-, mid- and high-dose males, respectively, and 58, 68, 60 and 60% in control, low-, mid- and high-dose females, respectively. The cause of the high mortality was not reported. At study termination, the mean body

weight in high-dose males was approximately 67% of controls and in high-dose females was approximately 90% of controls (data presented graphically; statistical analyses not performed by study authors). The incidence of focal hyperplasia of the renal tubules was significantly increased in high-dose males (14/60; the incidence was 0/60 in all other groups). The incidence of renal epithelial carcinomas (classified as solid or cystic papillary type) was also significantly increased in high-dose males (13/60; the incidence was 0/60 in all other groups). The incidence of renal adenomas (classified as solid or tubular type) was also significantly increased in high-dose males; the incidence was 0/60, 0/60, 1/60 and 5/60 in control, low-, mid- and high-dose males, respectively, and 0/60, 0/60, 0/60 and 1/60 in control low-, mid- and high-dose females, respectively. No metastases were seen in the animals. The incidences of a variety of non-neoplastic lesions were increased in the high-dose mice including the following: sensory neuropathy; neuronal necrosis in the cerebrum; neuronal degeneration in the cerebellum; and chronic nephropathy of the kidney. Males exhibited tubular atrophy of the testis (1/60, 5/60, 2/60 and 54/60 in control, low-, mid- and high-dose, respectively) and ulceration of the glandular stomach (1/60, 1/60, 0/60 and 7/60 in control, low-, mid- and high-dose males, respectively). An MTD was achieved in mid-dose males and high-dose females. High mortality in high-dose males indicated that the MTD was exceeded in this group.

Mitsumori et al. (1981) administered 0, 15 or 30 ppm of methylmercuric chloride (>99% pure) in the diet (0, 1.6 and 3.1 (mg/kg)/day) to 60 ICR mice/sex/group for 78 weeks. Interim sacrifices of up to 6/sex/group were conducted at weeks 26 and 52. Kidneys were microscopically examined from all animals that died or became moribund after week 53 or were killed at study termination. Lungs from mice with renal masses and renal lymph nodes showing gross abnormalities were also examined. Survival was decreased in a dose-related manner; at week 78 survival was 40, 10 and 0% in control, low- and high-dose males, respectively, and 55, 30 and 0%, in control, low- and high-dose females, respectively (statistical analyses not performed). The majority of high-dose mice (85% males and 98% females) died by week 26 of the study. Examination of the kidneys of mice that died or were sacrificed after 53 weeks showed a significant increase in renal tumors in low-dose males (13/16 versus 1/37 in controls). The incidence of renal epithelial adenocarcinomas in control and low-dose males was 0/37 and 11/16, respectively. The incidence of renal epithelial adenomas in control and low-dose males was 1/37 and 5/16, respectively. No renal tumors were observed in females in any group. No metastases to the lung or renal lymph nodes were observed. Evidence of neurotoxicity and renal pathology were observed in the treated mice at both dose levels. The high mortality in both groups of treated males and in high-dose females indicated that the MTD was exceeded in these groups. (Note: Hirano et al. (1986) was a followup to this study.)

Mitsumori et al. (1983, 1984) administered diets containing 0, 0.4, 2 or 10 ppm of methylmercuric chloride (0, 0.011, 0.05 and 0.28 (mg/kg)/day in males; 0, 0.014, 0.064 and 0.34 (mg/kg)/day in females) to 56/sex/group Sprague-Dawley rats for up to 130 weeks. Interim sacrifices of 10/group (either sex) were conducted at weeks 13 and 26 and of 6/group (either sex) at weeks 52 and 78. Mortality was increased in high-dose males and females. At week 104, survival was approximately 55, 45, 75 and 10% in control, low-, mid-and high-dose males, respectively, and 70, 75, 75 and 30% in control, low-, mid- and high-dose females, respectively (data presented graphically). All males in the high-dose group had died by week 119. Body weight gain was significantly decreased in high-dose males starting after week 44 and females
after 44 weeks (approximately 10-20%, data presented graphically). No increase in tumor incidence was observed in either males or females. Noncarcinogenic lesions that were significantly increased in high-dose rats included the following: degeneration in peripheral nerves and the spinal cord (both sexes); degeneration of the proximal tubular epithelium (both sexes); severe chronic nephropathy

(females); parathyroid hyperplasia (both sexes); polyarteritis nodosa and calcification of arterial wall (females); fibrosis of bone (females); bile duct hyperplasia (males); and hemosiderosis and extramedullary hematopoiesis in the spleen (males). Mid-dose males exhibited significantly increased degeneration of the proximal tubular epithelium and hyperplasia of the parathyroid. An MTD was achieved in mid-dose males and exceeded in high-dose males and high-dose females.

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

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

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

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

II.A.4 SUPPORTING DATA FOR CARCINOGENICITY

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

Humans ingesting methylmercury-contaminated foods have been reported to experience chromosomal aberrations (Skerfving et al., 1970, 1974) or SCE (Wulf et al., 1986); however, interpretation of these studies is limited by methodological deficiencies.

As reviewed in WHO (1990), methylmercury is not a potent mutagen but appears to be capable of causing chromosome damage and nuclear perturbations in a variety of systems. In Bacillus subtilis, methylmercury produced DNA damage (Kanematsu et al., 1980). Methylmercury produced chromosomal aberrations and aneuploidy in human peripheral lymphocytes (Betti et al., 1992), SCE in human lymphocytes (Morimoto et al., 1982), and DNA damage in human nerve and lung cells as well as Chinese hamster V-79 cells and rat glioblastoma cells (Costa et al., 1991).

Bone marrow cells of cats treated with methylmercury in a study by Charbonneau et al. (1976) were examined by Miller et al. (1979). The methylmercury treatment resulted in an increased number of nuclear abnormalities and an inhibition of DNA repair capacity. Methylmercury induced a weak mutagenic response in Chinese hamster V-79 cells (Fiskesjo, 1979). Methylmercury also induced histone protein perturbations and influenced factors regulating nucleolus-organizing activity (WHO, 1990). Moreover, methylmercury has been reported to interfere with gene expression in cultures of glioma cells (WHO, 1990). Mailhes (1983) reported a significant increase in the number of hyperploid oocysts in Lak:LVG Syrian hamsters fed methylmercury; however, no evidence of chromosomal damage was reported. Suter (1975) concluded that strain-specific differences exist with respect to the ability of methylmercury to produce dominant lethal effects in mice. Nondisjunction and sex-linked recessive lethal mutations were observed in Drosophila melanogaster treated with methylmercury (Ramel, 1972 as cited in U.S. EPA, 1985). Methylmercury produced single strand breaks in DNA in cultured L5178Y cells (Nakazawa et al., 1975).

Negative studies have also been reported. Methylmercury acetate was reported to be negative in a Salmonella typhimurium assay and a mouse micronucleus assay (Heddle and Bruce, 1977, as reported in Jenssen and Ramel, 1980). Methylmercury was not mutagenic and did not cause recombination in Saccharomyces cerevisiae but did slightly increase chromosomal nondisjunction (Nakai and Machida, 1973). Matsumoto and Spindle (1982) reported no significant increase in SCE in developing mouse embryos; they did report, however, that the developing mouse embryos were highly sensitive to in vitro treatment with methylmercury.

_II.B QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

None. The two studies by Mitsumori et al. (1981, 1990) were limited by high mortality in the high-dose males, the only group to exhibit a statistically significant increase in tumor incidence. Tumors were observed only in those dose groups in which the MTD had been exceeded. The study by Hirano et al. (1986) was not limited by low survival, but the tumors were observed in conjunction with nephrotoxicity and, thus, their incidence may have been a high-dose phenomenon that would not be expected to occur at low doses. The tumors appeared to originate from focal hyperplasia of the tubular epithelium induced as a reparative change. The hyperplasia was not observed in tubular epithelium that was undergoing early degenerative changes. Thus, the tumors may not occur where degenerative changes do not occur. The genotoxicity data indicate that methylmercury is not a potent mutagen but may produce chromosomal damage; these data do not support a hypothesis that methylmercury is a genotoxic carcinogen. It appears, rather, that methylmercury exerts its carcinogenic effect only at high dose, at or above an MTD. Because the linearized multistage procedure is based on the assumption of linearity at low doses, the relevance of deriving a slope factor based on data for which a threshold may exist is questionable.

It is likely that systemic non-cancer effects would be seen at methylmercury exposures lower than those required for tumor formation. Long-term administration of methylmercury to experimental animals produces overt symptoms of neurotoxicity at daily doses an order of magnitude lower than those required to induce tumors in mice.

_II.C QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM INHALATION EXPOSURE

None.

_II.D EPA DOCUMENTATION, REVIEW, AND CONTACTS (CARCINOGENICITY ASSESSMENT)

__II.D.1 EPA DOCUMENTATION

Source Documents -- U.S. EPA, 1995

This IRIS summary is included in The Mercury Study Report to Congress which was reviewed by OHEA and EPA's Mercury Work Group in November 1994. An interagency review by scientists from other federal agencies took place in January 1995. The report was also reviewed by a panel of non-federal external scientists in January 1995 who met in a public meeting on January 25-26. All reviewers comments have been carefully evaluated and considered in the revision and finalization of this IRIS summary. A record of these comments is summarized in the IRIS documentation files.

_II.D.2 REVIEW (CARCINOGENICITY ASSESSMENT)

Agency Work Group Review -- 03/03/94

Verification Date -- 03/03/94

_II.D.3 U.S. EPA CONTACTS (CARCINOGENICITY ASSESSMENT)

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APPENDIX C

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APPENDIX D

HEALTH EFFECTS OF MERCURY AND MERCURY COMPOUNDS
UNCERTAINTY ANALYSIS OF THE METHYLMERCURY RfD

D.1 Introduction and Background

The purpose of the analysis in this appendix is two-fold: first, to determine plausible bounds on uncertainty associated with the data and dose conversions used to derive the methylmercury Reference Dose (RfD); second, to compare the RfD to estimated distributions of human population thresholds for adverse effects. The analysis presented in this appendix is a modeled estimate of the human threshold for specific health effects attributable to methylmercury exposure. The basis for the analysis and the RfD is the data from the 1971 Iraqi methylmercury poisoning incident, specifically the data from the Marsh *et al.* (1987) study. The analysis also includes studies pertinent to the conversion of mercury concentrations in hair to estimated ingestion levels. The population studied in Marsh *et al.* (1987) is hereafter referred to as the Iraqi cohort. The methylmercury RfD was based on a benchmark dose calculated from the combined developmental effects of late walking, late talking, mental effects, seizures and neurological effects (scores greater than 3 on a test) in children of women exposed during pregnancy; benchmark doses for the individual developmental effects and for adult paresthesia were also calculated. All the benchmark doses for developmental endpoints were calculated from the Iraqi cohort data. The adult paresthesia benchmark dose was calculated from data presented in Bakir *et al.* (1973). The studies and their use in the calculation of the RfD for methylmercury are described in detail in chapters 3 and 6 of Volume IV of this Report.

The approach used in this analysis and the EPA's RfD methodology presuppose the existence of thresholds for certain health effects. The RfD is defined by the U.S. EPA (U.S. EPA, 1995) as

> an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

This definition implies that the RfD is an exposure level that is below the threshold for adverse effects in a sensitive subpopulation. For purposes of this analysis, the human population threshold is defined as the threshold for the most sensitive individual of an identified sensitive subpopulation. The definition of sensitive subpopulations excludes hypersensitive individuals whose susceptibilities fall far outside the normal range. A threshold is defined as the level of exposure to an agent or substance below which a specific effect is not expected to occur. The definition of threshold does not include concurrent exposure to other agents eliciting the same effect by the same mechanism of action. In other words, there is an assumption that the induced response is entirely a result of exposure to a single agent. The adverse health endpoints for the methylmercury RfD as determined by the RfD/RfC Workgroup are the specific clinically-observed endpoints reported in Marsh *et al.* (1987). The uncertainty analysis was confined to those endpoints. The 81 pregnant female/offspring pairs comprising the Iraqi cohort were taken as a surrogate for the most sensitive subpopulation expected in the general U.S. fish consuming population. The sensitive subpopulation was specifically identified as humans exposed to methylmercury *in utero*.

Other analyses of the Iraqi cohort data are available in the literature but are not directly applicable to the estimation of threshold distributions. An analysis presented in the Seafood Safety report (NAS, 1991) groups the Iraqi cohort observations by ranges of measured mercury concentrations in hair in order to estimate the cumulative response distribution. The response data grouped by hair mercury concentrations groupings were used to calculate the benchmark dose levels on which the methylmercury RfD was based. As any grouping of data introduces an additional level of uncertainty, this threshold analysis was based on the ungrouped observations.

Cox *et al.* (1989) presented estimates of thresholds based on the ungrouped observations of the Iraqi cohort for two of the five developmental endpoints considered by the U.S. EPA in the derivation of the methylmercury RfD. Cox *et al.* (1989) used a threshold model that included the threshold as a parameter. The value of the threshold parameter was estimated by a statistical method that optimized the likelihood at different values of the threshold. The estimated threshold for late walking in offspring (first walking after 18 months) was 7.3 ppm mercury in hair with an upper 95% confidence limit of 14 ppm. This threshold value was based on the best (optimized likelihood) estimate for background incidence of late walking of 0%. The upper 95% confidence limit was highly sensitive to the value of the background parameter, increasing to 190 ppm mercury in hair for a background of 4%. The optimized likelihood threshold for neurological effects (neurological scores > 3) based on a background incidence of 9% was 10 ppm mercury in hair with an upper 95% confidence limit of 287 ppm.

The analysis examined the major sources of uncertainty explicitly and implicitly inherent to the methylmercury RfD and attempted to bound them quantitatively. There are a number of sources of uncertainty in the estimation of either a human threshold or an RfD from the Iraqi cohort data and from the dose conversion used to estimate ingestion dose levels from hair mercury concentrations. The principal uncertainties arise from the following sources: the variability of susceptibilities within the Iraqi cohort; population variability in the pharmacokinetic processes reflected in the dose conversion; response classification error; and exposure classification error.

The data show a very broad range of susceptibilities in the 81 individuals of the Iraqi cohort. An analysis of the response rates based on hair mercury concentrations showed up to a 10,000-fold span between the 5^{th} and 95^{th} percentiles when projected to the general population (Hattis and Silver, 1994). Uncertainty in threshold estimates arising from the variability in individual susceptibilities was estimated by calculating a distribution of thresholds from a regression model for repeated bootstrap samples of the original Iraqi cohort data set. The bootstrap procedure and regression model are described in section D.2.1. The bootstrap procedure results in a distribution of population thresholds for specific effects in units of ppm mercury in hair.

