2.5 RELEVANCE TO PUBLIC HEALTH

OVERVIEW

The nature and severity of the toxicity that may result from mercury exposure are functions of the magnitude and duration of exposure, the route of exposure, and the form of the mercury or mercury compound to which exposure occurs. Since the ultimate toxic species for all mercury compounds is thought to be the mercuric ion, the kinetics of the parent compound are the primary determinant of the severity of parent compound toxicity. It is differences in the delivery to target sites that result in the spectrum of effects. Thus, mercury, in both inorganic and organic forms, can be toxic to humans and other animals.

Ingestion of methylmercuric chloride, for example, is more harmful than ingestion of an equal amount of inorganic salts (e.g., mercuric chloride or mercuric acetate), since methylmercury is more readily absorbed through the intestinal tract (about 95%) than are mercuric salts (about 10–30%). In turn, ingestion of inorganic mercury salts is more harmful than ingestion of an equal amount of liquid metallic mercury, because of negligible absorption of liquid metallic mercury (about 0.01%) from the gastrointestinal tract. There is insufficient information to develop a complete matrix of effects for different mercury forms by route of exposure. The information on inhalation exposure to mercury is limited primarily to metallic mercury; only a few case studies are available for exposure to inorganic dusts or volatile organomercurials.

Inorganic salts of mercury do not readily cross the blood-brain barrier or the placenta. They are, therefore, ultimately less toxic to the central nervous system and the developing fetus than either absorbed metallic mercury or organic mercury compounds. Metallic mercury is more readily oxidized to mercuric mercury than is methylmercury, so its transport across the placenta and into the brain may be more limited than that of methylmercury. Once in the central nervous system, however, metallic mercury vapor is oxidized to the mercuric ion (Hg^{++}), which is then trapped in the central nervous system due to the limited ability of the mercuric ion to cross the blood-brain barrier. Mercurous salts are relatively unstable in the presence of sulfhydryl groups and readily transform to metallic mercury and mercuric mercury. Thus, mercurous forms of mercury will possess the toxic characteristics of both metallic and mercuric mercury. All mercury compounds may ultimately be oxidized to divalent (or mercuric) mercury, which preferentially deposits in the kidneys, and all mercury compounds may cause some degree of renal toxicity. While this is not

typically the first effect noted in all forms of mercury exposure, it can be an ultimate effect of either low-dose chronic intake or high-dose acute mercury exposure.

The most sensitive end point following oral exposure of any duration to inorganic salts of mercury appears to be the kidneys. Liquid metallic mercury can volatilize at ambient temperatures. The absorption of metallic mercury vapors from lungs is high (about 80%) (Hursh et al. 1976), and the most sensitive target following inhalation exposure to metallic mercury is the central nervous system. Absorbed metallic mercury crosses the placenta, and the fetal blood may concentrate mercury to levels 10 or more times the levels found in the maternal blood. Therefore, the developing fetal nervous system may be quite sensitive to maternal exposures to mercury vapors.

Salts of mercury and organic mercury compounds are far less volatile than liquid mercury under most conditions. Inhalation of mercury vapors from these forms is not considered a major source of exposure. While inhalation of particulate matter containing mercury salts and/or organic compounds is possible, intestinal absorption is a more likely route of exposure. The most sensitive end point for oral exposure to alkyl mercury compounds (e.g., methylmercuric chloride or ethylmercurials) is the developing nervous system, but toxicity to the adult nervous system may also result from prolonged low-dose exposures. Mercury may adversely affect a wide range of other organ systems, if exposures are sufficiently high. These effects may result from the mercuric ion’s affinity for sulfhydryl groups, which are ubiquitous in animal tissue.

Pharmacokinetic studies indicate that repeated or continuous exposure to any form of mercury can result in the accumulation of mercury in the body. Numerous studies using laboratory animals have shown that retention of mercury in the brain may persist long after cessation of short- and long-term exposures. Mercury is unusual in its ability to induce delayed neurological effects. This is especially prevalent with exposure to alkyl mercury compounds. In such cases, the onset of adverse effects may be delayed for months after the initial exposure. The delayed effects of methyl- and dimethylmercury reported in human poisonings are thought, in part, to result from binding to red blood cells, and subsequent slow release. Methylmercury also forms a complex in plasma with the amino acid cysteine, which is structurally similar to the essential amino acid methionine (Aschner and Clarkson 1988). Clarkson (1995) proposed that methylmercury can cross the blood-brain barrier "disguised" as an amino acid via a carrier-mediated system (i.e., transport is not solely the result of methylmercury’s lipid solubility).

Phenylmercuric acetate is another form of organic mercury to which the general public may be exposed. Although phenylmercury compounds are considered organomercurials, they are absorbed less efficiently by the gastrointestinal tract than is methylmercury. Once inside the body, phenylmercury is rapidly metabolized to Hg^{++}, and its effects are, therefore, similar to those of mercuric salts.

Dimethylmercury is an extremely toxic form of organic mercury, and very small exposures can cause se vere and irreversible delayed neurotoxicity, including death. Dimethylmercury is thought to be metabolized to methylmercury prior to crossing the blood-brain barrier. Dimethylmercury is used in the calibration of laboratory equipment, as a reagent, and in the manufacture of other chemicals. Unlike other forms of mercury, dimethylmercury is quickly absorbed through intact skin, and it will penetrate latex or polyvinyl gloves. It is highly volatile, will readily evaporate, and can be inhaled. Based on its vapor pressure of 58.8 mm at 23.7 EC, Toribara et al. (1997) estimated that a cubic meter of saturated air could hold more than 600 g of dimethylmercury. A recent case history of a chemist who died from an accidental spill of dimethylmercury is prompting calls for its removal as an analytical standard as a safety precaution to prevent further accidents.

Upon significant inhalation exposure to metallic mercury vapors, some people (primarily children) may exhibit a syndrome known as acrodynia, or pink disease. Acrodynia is often characterized by severe leg cramps; irritability; and erythema and subsequent peeling of the hands, nose, and soles of the feet. Itching, swelling, fever, tachycardia, elevated blood pressure, excessive salivation or perspiration, morbilliform rashes, fretfulness, sleeplessness, and/or weakness may also be present. It was formerly thought that this syndrome occurred exclusively in children, but recent reported cases in teenagers and adults have shown that these groups are also susceptible.

Occupational mercury exposures generally occur when workers inhale metallic mercury vapors. Some dermal absorption may occur from skin contact with contaminated air, but the rate is low (less than 3% of the inhaled dose). Dialkyl mercury compounds, which are not normally found in hazardous waste sites, are rapidly and extensively absorbed from both dermal and inhalation routes of exposure.

Mercury is a naturally occurring element in the earth’s crust. It is considered to have been a component of the lithosphere since the planet was formed approximately 4.5 billion years ago. However, levels of mercury at or near the earth’s surface (environmental background levels) are increasing as mercury continues to be released from the earth’s crust by both natural (weathering, volcanoes) and human (mining,

burning of fossil fuels) activities. Background levels, however, are considerable below harmful levels. There are a number of possible pathways for exposure to mercury. For a hazardous waste site that contains mercury that is being released to the environment, pathways that could result in human exposure to mercury include: (1) eating fish or wild game near the top of the food chain (i.e., larger fish, larger mammals) that have accumulated mercury in their tissues from living at or near the site; (2) playing on or in contaminated surface soils; (3) playing with liquid mercury from broken electrical switches, thermometers, blood pressure monitors etc.; or (4) bringing any liquid mercury or broken mercury device into the home, where vapors might build up in indoor air. Other potentially harmful exposure pathways include the excessive use of skin ointments or creams (e.g., skin lightening creams, antiseptic creams) that contain mercury compounds, the use of mercury fungicides (breathing vapors or contact of the skin with the fungicide), or the use of liquid mercury in herbal remedies or religious practices, especially if used indoors. If swallowed, liquid mercury is not very harmful, because it is not easily absorbed into the body from the gastrointestinal tract. However, small amounts of liquid mercury evaporate at room temperature, and the inhaled vapors are harmful.

The developing fetus and breast-fed infants are vulnerable to the harmful effects of mercury. The fetus can be exposed to mercury from the pregnant woman's body through the placenta, and infants may be exposed from the nursing woman's milk. Both inhaled mercury vapors and ingested methylmercury can cross the placenta. Inorganic mercury, and to a lesser extent elemental mercury and methylmercury, will move into breast milk. Pregnant women and nursing women need to be extra cautious in their use of consumer products containing mercury (such as some religious or herbal remedies or skin lightening creams); they should also pay attention to possible exposures to mercury at work and at home.

The primary pathways of mercury exposure for the general population are from eating fish or marine mammals that contain methylmercury, or from breathing in or swallowing very small amounts of mercury that are released from the dental amalgam used for fillings. The relative contribution of mercury from these two main sources will vary considerably for different individuals, depending upon the amount of fish consumed, the level of mercury in the fish, the number of amalgam fillings, eating and chewing habits, and a number of other factors.

Methylmercury levels vary considerably between species and within species of fish (depending on water conditions and size), so there are wide ranges in estimates of the average exposure levels to mercury in the general population from consumption of fish. Some researchers estimate that the typical daily exposure to

mercury is 0.49 µg/day for infants (aged 6–11 months), 1.3 µg/d for 2-year-old children, 2.9 µg/day for females aged 25–30 years, and 3.9 µg/day for males 25–30 years of age. Expressed on a per body weight basis, the intake for all age groups, except for 2-year-old children, was approximately 0.05 µg/kg/day (Clarkson 1990; Gunderson 1988). More recently, MacIntosh et al. (1996) estimated mean dietary exposure of 8.2 µg/d (range, 0.37–203.5 µg/day) for females and 8.6 µg/day (range, 0.22–165.7 µg/day) for males. For an average body weight of 65 kg for women and 70 kg for men, the daily intakes of mercury would be 0.126 µg/kg/day (range, 5.7–3,131 ng/kg/day) for women and 0.123 µg/kg/day (range, 3.1–2,367 ng/kg/day) for men, respectively. Lack of data about the actual amount of food consumed accounted for 95% of the total uncertainty for mercury. This was especially true for consumption levels of canned tuna and other fish (MacIntosh et al. 1996)

The Food and Drug Administration (FDA, 1996) has posted on the Internet advice for consumers recommending that pregnant women and women of childbearing age, who may become pregnant, limit their consumption of shark and swordfish to no more that one meal per month. This advice is given because methylmercury levels are relatively high in these fish species. The FDA's advice covers both pregnant women and women of child-bearing age who might become pregnant, since dietary practices immediately before the pregnancy could have a direct bearing on fetal exposure, particularly during the first trimester of pregnancy. The FDA also states that nursing women who follow this advice will not expose their infants to increased health risks from methylmercury (FDA 1996). For the general population (other than pregnant women and women of child-bearing age), the FDA advises limiting the regular consumption of shark and swordfish (which typically contain methylmercury at 1 ppm) to about 7 ounces per week (about one serving). This level of consumption results in methylmercury exposures below the U.S. FDA acceptable daily intake level for mercury. For fish species with methylmercury levels averaging 0.5 ppm, regular consumption should be limited to 14 ounces per week. Recreational and subsistence fishers who eat larger amounts of fish than the general population and routinely fish the same waters may have a higher exposure to methylmercury if these waters are contaminated (EPA 1995). People who consume greater than 100 grams of fish per day are considered high-end consumers. This is over 10 times the amount of fish consumed by members of the general population (6.5 g/day) (EPA 1995). No consumption advice is necessary for the top 10 seafood species, which make up about 80% of the seafood market: canned tuna, shrimp, pollock, salmon, cod, catfish, clams, flatfish, crabs, and scallops. The methylmercury in these species are generally less than 0.2 ppm, and few people eat more than the suggested weekly limit of fish (i.e., 2.2 pounds). More information on exposure to methylmercury and the levels in fish can be found in Section 5.5, General Population and Occupational Exposures.

Estimating mercury exposure from dental amalgams is also difficult because of high variability in the number of amalgam fillings per individual and the differences in chewing, eating, and breathing habits. Dental amalgams, however, would be the most significant source of mercury exposure in the absence of fish consumption or proximity to a waste site or incinerator. A report from the Committee to Coordinate Environmental Health and Related Programs (CCEHRP) of the Department of Health and Human Services determined a level of from 1 to 5 µg Hg/day from dental amalgam for people with 7–10 fillings (DHHS 1993). The World Health Organization reported a consensus average estimate of 10 µg amalgam Hg/day (range: 3–17 µg/day) (WHO 1991). Weiner and Nylander (1995) estimated the average uptake of mercury from amalgam fillings in Swedish subjects to be within the range of 4–19 µg/day. Skare and Engqvist (1994) estimated that the systemic uptake of mercury from amalgams in middle-aged Swedish individuals with a moderate amalgam load (30 surfaces) was, on the average, 12 µg/day, an amount said to be equivalent to a daily occupational air mercury exposure concentration of 2 $\mu g/m^3$. Other researchers have estimated the average daily absorption of Hg from amalgam at 1–27 µg/day, with levels for some individuals being as high as 100 µg/day (Björkman et al. 1997; Lorscheider et al. 1995).

Richardson et al. (1995) estimated total mercury exposure for Canadian populations of different ages to be 3.3 µg/day in toddlers (3–4 years old), 5.6 µg/day in children (5–11 years old), 6.7 µg/day in teens (12–19 years old), 9.4 µg/day in adults (20–59 years old), and 6.8 µg/day in seniors (aged 60+). Of this exposure, amalgam was estimated to contribute 50% to the total Hg in adults and 32–42% for other age groups. Estimates based on 2 independent models of exposure from amalgam alone were 0.8–1.4 µg/day in toddlers), 1.1–1.7 µg/day in children, 1.9–2.5 µg/day in teens; 3.4–3.7 µg/day in adults, and 2.1–2.8 µg/day in seniors (Richardson 1995).

Higher levels of mercury exposure can occur in individuals who chew gum or show bruxism, a rhythmic or spasmodic grinding of the teeth other than chewing and typically occurring during sleep (Barregard et al. 1995; Enestrom and Hultman 1995). Richardson (1995) reported a transient 5.3-fold increase in levels of mercury upon stimulation by chewing, eating, or tooth brushing. Sallsten et al. (1996) also reported over a 5-fold increase in plasma and urinary mercury levels (27 and 6.5 nmol/mmol creatinine versus 4.9 and 1.2 nmol/mmol creatinine, respectively) in a sample of 18 people who regularly chewed nicotine chewing gum (median values of 10 sticks per day for 27 months), compared to a control group.

Berdouses et al. (1995) studied mercury release from dental amalgams using an artificial mouth under controlled conditions of brushing and chewing and found that although the release of mercury during initial

nonsteady-state conditions was influenced by both the age of the amalgam and the amalgam type, the steady-state value of the mercury dose released by the amalgam was only 0.03 µg/day.

Sandborgh-Englund et al. (1998) evaluated the absorption, blood levels, and excretion of mercury in nine healthy volunteers (2 males, 7 females) exposed to 400 µg /m^3 mercury vapor in air for 15 minutes. This exposure corresponded to a dose of 5.5 nmol Hg/kg body weight. Samples of exhaled air, blood and urine were collected for 30 days after exposure. The median retention of elemental Hg was 69% of the inhaled dose. To evaluate the chronic exposure to mercury from dental amalgam in the general population, the daily Hg dose from fillings was estimated based on the plasma Hg levels of subjects with amalgam fillings and the plasma clearance obtained in this study. The daily dose was estimated to be from 5 to 9 µg/day in subjects with an "average" number (20–35 amalgam surfaces) of amalgam fillings (Sandborgh-Englund et al. 1998)

Halbach (1994) examined the data from 14 independent studies and concluded that the probable mercury dose from amalgam is less than 10 µg/day. When combined with the 2.6 µg/day background intake estimated by WHO (1990) for persons without amalgam fillings and with an estimated methylmercury intake of 5 µg/day from food, Halbach noted that the sum of all those inputs still falls within the WHO's 40 µg/day acceptable daily intake (ADI) level for total mercury. For the ADI of 40 µg total mercury exposure inhaled, approximately 30 µg would be absorbed, assuming 80% absorption (Halbach 1994; WHO 1976).

Whether adverse health effects result from exposure to mercury from amalgams at the levels reported above is currently a topic of on-going research and considerable discussion. A thorough review of this subject is beyond the scope of this profile. Readers are referred to the end of this section (see More on the Effects of Dental Amalgam) for a discussion of some recent reviews of this topic, and a few examples of studies on the putative toxic effects or the lack thereof from continued use of amalgam.

Other Uses of Metallic Mercury

A less well-documented source of exposure to metallic mercury among the general population is its use in ethnic religious, magical, and ritualistic practices, and in herbal remedies. Mercury has long been used for medicinal purposes in Chinese herbal preparations and is also used in some Hispanic practices for medical and/or religious reasons. Espinoza et al. (1996) analyzed 12 types of commercially produced herbal ball preparations used in traditional Chinese medicine. Mercury levels were found to range from 7.8 to

621.3 mg per ball. Since the minimum recommended adult dosage is 2 such balls daily, intake levels of up to 1.2 mg of mercury (presumed to be mercury sulfide) might be a daily dosage.

Some religions have practices that may include the use of metallic mercury. Examples of these religions include Santeria (a Cuban-based religion that worships both African deities and Catholic saints), Voodoo (a Haitian-based set of beliefs and rituals), Palo Mayombe (a secret form of ancestor worship practiced mainly in the Caribbean), and Espiritismo (a spiritual belief system native to Puerto Rico). Not all people who observe these religions use mercury, but when mercury is used in religious, folk, or ritualistic practices, exposure to mercury may occur both at the time of the practice and afterwards from breathing in contaminated indoor air. Metallic mercury is sold under the name "azogue" (pronounced ah-SEW-gay) in stores called “botanicas.” Botanicas are common in Hispanic and Haitian communities, where azogue may be sold as an herbal remedy or for spiritual practices. The metallic mercury is often sold in capsules or in glass containers. It may be placed in a sealed pouch to be worn on a necklace or carried in a pocket, or it may be sprinkled in the home or car. Some store owners may also suggest mixing azogue in bath water or perfume, and some people place azogue in devotional candles. The use of metallic mercury in a home or apartment not only threatens the health of the current residents, but also poses health risks to future residents, who may unknowingly be exposed to further release of mercury vapors from contaminated floors, carpeting, or walls.

Due to the increased number of reported metallic mercury poisonings and to the widespread potential for exposure to liquid/metallic mercury in school chemistry and science laboratories and other places accessible to the general public, the EPA and ATSDR issued a joint mercury alert in June 1997, alerting school and public health officials to the potential toxicity of this substance. This joint mercury alert also advised restricting access to mercury-containing spaces and storage rooms, and the use of alternative substances or chemicals for purposes for which liquid/metallic mercury is currently used.

Issues relevant to children are explicitly discussed in Sections 2.6, Children’s Susceptibility, and 5.6, Exposures of Children.

Minimal Risk Levels for Mercury

A common misconception is that health guidance values, such as the MRL, represent a level above which toxicity is likely to occur. This misconception has occasionally led to unwarranted concern and public

apprehension about relatively benign exposures to environmental substances. The MRL is neither a threshold for toxicity, nor a level beyond which toxicity is likely to occur. MRLs are established solely as screening tools for public health officials to use when determining whether further evaluation of potential exposure at a hazardous waste site is warranted. The relevance of the MRL to public health is discussed further in the following sections concerning the derivation of the respective mercury MRLs.

ATSDR has established a chronic inhalation MRL of 0.2 μg/m^3 for metallic mercury. Assuming a ventilation rate of 20 m^3/day for an average adult, and assuming complete absorption, exposure at the level of the MRL would result in a daily dose of 4 μg. This level of exposure is thought to represent no health risk to any element of the human population. No other inhalation MRLs have been derived for mercury or its compounds.

Oral MRLs have been established for acute (0.007 mg/kg/day) and intermediate (0.002 mg/kg/day) duration exposures to inorganic mercury. ATSDR has also established a chronic oral MRL of 0.0003 mg/kg/day (equivalent to 21 μg/day for a 70-kg adult) for methylmercury. This MRL is at least four times the estimated average daily intake level for methylmercury from the diet. The FDA has estimated that, on average, the intake rate for total mercury (both inorganic and organic) is 50–100 ng/kg/day (equivalent to 0.05–0.1 μg/kg/day or 3.5–7 μg/day for a 70-kg adult). This figure is based on the FDA total diet study of 1982–1984 (Gunderson 1988). Approximately 80–90% of the mercury in the FDA estimate would be expected to be in the form of methylmercury. A separate estimate of the average intake of methylmercury alone, based on a survey of fish eaters and on average levels of methylmercury in fish, places the average intake of methylmercury at 36 ng/kg/day (equivalent to 0.036 μg/kg/day or 2.52 μg/day for a 70-kg adult), with a 99% upper-bound estimate at 243 ng/kg/day (equivalent to 0.243 μg/kg/day or 17 μg/day for a 70-kg adult) (Clarkson 1990). These results indicate that an assessment of total methylmercury intake and body burden should be conducted when estimating exposure to mercury in populations (especially sensitive populations) living near hazardous waste sites that have the potential to release mercury to the environment.

Inhalation MRLs

No inhalation MRLs were derived for inorganic mercury salts or organic mercury compounds due to the absence of data or to the lack of sufficient information regarding exposure levels associated with the reported observed effects.

No MRLs were derived for acute- or intermediate-duration inhalation exposure to metallic mercury vapors. Available studies were either deficient in their reporting of details of experimental protocols and results, used an insufficient number of experimental animals, or tested only one dose/concentration level.

- An MRL of 0.0002 mg/m^3 has been derived for chronic-duration inhalation exposure (365 days or longer) to metallic mercury vapor.

A significant increase in the average velocity of naturally occurring tremors compared to controls was observed in a group of 26 mercury-exposed workers (from 3 industries) exposed to low levels of mercury for an average of 15.3 years (range, 1–41 years) (Fawer et al. 1983). To estimate an equivalent continuous exposure concentration, the average concentration assumed for the 8 hour/day exposures was multiplied by 8/24 and 5/7 (0.026 mg/m^3 x 8/24 hours/day x 5/7 days/week=0.0062 mg/m^3). Uncertainty factors of 10 for variability in sensitivity to mercury within the human population and 3 for use of a minimal-effect LOAEL in MRL derivation were then applied to the calculated 0.0062 mg/m^3 value, yielding a chronic inhalation MRL of 0.2 µg/m^3. Although this MRL is based on experimental data from an adult working population, there is no experimental or clinical evidence to suggest that it would not also be sufficiently protective of neurodevelopmental effects in developing embryos/fetuses and children, the most sensitive subgroups for metallic mercury toxicity.

Inhaled metallic mercury is quickly absorbed through the lungs into the blood, and 70–80% is retained. Its biological half-life in humans is approximately 60 days. The half-life is different for different physiological compartments (e.g., 21 days in the head versus 64 days in the kidneys) (Hursh et al. 1976). Since the duration of exposure influences the level of mercury in the body, the exposure level reported in the Fawer et al. (1983) occupational study was extrapolated from an 8-hour day, 40-hour workweek exposure to a level equivalent to a continuous 24 hour/day, 7 day/week exposure, as might be encountered near a hazardous waste site containing metallic mercury.

Gentry et al. (1998) used the neurobehavioral information on a control group and one exposure group from the Fawer et al. (1983) study to derive an inhalation MRL for elemental mercury based upon a benchmark dose (BMD) analysis. Dose-response analysis could be performed on four measures of hand tremor, with tasks performed both at rest and with a load. The exposure level of the exposed group to metallic mercury was assumed to be the mean TWA exposure of 0.026 mg/m^3. A physiologically based pharmacokinetic model for metallic mercury vapor was found to be linear through the region of concern from the LOAEL to

the MRL; that is, the relationship between inhaled concentration and target tissue concentration at the LOAEL and at lower levels (including the MRL) did not differ. Therefore, exposure concentrations were used directly for the analysis. Gentry et al. (1998) also assumed that 1% of the unexposed population would be considered in the adverse response range. The BMD_{10} was the dose at which the probability of exceeding the 1% adverse response level was 10% greater than in unexposed individuals, and the $BMDL_{10}$ is the 95% lower bound confidence level on that dose. A simple linear model sufficed to describe the dose-response. A $BMDL_{10}$ of 0.017 mg metallic mercury/m^3 was derived as a reasonable representation of the sparse data. This level would be equivalent to a NOAEL (i.e., no LOAEL to NOAEL uncertainty factor is needed). Using a PBPK model to estimate target tissue doses from inhaled mercury vapor and adjusting for continuous exposure and interhuman variability (with an uncertainty factor of 10), an MRL of 0.0004 mg/m^3 (based on target tissue dose) was derived which is about two times the ATSDR derived MRL of 0.0002 mg/m^3 based upon the Fawer et al. (1983) LOAEL.

The ability of long-term, low-level exposure to metallic mercury to produce a degradation in neurological performance was also demonstrated in other studies. One such study (Ngim et al. 1992) attributed adverse neurological effects to a lower average level of exposure than did the Fawer et al. (1983) study; however, this study was not used in deriving a chronic inhalation MRL due to uncertainties concerning the study protocol, including methodological and reporting deficiencies. In the Ngim et al. (1992) study, dentists with an average of 5.5 years of exposure to low levels of metallic mercury were reported to have impaired performance on several neurobehavioral tests. Exposure levels measured at the time of the study ranged from 0.0007 to 0.042 mg/m^3, with an average of 0.014 mg/m^3. Mean blood mercury levels among the dentists ranged from 0.6 to 57 µg/L, with a geometric mean of 9.8 µg/L. The performance of the dentists on finger tapping (measures digital motor speed), trail-making (measures visual scanning and motor speed), digit symbol (measures visuomotor coordination and concentration), digit span, logical memory delayed recall (measure of verbal memory), and Bender-Gestalt time (measures visual construction) tests was significantly poorer than controls. The exposed dentists also showed higher aggression than did controls. Furthermore, within the group of exposed dentists, significant differences were observed between a subgroup with high mercury exposure compared to a subgroup with lower exposure. These exposure severity subgroups were not compared to controls, and average exposure levels for the subgroups were not reported. The design and reporting of this study limits its usefulness in deriving an MRL for metallic mercury. The exposure status of the subjects was known to the investigator during testing, mercury levels were not reported for controls, and methods used to adjust for potential contributions other than mercury from amalgams to the study results (such as the possible use in this population of traditional medicines

containing mercury) were not reported. It was also unclear whether the results for the mercury exposure group were inordinately influenced or skewed by the individual dentists with the highest exposures and/or blood levels. These confounding factors precluded the use of the Ngim et al. (1992) study for the derivation of an MRL, but the study does provide support both for the premise that low-dose chronic exposure to metallic mercury can result in adverse health sequelae and for the chronic inhalation MRL that is based on the Fawer et al. (1983) study of occupationally exposed individuals.