The methylmercury RfD used a dose conversion formula (section 6.3.1.1 of Volume IV of this report) to estimate the ingestion dose in mg methylmercury per kg body weight per day (mg/kg-day) that would result in a specified mercury concentration in hair. This formula comprises a number of variables that are associated with biological processes. There are measured ranges for each variable which can be attributed to interindividual variability in pharmacokinetics and to experimental variation.

The response classification is the assignment of an individual observation to one of two categories -- responder or nonresponder. The response classification for each of the developmental endpoints reported in Marsh *et al.* (1987) is based on a fixed value (response decision point) that constitutes a response when exceeded. It is possible that some observations, particularly those that represent responses in the immediate vicinity of the response decision point, were misclassified; a responder may have been classified as a nonresponder or *vice versa*. The response classifications for late walking and late talking are particularly susceptible to this type of error. The response estimates were based on subject recall in members of a population that does not traditionally record these events. The classification of neurological test battery scores is more objective but still susceptible to some degree of investigator interpretation and misclassification.

Exposure classification error is the inclusion of individuals in the exposure group who had been exposed outside a critical period. This type of error is a source of uncertainty for all developmental endpoints that have a critical period of exposure combined with uncertainty about the actual timing of the

gestational period. The result of this type of error is the misclassification of an unexposed individual as an exposed individual. The consequence of this misclassification is an overestimation of the exposure level associated with a given response or nonresponse and subsequent overestimation of population variability. For example, in the Iraqi cohort it is noted that an individual with the highest estimated mercury exposure is a non-responder for developmental effects on the nervous system. This may be due to differences in individual susceptibility to methyl mercury toxicity, or it may be a consequence of misclassification; the individual may have been exposed during a period of time which is not critical to development. There is potential for misclassification as the determination of the correspondence of gestational period and exposure was dependent on subject recall. Data pertaining to this type of uncertainty are not yet available.

Other areas of uncertainty are those directly related to the RfD methodology. Specifically, it was concluded by an Agency Work Group that there were no adequate chronic or reproductive studies. An uncertainty factor of 10 is generally applied when chronic studies are not available. This uncertainty factor is based on an assumption inherent to the RfD methodology that increased exposure duration will lower the dose required for observation of the effect. Support for this assumption has been published (Weil and McCollister, 1963; Dourson and Stara, 1989) and is discussed in section D.2.2.2 of this Appendix. An uncertainty factor of 3 is generally applied if reproductive studies are not available. No-Observed-Adverse-Effect Levels (NOAELs) for reproductive studies are generally 2-fold to 3-fold higher than NOAELs for chronic studies and are not expected to be the basis for the RfD more than 5% of the time (Dourson, Knauf and Swartout, 1992).

D.2 Methods

Thresholds were estimated in a two-stage process. The first stage was the estimation of threshold distributions based on hair mercury concentrations, which was accomplished by applying a regression model to successive bootstrap samples of the observations in Marsh *et al.* (1987). This process is detailed in section D.2.1. The second stage was the conversion of the thresholds expressed as ppm mercury in hair to mg methylmercury per kg body weight per day (mg/kg-day); this involved a Monte Carlo analysis of the variability of the underlying biological processes. The dose-conversion model is described in section D.2.2.

For the uncertainty analysis thresholds for four of the six endpoints evaluated for the methylmercury RfD and for combined developmental effects were estimated. The developmental effects included in the threshold analysis were late walking, late talking and neurological effects. Thresholds for seizures and mental symptoms were not estimated because these effects occurred at 5-fold higher hair-mercury concentrations than did the other effects. As the resulting thresholds would be much higher than the others they would not be expected to contribute significantly to the combined developmental effects threshold distribution as defined for this analysis (the lowest of the individual effect thresholds for each bootstrap sample). Response rates for seizures and mental symptoms would be expected to influence the benchmark dose, however, as the benchmark dose is a function of all responses. The data used to estimate thresholds for adult paresthesia were not the same as those used to calculate the benchmark dose in the derivation of the methylmercury RfD. The benchmark dose was calculated from the data presented in Bakir *et al.* (1973). The threshold estimates were calculated from the Iraqi cohort data so that all thresholds would be estimated from the same group of individuals to enable a direct comparison. The Iraqi cohort data are summarized in Table D-1. A plus (+) in Table D-1 indicates a positive response. A positive response for neurological effects was a neurological score greater than 3 as defined in Marsh *et al.* (1987). Positive responses for late walking and late talking were 18 months and 24 months (after birth), respectively (Marsh *et al.*, 1987).

Table D-1
Incidence of Developmental and Adult Effects as reported in Marsh, *et al.*, 1987

max ppm mercury in hair	neuro test scores > 3	late walking [a]	late talking [b]	adult pares-thesia	max ppm mercury in hair	neuro test scores > 3	late walking	late talking	adult pares-thesia
1					23				+
1					26				
1					38	+	+		
1					45	+			
1					48				
1			+		52				
1	+				59			+	+
1			+		60	+	+		
1					62				
1					72				
1					74			+	+
1					75				+
1					78	+		+	+
1	+				86	+		+	
1					98				
1	+				104	+			
2					114			+	
2					118				
2					154		+	+	+
2					196				
2					202				
2					242				
2					263	+			
2					269				
2					294		+	+	+
3					336		+	+	+
3					339	+	+	+	+
5					357		+	+	+
6					362	+			+
6					376		+	+	+
7					399				
8					404	+	+	+	
9					405	+	+	+	
10					418	+	+	+	
10					443	+	+	+	
12					468	+			
12					557		+	+	
14		+		+	568	+	+	+	
16	+				598	+	+		
18		+	+		674		+	+	+
19									

[a] defined as first walking after 18 months
[b] defined as first talking after 24 months

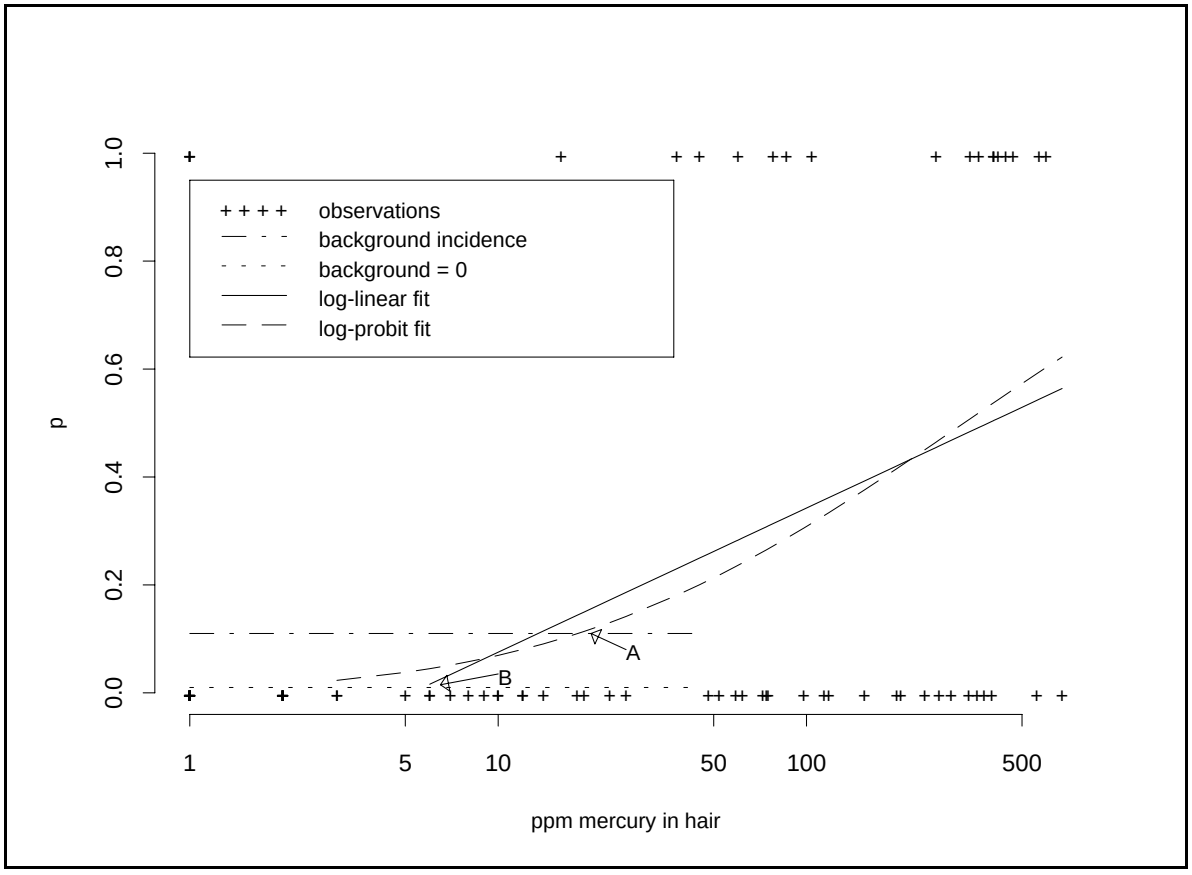
All threshold calculations and Monte Carlo simulations were performed in S-PLUS® (ver 3.2) for Microsoft® Windows® (ver 3.1) on several microprocessors based on the Intel® 486DX2/66 microprocessors. Sensitivity analyses were performed in Crystal Ball® (ver 3.0) and Excel® (ver 4.0) for Windows®.

D.2.1 Estimation of Thresholds Based on ppm Mercury in Hair

Hair mercury concentrations at the thresholds for adult paresthesia, three developmental endpoints and combined developmental effects were estimated from the Iraqi cohort data. Threshold estimation was accomplished by applying a regression model to successive bootstrap samples of the 81 observations. The bootstrap method (Efron and Tibshirani, 1991; 1993) is a nonparametric approach that can be used for estimation of confidence intervals on a given variable without assuming a specific parametric distribution for that variable. The bootstrap method is based on the assumption that the observed sample is a random sample of a larger population and provides an estimate of sample-size uncertainty. The bootstrap process consists of taking a random sample of the same size as the observed sample distribution from the original sample distribution. The sampling is conducted with replacement of selected observations prior to the next random selection such that individual observations may appear more than once in any given sample. In this case, the bootstrap approach was applied in order to allow estimation of confidence intervals on the dose associated with a given response. For this analysis, 5000 bootstrap samples were generated. Thresholds, in units of ppm mercury in hair, for each endpoint were calculated for each bootstrap sample. The output was a distribution of 5000 thresholds for each endpoint representing the variability in the population threshold as estimated from the Iraqi cohort data. The threshold distribution for combined effects was defined as the minimum of the single-effect threshold hair-mercury concentrations calculated at each bootstrap iteration; this definition is based on the assumption that the endpoints were independent. The stability of the bootstrap was evaluated by determining the change in the 5^{th} and 95^{th} percentiles, and their ratio, for each doubling of the number of iterations. The bootstrap was considered to be stable if successive estimates were within 5% of each other.

The threshold model used in this analysis treated background incidence and response related to exposure (induced response) independently. The procedure is illustrated in Figure D-1, which shows the Marsh *et al.*, 1987 data and regression lines for developmental neurological effects (neurological score > 3). Figure D-1 is an example of a threshold determination from a single iteration of the bootstrap procedure. All of the response data were binary; that is, individuals were either responders or nonresponders for a given effect. The binary responses associated with each hair mercury concentration are indicated by a "+" at the top and bottom of the chart for responders and nonresponders, respectively. The mean background and fitted regression lines for induced response are shown. The threshold was defined in the regression model as the concentration of mercury in hair corresponding to the fitted response equal to the background incidence. This is equivalent to the point of intersection of the background and induced response line indicated as point A in Figure D-1. This model was chosen so that the threshold estimate was a consequence of, rather than a contribution to, the estimation of background and induced response. Other threshold models that could have been used, such as the one used by Cox *et al*. (1989), include the threshold as a parameter to be simultaneously estimated with all other model parameters. Also the maximum likelihood approach for parameter estimation was not used because of the apparent extreme sensitivity of the upper 95% confidence limit on the threshold to variation in the background estimate (Cox *et al*., 1989).

Figure D-1
Regression Model for Determination of Bootstrap Thresholds



lognormal (GM = 250, GSD = 1.35)
pd = probability density

In the regression model, response was regressed on the logarithm of dose using the probit function (log-probit model). In those cases where the log-probit model-predicted responses were always greater than background, a log-linear regression model was used to determine the threshold (point B in Figure D-1). The log-probit and log-linear fitted regression lines are shown for one bootstrap sample in Figure D-1.

Hair mercury concentrations of 1 ppm were assumed to represent background exposure levels (Katz and Katz, 1992). All other observations, the first of which was at 14 ppm for any effect, were included in the estimation of background and induced response rate as follows. Background incidence for each effect was estimated directly from each bootstrap sample by performing repeated regressions of response on hair mercury concentrations, starting with the assumed background range and successively adding data points at the next higher hair mercury concentration until the regression slope was near zero and was least statistically significant. Background incidence was defined as the mean of the fitted values of the resulting regression. The induced response regression slope was calculated in a similar fashion, starting with all observations above concentrations of 12 ppm hair-mercury and successively adding data points at the next lower hair mercury concentration until the regression slope was maximized and statistically significant ($p < 0.05$).

D.2.2 Estimation of Ingestion Dose Levels in mg/kg-day

D.2.2.1 Estimation of Dose Conversion Uncertainty

The uncertainty arising from the calculation of ingestion dose levels, in mg/kg-day, corresponding to measured concentrations of mercury in hair was estimated through analysis of the dose conversion formula. The formula, which estimates ingested dose levels corresponding to the measured methylmercury concentration in hair, incorporates the formula used in the derivation of the RfD with the inclusion of an additional term to account for the hair to blood concentration ratio for methylmercury and the conversion of elimination constants to their equivalent half-lives. The latter was done as a matter of convenience as most of the studies reported half-lives rather than elimination constants. The formula used in the derivation of the RfD is described in Chapter 6 (section 6.3.1.1) of Volume IV of this report and is reproduced here as equation 1.

$$d = \frac{C \times b \times V}{A \times f \times bw} \qquad (1)$$

where

d is the daily dietary intake in mg/kg-day,
C is the concentration of methylmercury in the blood in µg/liter,
b is the elimination constant (of methylmercury from the blood) in $days^{-1}$,
V is the volume of blood in the body in liters,
A is the fraction of mercury in the diet that is absorbed,
f is the fraction of absorbed mercury that is found in the blood.
bw is body weight in kg.

Variable **C** in formula 1 can be related to the concentration of mercury in hair by the formula given in equation 2.

$$C = \frac{Hg_h}{hb} \qquad (2)$$

where

$\mathbf{Hg_h}$ is the concentration of mercury in hair in ppm (µg mercury/g hair),
hb is the hair to blood concentration ratio for methylmercury in µg mercury/g hair/(µg mercury/ml blood).

Variable **b** in formula 1, which is a first-order elimination rate constant, and the clearance half-life arerelated by the formula given in equation 3.

$$b = \frac{\log_e 2}{t_{1/2}}$$

(3)

where

b is the elimination constant,
$\log_e$ 2 is the natural logarithm of 2 (= 0.693),
$t_{½}$ is the half-life of methylmercury in the blood.

Substituting for **C** and **b** in equation 1 from equations 2 and 3, respectively, gives the formula for ingestion levels based on mercury concentrations in hair (equation 4).

$$d = \frac{\log_e 2 \times Hg_h \times V}{hb \times t_{½} \times f \times A \times bw} \quad (4)$$

Dividing both sides of equation 4 by $\mathbf{Hg_h}$ gives the dose conversion factor, which when multiplied by a hair mercury concentration gives the corresponding ingestion level in mg/kg-day (equation 5).

$$DCF = \frac{\log_e 2 \times V}{hb \times t_{½} \times f \times A \times bw} \quad (5)$$

where

DCF is the dose conversion factor in ppm mercury in hair/(mg/kg-day).

Lower levels of exposure to methylmercury were expected to be associated with the observation of effects in adults for exposure durations longer than those observed for the Iraqi cohort (U.S. EPA, 1995; Barnes and Dourson, 1988). The potential effect of exposure duration on the dose eliciting chronic effects is given in equation 6.

$$DCF_{eda} = \frac{DCF}{U_D} \quad (6)$$

where

DCF_{eda} is the exposure-duration adjusted dose conversion factor in ppm mercury in hair/(mg/kg-day),
DCF is the dose conversion factor (from equation 5),
U_D is a unitless adjustment for uncertainty arising from limited exposure duration.

Monte Carlo simulations were conducted for equations 5 and 6. The output of these simulations were used to calculate a family of ingestion threshold distributions (in mg/kg-day) for each endpoint. This was done by multiplying the bootstrap threshold distribution for a given endpoint by specific percentiles of the appropriate dose conversion distribution. Each member of a family of distributions was associated with a specific probability dependent on the relative likelihood of the DCF.

D.2.2.2 Input Variable Distributions

Distributions were assigned to each variable in equations 5 and 6 based on the data available in the literature. The general form of the distribution, whether triangular, normal or lognormal, was determined by examination of the shape of the distribution of empirical data and by consideration of the underlying biological and physical processes. A triangular distribution was used when a judgement was made that the value of the variable fell within identifiable absolute limits. Many of the variables reflect underlying exponential processes and would be distributed as the logarithm of the nominal values. Such variables were described as being distributed in log space. In these cases a lognormal, or log-triangular distribution was chosen to represent the variable.

Determination of the distribution parameters focused on identifying the median (50^{th} percentile) and extreme percentiles from the available data. The focus was on the median, rather than the mean, in order to specify percentiles of the distribution. In general, the mean value of the Monte Carlo output is more closely related to the median, rather than the mean, of the inputs. The extreme percentiles were those corresponding to the lowest and highest observations and were determined by multiplying the rank order of the observation by 100/(n + 1), where n is the total number of observations. For example, the lowest and highest observations in a sample size of 9 define the 10^{th} and 90^{th} percentiles (80% confidence interval), respectively. Calculating the percentiles on the basis of n + 1, rather than n, allowed for the possibility of obtaining more extreme values in additional samples. The median and extreme percentiles were preserved in the final distribution whenever possible by adjusting the distribution parameters accordingly. The distribution assigned to each variable is given in Table D-2.

Table D-2
Dose Conversion Monte Carlo Simulation Input Variable Distributions

Variable	Form	Nominal [a] Value	Parameters [b]
hb [c]	lognormal	250 [f]	GM =250, GSD = 1.5
$\mathbf{t}_{½}$	log triangular	53 days	min = 1.455, mode = 1.676, max = 2.085 ($\log_{10}$)
V [d]	triangular	5 liters	min = 3.63, mode = 5.0, max = 6.37
f [e]	log triangular	0.059 [g]	min = -1.41, mode = -1.30, max = -0.934 ($\log_{10}$)
A	triangular	0.95 [g]	min = 0.90, mode = 0.95, max = 1.0
bw	lognormal	55 kg	GM = 55, GSD = 1.13

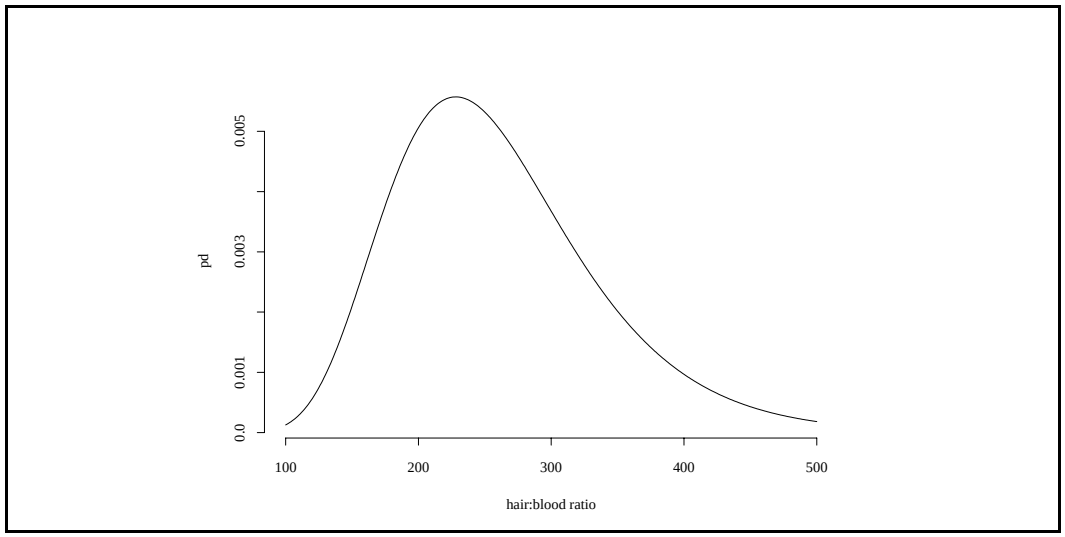
[a] median or geometric median
[b] Key: GM - geometric mean, GSD - geometric standard deviation; min - absolute minimum, mode - most likely value, max = absolute maximum
[c] correlated with $\mathbf{t}_{½}$ [correlation coefficient (r) = -0.5]
[d] correlated with **bw** (r = -0.47)
[e] correlated with **bw** (r = +0.57)
[f] µg Hg/g hair/mg Hg/l blood
[g] unitless ratio

*Hair to Blood Concentration Ratio for methylmercury (**hb**)*

This variable represents variation in a population of the ratio of the concentration of methylmercury in hair to the concentration of methylmercury in blood. The distribution for this variable was based on the EPA RfD/RfC Work Group's analysis of the available data, which is presented in Chapter 6 (section 6.3.1.1) of Volume IV of this report. The EPA RfD/RfC Work Group has judged that the most appropriate value for this variable lies between 200 and 300 based on results published by Phelps *et al.* (1980), with 250 selected as the point estimate. The value of 250 was used as the geometric mean of the distribution for **hb**. The data given in Phelps *et al.* (1980) were not detailed enough to allow determination of the shape or variance of the distribution. The lognormal form for the distribution was chosen as most representative of the empirical data reported in Sherlock *et al.* (1982). The geometric standard deviation (GSD) of 1.5 was estimated in this analysis from the same data (Sherlock *et al.*, 1982). The distribution is shown in Figure D-2.

A correlation coefficient (r) of -0.5 was assumed between **hb** and $\mathbf{t}_{½}$ in the Monte Carlo simulation of equation 5. The amount of mercury in the hair should be at least partially dependent on how quickly methylmercury is eliminated from the blood; that is, the faster that methylmercury is eliminated from the blood, the greater would be the difference between the concentration of mercury in the hair and mercury in the blood. The relationship between $\mathbf{t}_{½}$ and **hb** would be expected to be inverse (negative correlation); high values of $\mathbf{t}_{½}$ would be associated with low values of **hb**. The magnitude of the correlation coefficient was judged by the U.S. EPA to be at least as strong as -0.5. The data available for the calculation of the correlation between $\mathbf{t}_{½}$ and **hb** are extremely limited. A correlation coefficient between hair:blood concentration and half-life of about -0.3 was calculated in this analysis from data on four individuals (Kershaw *et al.*, 1980).

Figure D-2
Probability Density Function for Hair-to-Blood Concentration Ratio (hb)



lognormal (GM = 250, GSD = 1.35)
pd = probability density

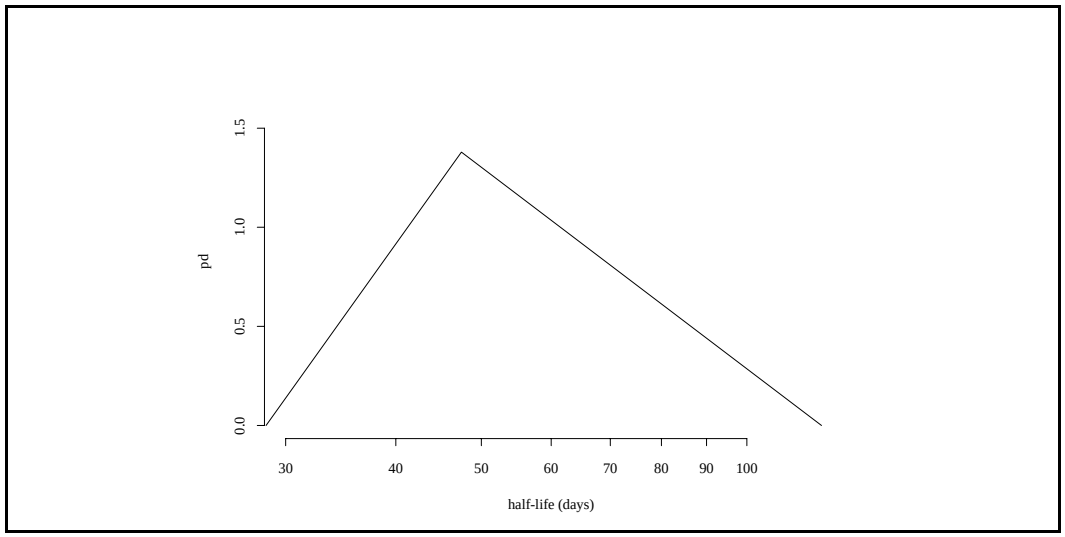
Half-Life of Methylmercury in the Blood ($t_{½}$)

Several human studies reported clearance half-lives for methylmercury from blood in the range of 32-105 days with averages of 45-70 days (Miettinen *et al.*, 1971; Bakir *et al.*, 1973; Greenwood et al., 1978; Kershaw *et al.*, 1980; Smith *et al.*, 1994). An average elimination constant for methylmercury from the blood of 0.014 with a range of 0.0099 to 0.0165 was reported by Sherlock, *et al.* (1984) corresponding to an average half-life of 50 days with a range of 42-70 days. Table D-3 gives the average and range of reported half-lives or half-lives calculated from equation 3 for each of the five studies. The values in Table D-3 were for male and female adults, combined. Average half-lives of methylmercury in the blood, as reported by Greenwood, *et al.* (1984), were somewhat shorter for lactating women (46 days) and longer for children (90 days) than for the adult average of 70 days. A histogram of the combined data was highly skewed and roughly triangular in shape. The log-triangular distribution (Figure D-3) was chosen as best representative of the empirical data. The median value of this distribution was 53 days; this was slightly higher than that used in the derivation of the methylmercury RfD (50 days), which would result in slightly lower dose conversion values.

Table D-3
Half-Life of Methylmercury in the Blood (days)

Reference	Low	Average	High
Smith et al. 1994	31.9	45.3	60
Sherlock et al. 1984	42	49.5	70
Kershaw et al. 1980	46.7	51.9	66.5
Bakir et al. 1973	40	65	105
Greenwood et al. 1978	49	70	95

Figure D-3
Probability Density Function for the Half Life of Methylmercury in the Blood ($t_{½}$)



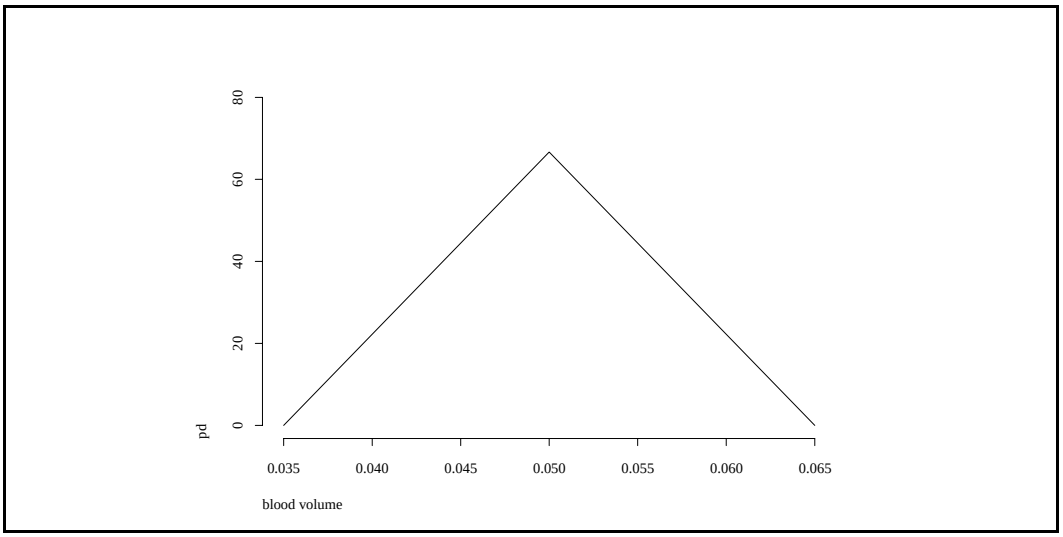
log triangular {min = 1.455, mode = 1.676, max = 2.085 ($\log_{10}$)}
pd = probability density

*Volume of Blood in the Body (**V**)*

This variable represents the variation in a population of the total volume of blood in the body. The distribution was based on published values of estimated whole blood volumes for a cohort of 20 pregnant Nigerian women (Harrison, 1966). Whole blood volumes in the third trimester of pregnancy ranged from 4.0 to 6.0 liters ; the mean and median values were both 5.0 liters (Harrison, 1966). The distribution of empirical data was roughly triangular and symmetrical. The minimum and maximum values were adjusted so that the range of observed values fell within the 90% confidence interval (n = 20). The distribution is shown in Figure D-4.