Other occupational studies further support the ability of metallic mercury to induce neurological deficits. Several studies have reported significant effects on tremor or cognitive skills among groups exposed occupationally to comparable or slightly higher (up to 0.076 mg/m^3) levels (Ehrenberg et al. 1991; Piikivi et al. 1984; Roels et al. 1982). Difficulty with heel-to-toe gait was observed in thermometer plant workers subjected to mean personal breathing zone air concentrations of 0.076 mg/m^3 (range, 0.026–0.27 mg/m^3) (Ehrenberg et al. 1991). Tremors have also been reported in occupationally exposed workers with urinary mercury concentrations of 50–100 µg/g creatinine and blood levels of 10–20 µg/L (Roels et al. 1982). By comparison, blood mercury levels in the Fawer et al. (1983) study averaged 41.3 and 16.6 µmol Hg/L for the exposed and control groups, respectively. Urinary mercury levels for the exposed workers in the Fawer et al. (1983) study averaged 11.3 µmol Hg/mol creatinine (about 20 µg/g creatinine), compared with 3.4 µmol/mol creatinine in the controls. Piikivi et al. (1984) found decreases in performance on tests that measured intelligence (based upon a similarities test) and memory (evaluating digit span and visual reproduction) in chloralkali workers exposed for an average of 16.9 years (range, 10–37 years) to low levels of mercury, when compared to an age-matched control group. In this study, significant differences from controls were observed on these tests among 16 workers with blood levels ranging from 75 nmol/L to 344 nmol/L and urine levels ranging from 280 nmol/L (about 56 µg/L) to 663 nmol/L. Abnormal nerve conduction velocities have also been observed in chloralkali plant workers at a mean urine concentration of 450 µg/L (Levine et al. 1982). These workers also experienced weakness, paresthesias, and muscle cramps. Prolongation of brainstem auditory evoked potentials was observed in workers with urinary mercury levels of 325 µg/g creatinine (Discalzi et al. 1993). Prolonged somatosensory-evoked potentials were found in 28 subjects exposed to airborne mercury concentrations of 20–96 mg/m^3 (Langauer-Lewowicka and Kazibutowska 1989). All of these studies substantiate the ability of chronic, low- to moderate-level exposure to metallic mercury vapors to cause neurological deficiencies.

Employment of the Chronic Inhalation MRL for Metallic Mercury

ATSDR emphasizes that the MRL is not intended to be used as an estimation of a threshold level. Exceeding the MRL does not necessarily mean that a health threat exists. However, the greater the amount by which the MRL is exceeded and the longer or more frequent the individual exposures, the greater the likelihood that some adverse health outcome may occur. Secondly, the chronic inhalation MRL is, by definition, a level that is considered to be without appreciable (or significant) health risk over a lifetime of exposure at that level. It is further considered to be a "safe" level for all factions of the exposed human population, when exposure exists for 24 hours a day, 7 days a week for an extended period of years. The employment of the MRL, therefore, must be geared to the particular exposure scenario at hand. For example, people may be able to "tolerate" metallic mercury levels above the MRL for intermittent periods of exposure (e.g., 1 or 2 hours per day, 5 days per week) without any adverse health sequelae, either overt or covert. The use of the "contaminated area" (e.g., storage versus exercise room versus day care) will largely influence the use of the MRL. Finally, the MRL is intended primarily as a "screening value" for public health officials to use in their assessment of whether further evaluation of the potential risk to public health is warranted in a hazardous waste site scenario. The MRL is *not* intended, nor should it be indiscriminately used, as a clean-up or remediation level, or as a predictor of adverse health effects. While it is considered to afford an adequate degree of protection for the health of all potentially exposed individuals, it might be unnecessarily stringent for application to some exposure situations (i.e., higher air concentrations might afford a similar degree of protection in some exposure scenarios); thus, its relevance in any specific environmental situation is intended to be determined by an experienced public health or medical official.

Oral MRLs

Metallic Mercury

No oral MRLs were derived for metallic (elemental) mercury due to the lack of data. Oral exposure to liquid metallic mercury would be expected to present little health risk, since it is so poorly absorbed (<0.01%) through the healthy intestine. Sufficiently large quantities could, however, present a risk of intestinal blockage, and some could enter the systemic circulation (blood or lymphatic) through open lesions, presenting a risk of occlusion of smaller arteries, especially within the pulmonary circulation.

Inorganic Mercury

The acute- and intermediate-duration MRLs for oral exposure to inorganic mercury are based on kidney effects reported in a 1993 NTP study of mercuric chloride (NTP 1993). Most of the supporting studies of oral exposure to inorganic mercury also use mercuric chloride.

Mercuric sulfide (also known as cinnabar) is the predominant natural form of mercury in the environment and is a common ore from which metallic mercury is derived. Mercury released to the environment may be transformed into mercuric sulfide. Several studies suggest that the bioavailability of mercuric sulfide in animals is less than that of mercuric chloride (Sin et al. 1983, 1990; Yeoh et al. 1986, 1989). For example, Sin et al. (1983) found an increase in tissue levels of mercury in mice orally exposed to low doses of mercuric chloride, but elevated levels of mercury were not found in the tissues of mice fed an equivalent weight of mercuric sulfide. This finding indicates a difference in bioavailability between $HgCl_2$ and HgS in mice. However, a quantitative determination of the relative bioavailabilities of mercuric sulfide versus mercuric chloride has not been derived in the available studies, nor has the relative bioavailability of mercuric sulfide in humans been examined.

- An MRL of 0.007 mg Hg/kg/day has been derived for acute-duration oral exposure (14 days or less) to inorganic mercury.

The MRL was based on a NOAEL of 0.93 mg Hg/kg/day for renal effects in rats administered mercuric chloride 5 days a week for 2 weeks. The dose used in this study was duration-adjusted for a 5-day/week exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). Increased absolute and relative kidney weights were observed in male rats exposed to 1.9 mg Hg/kg/day as mercuric chloride (NTP 1993). At higher doses, an increased incidence and severity of tubular necrosis was observed.

Several other studies examining the effects of oral exposure to inorganic mercury salts have also shown renal toxicity in humans as a result of acute oral exposures. Kidney effects (i.e., heavy albuminuria, hypoalbuminemia, edema, and hypercholesterolemia) have been reported after therapeutic administration of inorganic mercury (Kazantzis et al. 1962). Acute renal failure has been observed in a number of case studies in which mercuric chloride had been ingested (Afonso and deAlvarez 1960; Murphy et al. 1979; Samuels et al. 1982). The autopsy of a 35-year-old man who ingested a lethal dose of mercuric chloride

and exhibited acute renal failure showed pale and swollen kidneys (Murphy et al. 1979). A case study reported acute renal failure characterized by oliguria, proteinuria, hematuria, and granular casts in a woman who ingested 30 mg Hg/kg body weight as mercuric chloride (Afonso and deAlvarez 1960). Another case study reported a dramatic increase in urinary protein secretion by a patient who ingested a single dose of 15.8 mg Hg/kg body weight as mercuric chloride (assuming a body weight of 70 kg) (Pesce et al. 1977). The authors of the report surmised that the increased excretion of both albumin and β_2-microglobulin were indicative of mercury-induced tubular and glomerular pathology. Acute renal failure that persisted for 10 days was also observed in a 19-month-old child who ingested an unknown amount of powdered mercuric chloride (Samuels et al. 1982). Decreased urine was also observed in a 22-year-old who attempted suicide by ingesting approximately 20 mg Hg/kg (Chugh et al. 1978).

- An MRL of 0.002 mg Hg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to inorganic mercury.

This MRL was based on a NOAEL of 0.23 mg Hg/kg/day for renal effects in rats administered mercuric chloride 5 days a week for 6 months (Dieter et al. 1992; NTP 1993). This dose was duration-adjusted for a 5 day/week exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). Increased absolute and relative kidney weights were observed in rats exposed to 0.46 mg Hg/kg/day, the next higher treatment level. At higher doses, an increased incidence of nephropathy (described as foci of tubular regeneration, thickened tubular basement membrane, and scattered dilated tubules containing hyaline casts) was observed. Renal toxicity is a sensitive end point for inorganic mercury toxicity, as seen in other intermediate-duration oral studies on rats and mice exposed to inorganic mercury (Carmignani et al. 1992; Jonker et al. 1993a; NTP 1993), as well as case reports of humans ingesting inorganic mercury for acute and chronic durations (Afonso and deAlvarez 1960; Davis et al. 1974; Kang-Yum and Oransky 1992; Nielsen et al. 1991; Pesce et al. 1977).

The relatively small difference between the acute-duration MRL (0.007 mg/kg/day) and the intermediate-duration MRL (0.002 mg/kg/day) is not meant, nor is it considered, to imply a high level of precision in the calculation of these health guidance values. Rather, this difference of 5 µg/kg/day reflects the increased toxicity of continued low-dose exposure for longer periods of time and is consistent with the known build-up of mercury levels in body tissues over a prolonged course of continued exposure. The actual precision of any derived (actually estimated) MRL is dependent upon an encompassing, but not sharply defined, area of uncertainty based upon the database used in its determination.

As a method of comparison to evaluate whether use of another method to derive an MRL might result in a different intermediate oral MRL value for inorganic mercury, ATSDR used the same data (Dieter et al. 1992; NTP 1993) to calculate a benchmark dose for inorganic mercury. Using the most sensitive end point identified in this study (relative kidney weight changes in rats), the experimental data were used to obtain a modeled dose-response curve. Benchmark doses were then determined for the 10% response level (0.38 mg/kg/day) and the 5% response level (0.20 mg/kg/day). After adjusting the 5-days/week exposures in the study to 7-days/week equivalent doses, the 10 and 5% response-base benchmarks became 0.27 and 0.15 mg/kg/day, respectively. Application of 10-fold uncertainty factors for each inter- and intraspecies variability resulted in estimated human benchmark doses of 0.003 mg/kg/day for the 10% response level and 0.002 mg/kg/day for the 5% response level. These values strongly support the current existing intermediate oral MRL of 0.002 mg/kg/day for inorganic mercury.

No MRL for chronic-duration oral exposure to inorganic mercury was derived, because the study results showed decreased survival rate for male rats at all LOAELs.

Organic Mercury

Acute, Intermediate, or Chronic Inhalation MRLs: No inhalation MRLs were derived for organic mercury compounds, due to the absence of data or to the lack of sufficient information regarding exposure levels associated with the reported observed effects.

Acute and Intermediate Oral MRLs: No MRLs were derived for acute or intermediate oral exposure to organic mercury compounds due to the absence of data or to the lack of sufficient information regarding exposure levels associated with the reported observed effects.

Chronic Oral MRL for Methylmercury: Hair levels are typically used as an index of exposure to methymercury. A number of studies report that hair mercury levels correlate with total intake levels and with organ-specific levels of mercury. Suzuki et al. (1993) analyzed 46 human autopsies in Tokyo, Japan and reported that hair mercury levels were highly significantly correlated with organ Hg levels in the cerebrum, cerebellum, heart, spleen, liver, kidney cortex, and kidney medulla, when the total mercury or methyl mercury value in the organ was compared with the hair total mercury or organic mercury, respectively.

Nakagawa (1995) analyzed total mercury in hair samples from 365 volunteers in Tokyo, and reported higher mercury levels in those who preferred fish in their diet, compared to those who preferred other foods (preference choices were fish, fish and meat, meat, and vegetables). The mean hair mercury levels were 4 ppm in men who preferred fish and 2.7 ppm in women who preferred fish. The lowest hair mercury levels were seen in men and women who preferred vegetables, 2.27 and 1.31 ppm, respectively. The mean hair level for the whole group was 2.23 ppm (median 1.98).

Drasch et al. (1997) assayed tissue samples of 150 human cadavers (75 males, and 75 females) from a "normal" European (German) population, i.e., there were no occupational or higher than average exposures to metals found in any of the biographies of the deceased. The objective was to evaluate the validity of blood, urine, hair, and muscle as biomarkers for internal burdens of mercury, lead, and cadmium in the general population. All individuals died suddenly and not as a result of chronic ailments. Age ranged from 16 to 93 years, and every decade was represented by approximately 10 males and 10 females. Tissues sampled included kidney cortex, liver, cerebral cortex, cerebellum, petrous portion of the temporal bone, (pars petrosis ossis temporalis), pelvic bone (spina iliaca anterior-superior), muscle (musculus gluteus), blood (heart blood), urine, and hair (scalp-hair). Statistically significant rank correlations between biomarker levels and tissues were observed, but with large confidence intervals for the regressions. The authors conclude that specific biomarkers relative to each metal are useful in estimating body burdens and trends in groups, but are not useful for determining the body burden (and therefore the health risks) in individuals. A notable exception was for correlation to brain mercury. By comparison to a generally poor correlation of cadmium, lead, and mercury between hair and tissue, there was a strong correlation between mercury in hair and mercury in brain (cerebrum and cerebellum). The authors state that this may be due to the high lipophilicity of elemental and short-chain alkyl mercury compounds. As seen in other studies comparing European to Japanese hair mercury levels, the mercury hair levels reported by Nakagawa (1995) of 2–4 ppm for a Japanese population are 10–20 times higher than total mercury levels observed in the Drasch et al. (1997) study (median, 0.247 µg/g in hair; range, 0.43–2.5 µg/g).

Other studies have confirmed a good correlation between hair mercury and brain mercury levels. In a study on the Seychelles Islands cohort, Cernichiari et al. (1995b) compared maternal hair levels, maternal blood levels, fetal blood levels, and fetal brain levels. Autopsy brains were obtained from infants dying from a variety of causes. The concentrations of total mercury in six major regions of the brain were highly correlated with maternal hair levels. This correlation was confirmed by a sequence of comparisons among the four measurements. Maternal hair levels correlated to maternal blood levels (r=0.82) and infant brain

levels (r=0.6–0.8). Concentrations in maternal blood correlated with infant blood levels (r=0.65); and infant blood levels correlated to infant brain levels (r=0.4–0.8).

Accordingly, ATSDR used maternal hair mercury levels as the exposure measurement to derive a chronic MRL for methylmercury. While hair analysis can be confounded by outside sources of contamination (e.g., as might occur in certain occupational settings) (Hac and Krechniak 1993), the study population used as the basis of the chronic oral MRL for methylmercury is far removed from external or industrial sources of mercury, effectively eliminating this as a consideration for the following analysis.

- An MRL of 0.0003 mg Hg/kg/day has been derived for chronic-duration oral exposure (365 days or longer) to methylmercury.

The chronic oral MRL for methylmercury is based upon the Seychelles Child Development Study (SCDS), in which over 700 mother-infant pairs have, to date, been followed and tested from parturition through 66 months of age (Davidson et al. 1998). The SCDS was conducted as a double-blind study and used maternal hair mercury as the index of fetal exposure. Enrollees were recruited by the head nurse/hospital midwife by asking the mothers if they wished to participate in the study when they arrived at the hospital for delivery. The first 779 who did not decline participation became the mothers in the study cohort. Of the initial 779 mothers enrolled in the study at parturition, 740 remained at the pre-determined child testing age of 6.5 months, 738 remained in the 19-month cohort, 736 remained at 29 months, and 711 remained for the 66-month neurobehavioral and developmental examinations.

The Seychellois were chosen as a study population for a number of reasons.

- (1) All fish contain some level of methylmercury (Davidson et al. 1998); and the Seychellois regularly consume a high quantity and variety of ocean fish, with 12 fish meals per week representing a typical methylmercury exposure.
- (2) The median total mercury concentration in 350 fish sampled from 25 species consumed by the Seychellois was <1 ppm (range, 0.004–0.75 ppm), comparable to the mercury concentration in commercially obtainable fish in the U.S. (It should be noted here that while the methylmercury levels in the Seychellois population are 10–20 times those in the U.S., it is not because they consume more highly contaminated fish, but rather because they consume more fish than the U.S. population.)
- (3) The Seychelles represents a relatively pristine environment, with no local industry for pollution, and are situated more than 1,000 miles from any continent or large population center.

- (4) The population is highly literate, cooperative, and has minimal immigration and emigration.

C (5) The Seychellois constitute a generally healthy population, with low maternal alcohol consumption and tobacco use (<2%).

C (6) The large sample size/study population (>700 mother-infant pairs).

C (7) Excellent retention of mother-infant pairs throughout the study (i.e., 711 of the initially enrolled 778 mother-infant pairs still participating at 66 months post-partum.

C (8) The use of standardized neurobehavioral tests.

The results of the 66-month testing in the SCDS revealed no evidence of adverse effects attributable to chronic ingestion of low levels of methylmercury in fish (Davidson et al. 1998). In this study, developing fetuses were exposed *in utero* through maternal fish ingestion before and during pregnancy (Davidson et al. 1998). Neonates continued to be exposed to maternal mercury during breastfeeding (i.e., some mercury is secreted in breast milk), and methylmercury exposure from the solid diet began after the gradual post-weaning shift to a fish diet. In the 66-month study cohort, the mean maternal hair level of total mercury during pregnancy was 6.8 ppm (range, 0.5–26.7 ppm; n=711), and the mean child hair level at the 66-month testing interval was 6.5 ppm (range, 0.9–25.8 ppm; n=708). The mean maternal hair mercury level in the highest exposed subgroup in the study was 15.3 ppm (range, 12–26.7; n=95). The 66-month test battery, which was designed to test multiple developmental domains, included the following primary measurements:

C (1) General Cognitive Index (GCI) of the McCarthy Scales of Children's Abilities (to estimate cognitive ability);

C (2) the Preschool Language Scale (PLS) total score (to measure both expressive and receptive language ability);

C (3) the Letter and Word Recognition and

C (4) Applied Problems subtests of the Woodcock-Johnson (W-J) Tests of Achievement (to measure reading and arithmetic achievement);

C (5) the Bender-Gestalt test (to measure visual-spatial ability); and

C (6) the total T score from the Child Behavior Checklist (CBCL) (to measure the child's social and adaptive behavior). Serum sampling revealed no detectable levels of PCBs (detection limit=0.2 ng/mL).

None of the tests indicated an adverse effect of methylmercury exposure. As evaluated through regression analyses, there was no reduction in performance with increasing maternal hair mercury levels for the neurobehavioral parameters examined. In contrast, scores were better for four of the six tests in the highest MeHg-exposed groups, compared with lower exposure groups for both prenatal and postnatal exposure (the four test were the (1) General Cognitive Index (GCI) of the McCarthy Scales of Children's `Abilities (to estimate cognitive ability); (2) the Preschool Language Scale (PLS) total score (to measure both expressive and receptive language ability); (3) the Letter and Word Recognition and (4) Applied Problems subtests of the Woodcock-Johnson (W-J) Tests of Achievement (to measure reading and arithmetic achievement). While the positive outcomes are not considered to indicate any beneficial effect of methylmercury on neurological development or behavior, they might be more appropriately attributed to the beneficial effects of omega-3 fatty acids or other constituents present in fish tissue, since the methylmercury levels in hair are known to correlate closely with fish intake. The slight decreases in the subjectively reported activity level of boys reported in the 29-month observations were not seen during the 66-month tests. The mean maternal hair level of 15.3 ppm in the highest exposed group in the 66-month test cohort is, therefore, considered a NOAEL for SCDS and is used by ATSDR as the basis for derivation of a chronic oral MRL for methylmercury. A related study (Myers et al. 1997) by some members of the same team of researchers from the University of Rochester examined the Seychellois children for attainment of the same developmental milestones reported to have been delayed in the Iraqi poisoning incident in the early 1970s (Cox et al. 1989); however, unlike the Iraqi study, no delays in the age of first walking and talking was seen in the Seychellois children exposed in utero.

Sensitivity of Neurobehavioral Measures /Reliability of Tests

The neurobehavioral test battery used in the 66-month Seychelles study was designed to assess multiple developmental domains (Davidson et al. 1998). The tests were considered to be sufficiently sensitive and accurate to detect neurotoxicity in the presence of a number of statistical covariates. On-site test administration reliability was assessed by an independent scorer, and mean interclass correlations for interscorer reliability were 0.96–0.97 (Davidson et al. 1998). The sample size was determined to be sufficient to detect a 5.7-point difference on any test with a mean (SD) of 100 (16) between low (0–3 ppm) and high (>12 ppm) hair mercury concentration groups for a 2-sided test (A = 0.05 at 80% power).

Supporting Studies

Crump et al. (1998) conducted benchmark dose (BMD) calculations and additional regression analyses of data collected in a study in which a series of scholastic and psychological tests were administered to children whose mothers had been exposed to methylmercury during pregnancy. Hair samples were collected from 10,970 new mothers in New Zealand in 1977 and 1978. High hair mercury levels were considered to be those over 6 ppm, which was the hair level predicted to result at steady state from consumption of mercury at the WHO/FAO Provisional Tolerable Weekly Intake of 0.3 mg total mercury/week and 0.2 mg methylmercury/week. By this criterion, 73 of approximately 1,000 mothers who had consumed fish more than 3 times/week during pregnancy were determined to have high hair mercury levels. In 1985, when the children were 6 to 7 years of age, 61 children (1 set of twins) of the 73 mothers in the high hair mercury group were located; these children constituted the high exposure group, which was matched with three control groups (one with 3–6 ppm maternal hair mercury levels, one with 0–3 ppm whose mothers had been high fish consumers, and one with 0–3 ppm whose mothers had not been high fish consumers). The entire study cohort consisted of 237 children. A battery of 26 psychological and scholastic tests were administered to the children at school during the year 1985. Mothers were interviewed at the time of test administration to obtain additional data on social and environmental factors. In the high exposure group of children, one boy's mother had a hair mercury level of 86 ppm, which was more than four times higher than the next highest hair mercury level of 20 ppm. BMDs (10% response rate) calculated from five tests ranged from 32 to 73 ppm, when the 86 ppm mother's child was included. This corresponded to a BMDL range of 17 to 24 ppm. Although none of the 86 ppm child's test scores was an outlier according to the definition used in the analyses, his scores were significantly influential in the analyses. When this child was omitted from the analyses, BMDs ranged from 13 to 21, with corresponding BMDLs of 7.4 to 10 ppm.

Developing fetuses in the SCDS were exposed through maternal fish ingestion before and during pregnancy. Each child was evaluated at 19 months and again at 29 months (±2 weeks) for infant intelligence (Bayley Scales of Infant Development [BSID] Mental and Psychomotor Scales), with a modified version of the BSID Infant Behavior Record to measure adaptive behaviors at 29 months (Davidson et al. 1995b). Testing was performed by a team of Seychellois nurses extensively trained in administration of the BSID. Maternal hair concentrations, measured in hair segments that corresponded to pregnancy, ranged from 0.5 to 26.7 ppm, with a median exposure of 5.9 ppm for the entire study group. The mean BSID Mental Scale Indices determined at both 19 and 29 months were found to be comparable

to the mean performance of U.S. children. The BSID Psychomotor Scale Indices at both measurement intervals were two standard deviation units above U.S. norms, but were still consistent with previous findings of motor precocity in children reared in African countries. The study found no effect that could be attributed to mercury on the BSID scores obtained at either the 19- or 29-month measurement/testing interval. The 29-month cohort represented 94% of the 779 mother-infant pairs initially enrolled in the study, and approximately 50% of all live births in the Seychelles in 1989.

The only observation in the 29-month testing that might be attributable to prenatal mercury exposure was a slight decrease in the activity level in boys (but not girls) as determined by the Bayley Infant Behavior Record (subjective observation). Whereas this decrease was significant in males ($p = 0.0004$), it was not statistically significant in females ($p = 0.87$). When the subjective activity scores for male and female children were evaluated collectively, no statistically significant or remarkable decrease in activity was apparent outside the >12 ppm maternal hair concentration group. The affect on activity level in boys is not considered an adverse effect by the authors of the study.

Grandjean et al. (1997b, 1998) reported another epidemiological study of methylmercury exposure for a population in the Faroe Islands. Although the Faroese are a fishing culture, the major source of methylmercury exposure for this population is pilot whale meat, which is intermittently consumed as part of the cultural tradition. The initial study cohort consisted of 1,022 singleton births occurring in a 21-month window during 1986-1987. At approximately 7 years of age, neurobehavioral testing was conducted on 917 of the remaining cohort members. No abnormalities attributable to mercury were found during clinical examinations or neurophysiological testing. A neuropsychological test battery was also conducted, which included the following: Finger Tapping; Hand-Eye Coordination; reaction time on a Continuous Performance Test; Wechsler Intelligence Scale for Children - Revised Digit Spans, Similarities, and Block Designs; Bender Visual Motor Gestalt Test; Boston Naming Test; and California Verbal Learning Test (Children). Neuropsychological tests emphasized motor coordination, perceptual-motor performance, and visual acuity. Pattern reversal visual evoked potentials (VEP) with binocular full-field stimulation, brain stem auditory evoked potentials (BAEP), postural sway, and the coefficient of variation for R-R inter-peak intervals (CVRR) on the electrocardiogram were all measured. The neuropsychological testing indicated mercury-related dysfunction in the domains of language, attention, memory, and visuospatial and motor function (to a lesser extent), which the authors considered to remain after the children of women with maternal hair mercury concentrations above 10 µg/g (10 ppm) were excluded. While this study represents a significant contribution to the human database for methylmercury exposure

and effects, a number of potentially influential factors not fully considered as possible covariates somewhat cloud the interpretation of the results.

These differences between the neuropsychological effects observed in the Faroe Island cohort and the absence of effects reported in the Seychelles Island cohort might result from a variety of factors. The Faroe Island children were older (7–8 years versus 5.5 in the SCDS). Some of the measurement instruments (i.e., the neuropsychological test administered) were also different. Since the first neuropsychological testing in the Faroe study was not conducted until 7 years of age, it is not known whether the observed effects might have been apparent at an earlier age. Ongoing and planned future testing of the Seychelles population will provide additional information on the progression of any observed effects. Further examination of the Seychelles population using the neuropsychological test that showed positive results in the Faroe Island population will also allow a more direct comparison of results.

The diet in the two studies was also considerably different. The majority of the mercury exposure to the Faroe Island population came from whale meat (estimated at about 3 ppm in muscle tissue) with a relatively small portion coming from fish. Some of the mercury in whale meat is in the form of inorganic mercury. In the Seychelles study, all of the mercury came from fish as methylmercury with concentrations of around 0.3 ppm. Whale meat blubber is widely consumed in the Faroe Islands and also contains polychlorinated biphenyls (PCBs). Grandjean et al. (1995b) estimated a daily intake of 200 µg of PCB. This value can be compared to the Tolerable Daily Intake of PCBs established by the FDA of 60–70 µg/day for an adult. Further statistical analysis of the possible influence of PCBs on the observed study results needs to be conducted (see the discussion below on Peer Panel 1Review of Key Studies for additional comments).

The primary biomarker used to estimate mercury exposure was also different in the two studies. The Faroe Island analysis used cord blood, and the Seychelles study used maternal hair level. The use of mercury in cord blood has the advantage of being a more direct measure of exposure to the fetus, but the levels at term may not reflect exposures at earlier developmental stages. While Grandjean et al. (1997) did report maternal hair mercury levels, the mean hair level for the interquartile range of 2.6–7.7 ppm was reported only as a geometric average (4.27 ppm). In contrast, the Seychelles study reported only an arithmetic mean level for the entire study population (6.8 ppm). While both are valid measures, a direct comparison of "average" values for the two studies is not possible without further statistical analysis of both data sets.

In the case of the Faroe study, no data were presented in the peer-reviewed publications to address variability of food/whale meat or blubber intake among the Faroe Islanders, making it difficult to evaluate the possibility of peak intake levels during critical development phases. Consumption data were reported only as <1 pilot whale meat meal/month and 1–2 fish meals per week. In contrast, the Seychelles dietary habits provide a relatively stable intake, and a high degree of correlation was found between mean hair levels in samples covering each trimester and levels in samples for the entire pregnancy (Cernichiari et al. 1995a). Cernichiari et al. (1995b) also report a good correlation between levels of total mercury in neonatal brain and levels in the corresponding maternal hair. While the contribution of continued mercury exposure through breast feeding or post-weaning diet was not fully addressed in the Seychelles study reports (Davidson 1995, 1998), that is not considered a significant drawback of the study, since no effects on neurobehavioral/neuropsychological testing were seen at any maternal hair level. In the Faroese assessment of latent neuropsychological effects from an *in utero* exposure to mercury, however, the role of continuing postnatal exposure to mercury either from breast milk or from ingestion of methylmercury-containing foods (e.g., pilot whale meat) is less clear. Specifically, it is not known what proportion, if any, of the neuropsychological effects reported in the Faroe Islands population could be attributed to 7 years of postnatal exposure to methylmercury in food. The variability and magnitude of this postnatal exposure should, therefore, be further evaluated.

Peer Panel Review of Key Studies

In addition to the traditional peer review process that precedes publication in most scientific journals, the studies considered by ATSDR for use in estimating a chronic oral MRL for methylmercury underwent two stringent reviews by recognized experts in the environmental health field.

On July 20 and 21, 1998, ATSDR assembled a panel of 18 experts from the scientific and medical communities to review current issues and the relevant literature on mercury and its compounds, including methylmercury (ATSDR 1999). Several members of each of the respective research teams that conducted the Iraqi, Seychelles, Faroe, and Madeira studies were included among the expert panelists, and provided extensive overviews of their studies. The presentations were followed by an open, wide-ranging scientific discussion of the merits and interpretations of the currently available studies. Topics of significant discussion included the relative merits of the respective study populations, exposure regimens, sensitivity of neurobehavioral measures, and determination of an uncertainty factor. While it was unanimously agreed that the Seychelles and Faroe studies were both excellent studies that provided a significant contribution to

the human database for methylmercury exposure and effects, a number of factors that could have contributed to the study results, but were not considered as possible statistical covariates, were discussed. In the case of the Faroe study, the consumption of whale blubber, which is known to be contaminated with PCBs, DDT, and possibly other organochlorines, introduces a potentially significant influence on the study results. Weihe et al. (1996) reported that the PCB and DDT concentrations in blubber of pilot whales taken in Faroese waters are about 30 ppm and 20 ppm, respectively. In contrast, the Seychellois population does not eat marine mammals at all. In addition, the Faroe study did not address other possible statistical covariates, such as the dietary and nutritional status of the study population and the use of tobacco during pregnancy, further complicating the interpretation of the neuropsychological test results.

On November 18–20, 1998, a workshop on Scientific Issues Relevant to the Assessment of Health Effects from Exposure to Methylmercury was conducted in Raleigh, North Carolina. The workshop was jointly sponsored by the U.S. Department of Health and Human Services (DHHS), the National Institute of Environmental Health Sciences (NIEHS), the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA), the U.S. Environmental Protection Agency (EPA), the National Oceanic and Atmospheric Administration (NOAA), the Office of Science and Technology Policy (OSTP), the Office of Management and Budget (OMB), and ATSDR. The purpose of this workshop was to discuss and evaluate the major epidemiologic studies that associated methylmercury exposure and the results of an array of developmental measures in children. These studies monitored and evaluated exposed populations in Iraq, the Seychelles Islands, the Faroe Islands, and the Amazon River Basin. A number of animal studies were also considered in support of a human health risk assessment. Presentation of each study by the research team that conducted the study was followed by an expert panel evaluation that examined each study, taking into consideration the exposure data, experimental design and statistical analysis, potential confounders and variables, and neurobehavioral end points evaluated. A fifth panel evaluated the results of relevant animal studies. Significant issues that were discussed included the use of umbilical cord blood mercury levels versus hair mercury concentrations as an index of methylmercury exposure during pregnancy, the patterns of exposure, the dietary/health status of study populations, other potentially relevant exposures, other confounding influences, and the adjustments made for statistical covariates. All five panels at this workshop commended the efforts of the investigators and respective staffs of the Seychelles and Faroe studies for conducting highly sophisticated investigations under difficult conditions. However, specific findings of several of the panels raise issues that, at present, preclude the Faroe data from consideration as a starting point for MRL derivation.

In addressing the potential influence of concurrent PCB exposure on the Faroe results, the Confounders and Variables (Epidemiology) panel indicated that with respect to four of the prenatal outcomes (related primarily to verbal and memory performance), when PCBs were included in the model, only one of these outcomes is specifically related to mercury exposure. Concerning this matter, the panel wrote that "... the most likely explanation is that both (mercury and PCBs)... affect these three outcomes, but their relative contributions cannot be determined given their concurrence in this population." The Neurobehavioral Endpoints Panel also looked at this issue, and noted that "PCB exposure might act as an effect modifier, increasing the susceptibility to MeHg"; however, this panel further indicated that it did not believe that the effects seen in the Faroe Islands were due to uncontrolled confounding by PCBs. A third panel that addressed the issue of concurrent PCB exposures, the Statistics/Design Panel, noted that only 3 of 208 PCB congeners were measured in the Faroe study, and stated that it "seems likely that mercury was measured more accurately than the biologically relevant PCB exposure. Consequently even if the neurological effects seen in this study were caused entirely by PCBs, it is possible that mercury would still be more highly correlated with these effects than PCBs." The Statistics/Design Panel also said that "the best method to deal with this problem would be to study a population where exposure to PCBs is not an issue." This statement points directly to the Seychelles study as the study most appropriate for MRL derivation.

Another issue raised at Raleigh workshop concerned the taking of hair samples for determining pre-natal exposure. In the Seychelles, hair samples were collected 6 months post-partum, and segments corresponding to pregnancy were selected for analysis. In the case of the Faroese, hair samples were taken at the scalp. Regarding that, the Confounders and Variables (Epidemiology) panel stated that "Given the time it takes the Hg to be excreted into the hair, we can assume that samples collected at parturition do not cover the last 6 weeks of gestation, during which critically important neuronal proliferation and differentiation is taking place."

Regarding the Seychelles and Faroe studies, the Neurobehavioral Endpoints Panel found "no specific neurobehavioral signature injury from MeHg" in the data from either study (Seychelles or Faroe). The same panel also noted that episodic exposure in the Faroese (1–2 fish meals/week and <1 pilot whale meal/month) "may reduce the likelihood of detecting a consistent 'neurobehavioral signature injury' specific to MeHg and may account for different observations in children with the same average exposure."

Based upon the discussions at the Raleigh workshop and the individual panel findings, as well as the aforementioned Atlanta expert panel review, ATSDR has determined that the Seychelles study represents

the most appropriate and reliable database currently available for calculation of a chronic oral MRL from a population exposed only to methylmercury by a relevant route of exposure for the overall U.S. population.

Again, ATSDR would like to strongly emphasize that both the Seychelles study and the Faroese study represent credible scientific contributions by widely respected research teams. Similarly, both studies extend our knowledge base well beyond that provided by the Iraqi study and make significant contributions to our understanding of the effects of low-level exposure to methylmercury by an exposure route and vehicle (i.e., food) relevant to U.S. populations. The continuing monitoring and evaluation of the Seychellois and Faroese populations with more comparable neurobehavioral indices should help strengthen our understanding of the effects of low-level chronic methylmercury exposure and should reduce the uncertainty regarding the public health implications of exposure.

Other Key Studies Reviewed by ATSDR

Other epidemiology studies were also considered by ATSDR in evaluating the database on human exposure to methylmercury. Lebel et al. (1996) evaluated a fish-eating populations in the Amazon River Basin with a neurofunctional test battery and clinical manifestations of nervous system dysfunction in relation to hair mercury concentrations. The villagers examined live along the Tapajos River, a tributary of the Amazon. The study population consisted of 91 adult inhabitants 15–31 years of age. Hair mercury levels were below 50 µg/g (ppm). Clinical examinations were essentially normal, although persons displaying disorganized movements on an alternating movement task and those with restricted visual fields generally had higher hair mercury levels. Near visual contrast, sensitivity, and manual dexterity (adjusted for age) were found to decrease significantly with increasing mercury levels, while a tendency for muscular fatigue and decreasing strength were observed in women. The authors suggested that dose-dependent nervous system alterations might be associated with hair mercury levels below 50 ppm. This study, however, also had a number of potentially confounding factors. The impact of parasitic and other diseases endemic to the study area is of primary concern in the interpretation of the Lebel et al. (1996) results. In addition, the overall nutritional status of the study population was not known or reported, and the use of neuroactive drugs (from local herbs, plants, roots, or mushrooms) was not considered as a potential confounder or covariate. The previous mercury exposure history of the study cohort is also unclear. This is of particular importance because gold mining procedures that use metallic mercury have been commonly practiced along the Amazon Basin for decades. Finally, the end points of the Lebel et al. (1977) study evaluated adult toxicity and not effects in the developing fetus or the newborn (i.e., the most sensitive human population).

Myers et al. (1997) evaluated the population of the SCDS for developmental milestones similar to those determined in Iraq. As part of this ongoing study, cohort children were evaluated at 6.5, 19, 29, and 66 months of age. At 19 months care-givers were asked at what age the child walked (n=720 out of 738) and talked (n=680). Prenatal mercury exposure was determined by atomic absorption analysis of maternal hair segments corresponding to hair growth during the pregnancy. The median mercury level in maternal hair for the cohort in this analysis was 5.8 ppm, with a range of 0.5–26.7 ppm. The mean age (in months) at walking was 10.7 (SD=1.9) for females and 10.6 (SD=2.0) for males. The mean age for talking (in months) was 10.5 (SD=2.6) for females, and 11 (SD=2.9) for males. After adjusting for covariates and statistical outliers, no association was found between the age at which Seychellois children walked or talked and prenatal exposure to mercury. The ages for achievement of the developmental milestones were normal for walking and talking in the Seychellois toddlers following prenatal exposure to methylmercury from a maternal fish diet.

Clarkson (1995) raised some interesting issues concerning whether is it reasonable to apply health effects data based on an acute exposure to methylmercury fungicide eaten in homemade bread (in the 1971–1972 Iraq incident) to fish-eating populations having chronic exposure to much lower concentrations of methyl-mercury. He addressed two specific issues. The first regards the body's "defense mechanisms" that serve to mitigate the potential damage from mercury. One such mechanism in the case of methylmercury involves an enterohepatic cycling process in which methylmercury from dietary sources absorbed through the intestine is carried to the liver, where substantial quantities are secreted back into the bile and returned to the intestinal tract. During the residence time in the gut, microflora break the carbon-mercury bond, converting methylmercury into inorganic mercury, which in turn is poorly absorbed and is excreted in the feces. This creates an effective detoxification pathway for low-dose dietary exposures to methylmercury, but probably not for acute, high-dose exposures, such as occurred in Iraq. Secondly, the transport of methylmercury into brain tissue is inhibited by the presence of many amino acids, including leucine, methionine, and phenylalanine. Thus, it is possible that the rising plasma concentrations of amino acids from ingestion of fish protein may serve to depress the uptake of methylmercury by the brain.

While both of these issues need further laboratory/clinical investigation, they do raise appropriate questions concerning the relevance of the relatively short-term (i.e., about 6 weeks), high-level contaminated grain exposure scenario encountered in Iraq to the dietary methylmercury exposure scenarios encountered in many fish-eating populations (e.g., the Seychelles Islanders, Faroe Islanders, Peruvian villagers, and Inuit native people of Greenland). This position is supported by Cicmanec (1996), who reviewed data from the

Iraqi study, as well as data from studies of fish-consuming populations in the Faroe Islands, Seychelles Islands, and Peruvian fishing villages. Cicmanec concluded that the Iraqi population does not represent a sensitive subpopulation within a perinatal group; rather, the relatively lower threshold identified in that study was the result of confounders. Crump et al. (1995) reanalyzed the dose-response data from the Cox et al. (1989) report of the Iraqi incident and found the results to be potentially skewed by inadequacies in the study design and data-collection methods. Shortcomings or potentially confounding factors include: (1) the retrospective recall of developmental milestones by mothers and other family members; (2) the lack of precision in the determination of birth and other milestone dates; (3) and the possible biasing of the dose-response analysis by variation in symptom reporting and infant sex composition in the two study subcohorts. Crump et al. (1995) noted that perhaps the most serious limitation of the Iraqi study is the inability to assess the potential effects of low-level chronic-duration exposure to methylmercury, as these particular data are based on very high intake levels over a relatively brief period of time.

No increase in the frequency of neurodevelopmental abnormalities in early childhood was observed in a cohort of 131 infant-mother pairs in Mancora, Peru (Marsh et al. 1995b). The mean concentration of mercury in maternal hair was determined to be 8.3 ppm (range, 1.2–30 ppm), and the source of the mercury was believed to be from consumption of marine fish. Similarly, a study of 583 Faroe Island infants for the first 12 months after birth found no decrease in the age of attainment of sitting, creeping (crawling), and standing developmental milestones (Grandjean et al. 1995a). The age at which a child reached a particular developmental milestone was not only not found to be associated with prenatal mercury exposure, but infants that reached a milestone early were found to have significantly higher mercury concentrations in their hair at 12 months of age. It was also found that early milestone attainment was clearly associated with breast-feeding, which was in turn related to higher infant hair mercury levels. The authors (Grandjean et al. 1995a) concluded that the beneficial effects associated with breast-feeding seemed to overrule, or to compensate for, any neurotoxic effects on milestone development that could be due to the presence of contaminants (e.g., mercury) in human milk.

Additional studies have shown developmental toxicity after oral exposure of humans and animals to organic mercury compounds (Amin-Zaki et al. 1974; Bakir et al. 1973; Bornhausen et al. 1980; Cagiano et al. 1990; Elsner 1991; Engleson and Herner 1952; Fowler and Woods 1977; Guidetti et al. 1992; Harada 1978; Hughes and Annau 1976; Ilback et al. 1991; Inouye and Kajiwara 1988; Khera and Tabacova 1973; Lindstrom et al. 1991; McKeown-Eyssen et al. 1983; Nolen et al. 1972; Olson and Boush 1975; Rice 1992; Rice and Gilbert 1990; Snyder and Seelinger 1976; Stoltenburg-Didinger and Markwort 1990).

The accumulation of mercury is greater in larger fish and in fish higher in the food chain. The tendency for increased mercury concentration with increasing fish body weight is particularly noticeable in carnivorous fish species. Malm et al. (1995) analyzed mercury concentrations in 16 species of carnivorous fish from the Tapajos River basin in Brazil and hair samples from local populations who regularly ate such fish. Mercury levels in the fish averaged 0.55 ppm (range, 0.04–3.77 ppm), and the mercury levels in the hair of the affected fish-eating populations averaged approximately 25 ppm. In one population that consumed higher quantities of large carnivorous fish at the end of the local rainy season, 8 of 29 persons evaluated had hair mercury levels above 40 ppm, and one individual had a hair mercury concentration of 151 ppm. Some villages along the river can have per capita daily fish consumption rates around 200 g or more, which would greatly impact the human body burden and hair levels of mercury in such populations.

Hair-to-Blood Concentration Ratio

The hair:blood concentration ratio for total mercury is frequently cited as 250. However, a precise basis for this particular value is unclear. Ratios reported in the literature range from 140 to 416, a difference of more than a factor of 2.5 (see Table 2-9). Differences in the location of hair sampled (head versus chest, distance of sample from head or skin) may contribute to differences in observed ratios between studies. For example, 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). Thus, it is important to obtain hair samples as close to the follicle as possible to obtain an estimate of recent blood levels. Large errors (the direction of which depends on whether samples were taken while blood levels were falling or rising) could result if hair samples are not taken close to the scalp. Several studies did not report the distance to the scalp for the hair samples taken. The high slope reported by Tsubaki (1971a) may have reflected the fact that mercury levels were declining at the time of sampling (Berglund et al. 1971), so the hair levels may reflect earlier, higher blood levels. Hair taken from different parts of the body also may yield different ratios. In 26 subjects with moderate-to-high fish consumption, axillary hair (i.e., from the armpit area) was found to contain an average of 23% less mercury than head hair (Skerfving et al. 1974).

Phelps et al. (1980) obtained multiple blood samples and sequentially analyzed lengths of hair from 339 individuals in Northwestern Ontario. The large sample size and the attention to sampling and analysis with regard to the hair:blood relationship make the results from this study the most appropriate to use for estimating the mercury blood levels of the Seychellois women during pregnancy. The actual ratio Phelps et

Table 2-9. Available Data on Hair:Blood Ratio (total Hg)

Reference	Hair to blood ratio	Number of subjects	Hg range in whole blood (µg/L)	Hg range in hair (ppm)	Hair sample	
					Length (mm)	Distance to scalp
Sumari et al. 1969[a]	140	50	5–270	1–56	–	–
Soria et al. 1992	218	16	2.4–9.1	0.15–20	–	At scalp
Tejning 1967[a]	230	51	4–110	1–30	–	Axillary
Skerfving 1974	230	60	44–550	1–142	5	At scalp
Haxton et al. 1979	250	173	0.4–26	0.1–11.3	20	–
Tsubaki 1971b[b]	260	45	2–800	20–325	–	–
Birke et al. 1972[b]	280[c]	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[d]	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	–
Tsubaki 1971a[a]	370	≈25	–	–	"Longer tuft"[a]	–

[a] As cited in Berglund et al. 1971
[b] As cited in WHO 1976
[c] Ratio of methylmercury in hair to methylmercury in blood
[d] 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

al. (1980) observed between the total mercury 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 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. Based on these assumptions, they calculated that if the actual hair:blood ratio were 200, they would have observed a ratio of 290 (i.e., essentially equivalent to the observed value of 296). Based on these and other considerations, Phelps et al. (1980) state that the actual ratio is "probably higher than 200, but less than the observed value of 296." As the authors point out, two-thirds of the study population were sampled during the falling phase of the seasonal variation and one-third or less in the rising phase. This fact would tend to result in a lower observed ratio; therefore, the actual average value is likely to be >200.

Phelps et al. (1980) also provide estimates assuming a 2-week lag for the appearance of the relevant level of mercury in the centimeter of hair nearest the scalp. For a 2-week lag time, an actual ratio of 250 would have resulted in an observed ratio of 301 (again, essentially identical to the observed value of 296). A study of ingestion of a large dose of mercuric chloride in one individual suggests that the lag time is longer than 2 weeks (Suzuki et al. 1992). Hair samples were taken at 41 and 95 days following ingestion of the mercuric chloride. In the 41-day hair sample, a large mercury peak occurred in the centimeter of hair closest to the scalp, with no elevation in mercury in the second centimeter of hair. Head hair grows at a rate of about 1.1 cm a month (Al-Shahristani and Shihab 1974; Cox et al. 1989). If emergence had occurred so that the elevation in mercury could be measured in the first centimeter of hair by 2 weeks after exposure, then by day 41 after exposure the peak should have moved into the second centimeter of hair, at least enough to raise the mercury level slightly in the second centimeter. Because no elevation was seen in the second centimeter of hair at 41 days, it would appear that emergence occurred at a lag of >2 weeks. In the hair sample taken at 95 days, the leading edge of the mercury peak occurred in the third centimeter of hair.

Based on the data presented in Phelps et al. (1980) and the lag time indicated in the individual studied by Suzuki et al. (1992), the actual average value is likely to be somewhere between 200 and 250. Because the data do not allow a more accurate determination of an average ratio, the value 250 is acceptable for the purpose of estimating average blood levels in the Seychellois population. Using 250 rather than a lower number results in a lower MRL. It should be noted that a wide range in hair:blood ratios has been reported for individuals in various studies: 137–342 in Soria et al. (1992), 171–270 in Phelps et al. (1980), 416 in

Cernichiari et al. (1995), and 137–585 in Birke et al. (1972). Therefore, this ratio (250) should not be used as the sole basis for determining levels of exposure and potential effect for individuals.

Calculation of dietary intake of mercury from blood concentration.

Fraction of mercury in diet that is absorbed (A_D). Radiolabeled methyl-mercuric nitrate 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 radiolabeled $MeHgNO_3$ to yield a methylmercury proteinate. The proteinate was then fed to fish that were killed after a week, cooked, and fed to volunteers after confirmation of the methylmercury in the fish. Mean uptake exceeded 94%. For the derivation of an MRL, an absorption factor of 0.95 is used.

Fraction of the absorbed dose that is found in the blood (A_B). The value 0.05 has been used for this parameter in the past (Berglund et al. 1971; WHO 1990). Three studies report observations of the fraction of the absorbed methylmercury dose distributed to blood volume in humans. Kershaw et al. (1980) report an average fraction of 0.059 of the absorbed dose in the 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 body burden was present in 1 L 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 methyl-mercury in fish over a period of 3 months (Sherlock et al. 1984). Average daily intake for the 4 groups observed in the study ranged from 43 to 233 µg/day. The authors report a dose-related effect on the estimated percentage of the absorbed dose in 1 kg of blood, with 1.26% of the absorbed dose in 1 kg of blood at an average daily intake of 43 µg/day and 1.03% of the absorbed dose in 1 kg of blood at an average daily intake of 233 µg/day. The average for all subjects in the study was 1.14%. When individual values for distribution to one kilogram of blood reported in the study are converted into the percentage of the absorbed dose in the total blood volume (assuming that blood is 7% of body weight [Best 1961] and using body weights reported for individuals in the study), the average value for A_B for all individuals is 0.056 (0.057 using the values for percentage in 1 kg normalized for body weight as reported in the study). The average value for A_B for 6 women as reported in Sherlock et al. (1984) is 0.048 (0.047 using values normalized for body weight). The average for 14 men is 0.059 (0.061 using values normalized for body weight).

The average values for A_B for all studies ranged from 0.047 to 0.061 (the values for women and men reported in Sherlock et al. [1984]). The data suggest that the average value of A_B for women may be lower than that for men, and they further suggest that 0.05 may be appropriate for modeling intake in a group of women (Sherlock et al. 1984). Based on these studies, the best estimate of A_B based on the available data is 0.05. Use of a higher value (i.e., 0.06 instead of 0.05) for this parameter would result in a lower MRL, but the sensitive populations are pregnant women and developing fetuses, making the 0.5 value more appropriate for the Seychelles study population.

Elimination constant (b). Reported clearance half-times for methylmercury from blood or hair range from 48 to 65 days (Table 2-5). The average elimination constant based on the six studies listed in Table 2-5 is 0.014. The average of the individual values for *b* reported for 20 volunteers ingesting 42–233 µg Hg/day in fish for 3 months (Sherlock et al. 1984) is also 0.014. Use of the value 0.014 for this parameter, rather than 0.01 (as used by WHO 1990), results in a higher MRL.

Volume of blood in body (V), and body weight. Blood volume is assumed to be 7% of body weight, with an increase to about 9% during pregnancy (Best 1961). Data for the body weight of the Seychelles Islands women were not found. Assuming an average body weight of 60 kg for women, the blood volume is 4.2 L (60 kg x 0.07 L/kg). The 9% of body weight value is not used because it is not representative of the blood volume throughout pregnancy. Blood volume does not begin to increase significantly from the 7% pre-pregnancy level until around the 27th week of pregnancy. It then sharply rises until week 40 or parturition (Guyton 1996). To use the 9% value would, therefore, be representative of the blood volume late in pregnancy (i.e., mid- to late- third trimester), but not throughout most of pregnancy. In contrast, the hair mercury level to which it is compared represents an average value throughout pregnancy. The use of the 9% value would result in a higher MRL, and is not considered appropriate in this instance.

Calculation of Exposure Dose

The concentration of mercury in hair is assumed to be 250 times the concentration in blood. ATSDR's peer-reviewed, published guidance for MRL derivation (Chou et al. 1998) calls for the use of the highest value at which no adverse effects were observed in the critical study. Using, therefore, the 15.3 ppm mean maternal hair (taken at parturition) value from the highest exposure group (range, 12–26.7 ppm) in the Seychellois test population as a NOAEL for the 66-month Seychelles testing (Davidson et al. 1998), the corresponding methylmercury concentration in blood would be: 1/250 x 15.3 µg/g x 1 mg/1,000 µg x 1,000 g/L = 0.061 mg/L.

Converting blood mercury concentration to daily intake.