Blood volume was assumed to be positively correlated with body weight; larger blood volumes would be associated with higher body weights. For this analysis a correlation coefficient of 0.57 between **V** and **bw** from the data given in Harrison (1966) was calculated. This correlation coefficient was assumed for the standard Monte Carlo simulation of equation 5.

Figure D-4
Probability Density for Blood Volume (V)



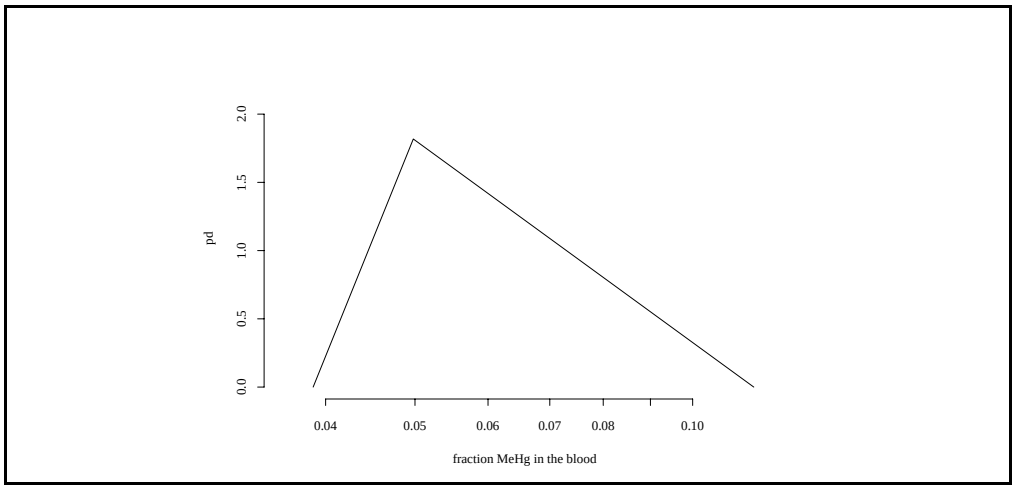
triangular {min = 3.5 liters, mode = 5.0 liters, max = 6.5 liters}
pd = probability density

*Fraction of Absorbed methylmercury in the Blood (**f**)*

This variable reflects the distribution and dilution of the absorbed methylmercury in all compartments of the body. The distribution on **f** was based on several human studies showing values in the range of 5-10% of the absorbed dose of methylmercury in the blood (Miettinen *et al.*, 1971; Kershaw *et al.*, 1980; Sherlock *et al.*, 1984). All of the measured values have been adjusted for an assumed total blood volume of 5 liters. The studies are summarized in Chapter 6 (section 6.3.1.1) of Volume IV of this report. The distribution is shown in Figure D-5. The median value of this distribution of 5.9% was higher than that used in the derivation of the methylmercury RfD (5%), which would result in lower dose conversion values.

Sherlock *et al.* (1984) reported that the fraction of methylmercury in the blood was negatively correlated with body weight; smaller fractions of methylmercury in the blood were associated with larger body weights. For this analysis the U.S. EPA calculated a correlation of -0.47 between **f** and **bw** from the data given in Sherlock *et al.* (1984). This correlation was assumed for the Monte Carlo simulation of equation 5.

Figure D-5
Probability Density for Fraction of Absorbed Methylmercury in the Blood (f)



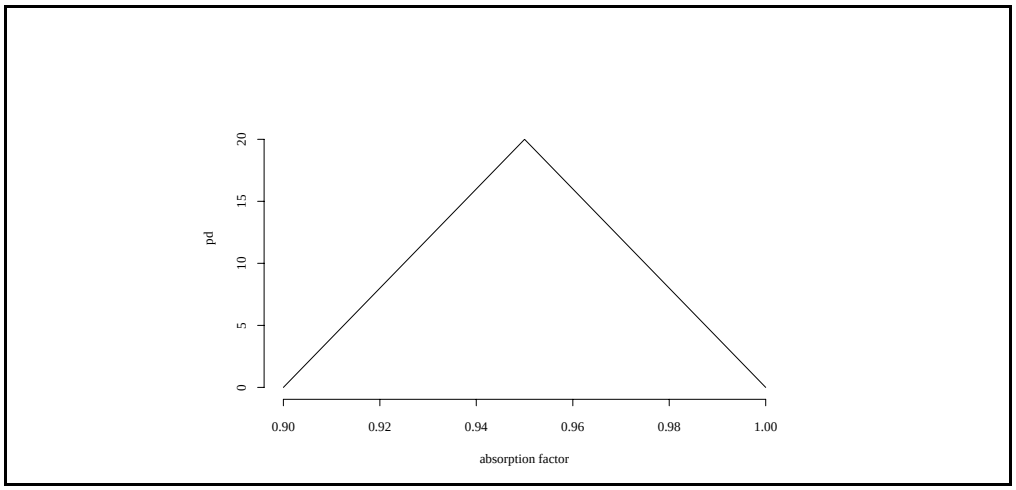
log triangular {min = -1.41, mode = -1.30, max = -0.934 ($\log_{10}$)}
pd = probability density

*Fraction of methylmercury in the Diet that is Absorbed (**A**)*

This distribution was based on the results of two human studies showing uptake of radio-labeled methylmercury of 95% and greater (Aberg *et al.*, 1969) and 94% and greater (Miettinen *et al.*, 1971) and animal studies showing 90% or greater absorption (summarized in Walsh, 1982). The distribution reflects the expectation that this value is close to 100 and will not vary much. The distribution is shown in Figure D-6.

Figure D-6

Probability Density for Fraction of Methylmercury Absorbed from the Gut (A)

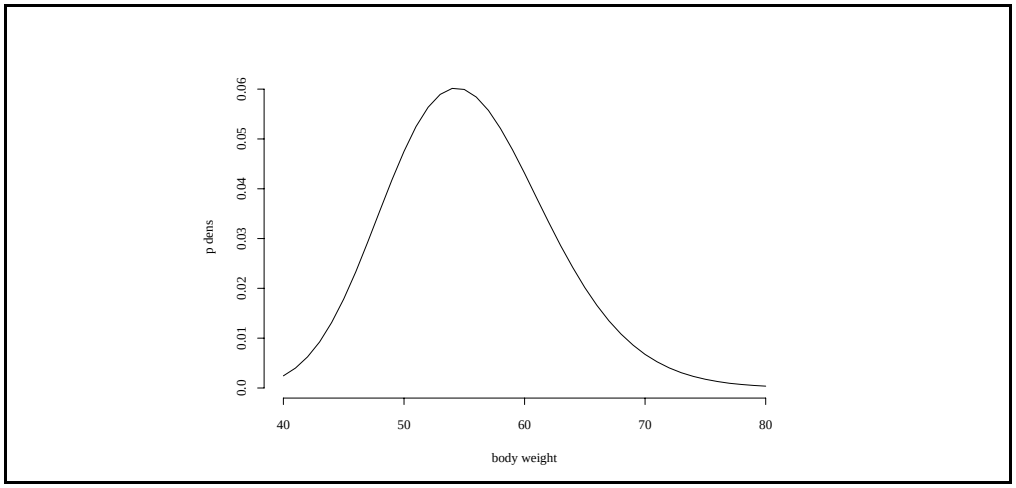


triangular {min = 0.90, mode = 0.95, max = 1.0}
pd = probability density

*Body Weight (**bw**)*

The distribution for body weight was based on Harrison (1966), previously described for the definition of **V**. The observed body weights during the third trimester of pregnancy ranged from 49.5 kg to 73.9 kg with a geometric mean of 55 kg (Harrison, 1966). A lognormal distribution was visually fitted to the data. The distribution is shown in Figure D-7. The median value of this distribution of 55 kg was lower than that used in the derivation of the methylmercury RfD (60 kg). Use of the lower value for **bw** which would result in higher dose conversion values.

Figure D-7
Probability Density Distribution for Body Weight (bw)



lognormal (GM = 55 kg, GSD = 1.13)
pd = probability density

Uncertainty Arising from Limited Exposure Duration (U_D)

This factor is an adjustment for the uncertain effects of exposure duration on the magnitude of the effective dose. It is based on the assumption that continuing exposure will result in the appearance of effects at exposure levels wherein there were no effects observed following shorter exposure durations. The U.S. EPA commonly applies an uncertainty factor of 10 when calculating a chronic RfD from a study of subchronic duration (U.S. EPA, 1995). In concept, the value of 10 for this uncertainty factor represents a high estimate of the uncertainty in order to maintain the protective nature of the RfD. An empirical analysis of the Weil and McCollister (1963) data by Dourson and Stara (1983) supports the use of an uncertainty factor of 10 as protective. About 50% of ratios of subchronic NOAELs to chronic NOAELS for rats exposed to a variety of substances other than methylmercury (as reported by Weil and McCollister, 1963) were below 3.5 and 95% were below 10 (Dourson and Stara, 1983).

The published data were insufficient for the estimation of a distribution for $\mathbf{U_D}$. A point estimate of 4.7 was made for $\mathbf{U_D}$ from a few studies of methylmercury toxicity in nonhuman primates. These studies are summarized in Table D-4. Table D-4 gives NOAELs and Lowest-Observed-Adverse-Effect Levels (LOAELs) for studies of short-term and long-term duration in monkeys. The neurologic endpoints were limited to clinically-observable effects in order to maintain approximate equivalence of effects across exposure durations. $\mathbf{U_D}$ was estimated by dividing the short-term LOAEL of 0.21 mg/kg-

day (Sato and Ikuta, 1975) by 0.045, the average of the two long-term exposure LOAELS of 0.04 and 0.05 mg/kg-day (Burbacher *et al.*, 1988; Rice and Gilber, 1992).

$\mathbf{U_D}$ was used in equation 6 to adjust the dose conversion factor for the estimation of the exposure level associated with chronic effects. Specifically, the exposure duration adjusted dose conversion factor ($\mathbf{DCF_{eda}}$) from equation 6 was multiplied by the adult paresthesia bootstrap threshold distribution to obtain an ingestion threshold distribution for chronic neurologic effects. The precise nature of the chronic effects was not specified because the effects observed in the monkey studies used to define $\mathbf{U_D}$ included a number of different neurologic effects. In this case the paresthesia observed in the Iraqi cohort was used as a surrogate for all possible adult neurologic effects that might occur following short-term exposure to methylmercury.

Table D-4
Methylmercury Toxicity in Animals

Reference	Exposure Duration	Effects	NOAEL	LOAEL
Sato & Ikuta 1975	36-132 days	ataxic gait, myoclonic seizures	0.07	0.21
Burbacher et al., 1988	3 years	slight tremor, motor incoordination, blindness	none	0.04
Rice & Gilbert, 1992	6.5-7 years	decreased fine motor performance, other	none	0.05

D.2.2.3 Correlation of Input Variables

Apart from the assumptions of correlation between individual input variables described previously (standard correlations), a simplifying assumption was that the susceptibility of any individual was independent of the value of the dose-conversion factor. It is very likely, however, that susceptibility and $\mathbf{t_{½}}$ are correlated. Longer residence times of methylmercury in the blood, corresponding to longer half-lives, should have a direct effect on toxicity. Thus, there would be some likelihood that the susceptibility of the individual at the population threshold (the most sensitive individual) would be related to larger values of $\mathbf{t_{½}}$. Monte Carlo analyses of equation 5 limiting $\mathbf{t_{½}}$ to values greater than 53 days (the median of the $\mathbf{t_{½}}$ distribution) or greater than 84 days (the 90th percentile of the $\mathbf{t_{½}}$ distribution) were also conducted. The latter simulation was included only to determine the sensitivity of the output to changes in the assumption and was not considered to be a realistic scenario. Standard input variable correlations were assumed for these simulations. Results of this simulation are presented in section D.3.

A sensitivity analysis was conducted to examine the effect of different correlation assumptions on the relative contribution of each input variable distribution to the variance of the Monte Carlo simulation output. The sensitivity analysis was performed for standard correlations, no correlations and for the alternate half-life ($\mathbf{t_{½}} > 53$ days or $\mathbf{t_{½}} > 84$ days) scenarios.

D.2.3 Estimation of Uncertainty Arising from Response Classification Error

A variable-response model was constructed to assess response classification error. The variable-response model is identical to the general threshold model except that responses presumed to be uncertain were given fractional values rather than 0 or 1 (for nonresponders and responders, respectively). Values of 0 or 1 were generated at each bootstrap sampling by comparison of the fractional value with random numbers drawn from a uniform distribution between 0 and 1. The uncertain observations were defined as those that fell close to the defined minimum response. For late walking the observations that fell into this category were those of 18 - 20 months (late walking = not walking by 18 months). For late talking the uncertain observations were 24 - 26 months (late talking = not talking by 24 months). A value of 0.5 was assigned to each of the observations for late walking and late talking that were designated uncertain; this represented the largest possible uncertainty in classification (50% classification error). A 50% classification error was judged to be plausible, given the highly variable factors involved in the original classifications in Marsh *et al*. (1987). A separate analysis was conducted for late walking assuming a 25% classification error. This was done to allow for the possibility that the large number of observations at exactly 18 months (22), was a result of 18 months being used as an upper bound, rather than an exact estimate. This could have occurred for observations that were uncertain but judged by the authors (Marsh *et al*., 1987) to be 18 months or less.

The determination of neurological scores in Marsh *et al*. (1987) was considered to be more objective than the determination of late walking or late talking. There is, however, a possibility that the distinction between adjacent scores is not absolute. The uncertain observations for neurological effects were scores of 3 or 4. Although there was no clear basis for determining classification error for this endpoint, the error was judged likely to be considerably less than for late walking and late talking. Simulations were run assuming a 10% or 20% classification error. A classification error rate of 20% was considered to be an upper bound.

There was no basis on which to determine the extent of classification error for adult paresthesia. In addition, there was no way to determine which responses (or nonresponses) were marginal. A 5% classification error was assumed for all observations to determine the sensitivity of the threshold simulation to small error rates.