The concentration of mercury in the blood may be converted to a daily intake by using the following equation from WHO (1990):

$$C = \frac{f(d}{b(V} \cdot \frac{A_D(A_B(d}{b(V}$$

Where:

C = concentration in blood
f = fraction of the daily intake taken up by the blood
d = daily dietary intake
b = elimination constant
A_D = percent of mercury intake in diet that is absorbed
A_B = percent of the absorbed amount that enters the blood
V = volume of blood in the body

where:

C = (0.95 x 0.05 x d)/(0.014 x 4.2)
C = 0.81 d
0.061 mg = 0.81 d
d = 0.075 mg/day

Using the assumed body weight of 60 kg for women, the estimated dose that would result in a hair level of 15.3 ppm is 0.075/60 kg = 0.0013 mg/kg/day. Therefore, the NOAEL derived from the highest exposure group (n = 95) at 66 months is 0.0013 mg/kg/day.

Consideration of Uncertainty

The standard/traditional areas of uncertainty addressed in any duration-specific MRL are: (1) interspecies variability (i.e., cross-species extrapolation of a NOAEL or LOAEL); (2) intra-human variability (i.e., differences in susceptibility to a substance or effect within the human population); (3) use of an LOAEL for MRL derivation when an NOAEL for the critical effect is not available; and (4) extrapolation from subchronic to chronic duration. In addition, a modifying factor may also be used when special circumstances exist that may contribute to, or introduce, uncertainty into the calculated health guidance value (MRL) in an area not typically covered by the traditional uncertainty factor approach.

The NOAEL of 15.3 ppm mercury in maternal hair from Davidson et al. (1998) used as the starting point for MRL derivation was based upon an unusually large study cohort of the population considered most sensitive to the neurodevelopmental effects of methylmercury, i.e., pregnant women and their developing fetuses. The negative results of this study are strongly supported by the BMD NOAEL range of 13 to 21 ppm calculated for the New Zealand cohort of 237 mother-child pairs (Crump et al. 1998). Consequently, much of the uncertainty normally present in the MRL derivation process does not exist in the case of methylmercury. Nonetheless, in view of the nature of the most susceptible group (developing fetuses) and some questions raised in the vast human data base for this chemical, an aggregate value of 4.5 was employed.

This value (4.5) was based upon three separate components, two of which are interrelated and the other independent. For the Seychelles data, a value of 1.5 was used to address variability in hair-to-blood ratios among women and fetuses in the U.S. population, as determined by pharmacokinetic modeling of actual data by Clewell et al. (1998); a second value of 1.5 was applied to address the remainder of any inter-individual variability (i.e., pharmacodynamics) in the U.S. population. A third, and independent, factor of 1.5 was employed to account for the possibility that the domain-specific tests, as employed extensively in the Faroe Islands, but not the Seychelles (which used primarily neurobehavioral tests of global function) might be able to detect very subtle neurological effects not tested for in the 66-month Seychelles cohort.

The World Health Organization (WHO, 1993, 1996) has defined the -kinetic and -dynamic components of intrahuman variability as being equal contributors to, and collectively constituting the total of, human variability. In order to assure a conservative approach, these two interdependent components were added to give a composite uncertainty factor of three (i.e., 1.5 + 1.5 = 3) to account for the full range of variability attributable to mercury in the Seychelles study. A modifying factor of 1.5 was also used to account for the possibility of domain-specific effects, as were seen in the Faroe study, being attributable to mercury. Since these effects were considered to be entirely separate or “independent” events, this modifying factor of 1.5 was multiplied by the uncertainty factor of 3.0 (for uncertainty attributable solely to the Seychelles study) to yield an aggregate uncertainty of 4.5 for chronic oral exposure to methylmercury.

While domain-specific tests from the Seychelles were reviewed at the North Carolina meeting in November 1998 and the results failed to demonstrate effects, the tests do not represent the full range of domain-specific tests that were administered in the Faroe Islands. For these reasons, and based on our consultation with our Board of Scientific Counselors about concerns for “missing” data sets (i.e., in relation to the Executive Order of children’s health and the agency’s efforts to protect the health of children, including the developing fetus), ATSDR determined that an additional factor of 1.5 should be used since the full range of

domain-specific neuropsychological test results from the Seychelles are not yet available. When these results become available and if they fail to show domain-specific effects, this additional factor of 1.5 would no longer be needed. At that time ATSDR will re-evaluate its MRL, as well as all other relevant data, in compliance with the agency's mandates and authorities.

Therefore, in the calculation of the chronic oral MRL for methylmercury, the NOAEL of 0.0013 mg/kg/day from the 66-month study (Davidson et al. 1998) is divided by 4.5, giving a chronic oral MRL for methylmercury of 0.0003 mg/kg/day [0.0013 mg/kg/day / 4.5 (UF) = 0.0003 mg/kg/day].

Alternative Derivations of the MRL

To ensure a health guidance value based upon the best use of the Seychelles study data (widely considered the most relevant data available), ATSDR evaluated alternate MRL derivation methods for methylmercury. One such approach is to use the mean total mercury level of 6.8 ppm in maternal hair for the entire Seychellois study cohort. Using the same formula as in the previous MRL calculation,

$C = (0.95 \times 0.05 \times d) / (0.014 \times 4.2)$

$C = 0.81\ d$

$(1/250 \times 6.8) = 0.027$

$0.027\ mg/L = 0.81\ d$

$d = 0.034\ mg/day$

$0.034\ mg/day / 60\ kg = 0.0006\ mg/kg/day$

In consideration of uncertainty factors for this MRL approach, multiple factors also apply. In this case, the mean value of 6.8 ppm for the NOAEL is for the entire study cohort at 66 months (n = 711). An uncertainty factor of 1.5 was used to account for the pharmacokinetically based variability of hair-to-blood ratios (95% confidence level) in pregnant women and fetuses in the U.S. population (Clewell et al. 1998, 1999). The extremely large size of the study population (n=711), in combination with an uncertainty factor of 1.5, is considered adequate to encompass the full range of pharmacokinetic and pharmacodynamic variability within the human population. An independent modifying factor of 1.5 was also used to take into consideration the positive results of the domain-specific tests administered in the Faroe study (Grandjean et al. 1997, 1998). The uncertainty factor of 1.5, multiplied by the modifying factor of 1.5, yields a total aggregate value of 2.25. Applying the factor of 2.25 to the daily intake calculated from the 6.8 ppm

NOAEL yields a chronic oral MRL value of 0.0003 mg/kg/day for methylmercury (0.0006 mg/kg/day divided by 2.25 = 0.0003 mg/kg/day).

A third approach to deriving a health guidance value is the use of bench mark dose (BMD) modeling. Clewell et al. (1998) used a benchmark dose analysis to determine a reference dose (RfD, a health guidance value used by the Environmental Protection Agency and, in some ways, the equivalent of ATSDR's chronic oral MRL). Clewell et al. (1998) used the data from the 29-month test in the Seychellois population (Davidson et al. 1995b) for their analysis (i.e., the 66-month study had not been published at the time of their benchmark dose analysis). The BMD is calculated by fitting a mathematical dose-response model to dose-response data. The bench mark dose level (BMDL) is a lower statistical confidence bound on the BMD and replaces the NOAEL in the calculation of a health guidance value. The BMD approach has been proposed as superior to the use of "average" or "grouped" exposure estimates when dose-response information is available, as is the case for the Seychelles study. Clewell et al. (1998) note that the Faroe Islands study reported by Grandjean et al. (1997b) could not be used for dose-response modeling due to inadequate reporting of the data and the confounding influence of co-exposure to PCBs.

For the 29-month Seychelles data, Clewell et al. (1998) used the 95% lower bound on the 10% benchmark dose level (BMDL), which represents a conservative estimate of the traditional NOAEL. The benchmark dose modeling over the entire range of neurological endpoints reported by Davidson et al. (1995b) yielded a lowest $BMDL_{10}$ of 21 ppm methylmercury in maternal hair. This $BMDL_{10}$ was then converted to an expected distribution of daily ingestion rates across a population of U.S. women of child-bearing age by using a Monte Carlo analysis with a physiologically based pharmacokinetic (PBPK) model of methyl-mercury developed by Gearhart et al. (1995). This analysis addresses the impact of interindividual pharmacokinetic variability on the relationship between ingestion rate and hair concentration for methyl-mercury. The resulting distribution had a geometric mean value of 0.00160 mg/kg/day (S.D. 0.00133). The 1st, 5th, and 10th percentiles of that distribution were 0.00086, 0.00104, and 0.00115 mg/kg/day, respectively. Clewell et al. (1998) suggested that the 5th percentile of 0.00104 mg/kg/day provides a scientifically based, conservative basis that incorporates the pharmacokinetic variability across the U.S. population of child-bearing women and that no other uncertainty factor for interindividual variability would be needed. To the benchmark-estimated NOAEL of 21 ppm derived from the Seychelles 29-month data, Clewell et al. (1998) applied an uncertainty factor of 3 to account for data base limitations. (Note: The 66-month Seychelles data was not yet published at the time; hence the reliance on the 29-month Seychelles

data for the benchmark analysis.) Consequently, Clewell et al. (1998) concluded that using a NOAEL of 7 ppm (21 ppm / 3 (UF) provides additional protection against the possibility that effects could occur at lower concentrations in some populations. Based upon this reasoning, Clewell and his colleagues recommended a health guidance value (i.e., an RfD) of 0.0004 mg/kg/day. If a modifying factor of 1.5 is used to further address the domain-specific findings in the Faroe study, a final MRL of 0.3 µg/kg/day results.

The above benchmark analysis of 29-month data from the Seychelles Child Development Study strongly supports the MRL of 0.0003 mg/kg/day calculated by ATSDR in this profile. Similarly, addressing the Seychellois 66-month data from the perspective of using the mean value (15.3 ppm) of the highest exposure group in the study, a method prescribed in ATSDR's published guidance for MRL development (Chou et al. 1998), also results in an identical MRL. ATSDR therefore has high confidence that this level is protective of the health of all potentially exposed human populations.

Employment of the Chronic Oral MRL for Methylmercury

It should be emphasized that the MRL is considered by ATSDR to be a level of exposure to a single chemical/substance which is considered "safe" for all potentially exposed populations for a specified duration of time (acute, intermediate, or chronic). It is not considered be a threshold for adverse effects, and not address the likelihood of adversity at any incremental level above the MRL value. ATSDR notes that the 0.3 µg/kg/day chronic oral MRL for methylmercury is in close agreement with the tolerable daily intake (ADI) levels of 0.47 and 0.48 µg/kg/day established by the FDA and WHO, respectively.

MRLs are, by definition (Chou et al. 1998), substance-specific and do not include effects attributable to interaction (whether additive, synergistic, or antagonistic) with other chemicals or environmental substances. Their relevance to the mission of ATSDR is to assist public health officials in the identification of chemicals/elements of potential health concern at hazardous waste sites. The ATSDR MRL is not intended to be used in the regulatory or site clean-up process, but is instead intended to serve as a basis of comparison with actual measured levels of environmental exposure. Further, the role of informed biomedical judgment is crucial in the application of any MRL, or the media-specific health guidance values (HGVs) derived from them, in any given exposure scenario (Risher and De Rosa 1997). MRLs for a particular substance are based upon the most sensitive effect/endpoint in that portion of the human population considered to be most susceptible to injury from exposure to that substance. Thus, the MRL has never been intended as a one-size-fits-all tool for all hazardous waste site exposure scenarios; rather, it is merely a starting point for further examination of potential health risk. Therefore, at sites

where methylmercury is present in combination with other known or suspected neurodevelopmental toxicants, such as lead or polychlorinated biphenyls (PCBs), and in which exposure is primarily episodic in nature, the health assessor might consider using a value below the chronic oral MRL for methylmercury as a starting point for determination of further site investigation. (A more complete description of the uses of MRLs and other HGVs can be found in Chou et al. 1998 and Risher and De Rosa 1997.)

Background and general population exposures relevant to the oral MRL for methylmercury

Mercury hair levels have been monitored in a variety of populations and generally range from 1 to 4 ppm, depending upon the level of fish consumption. Table 2-10 summarizes the mean (or median) values and the maximum value from a number of these studies.

Diet. Based on the FDA total diet study of 1982–1984 (Gunderson 1988), FDA estimated that the average intake for total mercury (both inorganic and organic) is 50–100 ng/kg/day. Based on the more recent 1989–1990 FDA total diet study, the estimated intake of total mercury is 27–60 ng/kg/day (Cramer 1994). An estimated 86% of the mercury in the total diet study is derived from fish (Tollefson and Cordle 1986). A separate estimate of the average intake of methylmercury alone, based on a survey of fish eaters and average levels of methylmercury in fish, places the average intake of methylmercury at 36 ng/kg/day, with a 99% upper bound at 243 ng/kg/day (Clarkson 1990).

Potential protective effect of selenium in fish. Selenium is known to bioconcentrate in fish, and selenium has been observed to correlate with mercury levels in the blood of fish consumed (Grandjean et al. 1992). Furthermore, there is evidence suggesting that consumption of methylmercury from fish, in conjunction with other beneficial constituents in fish (e.g., omega-3 fatty acids) may not result in the same toxicity dose-response relationship observed with methylmercury exposure from consumption of contaminated grain (as in the Iraqi population) (Davidson et al. 1998).

Regarding the bioavailability of methylmercury in fish, the available data indicate that methylmercury uptake is not affected by its presence in fish. Experimental studies on the metabolism of methylmercury in humans following the ingestion of contaminated fish (using methylmercury bound to fish muscle protein) have shown that absorption is almost complete (95% absorbed) (Miettinen 1973). Animal studies also support this absorption value. Data on cats given fish homogenates indicate absorptions of $90% of methylmercury, whether added to the homogenate, accumulated by fish *in vivo*, or from methylmercury proteinate (Berglund et al. 1971). Using blood and tissue levels as evidence of absorption, Charbonneau et al. (1976) concluded that there was no difference in the biological availability of methylmercury

Table 2-10. Concentration of Total Mercury in Hair

Mean (M) or median (m) (ppm)	Maximum (ppm)	N	Description of population	Reference
1.4 (M)	27.4	942	United Kingdom. Area chosen for "above average fish consumption"	Sherlock et al. 1982
1.35 (M)	5.8	55	United Kingdom. Fishing community, area with lesser contamination	Haxton et al. 1979
1.48 (M)	3.28	34	Islands of the central Adriatic (Yugoslavia). Women; degree of fish consumption not reported. Hair sampled at end of pregnancy	Horvat et al. 1988
2.0 (M)	11.3	119	United Kingdom. Fishing community, area with greater contamination. Fish consumption range 10–225 g/day with 50% eating greater than 50 g/day	Haxton et al. 1979
2.85 (M)	20	50	Spain. Women; degree of fish consumption not reported. Hair sampled at end of pregnancy	Soria et al. 1992
3.2 (M)	10.8	50	Sweden. High consumption of freshwater fish. Mercury levels in fish generally below 1 ppm	Oskarsson et al. 1990
3.9 (M)	21	98	United Kingdom. Consumed an average of 0.36 kg fish/week	Sherlock et al. 1982
5.6 (M)	20	35	Japan. Fish consumption not known	Suzuki et al. 1993
0.8 (m)	2[a]	18	Faroe Islands. No fish consumption	Grandjean et al. 1992
1.61 (m)	3.7	49	1 fish meal per week	
2.5 (m)	4.7	75	2 fish meals per week	
2.1 (m)	3.6	49	3 fish meals per week	
5.2 (m)	8	17	4 fish meals per week	
1.4 (M)			Eat fish once a month	Airey 1983b
1.9 (M)			Eat fish once every 2 weeks	
2.5 (M)			Eat fish once a week	
11.6 (M)			Eat fish once a day	
0.247 (m)	2.5	150	German sample from 150 cadavers (75 males, 75 females) from the general population (i.e., no occupational or unusual expsoures to metals.)	Drasch et al. 1997

[a] The "maximums" reported in this column for Grandjean et al. 1992 are the upper values for the "50% range" as reported in Grandjean et al. (1992).

administered to adult cats (0.003, 0.0084, 0.020, 0.046, 0.074, or 0.176 mg Hg/kg/day, 7 days a week for 2 years), either as pure methylmercuric chloride in corn oil added to a diet containing uncontaminated fish or as methylmercury-contaminated fish. In the two highest dose groups (0.074 and 0.176 mg Hg/kg body weight at 100 weeks of exposure), no significant differences were seen in total mercury concentrations in the blood between groups receiving the dose as methylmercuric chloride or as contaminated fish at the same dose level. In addition, monthly blood levels were comparable for all dose groups. No significant differences were seen at 100 weeks in total mercury concentrations in nervous tissue or other tissues (renal cortex, renal medulla, liver, spleen, adrenal, bladder, atria, ventricle, ovary, testis, muscle) between the two highest dose groups receiving the dose as methylmercuric chloride or as contaminated fish at the same dose level.

Regarding the effect of selenium on methylmercury toxicity, most studies have shown that the simultaneous administration of mercury and selenium in equimolar doses to animals has resulted in decreased toxicity of both elements for acute- and chronic-duration exposures. This effect has been observed with inorganic and organic mercury and with either inorganic or organic selenium compounds, although inorganic forms of selenium appear to be more effective than organic forms (Chang 1983; Skerfving 1978). Selenium protects against the acute nephrotoxicity of the mercuric ion and methylmercuric ion in rats (Ganther et al. 1972, 1980; Hansen 1988; Magos et al. 1987; Parizek and Ostadolva 1967) and possibly against acute neurotoxicity of methylmercuric ion in rats (Ohi et al. 1980).

Somewhat paradoxically, the protective effect of selenium has been associated with a higher whole-body retention of mercury (Hansen 1988; Magos et al. 1987). In a study of selenium excretion in workers exposed to low levels of metallic mercury vapor in a chloralkali plant, Ellingsen et al. (1995) found that even in a low-to-moderate occupational exposure, mercury may reduce the urinary selenium concentration in humans in a manner that is not yet fully known. Evidence from human autopsy tissues suggests that distribution of mercury throughout the body may be influenced by the presence of selenium (Suzuki et al. 1993). In this study, however, the level of selenium was found to negatively correlate with the level of mercury in some tissues including the cerebrum, spleen, and kidney cortex. Suzuki et al. (1993) also report that hair selenium values negatively correlated with total organ mercury and inorganic mercury levels. The association between concentrations of inorganic mercury and selenium in both the occipital lobe and the thalamus of the brains of methylmercury-exposed female monkeys was reported by Bjorkman et al. (1995). These authors observed a tendency to a "hockey stick-shaped" relationship between concentrations of selenium and inorganic mercury in the thalamus of monkeys with ongoing exposure to methylmercury, and

they postulated an important role for selenium in the retention of mercury in the brain. These studies indicate that selenium has an effect on mercury toxicokinetics, although more study is needed to determine the nature of the interaction with respect to different organs and exposure regimens.

Although the specific mechanism for the protection is not well understood, possible mechanisms for selenium's protective effect include redistribution of mercury (Mengel and Karlog 1980), competition by selenium for mercury-binding sites associated with toxicity, formation of a mercury-selenium complex that diverts mercury from sensitive targets (Hansen 1988; Magos et al. 1987; Naganuma and Imura 1981), and prevention of oxidative damage by increasing selenium available for the selenium-dependent glutathione peroxidase (Cuvin-Aralar and Furness 1991; Imura and Naganuma 1991; Nylander and Weiner 1991). One laboratory study showed that selenium-treated animals can remain unaffected, despite an accumulation of mercury in tissues to levels that are otherwise associated with toxic effects (Skerfving 1978). Support for the proposal that an inert complex is formed comes from the 1:1 ratio of selenium and mercury found in the livers of marine mammals and in the bodies of experimental animals administered compounds of mercury and compounds of selenium, regardless of the ratio of the injected doses (Hansen 1988).

Southworth et al. (1994) evaluated the elimination of slurried fly ash discharges into a water-filled quarry and found that the discharge was followed by a steady increase in concentrations of mercury in the axial muscle of resident largemouth bass *(Micropterus salmoides)*, increasing from 0.02 to 0.17 µg/g in a period of just 3 years. These authors also found that while selenium concentrations in the quarry decreased from 25 to <2 µg/L during the same period, selenium concentrations in bass remained at about 3 times the background levels. Southworth and his co-authors concluded that the results of their study suggest that selenium may also be effective at blocking the accumulation of methylmercury in harder, more alkaline waters.

SPECIFIC ADVERSE EFFECTS ATTRIBUTABLE TO MERCURY EXPOSURE

Death. Inhalation of sufficiently high concentrations of metallic and organic mercury vapors, ingestion of sufficiently high doses of organic and inorganic mercury, and exposure to dialkyl mercurials by any route can be fatal to humans and animals. In the cases of both inhalation and dermal exposure to dialkyl-organomercurials (e.g., diethyl- and dimethylmercury), acute exposures that appear innocuous or unremarkable at the time of exposure can result in death following a delay period of weeks or months. The tragic case of a delayed neurotoxicity and ultimately fatal poisoning 9 months after an acute dermal

exposure to only a few drops of dimethylmercury is striking example of the danger of these forms of organic mercury (Blayney et al. 1997; Nierenberg et al. 1998). At least 5 other deaths have been reported due to alkyl mercury exposure since its first synthesis in the mid-19th century (Toribara et al. 1997). These accidental poisoning cases also reveal a latency period of some months between the exposure and the onset of symptoms. In such cases, irreversible brain damage has already occurred by the time the first symptoms appeared.

No information was located regarding specific concentrations of elemental mercury vapor that may be lethal; however, lethal exposures have generally occurred as a result of exposure under conditions in which exposure levels are likely to be quite high (e.g., heating metallic mercury in a closed space). Death in these cases has generally been attributed to respiratory failure (Campbell 1948; Kanluen and Gottlieb 1991; Matthes et al. 1958; Rowens et al. 1991; Soni et al. 1992; Taueg et al. 1992; Teng and Brennan 1959; Tennant et al. 1961). Deaths resulting from inhalation exposure to organic mercury compounds have also been reported (Brown 1954; Hill 1943; Hook et al. 1954; Lundgren and Swensson 1949). Although the cause of death following inhalation of organic mercury was not reported, severe neurological dysfunction was observed prior to death.

Lethal doses for acute oral exposure to inorganic mercury have been estimated to be 29–50 mg Hg/kg (Troen et al. 1951). Deaths resulting from oral exposure to inorganic mercury have been attributed to renal failure, cardiovascular collapse, and severe gastrointestinal damage (Gleason et al. 1957; Kang-Yum and Oransky 1992; Troen et al. 1951).

Deaths from consumption of methylmercury-contaminated foods are well documented in outbreaks in Japan and Iraq, and lethal doses of 10–60 mg Hg/kg have been estimated from tissue concentrations (Bakir et al. 1973; Tsubaki and Takahashi 1986). Fatalities were attributed to central nervous system toxicity (Bakir et al. 1973; Tamashiro et al. 1984). Pneumonia and nonischemic heart disease were prominent secondary causes of death in the Japan epidemic (Tamashiro et al. 1984). Case reports of deaths associated solely with dermal mercury exposure to inorganic mercury are limited to a woman who died after inserting a mercuric chloride tablet into her vagina (Millar 1916) and a man who died after a 2-month treatment with a topical medicine containing mercurous chloride (Kang-Yum and Oransky 1992). Death was attributed to renal failure in one of these cases (Kang-Yum and Oransky 1992), and severe renal damage was noted at the autopsy in the other (Millar 1916).

Animal data support the findings from human studies and provide somewhat more information regarding lethal exposure levels. Deaths associated with acute-duration inhalation exposure to metallic mercury vapor have been observed in rats and rabbits at about 27–29 mg/m^3 (Ashe et al. 1953; Livardjani et al. 1991b). Severe pulmonary edema has been reported as the cause of death following such exposures (Christensen et al. 1937). Severe ataxia occurred prior to death in rats exposed to methylmercury iodide vapor for intermediate durations (Hunter et al. 1940). Acute oral LD_{50} values for inorganic mercury ranged from 25.9 to 77.7 mg Hg/kg, with the most sensitive LD_{50} for 2-week-old rats (Kostial et al. 1978). Increased mortality in chronic-duration oral studies has been observed at 1.9 mg Hg/kg in male rats gavaged 5 days a week (NTP 1993). Early deaths were attributed to renal toxicity. Oral exposure to methylmercuric compounds has resulted in increased mortality at 16 mg Hg/kg (single dose) (Yasutake et al. 1991b), 3.1 mg Hg/kg/day for 26 weeks (Mitsumori et al. 1981), and 0.69 mg Hg/kg/day for up to 2 years (Mitsumori et al. 1990).

Systemic Effects

Respiratory Effects. The evidence from case report studies suggests that inhalation of metallic mercury vapor may result in clinical respiratory symptoms (e.g., chest pains, dyspnea, cough, reduced vital capacity) (Bluhm et al. 1992a; Gore and Harding 1987; Haddad and Sternberg 1963; Hallee 1969; Kanluen and Gottlieb 1991; King 1954; Lilis et al. 1985; Matthes et al. 1958; McFarland and Reigel 1978; Milne et al. 1970; Rowens et al. 1991; Snodgrass et al. 1981; Soni et al. 1992; Taueg et al. 1992; Teng and Brennan 1959; Tennant et al. 1961). In the more severe cases, respiratory distress, pulmonary edema, lobar pneumonia, fibrosis, desquamation of the bronchiolar epithelium, and death due to respiratory failure have been observed (Campbell 1948; Gore and Harding 1987; Jaffe et al. 1983; Kanluen and Gottlieb 1991; Matthes et al. 1958; Taueg et al. 1992; Teng and Brennan 1959; Tennant et al. 1961). Acute- and intermediate-duration studies in rabbits appear to corroborate clinical symptoms observed in humans following inhalation exposure to metallic mercury vapors. Mild-to-moderate pathological changes (unspecified) were exhibited in the lungs of rabbits exposed to 6–28.8 mg/m^3 mercury vapor for up to 11 weeks (Ashe et al. 1953), and death due to asphyxiation has been observed in rats exposed to 27 mg/m^3 for 2 hours (Livardjani et al. 1991b). Lung congestion was observed after 100 hours of continuous exposure of rats to 1 mg/m^3 (Gage 1961). The potential for oral exposure was not quantified; however, it is likely that most of the exposure was through inhalation. Inconclusive evidence is available regarding respiratory effects due to inhalation of organic mercury (Brown 1954; Hunter et al. 1940), and there is no conclusive evidence indicating that oral or dermal exposure to inorganic or organic forms of mercury is

directly toxic to the respiratory system. Based on these results, it would appear that acute inhalation exposure of humans to high levels of metallic mercury may result in pulmonary effects.

Cardiovascular Effects. The evidence from clinical, occupational, and general population studies suggests that inhalation of metallic mercury may affect the cardiovascular system in humans, producing elevations in blood pressure and/or heart rate (Aronow et al. 1990; Bluhm et al. 1992a; Campbell 1948; Fagala and Wigg 1992; Foulds et al. 1987; Friberg et al. 1953; Haddad and Sternberg 1963; Hallee 1969; Jaffe et al. 1983; Karpathios et al. 1991; Siblerud 1990; Smith et al. 1970; Snodgrass et al. 1981; Soni et al. 1992; Taueg et al. 1992; Teng and Brennan 1959). Studies of workers chronically exposed to elemental mercury vapor have shown increased incidences of palpitations (Piikivi 1989), high incidences of hypertension (Vroom and Greer 1972), and increased likelihood of death due to ischemic heart and cerebrovascular disease (Barregard et al. 1990). Of particular interest is the study showing slightly higher blood pressure in persons with dental amalgams than in those with no mercury-containing amalgams (Siblerud 1990). Less information is available regarding inhalation of organic mercury, but one study showed elevated blood pressure in two men occupationally exposed to methylmercury compounds (Hook et al. 1954). Electrocardiographic abnormalities (ventricular ectopic beats, prolongation of the Q–T interval, S–T segment depression, and T-wave inversion) were reported in persons who ate foods contaminated with ethylmercury compounds or who ingested a large dose of mercuric chloride (Chugh et al. 1978; Cinca et al. 1979; Jalili and Abbasi 1961). It is unclear whether these electrocardiographic abnormalities were the result of direct cardiac toxicity or whether they were secondary to other toxicity.

A number of the above cases of mercury-related tachycardia and elevated blood pressure in children inhaling metallic mercury vapors (Aronow et al. 1990; Fagala and Wigg 1992; Foulds et al. 1987; Karpathios et al. 1991) are associated with acrodynia, a nonallergic hypersensitive reaction in children to mercury exposure. Similar elevations in heart rate and blood pressure have been reported in children ingesting mercurous chloride (calomel)-containing medications and in children dermally exposed to ammoniated mercury-containing ointments or diapers that had been rinsed in a mercuric chloride-containing solution (Warkany and Hubbard 1953).

Limited animal data are available regarding inhalation exposure to mercury, but studies indicate that mercury may have a toxic effect on the heart. Effects ranging from mild pathological changes to marked cellular degeneration of heart tissue were exhibited in rabbits inhaling 0.86–28.8 mg/m^3 mercury vapor for

acute and intermediate durations (Ashe et al. 1953). However, it is unclear whether these changes represent direct toxic effects on the heart or whether they were secondary to shock.

The bulk of information available regarding cardiovascular effects after oral exposure of animals to mercury generally supports findings seen in human inhalation studies. Oral administration of 7 mg Hg/kg/day as inorganic mercury (mercuric chloride) for a year or 0.4 mg Hg/kg/day as organic mercury (methylmercuric chloride) for up to 28 days in rats resulted in elevated blood pressure (Carmignani et al. 1989; Wakita 1987). At higher concentrations (28 mg Hg/kg/day as mercuric chloride for 180 days), decreases in cardiac contractility were observed; these effects were suggested to be due to direct myocardial toxicity (Carmignani et al. 1992). Biphasic effects on myocardial tissue have been demonstrated on isolated papillary muscles from rat ventricles (Oliveira and Vassallo 1992). At low mercury concentrations, an increase in contractile force was observed, whereas at 5–10-fold higher concentrations dose-related decreases in contractile force were observed. The increases in contractile force were suggested to be due to inhibition of Na+-K+-ATPase, and the decreases were suggested to be due to inhibition of Ca2+-ATPase of the sarcoplasmic reticulum. Based on these results, it would appear that children with hypersensitivity to mercury may exhibit tachycardia and elevated blood pressure following inhalation, oral, or dermal exposure to mercury or to mercury-containing compounds. In addition, low-level exposure to mercury for extended periods may cause elevated blood pressure in exposed populations. Chronic-duration inhalation exposures or intermediate-duration oral exposures may also be associated with increased mortality due to ischemic heart or cerebrovascular disease; however, the data supporting this conclusion are more limited.

Gastrointestinal Effects. Both inhalation and oral exposures to mercury have resulted in gastrointestinal toxicity. Mercurial stomatitis (inflammation of the oral mucosa, occasionally accompanied by excessive salivation) is a classic symptom of mercury toxicity and has been observed following inhalation exposure to both inorganic and organic mercury (Bluhm et al. 1992a; Brown 1954; Campbell 1948; Fagala and Wigg 1992; Garnier et al. 1981; Haddad and Sternberg 1963; Hallee 1969; Hill 1943; Hook et al. 1954; Karpathios et al. 1991; Sexton et al. 1976; Snodgrass et al. 1981; Tennant et al. 1961; Vroom and Greer 1972).

Mercuric chloride is caustic to the tissues of the gastrointestinal tract, and persons who have ingested large amounts of this form of mercury have exhibited blisters, ulceration, and hemorrhages throughout the gastro-intestinal tract (Afonso and deAlvarez 1960; Chugh et al. 1978; Murphy et al. 1979; Samuels et al.

1982). In some cases, gastrointestinal lesions have been observed after inhalation exposure to high concentrations of metallic mercury vapors. The autopsy of a young child who inhaled metallic mercury vapor revealed necrosis in the mucosa of the stomach and duodenum (Campbell 1948). Irritation of the oral mucosa has also been observed at the site of contact with dental amalgams that contain mercury (Veien 1990). However, this type of response appears to be a combination of stomatitis and a contact dermatitis.

Symptoms of abdominal cramps, diarrhea, and nausea have been reported following acute- and/or intermediate-duration inhalation, oral, and dermal exposures of persons to mercury (Afonso and deAlvarez 1960; Bluhm et al. 1992a; Campbell 1948; Cinca et al. 1979; Haddad and Sternberg 1963; Hallee 1969; Jalili and Abbasi 1961; Kang-Yum and Oransky 1992; Kanluen and Gottlieb 1991; Lilis et al. 1985; Milne et al. 1970; Sexton et al. 1976; Snodgrass et al. 1981; Soni et al. 1992; Taueg et al. 1992; Teng and Brennan 1959; Tennant et al. 1961; Warkany and Hubbard 1953).

Rabbits displayed mild pathological changes to marked cellular degeneration and some necrosis in the colon tissue after inhaling 28.8 mg/m^3 mercury for 4–30 hours (Ashe et al. 1953). Inflammation and necrosis of the glandular stomach were observed in mice given 59 mg Hg/kg as mercuric chloride by gavage 5 days a week for 2 weeks (NTP 1993). An increased incidence of forestomach hyperplasia was observed in male rats exposed to 1.9 mg Hg/kg/day as mercuric chloride for 2 years (NTP 1993). Necrosis and ulceration of the cecum have been observed in rats after chronic-duration exposure to 4.2 mg Hg/kg/day as phenylmercuric acetate in the drinking water (Fitzhugh et al. 1950; Solecki et al. 1991). Ulceration of the glandular stomach was observed in mice after 2 years of exposure to methylmercuric chloride (0.86 mg Hg/kg/day) in the diet. Acute-duration inhalation exposure or chronic-duration oral exposure to inorganic and organic mercury may, therefore, result in various gastrointestinal symptoms in humans, with possible damage to intestinal tissues.

Hematological Effects. Leukocytosis associated with a metal fume fever-like syndrome has been observed in persons exposed to high concentrations of metallic mercury vapor (Campbell 1948; Fagala and Wigg 1992; Haddad and Sternberg 1963; Hallee 1969; Jaffe et al. 1983; Lilis et al. 1985; Matthes et al. 1958; Rowens et al. 1991). It is probable that this effect is specific to inhalation exposure to mercury. Because of the high concentrations of mercury that have been involved in the studies reviewed, it is unlikely that persons exposed to mercury vapors at hazardous waste sites would be exposed to sufficiently high concentrations of mercury to result in leukocytosis. Other hematological effects associated with exposure to mercury include decreased hemoglobin and hematocrit in persons with dental amalgams (Siblerud 1990)

and decreased δ-aminolevulinic acid dehydratase activity in erythrocytes or increased serum proteins involved in the storage and transport of copper in workers exposed to mercury vapor (Bencko et al. 1990; Wada et al. 1969). Anemia was found in a man who ingested a lethal amount of mercuric chloride (Murphy et al. 1979). However, the anemia was most likely the result of massive gastrointestinal hemorrhaging. No reports of effects on blood parameters in humans were located after oral exposure to organic mercury. A decrease in red cell count, hemoglobin, and hematocrit and rupture of erythrocytes were observed after intraperitoneal injection of mice with 19.2 mg Hg/kg as methylmercuric chloride (Shaw et al. 1991). A decrease in hemoglobin, hematocrit, and red blood cell count was observed in rats that received phenylmercuric acetate in their drinking water for 2 years (Solecki et al. 1991). However, this effect was probably due to blood loss associated with intestinal ulcers. Thus, there is limited information that suggests that prolonged exposure of humans to high levels of mercury, possibly from living in the vicinity of hazardous waste sites, may result in hematological changes.

Musculoskeletal Effects. Increases in tremors, muscle fasciculations, myoclonus, or muscle pains have been reported in persons exposed to unspecified concentrations of elemental mercury vapor (Adams et al. 1983; Albers et al. 1982, 1988; Aronow et al. 1990; Barber 1978; Bidstrup et al. 1951; Bluhm et al. 1992a; Chaffin et al. 1973; Chapman et al. 1990; Fawer et al. 1983; Karpathios et al. 1991; McFarland and Reigel 1978; Sexton et al. 1976; Smith et al. 1970; Taueg et al. 1992; Verberk et al. 1986; Vroom and Greer 1972; Williamson et al. 1982), in individuals inhaling alkyl mercury compounds (Brown 1954; Hook et al. 1954; Hunter et al. 1940), and in persons ingesting mercurous chloride (Warkany and Hubbard 1953) or ethyl-mercury compounds (Jalili and Abbasi 1961). These muscular effects are probably the result of peripheral nervous system dysfunction. It is probable that persons exposed to sufficiently high concentrations of mercury in the air or in foodstuffs (e.g., contaminated fish) at hazardous waste sites may also experience symptoms of tremors, myoclonus, muscle fasciculations, or muscle pains. A single report was identified that found evidence of rhabdomyolysis (destruction of the skeletal muscle) in a 22-year-old man who attempted suicide by ingesting 2 g of mercuric chloride (Chugh et al. 1978). It is extremely unlikely that persons at hazardous waste sites would be exposed to similarly high concentrations of mercuric chloride.

Hepatic Effects. Elevated serum glutamic pyruvic transaminase (SGPT), ornithine carbamyl transferase, and serum bilirubin, as well as evidence of decreased synthesis of hepatic coagulation factors, were reported in a case study of a child who inhaled an unspecified concentration of metallic mercury vapor (Jaffe et al. 1983). Similarly, hepatomegaly and hepatocellular vacuolation were observed in a man who

died following acute-duration, high-level exposure to elemental mercury vapor (Kanluen and Gottlieb 1991; Rowens et al. 1991). A lethal oral dose of mercuric chloride in a 35-year-old man also resulted in jaundice, an enlarged liver, and elevated aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, and bilirubin (Murphy et al. 1979).

Inhalation of 6–28.8 mg/m^3 mercury vapor for 6 hours to 11 weeks by rabbits produced effects ranging from mild pathological changes to severe necrosis in the liver, including necrosis and degeneration; effects were less severe at the shorter durations (Ashe et al. 1953). Intermediate-duration oral exposure to inorganic mercury has also been associated with increases in hepatic lipid peroxidation (Rana and Boora 1992) and in serum alkaline phosphatase (Jonker et al. 1993a). It is unclear to what extent these effects were due to the direct toxic effects of mercury on the liver or were secondary to shock in the exposed animals. Reliable information regarding hepatic effects following organic mercury exposure was not located. These limited data suggest the potential hepatic toxicity of short-term inhalation of high concentrations of mercury vapor to humans. It is unlikely that persons at hazardous waste sites would ingest sufficiently large amounts of mercuric chloride to cause hepatic toxicity.

Renal Effects. The kidney is one of the major target organs of mercury-induced toxicity. Adverse renal effects have been reported following exposure to metallic, inorganic, and organic forms of mercury in both humans and experimental animals. The nephrotic syndrome in humans associated with the ingestion, inhalation, or dermal application of mercury is primarily identified as an increase in excretion of urinary protein, although depending on the severity of the renal toxicity, hematuria, oliguria, urinary casts, edema, inability to concentrate the urine, and hypercholesterolemia may also be observed (Agner and Jans 1978; Afonso and deAlvarez 1960; Anneroth et al. 1992; Barr et al. 1972; Buchet et al. 1980; Campbell 1948; Cinca et al. 1979; Danziger and Possick 1973; Dyall-Smith and Scurry 1990; Engleson and Herner 1952; Friberg et al. 1953; Hallee 1969; Jaffe et al. 1983; Jalili and Abbasi 1961; Kang-Yum and Oransky 1992; Kanluen and Gottlieb 1991; Kazantzis et al. 1962; Langworth et al. 1992b; Murphy et al. 1979; Pesce et al. 1977; Piikivi and Ruokonen 1989; Roels et al. 1982; Rowens et al. 1991; Samuels et al. 1982; Snodgrass et al. 1981; Soni et al. 1992; Stewart et al. 1977; Tubbs et al. 1982). These effects are usually reversible. However, in the most severe cases, acute renal failure has been observed (Afonso and deAlvarez 1960; Davis et al. 1974; Jaffe et al. 1983; Kang-Yum and Oransky 1992; Murphy et al. 1979; Samuels et al. 1982). Renal biopsies and/or autopsy results have primarily

Rowens et al. 1991), but glomerular changes have also been reported (Kazantzis et al. 1962; Tubbs et al. 1982).

Although the primary effect of mercury on the kidneys appears to be a direct toxic effect on the renal tubules, there is also evidence that implicates an immune mechanism of action for mercury-induced glomerular toxicity in some persons. In support of this theory, a few human case studies have reported deposition of IgG, immune complexes, and/or complement C3 along the glomerular basement membrane (Lindqvist et al. 1974; Tubbs et al. 1982).

Studies in animals support the conclusion that the primary toxic effect of both inorganic and organic mercury in the kidneys is on the epithelial cells of the renal proximal tubules. The changes observed in these studies were comparable with those observed in humans (i.e., proteinuria, oliguria, increases in urinary excretion of tubular enzymes, proteinaceous casts, decreased ability to concentrate the urine, decreased phenolsulfonphthalein excretion, increased plasma creatinine) (Bernard et al. 1992; Chan et al. 1992; Dieter et al. 1992; Girardi and Elias 1991; Jonker et al. 1993a; Kirschbaum et al. 1980; Nielsen et al. 1991; NTP 1993; Yasutake et al. 1991b). In addition, the animal studies provided detailed information regarding the histopathological changes occurring in the kidneys (Carmignani et al. 1989, 1992; Chan et al. 1992; Dieter et al. 1992; Falk et al. 1974; Fitzhugh et al. 1950; Fowler 1972; Goering et al. 1992; Hirano et al. 1986; Jonker et al. 1993a; Klein et al. 1973; Magos and Butler 1972; Magos et al. 1985; Mitsumori et al. 1990; Nielsen et al. 1991; NTP 1993; Yasutake et al. 1991b). The progression of renal toxicity included initial degenerative changes in the epithelial cells of the proximal tubules (nuclear swelling, increased eosinophilia/basophilia, vacuolization, and cellular hypertrophy). In the early stages, these degenerative changes were accompanied by tubular regeneration. Occasionally, when there is minor toxic damage, only the regenerative changes were observed. As the lesions progressed, tubular dilation, desquamation of the epithelial cells, and thickening of the tubular basement membrane were observed. Fibrosis, inflammation, necrosis, and atrophy of the tubules and glomerular changes (i.e., hypercellularity, thickening of the glomerular basement membrane) were then observed.

Several investigators have suggested that the renal toxicity exhibited after administration of organic forms of mercury (e.g., methylmercury) may actually result from the *in vivo* metabolism of this form to inorganic mercury (Fowler 1972; Klein et al. 1973; Magos et al. 1985). This hypothesis is supported by the increase in the smooth endoplasmic reticulum, a potential site for this metabolic conversion, and the measurement of

substantial levels of inorganic mercury in the kidneys following exposure to methylmercury (Fowler 1972; Klein et al. 1973).

In New Zealand White rabbits and in certain strains of mice and rats, a membranous glomerulonephropathy was the predominant finding in the absence of significant tubular damage. This syndrome was characterized by proteinuria, deposition of immune material (i.e., IgG and complement C3) in the renal mesangium and glomerular blood vessels, and minimal glomerular cell hyperplasia (Aten et al. 1992; Druet et al. 1978; Hirszel et al. 1985; Hultman and Enestrom 1992; Matsuo et al. 1989; Michaelson et al. 1985; Pelletier et al. 1990; Pusey et al. 1990; Roman-Franco et al. 1978; van der Meide et al. 1993). Deposition of antiglomerular basement membrane antibodies has been observed in a susceptible strain of rat at subcutaneous doses of mercuric chloride as low as 0.15 mg Hg/kg 4 days a week for 2 weeks (Michaelson et al. 1985). Increases in urinary protein were not observed until 0.74 mg Hg/kg 4 days a week for 2 weeks. In mice, autoantibodies to glomerular basement membrane were not observed, but deposition of IgG in the kidneys occurs as a result of autoantibodies to nucleolar antigens (Hultman and Enestrom 1988). The immune basis for these responses is covered in the section on immunological effects below. The susceptibility to this form of renal toxicity appears to be governed by both MHC genes and nonMHC genes (Aten et al. 1991; Sapin et al. 1984). Among rat strains, Brown-Norway, MAXX, and DZB strains showed susceptibility to renal damage, whereas Lewis, M520, and AO rats did not (Aten et al. 1991; Druet et al. 1978; Michaelson et al. 1985). Among mouse strains, SJL/N mice are susceptible to renal toxicity, whereas DBA, C57BL, and Balb/c mice are not (Hultman and Enestrom 1992; Hultman et al. 1992). The apparent genetic basis for susceptibility to mercury-induced nephrotoxicity in experimental animals has important implications with regard to susceptible subpopulations of humans.

Based on the above information, it is likely that persons exposed to sufficiently high concentrations of mercury may experience renal tubular toxicity. Certain persons who are genetically predisposed may also develop an immunologically based membranous glomerulonephritis.

Dermal Effects. Dermal reactions have been observed in persons exposed to inorganic and organic mercury following inhalation, oral, and/or dermal exposures. The predominant skin reaction is erythematous and pruritic skin rashes (Al-Mufti et al. 1976; Aronow et al. 1990; Bagley et al. 1987; Biro and Klein 1967; Bluhm et al. 1992a; Engleson and Herner 1952; Faria and Freitas 1992; Foulds et al. 1987; Goh and Ng 1988; Hunter et al. 1940; Jalili and Abbasi 1961; Kang-Yum and Oransky 1992; Karpathios et al. 1991; Morris 1960; Pambor and Timmel 1989; Schwartz et al. 1992; Sexton et al. 1976;

Tunnessen et al. 1987; Veien 1990; Warkany and Hubbard 1953). In many of the dermal cases, a contact dermatitis type of response was observed. However, a nonallergic pruritus is characteristic of acrodynia, a hypersensitive reaction to mercury exposure observed primarily in children, and several of the above cases may have been attributable to this syndrome (Aronow et al. 1990; Engleson and Herner 1952; Foulds et al. 1987; Jalili and Abbasi 1961; Karpathios et al. 1991; Tunnessen et al. 1987; Warkany and Hubbard 1953). Other dermal reactions characteristic of acrodynia include heavy perspiration (Aronow et al. 1990; Fagala and Wigg 1992; Karpathios et al. 1991; Sexton et al. 1976; Warkany and Hubbard 1953) and itching, reddened, swollen and/or peeling skin on the palms of the hands and soles of the feet (Aronow et al. 1990; Fagala and Wigg 1992; Jalili and Abbasi 1961; Karpathios et al. 1991; Tunnessen et al. 1987; Warkany and Hubbard 1953). No animal studies were located to support these findings. However, these results demonstrate that two populations may experience dermal effects as a result of mercury exposure. One is those persons who develop an allergic reaction to mercury. The other is those who are hypersensitive to mercury and who develop acrodynia upon exposure. It is unknown whether sufficiently high concentrations of inorganic mercury in soil or methylmercury in fish may exist at hazardous waste sites to trigger allergic dermatitis in sensitive persons or acrodynia in those predisposed to develop this syndrome.

Ocular Effects. Ocular effects have been observed in persons exposed to high concentrations of metallic mercury vapors. These effects are probably due to direct contact of the mercury vapor with the eyes. The observed effects include red and burning eyes, conjunctivitis (Bluhm et al. 1992a; Foulds et al. 1987; Karpathios et al. 1991; Schwartz et al. 1992; Sexton et al. 1976), and a yellow haze on the lenses of the eye (Atkinson 1943; Bidstrup et al. 1951; Locket and Nazroo 1952). The yellow haze was associated with long-term occupational exposures. Animal studies were not available to support these findings. However, the evidence suggests that exposure to high levels of mercury vapor may result in ocular irritation.

Other Systemic Effects. Studies of workers exposed to mercury vapor found no effect on serum levels of thyroid-stimulating hormone (Erfurth et al. 1990; McGregor and Mason 1991). However, an enlarged thyroid, with elevated triiodothyronine and thyroxine, as well as reduced thyroid-stimulating hormone developed in a 13-year-old boy exposed to mercury vapor for 2 weeks (Karpathios et al. 1991). Animal studies generally support an effect of acute-duration high-level exposure on the thyroid, although the results have been somewhat variable (Goldman and Blackburn 1979; Sin and The 1992; Sin et al. 1990). A single intramuscular injection of 14.8 mg Hg/kg in rabbits resulted in increased thyroid peroxidase and triiodo-thyronine and decreased thyroxine (Ghosh and Bhattacharya 1992). A study in which rats received three

daily subcutaneous doses of methylmercuric chloride showed slight increases in thyroid weight and basal levels of thyroid-stimulating hormone and thyroxine (Kabuto 1991). However, it was unclear whether these changes were statistically significant. In contrast, a single subcutaneous dose of 6.4 mg/Hg as methyl-mercuric chloride resulted in significant decreases in serum thyroxine (Kabuto 1987). At higher doses (9.6 and 12.8 mg mercury), increases in prolactin and thyroid-stimulating hormone were observed. The reason for these differences is unclear, but the data suggest that thyroid function may be affected if persons are exposed to sufficiently high concentrations of mercury.

Animal studies also provide evidence of mercury-induced effects on the corticosteroid levels. Increased adrenal and plasma corticosterone levels were reported in rats receiving 2.6 mg Hg/kg/day as mercuric chloride in drinking water after 120 days (Agrawal and Chansouria 1989). At 180 days of exposure, these effects were not evident in the animals. The investigators suggested that mercuric chloride is a dose- and duration-dependent chemical stressor. Subchronic administration of methylmercury to rats caused a diminished secretory response of corticosterone and testosterone serum levels following adrenocorticotropin (ACTH) and human chorionic gonadotropin (HCG) stimulation, respectively (Burton and Meikle 1980). The adrenal glands showed marked hyperplasia and increased weight, and basal levels of these hormones were also depressed. The treated animals exhibited stress intolerance and decreased sexual activity. These results suggest that methylmercury may have an adverse effect on steroidogenesis in the adrenal cortex and testes. Based on these animal studies, inorganic and organic mercury may also act on the corticosteroid system to alter hormonal levels. It is unclear to what extent the effects observed are the result of generalized stress or direct toxic effects on the endocrine system regulating corticosteroid levels.

Inhalation of metallic mercury vapor may result in a metal fume fever-like syndrome characterized by fatigue, fever, chills, cough, and an elevated leukocyte count (Bluhm et al. 1992a; Garnier et al. 1981; Lilis et al. 1985; McFarland and Reigel 1978; Milne et al. 1970; Schwartz et al. 1992; Snodgrass et al. 1981). Also, children with acrodynia frequently exhibit low-grade intermittent fevers (Aronow et al. 1990; Warkany and Hubbard 1953). Animal data are not available to support this finding, but the human data suggest that exposure to sufficiently high concentrations of metallic mercury vapor may result in transient fever (see Hematological Effects).

Immunological Effects. As indicated in the section on dermal effects, allergic dermatological reactions occurred in persons exposed to inorganic mercury from dental amalgams, tattoos, or breakage of medical instruments (Anneroth et al. 1992; Bagley et al. 1987; Biro and Klein 1967; Faria and Freitas

1992; Goh and Ng 1988; Pambor and Timmel 1989; Skoglund and Egelrud 1991; Veien 1990). Additionally, mercury may cause either decreases in immune activity or an autoimmune response, depending on the genetic predisposition of the individual exposed. The human data are very limited, and only decreased IgG production has been observed in workers chronically exposed to metallic mercury vapor at chloralkali and ore production plants (Bencko et al. 1990; Moszczynski et al. 1990b). Neither of these studies, however, adjusted for smoking or alcohol. Increases in serum immunoglobulins (IgA, IgG, IgE, or IgM) and autoantibody titres (antilaminin or antiglomerular basement membrane antibodies) have not been observed in similarly exposed populations (Bernard et al. 1987; Cardenas et al. 1993; Langworth et al. 1992b). There is limited information in humans that suggests that certain individuals may develop an autoimmune response when exposed to mercury. Deposition of IgG and complement C3 have been observed in the glomeruli of two workers with mercury-induced proteinuria (Tubbs et al. 1982). Also, increased antiglomerular basement membrane antibodies and elevated antinuclear antibodies have been observed in a few persons with exposure to mercury in dental amalgams (Anneroth et al. 1992). After removal of one dental amalgam, a significant decrease in IgE levels was observed. Within the populations described above that showed no overall increase in immune parameters, individuals in these groups showed either increases in anti-DNA antibody titres or antiglomerular basement membrane responses (Cardenas et al. 1993; Langworth et al. 1992b). Moszczynski et al. (1995) studied workers exposed to mercury vapor and reported a positive correlation between the T-helper cell count and the duration of exposure. The combined stimulation of the T-cell line and an observed decrease in the helper/suppressor ratio were suggestive of an autoimmune response.