D.3 Results

D.3.1 Bootstrap Analysis

Figures D-8 to D-10 show the frequency distributions of thresholds for the individual developmental endpoints resulting from the bootstrap analysis. Figure D-11 is the threshold distribution for the occurrence of any developmental effect. The figures are histograms of the frequency of occurrence of calculated threshold mercury concentrations resulting from 5,000 iterations of the bootstrap procedure. The threshold values on the abscissa are given in $\log_{10}$ units. These distributions represent uncertainty in the estimation of population thresholds calculated from hair mercury concentrations. The small separate peaks at about 600 ppm in Figures D-8 to D-10 represent bootstrap samples that result in a nonsignificant ($p > 0.05$) log-probit regression slope. A nonsignificant slope implies that there was no relationship between hair mercury concentrations and observed response for that bootstrap sample. In these cases the threshold was defined as the largest hair mercury concentration in the sample. The frequency with which nonsignificant slopes occurred was interpreted as a measure of the reliability of the endpoint as a measure of methylmercury toxicity. This occurred in 0.4% of the bootstrap samples for neurological effects, 0.1% of the late walking bootstrap samples and in 0.2% of the

late walking bootstrap samples. These percentages effectively limit the upper end of the bootstrap confidence intervals that can be defined meaningfully. As an example, the upper limit on the bootstrap confidence interval for the neurological effects threshold was 99.6%. The maximum two-tailed symmetrical confidence interval on the median threshold for this endpoint that excluded nonsignificant slopes was 99.2%.

At the other end of the distributions, the log-probit model fails to estimate a threshold when the lowest log-probit response is greater than the background incidence (log-probit regression line fails to intersect background in Figure D-1). This would occur in all bootstrap samples where a zero background incidence was estimated or when the calculated log-probit response at 3 ppm mercury in hair was greater than the background incidence for that sample. The threshold was calculated from the log-linear regression model in these cases (point B in Figure D-1); this occurred in 6%, 13.5% and 100% of the bootstrap samples for neurological effects, late talking and late walking, respectively. The average background incidence, as estimated from the Iraqi cohort data for each bootstrap sample, was 10.7% for neurological effects and 8.6% for late talking. Background incidence for late walking was 0% for all samples.

Figure D-8
Bootstrap Threshold Distribution for Developmental Neurological Effects

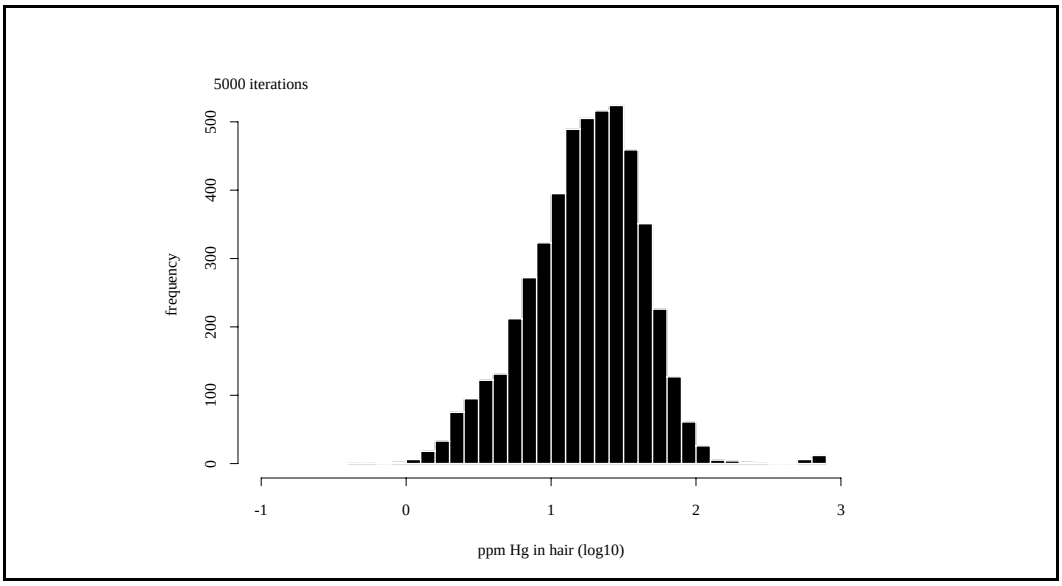


Figure D-9
Bootstrap Threshold Distribution for Late Walking

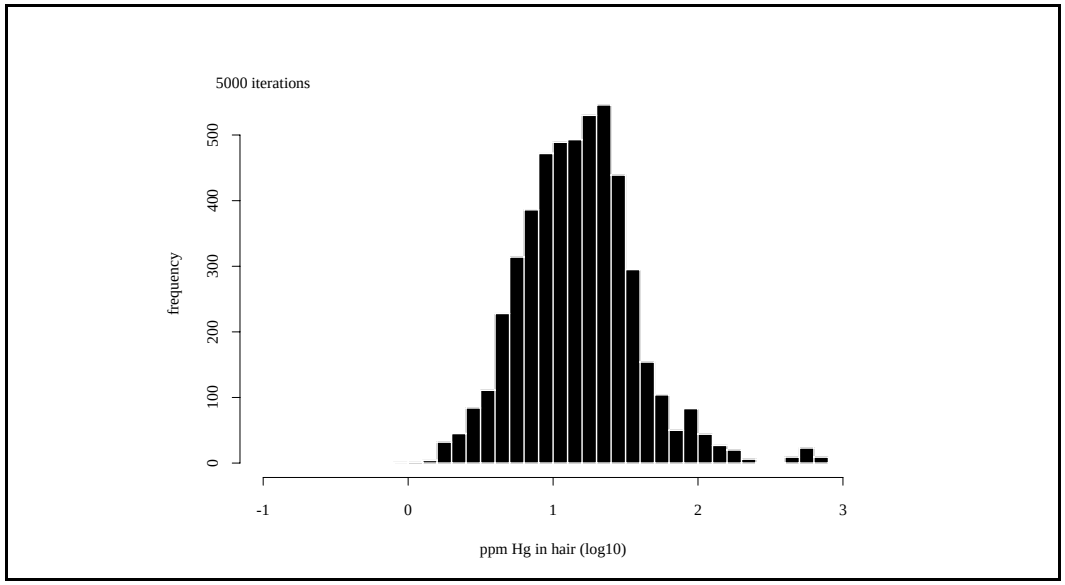


Figure D-10
Bootstrap Threshold Distribution for Late Talking

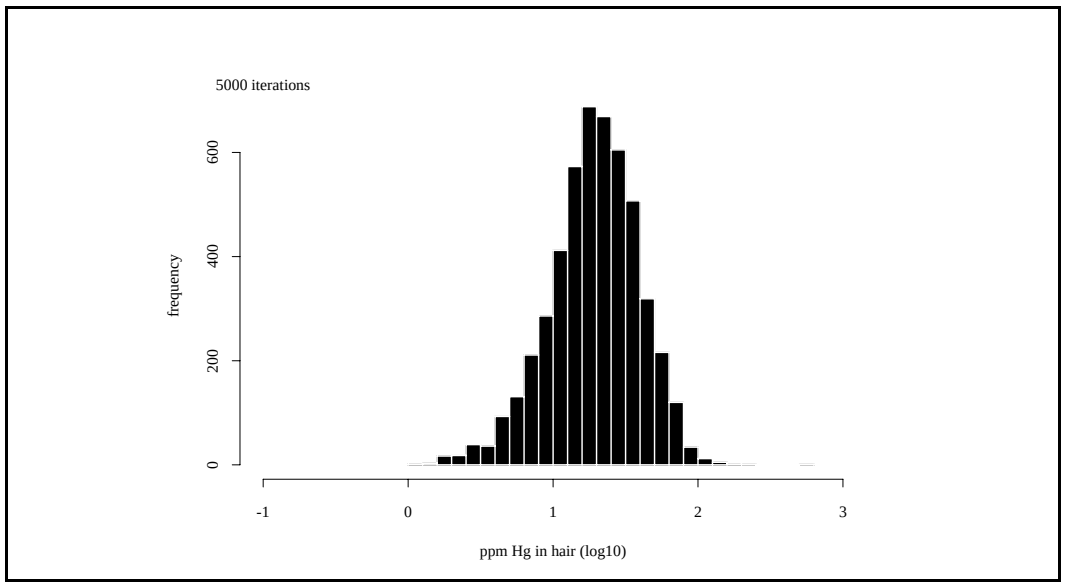


Figure D-11
Bootstrap Threshold Distribution for Combined Developmental Effects

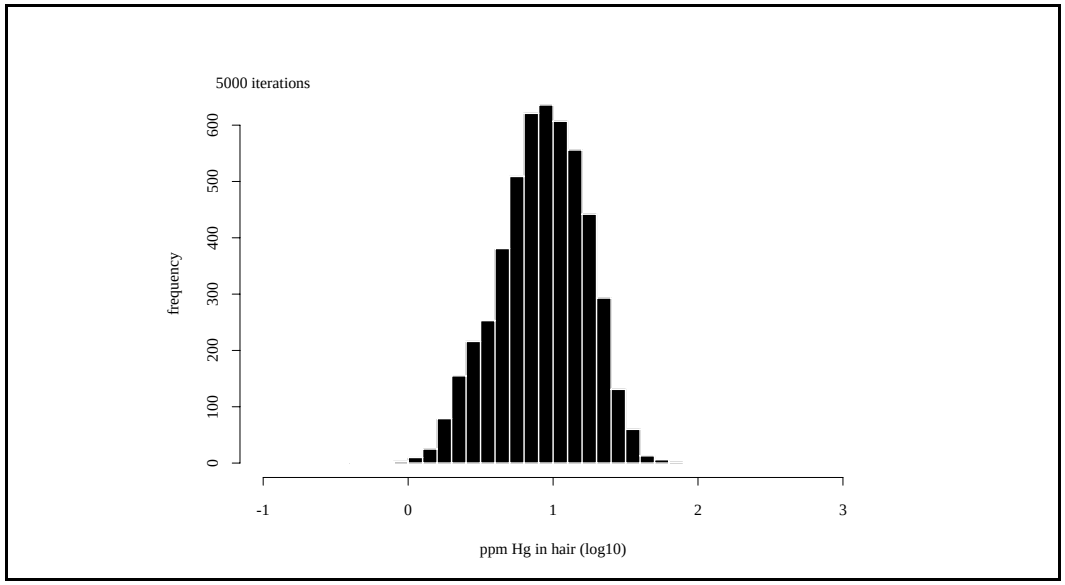


Figure D-12 shows the distribution of bootstrap thresholds for adult paresthesia . The distribution is a result of 5000 iterations of the bootstrap procedure. Nonsignificant regression slopes occurred in 7.5% of the samples as shown by the peak at around 2.8 (600 ppm) in Figure D-12. The largest confidence interval for the adult paresthesia threshold that excluded nonsignificant slopes was 85%. Background incidence for adult paresthesia was 0% for all samples.

Figure D-12
Bootstrap Threshold Distribution for Adult Paresthesia

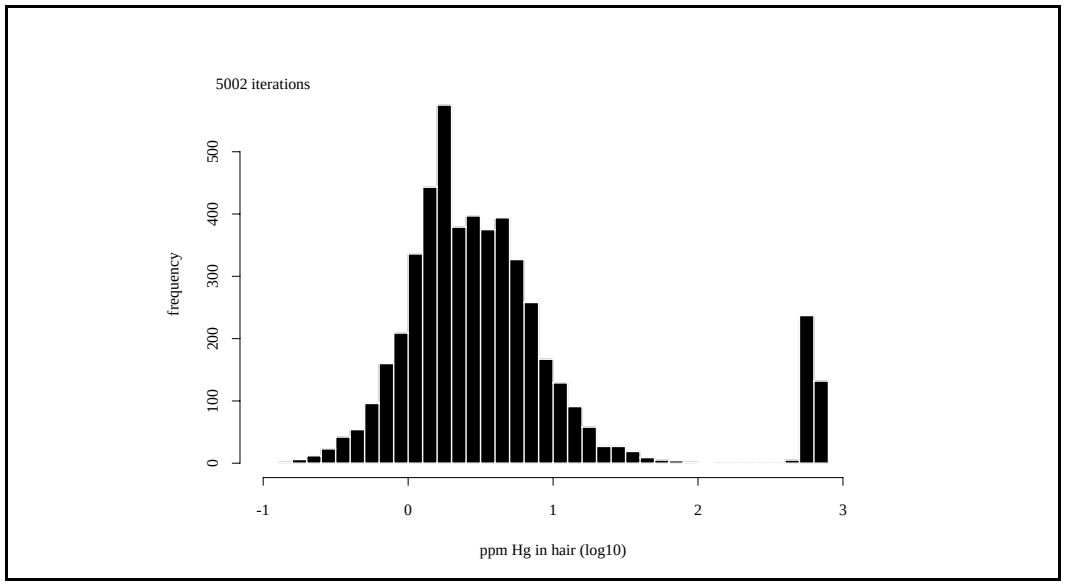


Table D-5 gives selected percentiles from the cumulative bootstrap threshold distributions for the developmental endpoints and adult paresthesia. The threshold values given in Table D-5 are given in units of ppm mercury in hair. The values given for the 5th and 95th percentiles in Table D-5 define the 90% bootstrap confidence interval for each threshold. The adult paresthesia thresholds were the lowest of all the endpoints modeled but showed the greatest variability. The late walking threshold was the lowest and most variable of the individual developmental endpoint thresholds. The combined-effects threshold was the least variable of all the thresholds as would be expected from the method of calculation (minimum of the three individual endpoint thresholds). For the combined developmental effects, the late walking threshold was the lowest of the three thresholds most often (45%) with neurological effects and late talking contributing the lowest threshold 31% and 24% of the time, respectively.

Table D-5
Bootstrap Threshold Distributions in ppm Mercury in Hair for All Effects

	Bootstrap Percentile				
Endpoint	5th	25th	50th	75th	95th
neurological effects [a]	3.8	10	19	33	63
late walking [b]	3.6	8.0	14	25	58
late talking [c]	5.5	13	20	31	57
combined developmental effects 24 [4]	2.5	5.3	8.7	14	24
adult paresthesia	0.64	1.5	2.8	5.9	> 500

[a] neurological test scores > 3 in children exposed *in utero*
[b] walking after 18 months
[c] talking after 24 months
[d] threshold for the occurrence of any developmental effect

All bootstraps stabilized within 4000 iterations as measured by the change in the 5th and 95th percentiles and the ratio of those percentiles. The largest change from 2000 to 4000 iterations in any of the stability measurements was 3.5%.

D.3.2 Response Classification Uncertainty

Table D-6 gives percentiles of the cumulative bootstrap threshold distributions resulting from the consideration of response classification error for each of the endpoints. The distributions were a result of 5000 iterations of the bootstrap procedure. Frequency plots for these distributions are shown in Figures D-13 and D-14 for late walking and adult paresthesia, respectively.