The immune system reaction to mercury has been extensively studied in animals. Although it has not been completely described, a great deal of information exists about the changes that occur in the immune system in response to mercury exposure (Bigazzi 1992; Goldman et al. 1991; Mathieson 1992). Animal strains that are susceptible or predisposed to develop an autoimmune response show a proliferation of autoreactive T-cells (specifically CD4+ T-cells) (Pelletier et al. 1986; Rossert et al. 1988). The fundamental change caused by mercury that results in the autoimmune response appears to be in these autoreactive T-cells, since transfer of these cells to an unexposed animal results in the development of the autoimmune response in the unexposed animal (Pelletier et al. 1988). A subset of the CD4+ T-cells, the Th2 cells, are activated and induce polyclonal B-cell activation (possibly through the release of interleukin-4 [IL-4]), which results in IgE production by the B-cells (Ochel et al. 1991). The increases in serum IgE are paralleled by increases in MHC molecule expression on the B-cells (Dubey et al. 1991a). These changes are accompanied by enlargement of the spleen and lymph nodes, an increase in the number of spleen cells

(thought to be associated with the B-cell proliferation) (Hirsch et al. 1982; Matsuo et al. 1989), and marked increases in serum levels of IgE (Dubey et al. 1991b; Hirsch et al. 1986; Lymberi et al. 1986; Prouvost-Danon et al. 1981). Increases in the production of autoantibodies (IgG) to glomerular basement membrane, thyroglobulin, collagen types I and II, and DNA also occur (Pusey et al. 1990). Immune complex deposits occur in blood vessels in several organs (Hultman et al. 1992), and deposition of these autoantibodies and complement in the renal glomerulus ultimately lead to membranous glomerulonephropathy, although the deposition of the IgG alone does not appear to be sufficient to induce renal dysfunction (Michaelson et al. 1985). In rodents, the autoimmune response spontaneously resolves within a few weeks. The mechanism underlying the resolution is unknown, but antiidiotypic antibodies and a change in the balance between Th2 and Th1 (another subset of the CD4+ T-cells) cell activation (see below) have been proposed (Mathieson 1992). After this resolution phase has occurred, affected individuals develop a resistance to future autoimmune toxicity (Bowman et al. 1984). The resistance appears to be mediated by CD8+ T-cells, since depletion of these cells reverses the resistance (Mathieson et al. 1991).

The so-called resistant strains, however, show a different response to mercury exposure. These resistant strains also show an increase in MHC expression molecules on B-cells, but this response is extremely short-lived, and increases in serum IgE were not observed (Dubey et al. 1991a; Prouvost-Danon et al. 1981). The difference in the responses of the so-called resistant and susceptible strains may be found in the activation of Th1 cells and the increase in secretion of γ-interferon by the Th1 cells of resistant animals (van der Meide et al. 1993). The susceptible strains do not show an increase in γ-interferon production with mercury exposure. Because γ-interferon inhibits the proliferation of Th2 cells, the absence of this response in the susceptible strains may allow the Th2 cell-stimulated production of autoantibodies to occur, whereas in the resistant strains the production of antibodies is curtailed. Thus, differences in the activation of Th1 versus Th2 cells may underlie the differences in susceptibility of various individuals. Studies using in-bred strains of mice and rats have determined that the susceptibility to the different immune reactions is governed by both MHC genes as well as other genes (Aten et al. 1991; Druet et al. 1977; Mirtcheva et al. 1989; Sapin et al. 1984). As indicated in the section on renal effects, Brown-Norway, MAXX, and DZB rat strains showed susceptibility, whereas Lewis, M520, and AO rats did not (Aten et al. 1991; Druet et al. 1978; Michaelson et al. 1985). Among mouse strains, SJL/N mice are susceptible and DBA, C57BL, and Balb/c mice are not (Hultman and Enestrom 1992; Hultman et al. 1992). In a resistant strain, the Balb/c mouse, immune suppression was manifested as decreased natural killer cell activity in mice administered a diet containing 0.5 mg Hg/kg/day as methylmercury (Ilback 1991).

Neurological Effects. The nervous system is the primary target organ for elemental and methyl-mercury-induced toxicity. Neurological and behavioral disorders in humans have been observed following inhalation of metallic mercury vapor and organic mercury compounds, ingestion or dermal application of inorganic mercury-containing medicinal products (e.g., teething powders, ointments, and laxatives), and ingestion or dermal exposure to organic mercury-containing pesticides or ingestion of contaminated seafood. A broad range of symptoms has been reported, and these symptoms are qualitatively similar, irrespective of the mercury compound to which one is exposed. Specific neurotoxic symptoms include tremors (initially affecting the hands and sometimes spreading to other parts of the body), emotional lability (characterized by irritability, excessive shyness, confidence loss, and nervousness), insomnia, memory loss, neuromuscular changes (weakness, muscle atrophy, and muscle twitching), headaches, polyneuropathy (paresthesias, stocking-glove sensory loss, hyperactive tendon reflexes, slowed sensory and motor nerve conduction velocities), and performance deficits in tests of cognitive and motor function (Adams et al. 1983; Albers et al. 1982, 1988; Aronow et al. 1990; Bakir et al. 1973; Barber 1978; Bidstrup et al. 1951; Bluhm et al. 1992a; Bourgeois et al. 1986; Chaffin et al. 1973; Chapman et al. 1990; Choi et al. 1978; Cinca et al. 1979; Davis et al. 1974; DeBont et al. 1986; Discalzi et al. 1993; Dyall-Smith and Scurry 1990; Ehrenberg et al. 1991; Fagala and Wigg 1992; Fawer et al. 1983; Foulds et al. 1987; Friberg et al. 1953; Hallee 1969; Harada 1978; Hook et al. 1954; Hunter et al. 1940; Iyer et al. 1976; Jaffe et al. 1983; Jalili and Abbasi 1961; Kang-Yum and Oransky 1992; Karpathios et al. 1991; Kutsuna 1968; Langauer-Lewowicka and Kazibutowska 1989; Kutsuna 1968; Langolf et al. 1978; Langworth et al. 1992a; Levine et al. 1982; Lilis et al. 1985; Lundgren and Swensson 1949; Matsumoto et al. 1965; McFarland and Reigel 1978; Melkonian and Baker 1988; Miyakawa et al. 1976; Ngim et al. 1992; Piikivi and Hanninen 1989; Piikivi and Tolonen 1989; Piikivi et al. 1984; Roels et al. 1982; Sexton et al. 1976; Shapiro et al. 1982; Snodgrass et al. 1981; Smith et al. 1970; Tamashiro et al. 1984; Taueg et al. 1992; Tsubaki and Takahashi 1986; Verberk et al. 1986; Vroom and Greer 1972; Warkany and Hubbard 1953; Williamson et al. 1982). Some individuals have also noted hearing loss, visual disturbances (visual field defects), and/or hallucinations (Bluhm et al. 1992a; Cinca et al. 1979; Fagala and Wigg 1992; Jalili and Abbasi 1961; Locket and Nazroo 1952; McFarland and Reigel 1978; Taueg et al. 1992). Although improvement has often been observed upon removal of persons from the source of exposure, it is possible that some changes may be irreversible. Autopsy findings of degenerative changes in the brains of poisoned patients exposed to mercury support the functional changes observed (Al-Saleem and the Clinical Committee on Mercury Poisoning 1976; Cinca et al. 1979; Davis et al. 1974; Miyakawa et al. 1976). Limited information was located regarding exposure levels associated with the above effects, but increased tremors and cognitive difficulties are sensitive end points for chronic low-level exposure to metallic mercury vapor (Fawer et al.

1983; Ngim et al. 1992). Photophobia has been reported exclusively in children with acrodynia (Fagala and Wigg 1992; Warkany and Hubbard 1953). The physiological basis for the photophobia is unknown.

The neurotoxicity of inorganic and organic mercury in experimental animals is manifested as functional, behavioral, and morphological changes, as well as alterations in brain neurochemistry (Arito and Takahashi 1991; Ashe et al. 1953; Berthoud et al. 1976; Burbacher et al. 1988; Cavanaugh and Chen 1971; Chang and Hartmann 1972a, 1972b; Chang et al. 1974; Charbonneau et al. 1976; Concas et al. 1983; Evans et al. 1977; Fukuda 1971; Fuyuta et al. 1978; Ganser and Kirschner 1985; Inouye and Murakami 1975; Jacobs et al. 1977; Kishi et al. 1978; Lehotzky and Meszaros 1974; Leyshon and Morgan 1991; MacDonald and Harbison 1977; Magos and Butler 1972; Magos et al. 1980, 1985; Mitsumori et al. 1981; Miyama et al. 1983; Post et al. 1973; Rice 1989c; Rice and Gilbert 1982, 1992; Salvaterra et al. 1973; Sharma et al. 1982; Tsuzuki 1981; Yip and Chang 1981).

Animal studies have shown damage to the cerebellar cortex and dorsal root ganglion cells following both mercuric chloride and methylmercuric chloride exposure (Chang and Hartmann 1972b). These structures appear to be especially sensitive to the toxic effects of mercury (Chang and Hartmann 1972a, 1972b; Chang et al. 1974; Charbonneau et al. 1976; Falk et al. 1974; Hirano et al. 1986; Jacobs et al. 1977; Leyshon and Morgan 1991; MacDonald and Harbison 1977; Magos and Butler 1972; Magos et al. 1980, 1985; Mitsumori et al. 1990; Yip and Chang 1981), although other areas (e.g., the cerebral cortex, corpus striatum, thalamus, hypothalamus, organ of Corti, and peripheral nerves) have also shown degenerative changes after exposure to methylmercury (Berthoud et al. 1976; Chang et al. 1974; Charbonneau et al. 1976; Falk et al. 1974; Fehling et al. 1975; Jacobs et al. 1977; Miyakawa et al. 1974, 1976; Yip and Chang 1981). Cats and monkeys appear to be more sensitive to the toxic effects than rodents and have shown signs of neurotoxicity at approximately 10-fold lower doses (0.05 mg Hg/kg/day) following long-term exposure to methylmercuric chloride (Charbonneau et al. 1976; Rice 1989c; Rice and Gilbert 1982, 1992).

Although it is unclear whether changes in neurochemical parameters are primary targets of mercury or whether the changes are secondary to degenerative changes in neurons, several neurotransmitter systems have been shown to be affected by mercury exposure. Cholinergic transmission at the neuromuscular junction has been shown to be affected by mercury exposure (Eldefrawi et al. 1977; Sager et al. 1982). Changes in GABA receptor activity and number have also been observed (Arakawa et al. 1991; Concas et al. 1983). Changes in the activities of enzymes involved in cholinergic, adrenergic, dopaminergic, and

serotonergic synthesis and/or catabolism have also been observed following mercury exposure (Sharma et al. 1982; Tsuzuki 1981).

Collectively, the above information shows the high sensitivity of the nervous system to mercury toxicity and indicates that persons exposed to sufficiently high amounts of mercury may experience adverse neurological symptoms.

Reproductive Effects. Studies in humans indicate that metallic mercury vapor does not cause infertility or malformations following paternal exposure (Alcser et al. 1989; Lauwerys et al. 1985) but may cause an increase in the rate of spontaneous abortions (Cordier et al. 1991). No correlation was observed between levels of testosterone, luteinizing hormone, or follicle-stimulating hormone and occupational exposure to metallic mercury vapor, indicating that the pituitary control of testosterone secretion was not affected (Erfurth et al. 1990; McGregor and Mason 1991). However, *in vitro* studies have shown that mercury can adversely affect human spermatozoa. Inorganic (mercuric chloride) and organic (methylmercuric chloride) mercury decreased the percentage of motile spermatozoa *in vitro* (Ernst and Lauritsen 1991). Incubation of human spermatozoa with inorganic mercury resulted in mercury deposits localized in the membranes of the midpiece and tailpiece. The lack of mercury grains in spermatozoa with methylmercury exposure may be due to the inability of spermatozoa or the semen plasma to demethylate methylmercury in the 15-minute incubation period (Ernst and Lauritsen 1991).

Female dentists and dental assistants exposed to metallic mercury vapors had increased reproductive failures (spontaneous abortions, stillbirths, and congenital malformations) and irregular, painful, or hemorrhagic menstrual disorders (Sikorski et al. 1987). Correlations were observed between the incidence of these effects and hair mercury levels.

Rowland et al. (1994) report that female dental assistants with a high occupational exposure to mercury were found to be less fertile than controls. The probability of conception with each menstrual cycle (called "fecundability" by the authors) in women who prepared 30 or more amalgams per week and who were evaluated as having 4 or more poor mercury-hygiene practices was only 63% of that of unexposed controls. Hygiene was incorporated into the evaluation of the results of this study because occupational groups with roughly the same potential for exposure often contain subjects whose actual exposures are quite different, depending on their particular work environment and their work (and personal) hygiene practices within that environment. Rowland et al. (1994) found that 20% of the women in their final evaluation who prepared

more than 30 amalgams a week had 4 or more poor mercury-hygiene factors. Among women preparing a comparable number of amalgams, there were differences in "fecundability," based on the number of self-reported poor hygiene factors. The study is limited in that a group of unexposed women had lower fertility than the low exposed group suggesting other unaccounted for exposures or confounding factors.

Animal data suggest that mercury may alter reproductive function and/or success when administered to either males or females. In males, mercury exposure results primarily in impaired spermatogenesis, sperm motility, and degeneration of seminiferous tubules. Oral administration of methylmercury to males has resulted in decreases in litter size due to preimplantation loss (presumably due to defective sperm) in rats (Khera 1973), decreases in sperm motility in monkeys (at 0.025 mg Hg/kg/day for 20 weeks) (Mohamed et al. 1987), and tubular atrophy and decreased spermatogenesis in mice after prolonged exposure (Hirano et al. 1986; Mitsumori et al. 1990). Parenteral administration of methylmercury has shown similar results. A single intraperitoneal injection of 10 mg/kg of methylmercury in male mice resulted in decreased implantations in females (Suter 1975), and a single intraperitoneal injection of 1 mg/kg of methylmercury resulted in a reversible failure of spermatogenesis and infertility in male mice (Lee and Dixon 1975). Repeated intraperitoneal injections of methylmercury (3.5 mg Hg/kg/day for 6 weeks) in male rats resulted in decreased sexual activity, depression of testosterone levels (Burton and Meikle 1980), and decreased spermatogenesis (0.004 mg Hg/kg/day for 15–90 days) (Vachhrajani et al. 1992). Less is known about the effects of inorganic mercury on the male reproductive system, but a single intraperitoneal injection of mercuric chloride (1 mg Hg/kg) in male rats resulted in decreased conceptions in females (Lee and Dixon 1975), and 0.74 mg Hg/kg resulted in tubular degeneration (Prem et al. 1992). An *in vitro* study (Mohamed et al. 1987) suggested that the decrease in sperm motility observed in monkeys may be due to interference with microtubule assembly or dynein/microtubule sliding function.

In females, mercury exposure results primarily in increases in resorptions and decreases in implantations. Inhalation exposure of female rats to metallic mercury vapor (2.5 mg/m^3, 6 hours a day, 5 days a week for 21 days) resulted in a prolongation of the estrous cycle (Baranski and Szymczyk 1973). Oral administration of mercuric acetate (22 mg Hg/kg) to pregnant hamsters resulted in an increase in resorptions (Gale 1974). Oral administration of methylmercury to pregnant guinea pigs (11.5 mg Hg/kg) resulted in an increase in abortions (Inouye and Kajiwara 1988), and 3 mg Hg/kg resulted in a decrease in the number of pups in the litter from pregnant mice (Hughes and Annau 1976). Pregnant mice given a single dose of 20 mg Hg/kg as methylmercuric chloride had increased resorptions, decreased live fetuses, and decreased fetuses per litter (Fuyuta et al. 1978). Repeated oral administration of methylmercury

(0.06 mg Hg/kg/day) to female monkeys resulted in an increase in the number of abortions and a decrease in conceptions (Burbacher et al. 1988). No effect on the monkeys' menstrual cycles was observed. Intraperitoneal administration of mercuric chloride (1.48 mg Hg/kg) to female mice resulted in decreases in litter size and number of litters/female and an increase in dead implants in some strains of mice, but these effects were strain-specific (Suter 1975). In female mice administered a single intraperitoneal dose of 1 mg Hg/kg as mercuric chloride, a decrease in mean implantation sites was observed (Kajiwara and Inouye 1992). Subcutaneous injection of female hamsters with 6.2–8.2 mg Hg/kg as mercuric chloride for 1–4 days resulted in a disruption of estrous (Lamperti and Printz 1973). Inhibition of follicular maturation and normal uterine hypertrophy, morphological prolongation of the corpora lutea, and alteration of progesterone levels were observed. Collectively, these results suggest that at sufficiently high mercury concentrations, men may experience some adverse effects on testicular function and women may experience increases in abortions, decreases in conceptions, or development of menstrual disorders.

Developmental Effects. Mercury is considered to be a developmental toxicant. Extremely limited information was located regarding human developmental effects associated with exposure to inorganic mercury (Alcser et al. 1989; Derobert and Tara 1950; Melkonian and Baker 1988; Thorpe et al. 1992). However, developmental toxicity in humans associated with oral exposure to organic forms of mercury is well recognized (Amin-Zaki et al. 1974; Bakir et al. 1973; Cox et al. 1989; Engleson and Herner 1952; Harada 1978; Marsh et al. 1980, 1981, 1987; McKeown-Eyssen et al. 1983; Snyder and Seelinger 1976). The symptoms observed in offspring of exposed mothers are primarily neurological in origin and have ranged from delays in motor and verbal development to severe brain damage. Subtle changes, such as small changes in intelligence or learning capacity are currently being tested in populations with low-level, chronic exposure to mercury in the diet (Davidson et al. 1998; Grandjean et al. 1997b, 1998). MRLs for acute- and intermediate-duration exposure to methylmercury have been developed based on the lowest observed peak hair level in a mother whose child was reported to have a delayed onset of walking (14 ppm in hair) (Cox et al. 1989; WHO 1990).

Animal studies suggest that both inorganic mercury and organic mercury cause developmental toxicity. Metallic mercury vapor may be transferred across the placenta (Greenwood et al. 1972). The placental transport of mercury in pregnant mice and its localization in the embryo and fetus were studied by autoradiography and gamma counting (Khayat and Dencker 1982). Retention of ^{203}Hg vapor following inhalation was compared to intravenous injection of ^{203}Hg as mercuric chloride. The authors reported that inhalation of mercury vapor resulted in a mercury concentration that was four times higher than the

concentration resulting from injection of mercuric chloride. Furthermore, the authors reported that metallic mercury appeared to oxidize to Hg^{+2} in the fetal tissues. Evidence that inhalation exposure may result in developmental toxicity comes from a study in which neonatal rats were exposed to metallic mercury vapor during a period of rapid brain development (this occurs postnatally in rodents but prenatally in humans), resulting in impaired spatial learning (Fredriksson et al. 1992). Oral administration of inorganic mercury salts to pregnant hamsters has been observed to produce an increase in the number of resorptions and small and edematous embryos (Gale 1974). Mercury-induced embryotoxicity in one non-inbred and five inbred stains of female hamsters was investigated by Gale and Ferm (1971). A single subcutaneous injection of 9.5 mg Hg/kg as mercuric acetate to dams on Gd 8 produced a variety of malformations, including cleft palate, hydrocephalus, and heart defects, and statistically significant interstrain differences in the embryotoxic response. Single doses of 1.3–2.5 mg Hg/kg as mercuric acetate injected intravenously into pregnant hamsters on Gd 8 produced growth retardation and edema of the fetuses at all 3 dose levels, while an increase in the number of abnormalities was detected at the two higher doses (Gale and Ferm 1971). The relative effectiveness of different exposure routes in hamsters was compared by Gale (1974). The following sequence of decreasing efficacy was noted for mercuric acetate: intraperitoneal > intravenous > subcutaneous > oral. The lowest doses used (2 mg/kg for intraperitoneal and 4 mg/kg for the other 3 routes) were all effective in causing increased resorption and an increased percentage of abnormalities. Intravenous injection of 1.5 mg Hg/kg/day as mercuric chloride also resulted in a significant increase in the number of abnormal preimplantation embryos (Kajiwara and Inouye 1986).

In animals, embryolethal, anatomical, and behavioral effects have been reported following oral exposure of pregnant dams to methylmercury (Bornhausen et al. 1980; Cagiano et al. 1990; Elsner 1991; Fowler and Woods 1977; Fuyuta et al. 1978, 1979; Guidetti et al. 1992; Gunderson et al. 1988; Hughes and Annau 1976; Ilback et al. 1991; Inouye and Kajiwara 1988; Inouye and Murakami 1975; Inouye et al. 1985; Khera and Tabacova 1973; Lindstrom et al. 1991; Nolen et al. 1972; Olson and Boush 1975; Reuhl et al. 1981a, 1981b; Rice 1992; Rice and Gilbert 1990; Stoltenburg-Didinger and Markwort 1990; Yasuda et al. 1985). Thus far, the most sensitive animal assay for developmental neurotoxicity has been a behavioral paradigm that examined the number of rewarded responses to differential reinforcement at high rates (Bornhausen et al. 1980). At doses of 0.008 mg Hg/kg/day and above, a dose-related decrease in rewarded responses was observed in 4-month-old offspring of rats treated on Gd 6–9. The effect was more pronounced in male offspring than females. Foster mothers were used to preclude consumption of contaminated milk during lactation.

Developmental toxicity has also been observed with parenteral exposure to methylmercury in pregnant dams during gestation. In mice given methylmercuric hydroxide subcutaneously daily from Gd 7–12, significant dose-related increases in the percentage of litters with resorptions were seen in groups receiving 3.45–8.6 mg Hg/kg/day (Su and Okita 1976). The frequency of cleft palate increased significantly in litters of the 3.45 and 4.3 mg Hg/kg/day groups only. A high incidence of delayed palate closure and cleft palate was also reported in mice injected subcutaneously with 5 mg Hg/kg of methylmercuric chloride on Gd 12 (Olson and Massaro 1977). Gross incoordination and decreased frequencies of defecation and urination in pups were observed following intraperitoneal administration of a single dose of methylmercury dicyandiamide (8 mg/kg/day) to pregnant mice on day 7 or 9 of pregnancy (Spyker et al. 1972). Degenerative changes were observed in the cerebellum and cerebral cortex of rat pups of maternal rats injected with 4 mg Hg/kg as methylmercuric chloride on Gd 8 (Chang et al. 1977). Degenerative renal changes (in epithelial cells of proximal tubules and Bowman's capsule of glomeruli) were reported in rat fetuses of dams exposed intraperitoneally to methylmercuric chloride during Gd 8 (Chang and Sprecher 1976). The studies by Spyker and Smithberg (1972) demonstrated strain differences in susceptibility to the developmental effects of methylmercury dicyandiamide. Intraperitoneal administration of single doses of methylmercury dicyandiamide (2, 4, or 8 mg/kg) to pregnant mice of strains 129 Sv/S1 and A/J during gestation resulted in retardation of fetal growth and increased resorption of implants in both strains. Teratogenic effects, primarily of the palate and jaw, were detected at all dose levels in 129 Sv/S1 mice, but only at the highest dose in strain A/J. The differential effects of methylmercury were dependent on the strain, the dose of the agent, and the stage of embryonic development.

Antilaminin antibodies induced by mercuric chloride have been demonstrated to be detrimental to the development of cultured rat embryos (Chambers and Klein 1993). Based upon that observation, those authors suggested that it might be possible for an autoimmune disease induced by a substance such as mercury at an early age to persist into later life, acting as a teratogen independent of both dose-response relationships and time of exposure, but that possibility remains to be experimentally demonstrated.

One developmental study of phenylmercury compounds was reported by Gale and Ferm (1971) in which hamsters were injected intravenously with phenylmercuric acetate at doses ranging from 5 to 10 mg/kg on Gd 8. With the exception of the lowest dose, all other doses induced increased resorption rates and edema, along with a few miscellaneous abnormalities including cleft palate and exencephaly.

The above information clearly indicates the possibility of developmental toxicity in offspring of mothers that ingest sufficient amounts of organic mercury. The animal data also suggest that exposure to sufficient amounts of inorganic mercury by inhalation of metallic mercury vapor or ingestion of inorganic mercury may result in developmental toxicity.

Genotoxic Effects. The overall findings from cytogenetic monitoring studies of workers occupationally exposed to mercury compounds by inhalation (Anwar and Gabal 1991; Barregard et al. 1991; Mabille et al. 1984; Popescu et al. 1979; Verschaeve et al. 1976, 1979) or accidentally exposed through ingestion (Wulf et al. 1986) provided no convincing evidence that mercury adversely affects the number or structure of chromosomes in human somatic cells. Studies reporting a positive result (Anwar and Gabal 1991; Barregard et al. 1991; Popescu et al. 1979; Skerfving et al. 1970, 1974; Verschaeve et al. 1976; Wulf et al. 1986) were compromised either by technical problems, a lack of consideration of confounding factors, or a failure to demonstrate a relationship between mercury exposure and induced aberrations. Therefore, none of these studies can be used to predict the potential genetic hazard to humans associated with exposure to mercury or mercury compounds.

A dose-related increase in chromosome aberrations was observed in the bone marrow of mice administered a single oral dose of mercuric chloride at levels of at least 4.4 mg Hg/kg (Ghosh et al. 1991). By contrast, there was no valid evidence of a genotoxic effect on somatic cells of cats chronically exposed to methyl-mercury orally (Miller et al. 1979). However, only minimal toxicity was observed at the high dose (0.046 mg Hg/kg/day) in this study. Doses of 0.86, 1.7, or 3.4 mg Hg/kg as methylmercury hydroxide administered once by intraperitoneal injection to groups of 2 male CBA mice did not cause an increase in micronucleated polychromatic erythrocytes harvested from bone marrow cells 24 hours after treatment (Jenssen and Ramel 1980). Similarly, there was no increase in structural chromosome aberrations in bone marrow cells collected from male Swiss OF_1 mice (3–4/group) 12, 24, 36, or 48 hours postexposure to single intraperitoneal doses of 0.7, 1.5, 3.0, or 4.4 mg Hg/kg as mercuric chloride (Poma et al. 1981). The lack of a clastogenic response, particularly with mercuric chloride, should not be viewed as a possible inability of this compound to penetrate somatic cell membranes. There are data from the study of Bryan et al. (1974) indicating that mercuric chloride can bind to chromatin in the livers of mice challenged with 38 mg Hg/kg/day as mercuric chloride for 1 month. Although the overall data are mixed, the findings from a well conducted study using oral dosing suggests that mercury can be clastogenic for somatic cells (Ghosh et al. 1991).

The intraperitoneal administration of mercuric chloride at levels comparable to those described above did not induce a clastogenic response in the spermatogonia of the same mouse strain (Poma et al. 1981). Structural chromosome aberrations were not produced in metaphase II oocytes of 15 virgin Syrian hamsters receiving a single intraperitoneal injection of 7.4 mg Hg/kg as methylmercury chloride (Mailhes 1983). However, the frequency of hyperploid cells in the treated animals was significantly ($p<0.01$) increased compared to the control. A borderline significant increase in hypoploid cells was also seen. By contrast, Jagiello and Lin (1973) found no evidence of aneuploidy in the oocytes of Swiss/Webster mice (6–8/group) for 3 days after receiving a single intravenous injection of dimethylmercury (140 mg Hg/kg) or mercuric acetate (2, 5, or 10 mg Hg/kg). The lack of concordance between these two studies could be related to the different mercurials that were utilized, the different routes of exposure, or the possible differences in species sensitivity. There are data from a series of dominant lethal assays suggesting that variable strain sensitivity to mercury compounds can affect the outcome of germinal cell cytogenetic investigations (Suter 1975). In this study, two strains of male mice, $(101\times C3H)F_1$ and $(SEC\times C57BL)F_1$, and one strain of female mice, $(101\times C3H)F_1$, received single intraperitoneal injections of 8.6 mg Hg/kg as methylmercuric hydroxide. An additional group of females was injected intraperitoneally with 1.5 mg Hg/kg as mercuric chloride. Males were sequentially mated with untreated females over the entire spermatogenic cycle; treated females were mated once with untreated males. Methylmercuric hydroxide had no effect on fertility and did not induce a clastogenic response in $(101\times C3H)F_1$ males. However, a comparable dose administered to $(SEC\times C57BL)F_1$ males adversely affected fertility and caused significant reductions in total and live implants accompanied by increases in the percentage of dead implants following the first two mating cycles. Suggestive evidence of poor reproductive performance and a dominant lethal effect was also seen in female $(101\times C3H)F_1$ mice treated with methylmercuric hydroxide (8.6 mg Hg/kg) and mercuric chloride (1.5 mg Hg/kg). It was noteworthy that an independent phase of the investigation examined reproduction in females in two additional strains, $(SEC\times C57BL)F_1$ and a mixed stock obtained by crossing $(SEC\times C57BL)F_1$ females with XGSY males. Neither compound had a detrimental effect on the fertility of these females. The single dominant lethal assay conducted with rats (strain not specified) showed that mercuric chloride, administered orally for 12 months (1.8×10^{-3} to 1.8×10^{-4} mg Hg/kg), induced a dose-related increase in dominant lethal mutations, as indicated by increased embryonic death (Zasukhina et al. 1983).

The overall findings from *in vivo* germinal cell assays suggest that mercury compounds are clastogenic for mammalian germ cells. However, the apparent differences in species sensitivity and, in some cases, strain

sensitivity preclude an extrapolation of the relevance of these findings to humans. Refer to Table 2-11 for a further summary of these results.

Several *in vitro* assays employing human cells were located. Both structural and numerical chromosomal aberrations were observed following the exposure of human lymphocytes to methylmercury chloride or dimethylmercury *in vitro* (Betti et al. 1992). Although the smoking status of the donor was not reported, all of the cells came from the same donor, and no aberrations were observed in the control cultures. Mercuric acetate caused single-strand breaks in DNA from human KB-cells (Williams et al. 1987). Methylmercuric chloride treatment of human lymphocytes resulted in the formation of chromosome and chromatid aberrations (Betti et al. 1993b). Further, it was found to be a weak inducer of sister chromatid exchange, but that effect did not increase with an increasing dosage. Methylmercuric chloride was also found to be capable of producing aneuploidy (particularly hyperdiploidy). At low doses, more chromosomal aberrations were observed in the second metaphases than in the first, suggesting that several premutational lesions induced by that organomercurial survived through one cell cycle. Thus, the damage produced by methylmercuric chloride appeared to be stable and could lead to chromosome segregation errors. Betti et al. (1993b) concluded that methylmercuric chloride was capable of producing long-lasting damage, which in turn gives rise to both structural and numerical chromosome abnormalities. Bala et al. (1993) reported that methyl-mercuric chloride in concentrations of 10^{-5}, 10^{-6}, and 10^{-7} M induced aberrant metaphases (including gaps) in cultured human peripheral lymphocytes in a dose-dependent manner ($p<0.05$). Methylmercuric chloride at the higher concentrations also induced a significant number of breaks. Further, methylmercuric chloride induced a significant number of SCEs per cell in a dose-dependent manner. However, cultures treated with gamma linolenic acid (GLA), a derivative of dietary essential fatty acid, did not differ from controls with respect to aberrations, and GLA reduced the frequency of SCEs induced by methylmercuric chloride in a dose-dependent manner ($p<0.05$).

Mercuric chloride was not mutagenic in the *Salmonella typhimurium* plate incorporation assay (Wong 1988). These negative results are not unexpected because the Ames test is not suitable for the detection of heavy metal mutagens. Oberly et al. (1982) reported, however, that doses of mercuric chloride (4.4 and 5.9 µg Hg/mL) approaching severely cytotoxic levels induced a weak mutagenic response in mouse lymphoma L5178Y cells but only in the presence of auxiliary metabolic activation.

In an *in vitro* study of the clastogenic effects of mercurials in animal cells, Howard et al. (1991) observed a dose-related increase in chromosome aberrations in Chinese hamster ovary (CHO) cells treated with

Table 2-11. Genotoxicity of Mercury *In Vivo*

Species (test system)	End point	Results	Reference
Somatic cells:			
CBA mouse (bone marrow cells)	Micronuclei induction	–	Jenssen and Ramel 1980
Swiss mouse (bone marrow cells)	Chromosome aberrations	–	Poma et al. 1981
Swiss mouse (bone marrow cells)	Chromosome aberrations	+	Ghosh et al. 1991
Swiss mouse (liver chromatin)	Chromatid binding	+	Bryan et al. 1974
Cat (bone marrow cells)	Chromsome aberrations	(+)	Miller et al. 1979
Human (peripheral lymphocytes)	Structural chromosome aberrations	(+)	Skerfving et al. 1970
	Aneuploidy	(+)	Skerfving et al. 1974
Human (peripheral lymphocytes)	Structural chromosome aberrations	(+)	Skerfving et al. 1974
	Aneuploidy	(+)	Skerfving et al. 1974
Human (peripheral lymphocytes)	Structural chromosome aberrations	(+)	Popescu et al. 1979
	Aneuploidy	–	Popescu et al. 1979
Human (peripheral lymphocytes)	Structural chromosome aberrations	(+)	Verschaeve et al. 1976
	Aneuploidy	(+)	Verschaeve et al. 1976
Human (peripheral lymphocytes)	Structural chromosome aberrations	–	Verschaeve et al. 1979
	Aneuploidy	–	Verschaeve et al. 1979
Human (peripheral lymphocytes)	Structural chromosome aberrations	–	Mabille et al. 1984
Human (peripheral lymphocytes)	Micronuclei induction	+[a]	Barregard et al. 1991
Human (peripheral lymphocytes)	Structural chromosome aberrations	+[b]	Anwar and Gabal 1991
	Micronuclei induction	+[b]	Anwar and Gabal 1991
	Aneuploidy	–	Anwar and Gabal 1991
Human (peripheral lymphocytes)	Sister chromatid exchange	(+)	Wulf et al. 1986

Table 2-11. Genotoxicity of Mercury *In Vivo* (continued)

Species (test system)	End point	Results	Reference
Germinal cells:			
Swiss mouse (spermatogonia)	Aneuploidy	–	Poma et al. 1981
Swiss mouse (oocytes)	Aneuploidy	–	Jagiello and Lin 1973
$(101\times C_3H)F_1$ mouse (spermatogonia)	Dominant lethal	–	Suter 1975
$(SEC\times C_{57}BL)F_1$ mouse (spermatogonia)	Dominant lethal	+	Suter 1975
$(101\times C_3H)F_1$ mouse (oocytes)	Dominant lethal	+/–	Suter 1975
Rat (spermatogonia)	Dominant lethal	+	Zasukhina et al. 1983
Syrian hamsters (oocytes)	Structural aberrations	–	Mailhes 1983
	Aneuploidy	+	Mailhes 1983

[a]Positive response only in stimulated T-lymphocytes
[b]Positive response but no correlation to urinary mercury levels or duration of exposure

– = negative results; + = positive results; (+) = reported as positive but study was either seriously compromised or findings did not provide valid evidence of a positive response; +/– = inconclusive

mercuric chloride. In a study of the potentiating effects of organomercurials on clastogen-induced chromosomal aberrations in cultured Chinese hamster cells, Yamada et al. (1993) investigated the effects of five organomercurial compounds (methylmercuric chloride, ethylmercuric chloride, phenylmercuric chloride, dimethylmercury, and diethylmercury) and found all to produce remarkable cytotoxicity. Fifty percent or more depression in the mitotic index was observed following treatment with methylmercuric chloride (2.5 µg/mL), ethylmercuric chloride (2.5 µg/mL), phenylmercuric chloride (1.25 µg/mL), and HgCl and $HgCl_2$ ($1.25 µg/mL). Post-treatment with methylmercuric chloride and ethylmercuric chloride increased the number of breakage and exchange-type aberrations induced by 4-nitroquinoline 1-oxide and methylmethane sulfonate but they did not show any clastogenic effects by themselves. Dimethylmercury, diethylmercury, mercurous chloride, and mercuric chloride did not show any potentiating effects. Following pretreatment with the 4-nitroquinoline 1-oxide or the DNA cross-linking agent mitomycin C, treatment with methylmercuric chloride during the G1 phase resulted in the enhancement of both breakage- and exchange-type aberrations. Ethylmercuric chloride treatment during the G1 phase also enhanced both types of aberrations induced by 4-nitroquinoline 1-oxide, but did not show any potentiating effect. When treatment was during the G2 phase, however, both methylmercuric chloride and ethylmercuric chloride enhanced breakage-type aberrations only. In the Yamada et al. (1993) study, the dialkyl mercury compounds dimethylmercury and diethylmercury did not show any cytotoxicity at 5–40 µg/mL, but they did cause a significant increase in the frequency of aberrant cells at the 40 µg/mL concentration. The authors of this study suggested three possible mechanisms for the observed potentiation of clastogenicity by monoalkylated mercurials: (1) they interfere with the repair of base lesions induced by 4-nitroquinoline 1-oxide and mitomycin C during the prereplication stage, thus increasing unrepaired DNA lesions that subsequently convert into DNA double-strand breaks in the S phase; (2) methylmercuric chloride (but not ethylmercuric chloride) inhibits the repair of cross-linking lesions during the prereplication stage; and (3) their G2 effects enhance breakage-type aberrations only. Yamada et al. (1993) concluded that because mercury compounds are known to react with protein thiol groups to inhibit protein activity, it is possible that they also inhibit some protein activities involved in the DNA repair process. The specific target protein for organomercurials and why the potentiation activities of methylmercury chloride and ethylmercury chloride differ remain to be identified.

There is a sizable database of studies investigating the DNA-damaging activity of mercuric chloride. The finding that mercuric chloride can damage DNA in rat and mouse embryo fibroblasts (Zasukhina et al. 1983), supports the *in vivo* evidence of species- and intraspecies-specific sensitivity to the genotoxic action of mercuric chloride. Marked conversion of DNA into the single-stranded form occurred at 10^{-6} M

mercuric chloride in rat fibroblasts, while 5×10^{-6} M mercuric chloride produced a comparable response in C57BL/6 mouse cells; at this level, the response in CBA mouse cells was marginal. Mercuric chloride can also bind to the chromatin of rat fibroblasts (Rozalski and Wierzbicki 1983) and Chinese hamster ovary cells (Cantoni et al. 1984a, 1984b; Christie et al. 1984, 1985). Using the alkaline elution assay with intact Chinese hamster ovary cells, several studies have demonstrated that mercuric chloride induces single-strand breaks in DNA (Cantoni and Costa 1983; Cantoni et al. 1982, 1984a, 1984b; Christie et al. 1984, 1985). Furthermore, Cantoni and Costa (1983) found that the DNA-damaging potential of mercuric chloride is enhanced by a concurrent inhibition of DNA repair mechanisms. Methylmercuric chloride induced single-strand breaks in the DNA of intact rat glioblastoma cells, Chinese hamster V79 (fetal lung) cells, human lung cells, and human nerve cells (Costa et al. 1991). Results of the *Bacillus subtilis* rec-assay (Kanematsu et al. 1980) and the sister chromatid exchange assay (Howard et al. 1991) provide additional support to the body of evidence suggesting that mercuric chloride is genotoxic. However, there is no clear evidence that mercury would cause DNA damage *in vivo*.

Two organic mercury compounds (methylmercury chloride at 0.08–0.4 µg Hg/mL and methoxyethyl mercury chloride at 0.04–0.23 µg Hg/mL) induced weak but dose-related mutagenic responses in Chinese hamster V-79 cells near the cytotoxic threshold (Fiskesjo 1979). Methylmercury was neither mutagenic nor caused recombination in *Saccharomyces cerevisiae*, but it did produce a slight increase in the frequency of chromosomal nondisjunction (Nakai and Machida 1973). Both methylmercury and phenylmercuric acetate induced primary DNA damage in the *B. subtilis* rec-assay (Kanematsu et al. 1980).

In contrast, high concentrations of methylmercury (1 or 2 µm) did not increase the frequency of sister chromatid exchanges in cultured blastocysts of early ICR mouse embryos (Matsumoto and Spindle 1982). Severe toxicity, which was more intense in blastocysts than in morulae, consisted of cessation of preimplantation development, blastocoel collapse, and mitotic delay.

In summary, the body of evidence showing the induction of primary DNA damage in mammalian and bacterial cells and weak mutagenesis in mammalian cells suggests that inorganic and organic mercury compounds have some genotoxic potential. Although the data on clastogenesis are less consistent, recent well conducted studies suggest that mercury compounds can be clastogenic. Refer to Table 2-12 for a further summary of these results.

Table 2-12. Genotoxicity of Mercury *In Vitro*

Species (test system	End point	Results: With activation	Results: Without activation	Reference
Inorganic Mercury Compounds				
Prokaryotic organisms:				
Salmonella typhimurium (TA1535, TA1537, TA98, TA102)	Gene mutation	–	–	Wong 1988
Bacillus subtilis (H17, M45)	DNA damage	NT	+	Kanematsu et al. 1980
Eukaryotic organisms: Mouse lymphoma cells L5178Y	Gene mutation	(+)	–	Oberly et al. 1982
Mouse embryo fibroblasts	DNA damage	NT	+	Zasukhina et al. 1983
Rat embryo fibroblasts	DNA damage	NT	+	Zasukhina et al. 1983
Chinese hamster ovary cells	Chromosome aberrations	NT	+	Howard et al. 1991
Chinese hamster ovary cells	Sister chromatid exchange	NT	+	Howard et al. 1991
Chinese hamster ovary cells	DNA damage	NT	+	Cantoni and Costa 1983
Chinese hamster ovary cells	DNA damage	NT	+	Cantoni et al. 1982, 1984a,b
Chinese hamster ovary cells	DNA damage	NT	+	Christie et al. 1984, 1986
Human KB cells	DNA damage	NT	+	Williams 1987
Rat embryo fibroblasts	Chromatin binding	NT	+	Rozalski and Wierzbicki 1983
Chinese hamster ovary cells	Chromatin binding	NT	+	Cantoni et al. 1984
Organic Mercury Compounds				
Prokaryotic organisms:				
B. subtilis (H17, M45)	DNA damage	NT	+	Kanematsu et al. 1980
Eukaryotic organisms:				
Early mouse embryos (blastocysts)	Sister chromatid exchange	NT	–	Matsumoto and Spindle 1982
Chinese hamster V79 cells	Gene mutation	NT	(+)	Fiskesjo 1979
Chinese hamster V79 cells	DNA damage	NT	+	Costa et al. 1991
Rat glioblastoma cells	DNA damage	NT	+	Costa et al. 1991
Saccharomyces cerevisiae	Gene mutation	NT	–	Nakai and Machida 1973

Table 2-12. Genotoxicity of Mercury *In Vitro* (continued)

Species (test system	End point	Results		Reference
		With activation	Without activation	
S. cerevisiae/intragenic and intergenic recombination	Recombination	NT	–	Nakai and Machida 1973
S. cerevisiae	Chromosome nondisjunction	NT	(+)	Nakai and Machida 1973
Human (peripheral lymphocytes)	Structural chromosome aberrations	NT	+	Betti et al. 1992
Human (peripheral lymphocytes)	Aneuploidy	NT	+	Betti et al. 1992
Human (nerve cells)	DNA damage	NT	+	Costa et al. 1991
Human (lung cells)	DNA damage	NT	+	Costa et al. 1991

(+) = weakly positive or marginal result; – = negative result; + = positive result; DNA = deoxyribonucleic acid; NT = not tested

Cancer. Mercury has not been determined to be carcinogenic in humans (Cragle et al. 1984; Kazantzis 1981). An excess of lung cancer (type not specified) was found in Swedish chloralkali workers, but these workers had also been exposed to asbestos (Barregard et al. 1990). A significant association between the farm use of mercury-containing fungicides and lymphocytic leukemia in cattle was presented by Janicki et al. (1987). However, this study is limited because exposure to other chemicals was not adequately addressed and risk estimates were not adjusted for other risk factors for leukemia.

Animal data, however, suggest that mercuric chloride, and methylmercuric chloride, phenylmercuric acetate are tumorigenic in rats and/or mice. In a 2-year NTP (1993) study, male Fischer 344 rats administered mercuric chloride by gavage had an increased incidence of squamous cell papillomas of the forestomach and an increased incidence of thyroid follicular cell carcinomas at 3.7 mg Hg/kg/day. There is equivocal evidence of carcinogenicity in female rats (a nonsignificant incidence of squamous cell papillomas) and in male $B6C3F_1$ mice (a nonsignificant incidence of renal tubule adenomas and carcinomas). Dietary exposure of ICR and $B6C3F_1$ mice to methylmercuric chloride resulted in significant increases in the incidences of renal epithelial cell adenomas and/or carcinomas in males at doses as low as 0.69–0.73 mg Hg/kg/day (Hirano et al. 1986; Mitsumori et al. 1981, 1990). Similar increases were not observed in females. Renal cell adenomas were also significantly increased in male Wistar rats that received 4.2 mg Hg/kg/day as phenylmercuric acetate in their drinking water (Solecki et al. 1991). This study is limited, however, because an insufficient number of animals were tested to adequately assess carcinogenicity.

Swiss mice were exposed for 15 weeks to drinking water containing methylmercuric chloride at concentrations of 0.038, 0.095, and 0.38 mg Hg/kg/day (Blakley 1984). Urethane (1.5 mg/g) was subsequently given intraperitoneally to the mice at week 3 of the study. Methylmercury exposures of 0.038 and 0.095 mg Hg/kg/day produced a significant increase in the incidence of urethane-induced pulmonary adenomas. The author suggested that methylmercury enhances the formation of pulmonary adenomas and that the immunosuppressive activity of methylmercury may be partially responsible for this tumor-enhancing effect. No other studies were located regarding carcinogenic effects in animals following oral exposure to mercury.

The Department of Health and Human Services (DHHS), and the International Agency for Research on Cancer (IARC) have not classified mercury as to its human carcinogenicity. The Environmental Protection Agency has determined that mercury chloride and methylmercury are possible human carcinogens.

More on Health Effects and Dental Amalgam.

A number of government sponsored scientific reviews of the literature on the health effects associated with the use of dental amalgam have concluded that the data do not demonstrate a health hazard for the large majority of individuals exposed to mercury vapor at levels commonly encountered from dental amalgam (DHHS 1993; Health Canada 1997). Governments that have restricted the use of amalgam or recommend limited use (e.g., Germany, Sweden, Denmark, and Canada) cite the need to minimize human exposure to all forms of mercury as much as possible and to reduce the release of mercury to the environment (DHHS 1993; Health Canada 1997). The restrictive actions, however are prospective, and none of the government reports recommend removing existing fillings in people who have no indication of adverse effects attributable to mercury exposure. Removal of existing amalgams, if improperly performed or not indicated, may result in unnecessarily high exposure to mercury. Levels of mercury release for various dental procedures have been reported by Eley (1997). Chelation therapy (used to remove metals from the body tissues) also may have adverse health effects (and varying levels of effectiveness), and should be considered only in consultation with a qualified physician.

In 1990 in the United States, over 200 million restorative procedures were provided of which dental amalgam accounted for roughly 96 million (DHHS 1993). In the 1970s, the use of amalgam was 38% higher. The use of mercury amalgam has been steadily declining and is expected to continue to decline due to improvements in dental hygiene and preventive care. Approximately 70% of the restorations placed annually are replacements. Advocates of the safety of amalgam emphasize the long history of use (over 150 years) and the large exposed population without apparent adverse effects as strong support for their position (ADA 1997). They also underscore the poor quality of the studies in the literature reporting adverse effects attributable to amalgam. Researchers concerned about the safety of mercury amalgams counter that sample sizes in the studies that support the safety of amalgams are also too small to detect low frequency effects in the general population, and that the absence of high quality studies simply reflects the relatively small amount of research effort that has gone into resolving this very important issue (Richardson 1995; Weiner et al. 1990).

The general public is also clearly concerned about the placement of mercury, a substance with demonstrated toxic effects, into their mouths. A survey conducted by the American Dental Association in 1991 demonstrated that nearly half of the 1,000 American adults surveyed believed that health problems could develop as a result of dental amalgam (ADA 1991). Increases in life expectancy and increases in the

numbers of older adults who still have their permanent teeth will result in longer mercury exposure durations from dental amalgam, which may result in new or increased severity of effects. Recent improvements in neurological measures of performance (especially cognitive and behavior tests) as well as immunological assays have also improved the ability to resolve more subtle or preclinical effects. In this context, DHHS (1993) and other summary reports on the health risks from the use of mercury amalgam generally support the need for further investigations.

Additional recommendations concerning the use of dental amalgam include minimizing exposure to populations susceptible to mercury toxicity including pregnant women and nursing women (to minimize the exposure to their developing young), young children up to the age of 6 (and especially up to the age of 3), people with impaired kidney function, and people with hypersensitive immune response to mercury. People who have higher than average exposures to mercury from other sources (e.g., people who consume large quantities of fish or who work in professions that expose them to mercury) should also consider their total mercury exposure in making their life style and health care decisions. In all cases, the choice not to use mercury amalgam should be made in consultation with a qualified dentists (and/or physician) and weighed against the risk of alternative practices and materials.

The DHHS (1993) report also strongly recommends educating the public on the risks and benefits of dental amalgam. To prevent misleading or unduly alarming the public, the layperson should be informed that the presence of metallic mercury in dental amalgams is, in itself, not sufficient to produce an adverse health effect. Toxic levels of mercury must first be released from the filling, absorbed into the body, and transported to target tissues where adverse effects are produced. What constitutes a “toxic level” from an amalgam exposure has been the focus of recent research. Uncertainty continues concerning the presence or absence of a threshold for adverse effects from low level chronic exposure to mercury. The above mentioned inadequacies in study size, the measures used for effects, the reproducibility of the results, and the subjective nature of some of the low level effects have precluded a consensus in the scientific community on the safety of mercury amalgam. In the absence of clearly defined toxicity from low level exposures, one approach has been to focus upon determining exposure levels from mercury amalgam, and whether these levels exceed recommended guidelines or regulations. Since these guidelines and regulations (including the MRL) are themselves extrapolated from the hazardous effects literature, there is some circularity in the argument that exposures of mercury from amalgam that exceed guidelines like the MRL (or other standard) “support” the position that mercury amalgams pose a health risk. This aspect of the

controversy will only be satisfactorily resolved with better toxicity and pharmacokinetic data for chronic low level mercury exposure from amalgams.

People who are concerned that their mercury exposure may be causing adverse effects can be tested for allergies to mercury or to other metals, or for the amount of mercury in their body. Tests that measure the amounts of mercury in hair and urine are available and provide some indication of the potential for adverse effects from mercury. For more information about the tests that are available, see Section 2.7, Biomarkers of Exposure and Effects.

The following studies supporting or refuting the adverse health effects from exposure to dental amalgam provide some examples from the recent literature of effects being evaluated and the procedures that are being used. Some of the studies depend upon the self-reporting of symptoms or may be weakly blinded (i.e., the patients were not completely unaware of the assignment to different exposure groups) which could bias the outcome, especially with respect to some of the end points. An exhaustive analysis of the results presented below, however, is beyond the scope of this profile, and the reader is referred to the cited references for a more complete discussion of the issues concerning the potential adverse effects from exposure to dental amalgam.

Studies reporting no association between adverse effects and mercury amalgam.

Berglund and Molin (1996) evaluated whether a group of patients with symptoms, self-related to their amalgam restorations, experienced an exposure to mercury vapor from their amalgam restorations that reached the range at which subtle symptoms have been reported in the literature. They further evaluated whether the mercury exposure for these patients was significantly higher than for controls with no reported health complaints. The symptom group consisted of 10 consecutively selected patients from a larger group. The larger group consisted of patients who were referred by their physicians for an investigation of a correlation between subjective symptoms and amalgam restorations. The control group consisted of 8 persons with no reported health complaints. The intra-oral release of mercury vapor was measured between 7:45 a.m. and 9:00 p.m. at intervals of 30–45 minutes, following a standardized schedule. The mercury levels in plasma, erythrocytes, and urine were also determined. The calculated daily uptake of inhaled mercury vapor, released from the amalgam restorations, was less than 5% of the daily uptake calculated at the lower concentration range given by the WHO (1991), at which subtle symptoms have been found in particularly sensitive individuals. The symptom group had neither a higher estimated daily uptake

of inhaled mercury vapor nor a higher mercury concentration in blood and urine than in the control group. The study provided no scientific support for the belief that the symptoms of the patients examined originated from an enhanced mercury release from their amalgam restorations.