Table D-6
Bootstrap Threshold Distributions in ppm Mercury in Hair with Inclusion of Response Classification Error

Endpoint	Bootstrap Percentile				
	5th	25th	50th	75th	95th
neurological effects (CE[a] = 10%)	2.6	8.4	16	30	71
neurological effects (CE = 20%)	2.3	7.4	15	30	> 600
late walking (CE = 25%)	0.74	2.5	5.7	15	> 600
late walking (CE = 50%)	0.79	2.6	5.0	12	> 600
late talking[b]	2.1	5.9	12	25	99
adult paresthesia[c]	0.50	1.5	3.3	15	> 600

[a] classification error assumption for responses at boundary of minimum value defining a positive response
[b] 50% classification error (boundary responses)
[c] 5% classification error assumed for all responses above background

Figure D-13
Bootstrap Threshold Distribution for Late Walking with Response-Classification Error

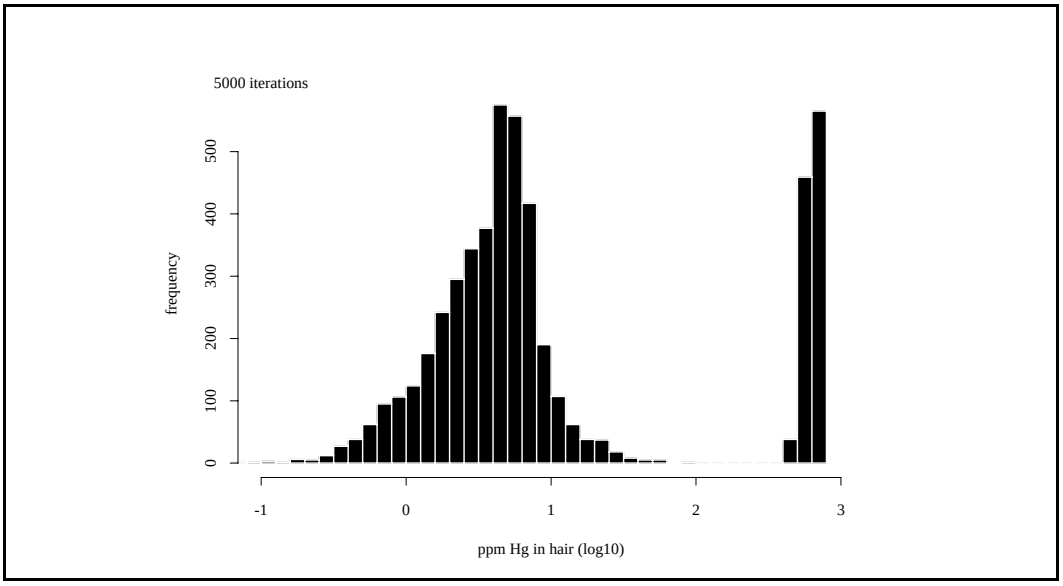
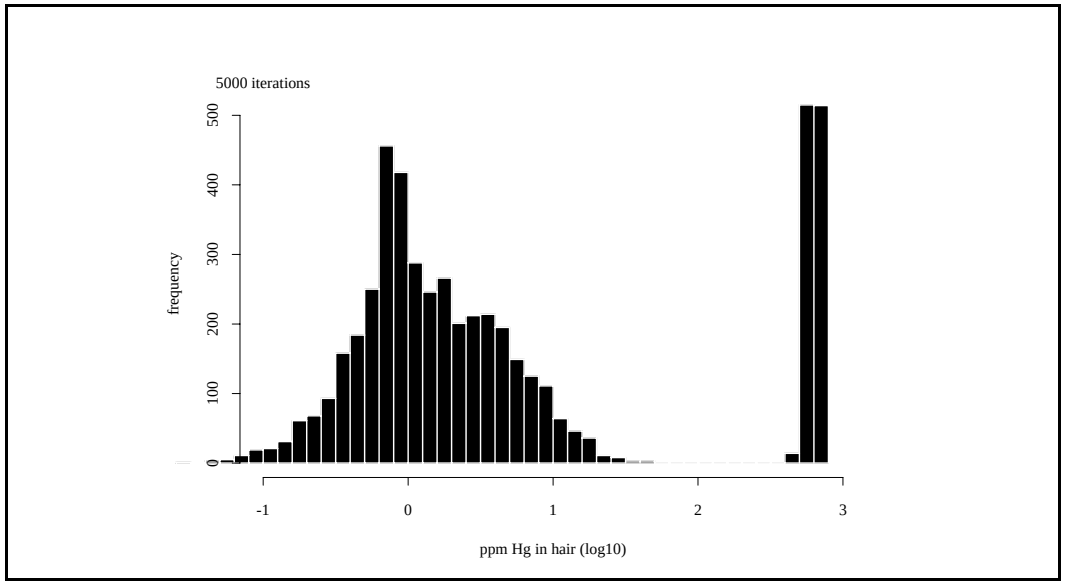


Figure D-14
Bootstrap Threshold Distribution for Adult Paresthesia with Response-Classification Error



The primary result of the assumptions of response classification error was an increase in the number of bootstrap samples resulting in a nonsignificant log-probit regression slope as shown in Table D-7. Late walking and adult paresthesia, for which over 20% of the regression slopes were nonsignificant, were the most sensitive to classification error. Bootstrap confidence intervals of less than 60% were the largest that excluded nonsignificant log-probit slopes for both endpoints. 6.1% of the slopes for neurological effects were nonsignificant when a 20% classification error was assumed; there was little effect when the error estimate was reduced to 10%. Only 4.6% of the slopes for late walking were nonsignificant with a classification error of 50%; the width of the 90% confidence interval, however, increased by a factor of 4. Eliminating late walking from the combined developmental effects resulted in a 53% increase in the median bootstrap threshold to 12 ppm; the 5^{th} and 95^{th} percentiles were increased to 2.7 and 32 ppm, respectively.

Table D-7
Percentage of Bootstrap Thresholds Resulting from Nonsignificant Log-Probit Regression Slopes

Endpoint	standard[1] regression	classification error regression	
neurological effects	0.4	CE[2] = 10% CE = 20%	2.1 6.1
late walking	0.8	CE = 25% CE = 50%	10.9 21.9
late talking	0.2	CE = 50%	4.6
adult paresthesia	7.5	CE = 5%[3]	20.9

[a] no classification error
[b] classification error assumption
[c] all observations

D.3.3 Dose Conversion Monte Carlo Simulation and Sensitivity Analysis

Table D-8 shows the results of the Monte Carlo simulation of the dose conversion factor (DCF in equation 5) for different correlation assumptions. Standard assumptions (scenario 1, Table D-8) were the distribution assignments and correlations described in section D.2.2.2 and summarized in Table D-2. Scenario 2 in Table D-8 assumed that all the variables in equation 5 were independent. Scenarios 3 and 4 included the standard assumptions and an additional assumption that an increased residence time of methylmercury in the blood contributed to the susceptibility of the most sensitive individual. Scenario 3 restricted $\mathbf{t}_{½}$ to the upper half of the standard distribution, representing a moderate association of half-life and susceptibility, while scenario 4 represented a stronger association, restricting $\mathbf{t}_{½}$ to the upper 10% of the standard distribution.

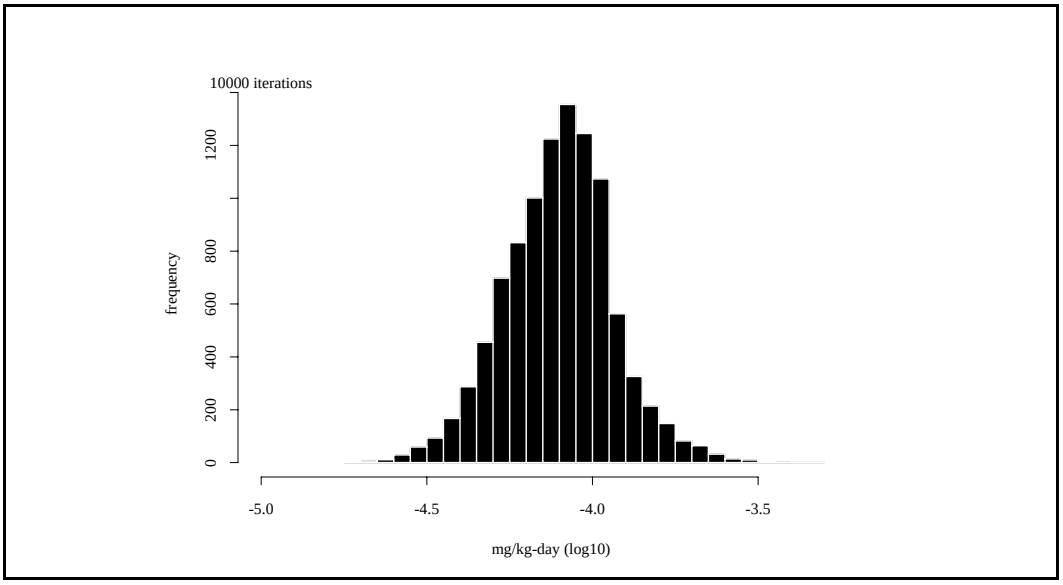
Figure D-15 shows the dose conversion frequency distributions for the standard dose conversion factor distribution (scenario 1, Table D-8). The values on the abscissa are given in ppm mercury in hair/(mg/kg-day) in $\log_{10}$ units. The distributions in Table D-8 and Figure D-15 represent the uncertainty in the ratio of the exposure level (in mg/kg-day) to hair mercury concentration for the most sensitive individual of the exposed population. The nominal dose conversion factor is defined here as the median value of the standard simulation (scenario 1, Table D-8). The median value for the standard simulation was 8.0×10^{-5} with a 90% confidence interval spanning a 3.57-fold range. The corresponding dose conversion value used in the derivation of the methylmercury RfD was 9.8×10^{-5}. That is, the methylmercury RfD would change very little if calculated using the median of the simulated dose conversion distribution. Using the nominal dose conversion factor, an exposure level of 1×10^{-4} mg/kg-day corresponds to a hair mercury concentration of 1.25 ppm, with a 90% confidence interval of 0.69 ppm to 2.36 ppm.

Table D-8
Dose Conversion Factor Monte Carlo Simulation Output for Different Correlation Assumptions in mg/kg-day

Scenario	Percentile				
	5th	25th	50th	75th	95th
1) standard correlations[a]	4.2×10^{-5}	6.2×10^{-5}	8.0×10^{-5}	1.0×10^{-4}	1.5×10^{-4}
2) no correlations	2.6×10^{-5}	5.0×10^{-5}	7.9×10^{-5}	1.2×10^{-4}	2.3×10^{-4}
3) $\mathbf{t}_{½}$ > 53 days[b] (std. correlations)	3.0×10^{-5}	4.7×10^{-5}	6.2×10^{-5}	8.3×10^{-5}	1.3×10^{-4}
4) $\mathbf{t}_{½}$ > 84 days[c] (std. correlations)	2.2×10^{-5}	3.4×10^{-5}	4.6×10^{-5}	6.1×10^{-5}	9.4×10^{-5}

[a] **hb** correlated with $\mathbf{t}_{½}$ (r = -0.5); **f** correlated with **bw** (r = -0.47); **V** correlated with **bw** (r = +0.57)
[b] 50th percentile of $\mathbf{t}_{½}$
[c] 90th percentile of $\mathbf{t}_{½}$

Figure D-15
Dose Conversion Distribution (standard assumptions)



Monte Carlo simulation of equation 4 with $\mathbf{Hg_H} = 1$
Standard assumptions as in Table D-2

The result of assuming correlations between input variables in equation 5 (Table D-8, scenario 1) is a 60% reduction of the width of the 90% confidence interval compared to assuming total independence of inputs (scenario 2). Conversely, restricting $\mathbf{t}_{½}$ to the upper half of the distribution (correlating susceptibility and $\mathbf{t}_{½}$) resulted in increased uncertainty around the DCF and lower dose conversion estimates. Reductions in the median dose conversion estimate were 22% and 42% for a moderate ($\mathbf{t}_{½}$ > 53 days) and a strong ($\mathbf{t}_{½}$ > 84 days) association of $\mathbf{t}_{½}$ and susceptibility, respectively;

the increase in the width of the 90% confidence interval, as measured by the ratio of the 95th and 5th percentiles, was about 20% in both cases.

Table D-9 shows the relative contribution of each dose conversion input variable to the variance of the Monte Carlo simulation output for selected scenarios from Table D-8. It can be seen from Table D-9 that **hb** contributed the most to the variance of the output across the scenarios, while **bw**, **V** and **A** contributed relatively little. The relative contribution to the output variance of $\mathbf{t}_{½}$ and **f** was highly sensitive to the correlation assumptions.

Table D-9
Sensitivity Analysis for Dose Conversion Monte Carlo Simulation:
Contribution of Each Input Variable to Output Variance (%)

Input Variable	Scenario		
	no correlations	standard correlations[a]	alternate $\mathbf{t}_{½}$[b]
hb	47.9	46.5	60.4
$\mathbf{t}_{½}$	26.7	7.9	0.0
f	16.3	33.3	29.0
V	4.7	9.6	8.3
bw	4.3	2.4	1.9
A	0	0.3	0.4

[a] **hb** correlated with $\mathbf{t}_{½}$ (r=-0.5); **f** correlated with **bw** (r=-0.47); **V** correlated with **bw** (r=+0.57)
[b] $t_{½}$ > 53 days

The variability of the dose conversion simulation was somewhat less than the contribution from the bootstrap procedure. The widths of the 90% bootstrap confidence intervals on the thresholds (in ppm mercury in hair) ranged from 1.1 to 1.3 orders of magnitude (12-20 fold difference in the 5th and 95th percentiles from Table D-5). The width of the 90% confidence interval for the standard dose conversion simulation spanned 0.55 orders of magnitude (Table D-8), or about 18-30% of that for the bootstrap confidence intervals.

D.3.4 Estimation of Ingestion Thresholds

The distributions given in Table D-7 were used to obtain dose-conversion confidence intervals for specific threshold estimates. Table D-10 gives values at selected percentiles for the distribution of dose-conversion uncertainty around the median ingestion threshold estimates. The values in Table D-10 are given in units of 10^{-4} mg/kg-day as a convenience for comparison with the RfD of 1 x 10^{-4} mg/kg-day. The distributions in Table D-10 were determined by multiplying the appropriate dose conversion distribution from Table D-7, as noted in Table D-10, by the median bootstrap threshold estimates for each of the endpoints given in Table D-5. That is, the distributions in Table D-10 represent the output of equations 5 or 6 with $\mathbf{Hg}_{\mathbf{h}}$ equal to the median of the indicated bootstrap distribution. As an example, the distribution for developmental neurological effects in Table D-10 was a result of multiplying the 5th, 25th, 50th, 75th and 95th percentiles of the standard DCF distribution by the median bootstrap threshold (19

ppm mercury in hair) for developmental neurological effects. The duration-adjusted adult paresthesia distribution was the dose conversion distribution for adult paresthesia given in Table D-11 divided by 4.7 ($\mathbf{U_D}$). Table D-11 is the equivalent of Table D-8 for the 5th percentile bootstrap threshold estimates from Table D-5.

Table D-10
Dose Conversion Distributions for Median Ingestion Threshold Estimates in mg/kg-day (x 10^4)

Endpoint	Percentile				
	5th	25th	50th	75th	95th
neurological effects[a]	7.9	12	15	19	27
late walking[a]	6.1	9.0	12	15	21
late talking[a]	8.4	12	16	20	29
combined developmental effects[a]	3.7	5.4	7.0	8.7	13
combined developmental effects ($t_{½}$ > 53 days)[b]	2.6	4.0	5.4	7.2	11
adult paresthesia[a]	1.2	1.7	2.2	2.8	4.0
duration-adjusted adult paresthesia[c]	0.26	0.36	0.47	0.60	0.86

[a] standard assumptions (scenario 1, Table D-9)
[b] scenario 3, Table D-9
[c] adult paresthesia distribution divided by $\mathbf{U_D}$

Table D-11
Dose Conversion Distributions for 5th Percentile Ingestion Threshold Estimates in mg/kg-day (x 10^4)

Endpoint	Percentile				
	5th	25th	50th	75th	95th
neurological effects[a]	1.6	2.4	3.1	3.8	5.6
late walking[a]	1.5	2.2	2.9	3.6	5.2
late talking[a]	2.3	3.4	4.4	5.5	8.0
combined developmental effects[a]	1.0	1.5	2.0	2.5	3.6
combined developmental effects[b] ($t_{½}$ > 53 days)	0.74	1.1	1.5	2.0	3.1
adult paresthesia[a]	0.27	0.40	0.52	0.65	0.94
duration-adjusted adult paresthesia[c]	0.058	0.086	0.11	0.14	0.20

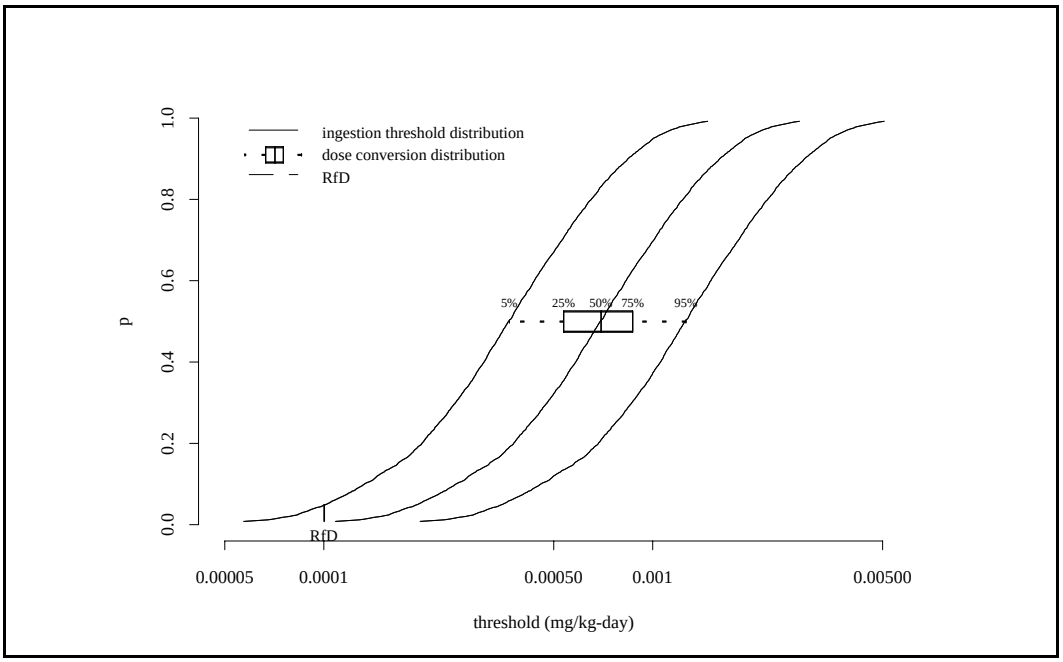
[a] standard assumptions (scenario 1, Table D-9)

[b] scenario 3, Table D-9
[c] adult paresthesia distribution divided by $\mathbf{U_D}$

The 5% and 95% columns in Tables D-10 and D-11 represent the 90% confidence intervals for specific percentiles of the thresholds expressed as ingestion levels in mg/kg-day. For example, with respect to the neurological effects distribution in Table D-10, there is 90% confidence that the true median threshold for neurological effects is between 7.8×10^{-4} and 2.7×10^{-3} mg/kg-day. Similarly, there is 90% confidence that the true 5th percentile of the neurological effects threshold distribution is between 1.4×10^{-4} and 4.8×10^{-4} mg/kg-day (Table D-11). The median ingestion threshold for duration-adjusted adult paresthesia was 1×10^{-4} mg/kg-day, with a 90% confidence interval of 2.3×10^{-5} mg/kg-day to 4×10^{-5} mg/kg-day.

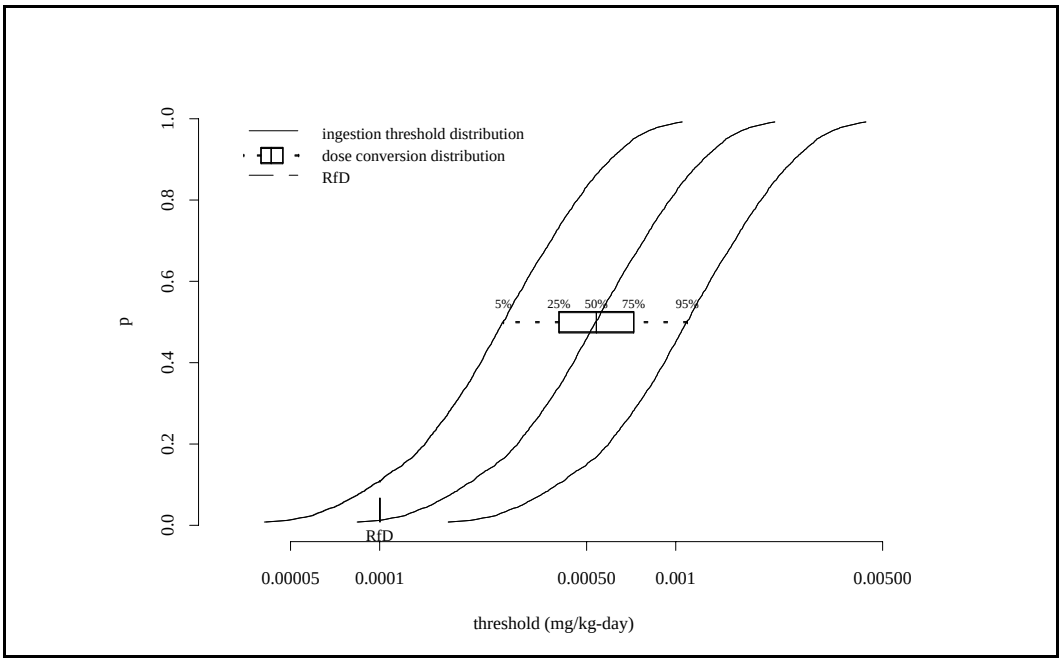
Figure D-16 is a plot of the cumulative bootstrap threshold distribution for combined developmental effects multiplied by values of the dose conversion distribution at selected percentiles. The plots from left to right in Figure D-16 represent different realizations of the distribution of ingestion thresholds based on the relative likelihood of specific values of the dose conversion factor (5th, 50th and 95th percentiles). The horizontal box and whisker plot corresponds to the dose conversion distribution multiplied by the median of the bootstrap threshold distribution for combined developmental effects as given in Table D-10; the box is the interquartile range (25th to 75th percentiles) and the whiskers are the 5th and 95th percentiles. Figure D-17 is the same plot for combined developmental effects with the assumption that $\mathbf{t_{½}}$ is greater than 53 days (Table D-8, scenario 3). Figures D-18 to D-22 are the equivalent plots for the individual-effect thresholds. Ingestion threshold distributions for adult effects are shown in Figures D-21 and D-22. Figure D-21 is the ingestion threshold distribution for the adult paresthesia observed in the Iraqi cohort. Figure D-22 is the ingestion threshold distribution for duration-adjusted adult paresthesia resulting from dividing the adult paresthesia ingestion threshold distribution by $\mathbf{U_D}$. That is, Figure D-22 is the distribution in Figure D-21 shifted to the left by a factor of 4.7.

Figure D-16
Cumulative Combined Developmental Effects Ingestion Threshold Distribution



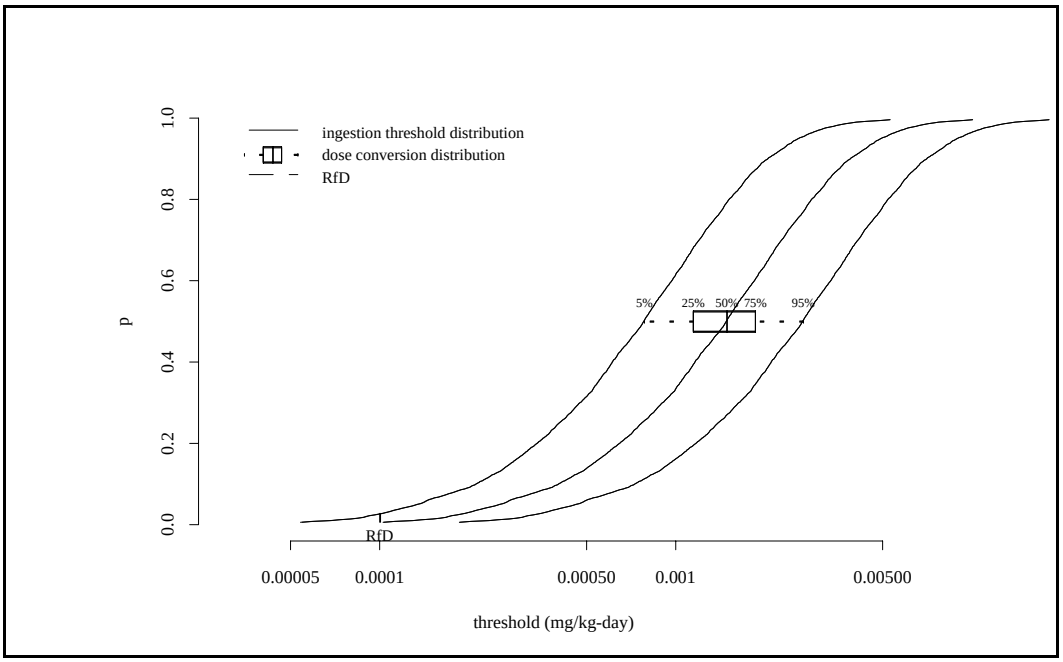
p = cumulative probability

Figure D-17
Cumulative Combined Developmental Effects Ingestion Threshold Distribution
($t_{½}$ > 53 days)



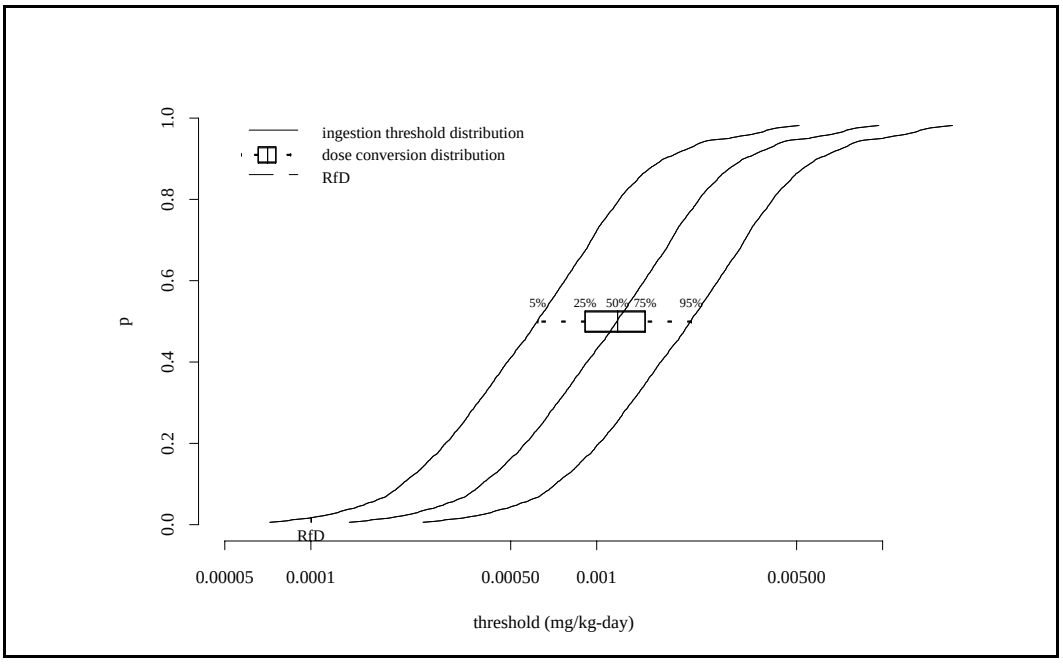
p = cumulative probability

Figure D-18
Cumulative Neurological Effects Ingestion Threshold Distribution



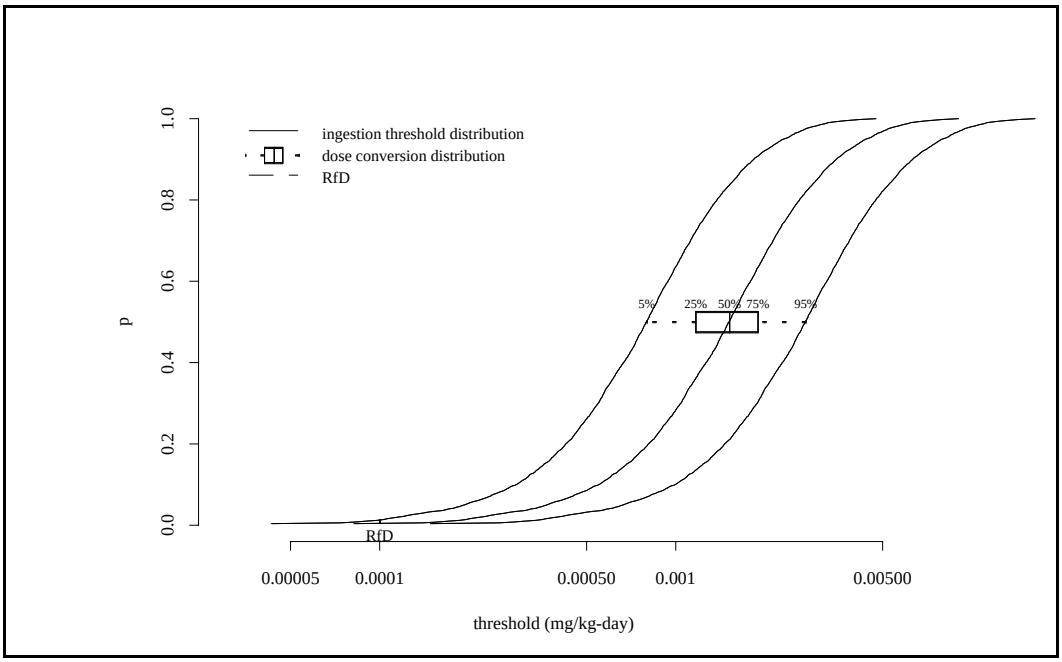
p = cumulative probability

Figure D-19
Cumulative Late Walking Ingestion Threshold Distribution



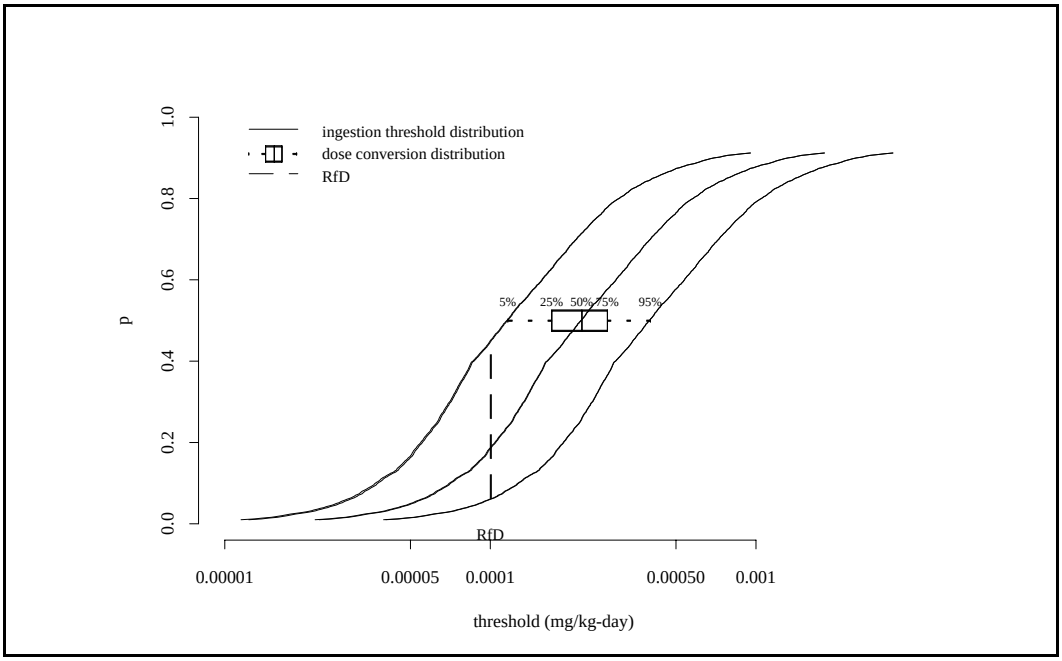
p = cumulative probability

Figure D-20
Cumulative Late Talking Ingestion Threshold Distribution



p = cumulative probability

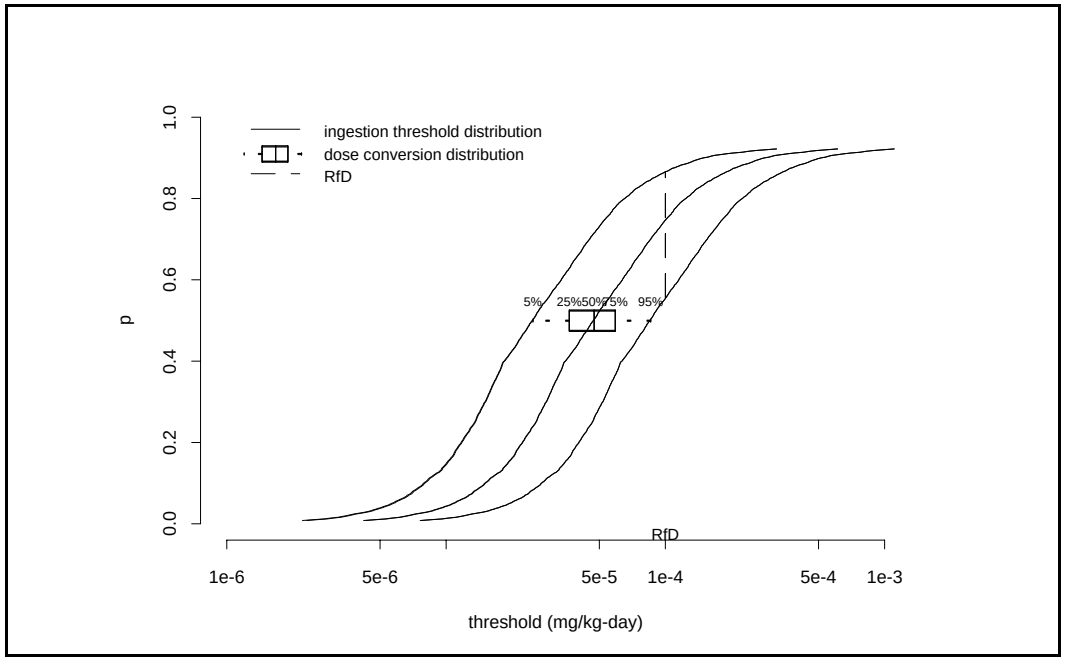
Figure D-21
Cumulative Adult Paresthesia Ingestion Threshold Distribution
(no exposure duration adjustment)



p = cumulative probability

Figure D-22

Cumulative Duration-Adjusted Adult Paresthesia Threshold Distribution



p = cumulative probability

The uncertainty around the location of the RfD within each of the distributions shown in Figures D-17 to D-22 is indicated by the vertical line at 1 x 10^{-4} mg/kg-day; this uncertainty came from the dose conversion variability. As an example, the RfD fell between the 0.035 and 4.5 percentiles of the combined developmental effects threshold distribution with 90% confidence as determined by the intersection of the RfD line with the 5 % and 95% ingestion threshold curves (Figure D-16). The median estimate of the location of the RfD in this distribution was the 0.25 percentile. Similarly, the RfD fell between the 39th and 91st percentile of the duration-adjusted adult paresthesia threshold distribution with the median at the 75th percentile (Figure D-22). The RfD was at the 18th percentile of the adult paresthesia threshold distribution and below the 1st percentile for all of the other threshold distributions.

D.4 Discussion of Uncertainty Analysis

Because the Iraqi cohort is considered to be a sensitive subgroup, as defined in the RfD methodology, the output distributions of the analysis are meant to reflect the uncertainty around an estimate of the thresholds for effects in humans including sensitive individuals. The results for each endpoint should be interpreted as the distribution of the uncertainty around the human population threshold. The results should not be interpreted as the distributions of individual thresholds within the population. Estimates of risk above the threshold cannot be obtained from this analysis.

This analysis has attempted to incorporate all areas of uncertainty involved in the derivation of the methylmercury RfD in Chapter 6 of Volume IV of this report. The 10-fold uncertainty factor (UF) includes a 3-fold ($10^{0.5}$) factor for human variability and a 3-fold factor for the lack of reproductive and chronic studies. The UF for human variability includes a consideration of both susceptibility and variation in methylmercury blood half-lives. The bootstrap threshold confidence intervals incorporate the former, and the latter is explicitly modeled ($\mathbf{t}_{½}$) in the dose conversion Monte Carlo analysis. Uncertainty arising from the lack of chronic data is estimated by $\mathbf{U_D}$; this uncertainty was a point estimate only, as the data were inadequate for defining a distribution for $\mathbf{U_D}$. Because $\mathbf{U_D}$ was derived as a scaling

factor for adult effects it is not directly comparable to the UF for chronic effects used in the derivation of the RfD, which was based on developmental endpoints. The only uncertainty included in the RfD and not addressed here is the uncertainty attributed to the lack of a reproductive study; there are no appropriate data for the estimation of this uncertainty. In general, reproductive NOAELs are slightly lower than developmental NOAELs for other substances, but much higher than chronic NOAELs (Dourson, Knauf and Swartout, 1992). That is, the uncertainty in the thresholds is expected to be much less for lack of a reproductive study than for lack of a chronic study.

The uncertainty analysis presented in this appendix was limited to only those data and formulae directly related to the derivation of the methylmercury RfD. Other data sets or models were not considered. A few sources of uncertainty in the data used to derive the methylmercury RfD have not been included in this analysis. Exposure classification error arising from uncertainty as to the correspondence of actual exposure and critical exposure period cannot be estimated from the data as published in Marsh *et al.*, 1987. This source of uncertainty could be a major contributor to the apparent extreme variability of susceptibilities in the Iraqi cohort. Variability in the interpretation of the definition of a response was not estimated in this analysis. That is, there would have been some differences in how individuals interpreted what constituted first walking or first talking, probably more so for the latter. The classification errors assumed for this analysis only account for uncertainty in the timing of the event given an unequivocal positive response. Also, the response decision points defining an adverse effect were accepted uncritically. For example, changing the definition of late walking to either greater than 16 months or greater than 20 months would have a significant effect on the analysis. Measurement error for hair mercury concentrations has not been estimated for this analysis; the necessary data are unavailable in the published reports (Marsh *et al.*, 1987; Cox *et al.*, 1989). In addition, the results of this analysis are conditional on a specific representation of population variability in the parameters of the dose conversion variables. That is, the choice of the form, and parameters for the distributions assigned to each of the variables is largely a matter of judgement. The particular set of parameters chosen for each distribution is only one option of a number of possible choices; uncertainty as to the value of the parameters is not included in the analysis.

The threshold analysis shows that adult paresthesia was the most sensitive individual effect observed for the Iraqi cohort, particularly when adjusted for the effects of continuing exposure. That is, in this analysis, paresthesia in adults was estimated to be observable at a lower exposure than the developmental endpoints. The absence of an observed background incidence for paresthesia in the Iraqi cohort partially contributed to the low threshold estimates. A background incidence for paresthesia would be expected in the general population. The adult paresthesia bootstrap thresholds were also the most unstable as measured by the frequency of nonsignificant slopes. The RfD fell between the 39^{th} and 91^{st} percentiles of the duration-adjusted adult paresthesia threshold distribution, a considerably larger range than that for any of the developmental effects. On the average, the RfD fell below the 1^{st} percentile for all developmental effects, with only a 5% chance that it was as high as the 16^{th} percentile.

The response-classification uncertainty analyses were based on hypothetical classification error rates. Assumptions of 50% response-classification error for late walking and late talking were worst-case for those values immediately adjacent to the response decision point value for any given effect. That is, for late walking, the values of 18 or 20 months for first walking and 24 or 26 months for first talking were assumed to be equally likely, resulting in misclassification 50% of the time. This would require an uncertainty in recall of these events of at least 2 months, which is not unlikely in this particular situation. The actual classification error was likely to be somewhat less than 50%, particularly as the large number of observations for late walking at 18 months (22 of the 81 individuals) suggests that 18 months may have been used as an upper bound in some cases. The response-classification error assumptions for late walking and late talking were best-case for all other values as no error is assumed. Even with a 25%

classification error, however, the results of the response-classification uncertainty analysis indicate that the late walking endpoint was unreliable as a measure of methylmercury toxicity. The exclusion of this endpoint would not have a very large impact on the combined developmental effects threshold distribution, increasing the thresholds by about 50%. Although the late talking threshold distribution is not grossly affected by response-classification error, variability in interpretation of the definition of the endpoint (first talking) likely would have been greater than that for walking; this uncertainty was not estimated in this analysis. The neurological effects thresholds were least sensitive to classification error, assuming that the true error was closer 10% than 20%. The assumption seems reasonable given the much greater objectivity of the measurement of the effect. Adult paresthesia was the most sensitive to classification error, showing extreme variability in the threshold estimates with a classification error rate as low as 5% (all observations). These results suggest that strong conclusions based on the late walking and adult paresthesia endpoints are unwarranted.

Results of the alternate scenarios (Table D-8) show that the primary effect of the correlation assumptions among the dose conversion input variables was a fairly large reduction in the variance of the Monte Carlo simulation output. The assumption of correlation of individual susceptibility and half-life of methylmercury in the blood did not have a marked effect on the simulation except for a 42% reduction in the median when a strong correlation was assumed ($\mathbf{t}_{½} > 84$ days). The latter scenario probably represented a worst-case situation although no data were found that directly address the magnitude of the hypothetical correlation.

The sensitivity analysis indicates that the variables that contribute the most to the dose conversion simulation variability are the hair:blood ratio (**hb**), the half-life of methylmercury in the blood ($\mathbf{t}_{½}$) and the fraction of absorbed methylmercury found in the blood (**f**). There is very little that can be done to reduce the uncertainty in these variables because appropriate data directly applicable to the Iraqi cohort are not available. These results could be of use in the experimental design and collection of data for estimates of ingestion levels from hair concentrations in the future.

2.4.5 Conclusions of Analysis of Uncertainty Around Human Health effects of Methylmercury

A major source of the variability was in the estimation of bootstrap thresholds from the Iraqi cohort data as evidenced by the 12-20 fold difference in the 5^{th} and 95^{th} percentiles of the bootstrap threshold distributions. The uncertainty arising from limited exposure duration contributed almost as much, with a 12.5-fold difference in the 5^{th} and 95^{th} percentiles. The corresponding spreads in the dose conversion distributions were 2.4-4.2 fold. Correlations between variables were important with respect to the variance of the Monte Carlo simulations but were not well-defined by empirical data. Additional areas of uncertainty remain to be modeled.

Of the developmental endpoints, the neurological effects, which are determined by a battery of tests and do not depend on subject recall, would seem to be the most objective measure of methylmercury toxicity. Late walking was not a reliable endpoint because of sensitivity to classification error.

The RfD of 1×10^{-4} mg/kg-day is very likely well below the threshold for developmental effects but may be above the threshold for exposure duration-adjusted adult paresthesia. Strong conclusions based on the latter result are not warranted because of the sensitivity of the adult paresthesia threshold to classification error and the general lack of data addressing the effects of exposure duration.

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