Bagedahl-Strindlund et al. (1997) evaluated Swedish patients with illnesses thought to be causally related to mercury release during dental restorations, and mapped the psychological/psychiatric, odontological, and medical aspects of the patients and their purportedly mercury-induced symptoms. A total of 67 consecutive patients and 64 controls matched for age, sex, and residential area were included in the study. Questionnaires were completed and a semi-structured psychiatric interview performed. The Comprehensive Psychopathological Rating Scale was used to record psychopatholgical symptoms. The Karolinska Scales of Personality (KSP) set was used to assess personality traits. The Toronto Alexithymia Scale and the Schalling-Sifneos Personality Scale were completed. The Whitely Index was used to assess hypochondriacal attitudes. The type and number of amalgam-filled surfaces was determined. The most striking result was the high prevalence of psychiatric disorders (predominantly somatoform disorders) in the patients (89%) compared to the controls (6%). The personality traits differentiating the patients according to the Karolinska Scales of Personality were somatic anxiety, muscular tension, psychasthenia, and low socialization. More patients than controls showed alexithymic traits. The prevalence of diagnosed somatic diseases was higher, but not sufficiently so to explain the large difference in perceived health. The multiple symptoms and signs of distress displayed by the patients could not be explained either by the odontological data or by the medical examination. These data indicate that the patients show sociodemographic and clinical patterns similar to those of somatizing patients. The number of amalgam-filled surfaces did not differ significantly between patients and controls; 19% of the patients lacked amalgam fillings.

Grandjean et al. (1997a) evaluated the effects of chelation therapy versus a placebo on patient improvement for patients who attribute their environmental illness to mercury from amalgam fillings. Succimer (meso-2, 3-dimercaptosuccinic acid) was given at a daily dose of 30 mg/kg for 5 days in a double-blind, randomized placebo-controlled trial. Treatment of patients who attribute their environmental illness to mercury from amalgam fillings is largely experimental. On the Symptom Check List, overall distress, and somatization, obsessive-compulsive, depression, and anxiety symptom dimensions, were increased in 50 consecutive patients examined, and Eysenck Personality Questionnaire scores suggested less extroversion and increased degree of emotional lability. Urinary excretion of mercury and lead was considerably increased in the patients who received the chelator. Immediately after the treatment and 5–6 weeks later, most distress

dimensions had improved considerably, but there was no difference between the succimer and placebo groups. These findings suggest that some patients with environmental illness may substantially benefit from placebo.

Stoz et al. (1995) studied 185 mothers with tooth amalgam filling surfaces ranging from 0 to 780 mm^2 and found no relationship between the blood values of the women and their children and the size of the surfaces of the amalgam fillings. All mothers gave birth to healthy children. Malt et al. (1997) evaluated the physical and mental symptomatology of 99 self-referred adult patients complaining of multiple somatic and mental symptoms attributed to dental amalgam fillings. These patients were compared with patients with known chronic medical disorders seen in alternative (n=93) and ordinary (n=99) medical family practices and patients with dental amalgam fillings (n=80) seen in an ordinary dental practice. The assessments included written self-reports, a 131-item somatic symptom checklist, Eysenck Personality Questionnaire, the General Health Questionnaire, and Toronto Alexithymia Scale. Somatic symptom complaints were categorized by exhaustion, and musculoskeletal, cardiovascular, and gastrointestinal effects. The mean number of silver fillings surfaces were 40.96 in self-referrents as compared to 36.61 in the dental practice patients. No correlation between number of dental fillings and symptomatology was found. Self-reports suggested that 62% suffered from chronic anxiety. Forty-seven percent suffered from major depression compared with none in the dental control sample. Symptoms suggesting somatization disorder were found in 29% of the dental amalgam sample compared with only one subject in the 272 comparison subjects; 37.5% of the dental amalgam patients reported symptoms of chronic fatigue syndrome compared with none in the dental control sample and only 2 and 6%, respectively, in the two clinical comparison samples. The dental amalgam group reported higher mean neuroticism and lower lie scores than the comparison groups. The authors concluded that self-referred patients with health complaints attributed to dental amalgam are a heterogeneous group of patients who suffer multiple symptoms and frequently have mental disorders. The authors report a striking similarity with the multiple chemical sensitivity syndrome.

An ad hoc review group of the DHHS Working Group on Dental Amalgam examined 175 literature articles concerning mercury amalgam (DHHS, 1997). The articles represented an assortment of literature from peer-reviewed journals and a variety of other print media. None of the 12 expert reviewers evaluating the articles suggested that any study under review would indicate that individuals with dental amalgam restorations would experience adverse health effects. Many of the reviewed articles were reported to suffer from inadequacy of experimental control, lack of dose-response information, poor measurement of exposure, and a variety of other experimental design inadequacies.

Studies that report an association between dental amalgam and adverse effects.

Echeverria et al. (1995) evaluated the behavioral effects of low-level exposure to Hg among dentists who had either been exposed to mercury or not as measured in a selection procedure where the exposed group was defined as those with urinary mercury levels greater than 19 µg/L. Exposure thresholds for health effects associated with elemental mercury exposure were examined by comparing behavioral test scores of 19 exposed (17 males, 2 females) with those of 20 unexposed dentists (14 males, 6 females). The mean urinary Hg of exposed dentists was 36.4 µg/L, which was 7 times greater than the 5 µg Hg/L mean level measured in a national sample of dentists (urinary Hg was below the level of detection in unexposed dentists for this study). To improve the distinction between recent and cumulative effects, the study also evaluated porphyrin concentrations in urine, which are correlated with renal Hg content (a measure of cumulative body burden). Significant urinary Hg dose-effects were found for poor mental concentration, emotional lability, somatosensory irritation, and mood scores (tension, fatigue, confusion). Individual tests evaluating cognitive and motor function changed in the expected directions but were not significantly associated with urinary Hg. However, the pooled sum of rank scores for combinations of tests within domains were significantly associated with urinary Hg, providing evidence of subtle preclinical changes in behavior associated with Hg exposure. Coproporphyrin, one of three urinary porphyrins altered by mercury exposure, was significantly associated with deficits in digit span and simple reaction time. Exposed dentists placed significantly more amalgams per week (28.0) than unexposed dentists (19.8). No significant differences were found between exposed and unexposed dentists for the overall number of years in practice or the number of amalgams removed per week.

Altmann et al. (1998) compared visual functions in 6-year-old children exposed to lead and mercury levels, in a cohort of 384 children (mean age 6.2 years) living in three different areas of East and West Germany. After adjusting for confounding effects, statistically significant lead-related changes were found only for some of the visually evoked potentials (VEP) interpeak latencies, while some of the contrast sensitivity values were significantly reduced with increasing mercury concentrations. All other outcome variables were not significantly related to lead or mercury levels. The authors concluded that even at blood lead levels in the range of 14–174 µg/L and at very low urinary mercury levels subtle changes in visual system functions can be measured. The geometric means of urinary mercury concentrations were 0.161, 0.203, and 0.075 µg Hg/24 hours for subjects of the three study areas (0.157 µg Hg/24 hours for the total study); the average numbers of amalgam fillings were 0.76, 1.10, and 1.88, respectively (1.15 amalgam fillings for the total study).

Siblerud and Kienholz (1997) investigated whether mercury from silver dental fillings (amalgam) may be an etiological factor in multiple sclerosis (MS). Blood findings were compared between MS subjects who had their amalgams removed (n=50) and MS subjects with amalgams (n=47). All subjects filled out a health survey, an MS health questionnaire, and a psychological profile; the MS amalgam removal group completed a health questionnaire comparing their health before and after amalgam removal. MS subjects with amalgams were found to have significantly lower levels of red blood cells, hemoglobin, and hematocrit compared to MS subjects with amalgam removal. Thyroxine (T-4) levels were also significantly lower in the MS amalgam group, which had significantly lower levels of total T-lymphocytes and T-8 (CD8) suppressor cells. The MS amalgam group had significantly higher blood urea nitrogen (BUN) and BUN/creatinine ratio, and lower serum IgG. Hair mercury was significantly higher in the MS subjects compared to the non-MS control group (2.08 versus 1.32 ppm). A health questionnaire found that MS subjects with amalgams had significantly more (33.7%) exacerbations during the past 12 months compared to the MS volunteers with amalgam removal: 31% of MS subjects felt their MS got better after amalgam removal, 7% felt it was eliminated, 33% felt no change, and 29% believed the condition got worse. In addition, 17% of the MS with amalgam group had more neuromuscular symptoms compared to the amalgam removal group.

Björkman et al. (1997) examined the mercury concentrations in saliva, feces, urine, whole blood, and plasma before and after removal of dental amalgam fillings in 10 human subjects. Before removal, the median mercury concentration in feces was more than 10 times higher than in samples taken from an amalgam-free reference group of 10 individuals. Two days following removal of all amalgams, a considerable increase in mercury appeared in the feces. This initial increase was followed by a significant decrease. In saliva, there was an exponential decline in the mercury concentration during the first 2 weeks after amalgam removal ($t_{1/2}$ of 1.8 days). The authors concluded that while mercury amalgam fillings are a significant source of mercury in saliva and feces, those levels decrease considerably following amalgam removal. Further, the gastrointestinal uptake of mercury seen in conjunction with removal of amalgam fillings appears to be low. Of 108 patients (all with amalgam dental fillings) presenting to an environmental toxicology service, the average salivary mercury level was 11 µg/L (range, <1–19 µg/L) before chewing and 38 µg/L (range, 6–500 µg/L) after chewing. Six of the 108 patients had salivary mercury concentrations >100 µg/L. Of 58 patients with suspected allergic disease, an epicutaneous test for amalgam was positive in 32 of them; however, direct involvement of dental amalgams in these sensitivities was not mentioned. Seventy-five of the total patients presenting with symptoms felt that amalgam fillings

or other dental materials were responsible, at least in part, for their symptoms, although no causal relationship was borne out by medical evaluation.

Bratel et al. (1996) investigated (1) healing of oral lichenoid reactions (OLR) following the selective replacement of restorations of dental amalgam, (2) whether there were differences in healing between contact lesions (CL) and oral lichen planus (OLP), and (3) whether there was a difference in healing potential when different materials were selected as a substitute for dental amalgam. Patients included in the study presented with OLR confined to areas of the oral mucosa in close contact with amalgam restorations (CL; n=142) or with OLR which involved other parts of the oral mucosa as well (OLP; n=19). After examination, restorations of dental amalgam which were in contact with OLR in both patient groups were replaced. The effect of replacement was evaluated at a follow-up after 6–12 months. In the CL group, the lesions showed a considerable improvement or had totally disappeared in 95% of the patients after replacement of the restorations of dental amalgam (n=474). This effect was paralleled by a disappearance of symptoms, in contrast to patients with persisting CL (5%) who did not report any significant improvement. The healing response was not found to correlate with age, gender, smoking habits, subjective dryness of the mouth or current medication. However, the healing effect in patients who received gold crowns was superior than in patients treated with metal-ceramic crowns (MC) ($p<0.05$). In the OLP group (n=19), 63% of the patients with amalgam-associated erosive and atrophic lesions showed an improvement following selective replacement. OLP lesions in sites not in contact with amalgams were not affected. Most of the patients (53%) with OLP reported symptoms also after replacement. From these data the authors conclude that in the vast majority of cases, CL resolves following selective replacement of restorations of dental amalgam, provided that a correct clinical diagnosis is established. The authors note that MC crowns did not facilitate healing of CL to the same extent as gold crowns.

Hultman et al. (1994) studied the effects of dental amalgams in in-bred mice genetically susceptible to mercury-induced immunotoxic effects. Following intraperitoneal implantation of a silver amalgam and observation for up to 6 months, chronic hyperimmunoglobulinemia, serum IgG autoantibodies targeting the nucleolar protein fibrillarin, and systemic immune-complex deposits developed in both a time- and dose-dependent manner. The functional capacity of splenic T- and B-cells was affected in a dose-dependent fashion. In this study, not only did the dental amalgam implantation cause chronic stimulation of the immune system with induction of systemic autoimmunity, but the implantation of silver alloy not containing mercury also induced autoimmunity, suggesting that other metals have the potential to induce autoimmunity in that genetically susceptible strain of mice. Accumulation of heavy metals from dental amalgams, as well

as from other sources, may lower the threshold of an individual metal to elicit immune aberrations, which could lead to overt autoimmunity.

2.6 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate due to maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 5.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both pre-natal and post-natal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns and at various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996).

Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in the newborn who has a low glomerular filtration rate and has not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility while others may decrease susceptibility to the same chemical. For example, the fact that infants breathe more air per kilogram of body weight than adults may be somewhat counterbalanced by their alveoli being less developed, so there is a disproportionately smaller surface area for absorption (NRC 1993).

Adverse health effects from different forms of mercury differ primarily because of differences in kinetics rather than mode of action. As discussed in the introduction to this section, children have different, and sometimes dramatically different, morphology or physiology that alters the way toxic compounds are absorbed and distributed throughout their bodies. For mercury compounds, preventing entry into the systemic circulation is the best means to prevent adverse effects. Once mercury enters the circulation, the tissues that end up as target sites are those that accumulate the most mercuric ion or the ones that are most often exposed to mercuric ion. That is why the kidney is a prime target site, for in fulfilling its major role of filtering and purifying the blood, the kidney is continually exposed to ionic mercury. The central nervous system is a major target site because mercuric ion also concentrates in the brain compartment. Ironically, it may be the blood-brain barrier that contributes to, rather than prevents, mercuric ion "trapping" in the brain. A current hypothesis is that once lipophilic forms of mercury cross the blood-brain barrier, they are oxidized to more hydrophilic species and become trapped inside the brain compartment. This "one way" only kinetic pathway results in continually increasing brain mercuric ion levels, as long as nonpolar forms are in the blood stream. Even small amounts of nonpolar mercury (<2 g) in the body may eventually lead to central nervous system damage (Neirenberg et al. 1998). The low capacity for central nervous system tissues to regenerate and the fact that even subtle damage to small areas of the brain can have profound overall effects, makes this tissue very susceptible to the highly toxic mercuric ion. These factors, and a slow but inevitable trapping of mercuric ions may lead to the mercury-induced delayed central nervous system toxicity observed months to years after exposure ceases (Neirenberg et al. 1998, Rice 1996a). Even potent chelators have not been effective in interfering with progressive central nervous

system damage once a nonpolar mercury compound gains access to the circulatory system and begins to concentrate in tissues (Neirenberg et al. 1998, Taueg et al. 1992).

For similar routes and forms of mercury, the adverse health effects seen in children are similar to the effects seen in adults. For example, a young child who was intoxicated with mercury vapor, died of pulmonary edema and had a grayish, necrotic mucosa of the stomach and duodenum (Campbell 1948). These effects are similar to those seen in adult populations occupationally exposures to inhaled metallic mercury vapors. Respiratory effects in adults from inhalation of metallic mercury vapor include pulmonary edema, lobar pneumonia, fibrosis, desquamation of the bronchiolar epithelium, and death in severe cases due to respiratory failure (Gore and Harding 1987; Jaffe et al. 1983; Kanluen and Gottlieb 1991; Matthes et al. 1958; Taueg et al. 1992; Teng and Brennan 1959; Tennant et al. 1961).

The majority of the information regarding cardiovascular effects comes from reports of children who were treated with mercurous chloride tablets for worms or mercurous chloride-containing powders for teething discomfort (Warkany and Hubbard 1953). These authors described multiple cases in which tachycardia and elevated blood pressure were observed in the affected children.

Electrocardiography in four family members who ate meat from a hog that had consumed seed treated with ethylmercuric chloride showed abnormal heart rhythms (ST segment depression and T wave inversion) (Cinca et al. 1979). Death of the two children in the family was attributed to cardiac arrest, and autopsy of these boys showed myocarditis. Cardiovascular abnormalities were also observed in severe cases of poisoning in the Iraqi epidemic of 1956, when widespread poisoning resulted from eating flour made from seed grains treated with ethylmercury *p*-toluene sulfonanilide (Jalili and Abbasi 1961). These abnormalities included irregular pulse, occasionally with bradycardia, and electrocardiograms showing ventricular ectopic beats, prolongation of the Q-T interval, depression of the S-T segment, and T inversion.

Several children who were treated with mercurous chloride for constipation, worms, or teething discomfort had swollen red gums, excessive salivation, anorexia, diarrhea, and/or abdominal pain (Warkany and Hubbard 1953). They also experienced muscle twitching or cramping in the legs and/or arms, but these muscular effects were probably secondary to changes in electrolyte balance (i.e., potassium imbalance due to fluid loss or renal wasting).

Acute renal failure that persisted for 10 days was observed in a 19-month-old child who ingested an unknown amount of powdered mercuric chloride (Samuels et al. 1982). Several children who were treated with medications containing mercurous chloride for constipation, worms, or teething discomfort exhibited flushing of the palms of the hands and soles of the feet (Warkany and Hubbard 1953). The flushing was frequently accompanied by itching, swelling, and desquamation of these areas. Morbilliform rashes, conjunctivitis, and excessive perspiration were also frequently observed in the affected children. Patch tests conducted in several children revealed that the rashes were not allergic reactions to the mercury. They also had irritability, fretfulness, sleeplessness, weakness, photophobia, muscle twitching, hyperactive or hypoactive tendon reflexes, and/or confusion.

A 13-month-old child who ingested porridge made from flour that had been treated with an alkyl mercury compound (specific mercury compound not reported) developed a measles-like rash, fever, and facial flushing (Engleson and Herner 1952). A 4-year-old boy who had been given a Chinese medicine containing mercurous chloride for 3 months developed drooling, dysphagia, irregular arm movements, and impaired gait (Kang-Yum and Oransky 1992). A number of children who were treated with an ammoniated mercury ointment or whose diapers had been rinsed in a mercuric chloride solution experienced tachycardia and elevated blood pressure, and anorexia (Warkany and Hubbard 1953).

In addition, rashes, conjunctivitis, and/or excessive perspiration were observed. These dermal and ocular reactions were not attributed to allergic-type reactions to the mercury. A 23-month-old boy who was exposed to an unspecified form of mercury also developed a "diffuse, pinpoint, erythematous, papular rash" and bright red finger tips "with large sheets of peeling skin" (Tunnessen et al. 1987).

A woman chronically exposed to an undetermined concentration of mercury vapor reported that her first pregnancy resulted in spontaneous abortion, and her second resulted in the death of the newborn soon after birth (Derobert and Tara 1950). It is unclear whether the reproductive toxicity experienced by the woman was due to the mercury exposure. However, after recovery from overt mercury poisoning, she gave birth to a healthy child. Not all exposures lead to immediate adverse effects. A woman occupationally exposed to mercury vapors for 2 years prior to pregnancy and throughout pregnancy was reported to have delivered a viable infant at term (Melkonian and Baker 1988). Urinary mercury in the woman at 15 weeks of pregnancy was 0.875 mg/L (normal levels are approximately 0.004 mg/L). A case report of a woman exposed to mercury vapors in her home during the first 17 weeks of pregnancy reported that the woman delivered a normal child who met all developmental milestones (although the child was not formally tested

for psychological development) (Thorpe et al. 1992). Mercury exposure was not measured, but the child was born with hair levels of 3 mg/kg (3 ppm) of mercury. This hair level was comparable to that observed in populations consuming fish once a week (WHO 1990) and suggests that exposure in this case may have been relatively low.

In the *in vivo* study by Sager et al. (1982), it was concluded that methylmercury may be acting on mitotic spindle microtubules leading to cell injury in the developing cerebellar cortex. Cell injury observed in the external granular layer of the cerebellar cortex of 2-day-old rats was attributed to a reduced percentage of late mitotic figures (arrested cell division) due to the loss of spindle microtubules. Mitosis and migration of granule cells in the cerebellum end within weeks following birth; therefore, this observation may suggest potential differences in the sensitivities of children and adults to mercury-induced neurotoxicity.

Regardless of whether mercury exposure is through inhalation of mercury vapors, ingestion of organic mercury or mercury salts, or dermal application of mercury-containing ointments, patients (primarily children) may exhibit a syndrome known as acrodynia, or pink disease. Acrodynia is often characterized by severe leg cramps; irritability; and erythema and subsequent peeling of the hands, nose, and soles of the feet. Itching, swelling, fever, tachycardia, elevated blood pressure, excessive salivation or perspiration, morbilliform rashes, fretfulness, sleeplessness, and/or weakness may also be present. It was formerly thought that this syndrome occurred exclusively in children, but recent reported cases in teenagers and adults have shown that these groups are also susceptible.

Developmental effects from prenatal or postnatal exposures to mercury are unique to children. During critical periods of structural and functional development in both prenatal and postnatal life, children are especially vulnerable to the toxic effects of mercury. Inhalation exposures are relatively rare outside of the occupational setting so the exposure route and form of mercury most commonly associated with a risk for development effects is the ingestion of methylmercury on the surface of contaminated foods (methylmercury used as a fungicide on seed grain) or accumulated within the food (methylmercury in fish, wild game, and marine mammals). The exposure route and form of mercury most commonly associated with maternal exposures is to foods contaminated with methylmercury fungicides (Bakir et al. 1973) or foods that contain high levels of methylmercury (Grandjean et al. 1997b, 1998; Tsubaki and Takahashi 1986).

The first such incident was reported in Sweden in 1952 when flour from grain treated with an unspecified alkyl mercury compound ingested by a pregnant woman was associated with developmental toxicity. An

apparently normal infant was born, but the infant later displayed brain damage manifested by mental retardation, incoordination, and inability to move (Engleson and Herner 1952). A 40-year-old woman, 3 months pregnant, consumed methylmercury-contaminated meat for an unspecified duration and subsequently delivered a male infant with elevated urinary mercury levels (Snyder and Seelinger 1976). At 3 months, the infant was hypotonic, irritable, and exhibited myoclonic seizures. At 6 years of age, the child displayed severe neurological impairment (e.g., blindness, myoclonic seizures, neuromuscular weakness, inability to speak) (Snyder and Seelinger 1976).

Another incidence of neurodevelopmental effects occurring as a result of *in utero* exposure to methylmercury was reported by Cox et al. (1989) and WHO (1990). The effect of concern was the delayed onset of walking in offspring in Iraqi children whose mothers were exposed to methylmercury through the consumption of seed grain treated with methylmercury as a fungicide (Al-Mufti et al. 1976; Bakir et al. 1973; Cox et al. 1989; Marsh et al. 1981, 1987).

A New Mexico family, including a pregnant woman, a 20-year-old female, and 2 children (a 13-year-old male and an 8-year-old female) ate meat from a hog inadvertently fed seed grain treated with a fungicide containing methylmercury and experienced severe, delayed neurological effects (Davis et al. 1994). Several months after the exposures, the children developed symptoms of neurological dysfunction. The newborn child of the exposed mother showed signs of central nervous system disorder from birth. Twenty-two years after the 3-month exposure period, the people who were 20 and 13 years old at time of exposure had developed cortical blindness or constricted visual fields, diminished hand proprioception, choreoathetosis, and attention deficits. MRI examination of these two revealed residual brain damage in the calcarine cortices, parietal cortices, and cerebellum. The brain of the person who was exposed at age 8 (who died of aspiration pneumonia with a superimposed *Klebsiella* bronchopneumonia and sepsis at age 29) showed cortical atrophy, neuronal loss, and gliosis, most pronounced in the paracentral and parieto-occipital regions. Regional brain mercury levels correlated with the extent of brain damage. The youngest (*in utero* at the time of exposure) developed quadriplegia, blindness, severe mental retardation, choreoathetosis, and seizures, and died at age 21. Since inorganic mercury crosses the blood-brain barrier poorly, biotransformation of the methylmercury to inorganic mercury may have occurred after the methylmercury crossed the blood-brain barrier, accounting for its observed persistence in the brain and its possible contribution to the brain damage.

More recently, Grandjean et al. (1997b, 1998) evaluated a cohort of 1,022 consecutive singleton births generated during 1986–1987 in the Faroe Islands. Increased methylmercury exposure from maternal consumption of pilot whale meat was indicated by mercury concentrations in cord blood and maternal hair. Neurophysiological tests emphasized motor coordination, perceptual-motor performance, and visual acuity; pattern reversal visual evoked potentials (VEP) with binocular full-field stimulation, brain stem auditory evoked potentials (BAEP), postural sway, and the coefficient of variation for R-R interpeak intervals (CVRR) on the electrocardiogram were measured. Clinical examination and neurophysiological testing did not reveal any clear-cut mercury-related abnormalities. However, mercury-related neuropsychological dysfunctions were most pronounced in the domains of language, attention, and memory, and to a lesser extent in visuospatial and motor functions. These associations remained after adjustment for covariates and after exclusion of children of mothers with maternal hair mercury concentrations above 10 μg/g (50 nmol/g). The effects on brain function associated with prenatal methylmercury exposure appear widespread, and early dysfunction is detectable at exposure levels currently considered safe.

There are differences in the outcomes of these epidemiology studies on low level chronic exposures to methylmercury in foods. Davidson et al. (1998) report no adverse developmental effects associated with prenatal and postnatal exposure to methylmercury in fish in a Seychelles Island cohort of children at age 66 months (n=708). The exposure levels are reflected in maternal hair levels of 6.8 ppm for the prenatal exposure (SD=4.5, n=711) and children's hair levels of 6.5 ppm (SD=3.3, n=708) for both the prenatal and subsequent postnatal exposure. The age-appropriate main outcome measures included: (1) the McCarthy Scales of Children's Abilities, (2) the Preschool Language Scale, (3) the Woodcock-Johnson Tests of Achievement - Letter and Word Recognition, (4) Woodcock-Johnson Tests of Achievement - Applied Problems and, (5) the Bender Gestalt test, and (6) the Child Behavior Checklist. The test results were similar to what would be expected from a healthy, well-developing U.S. population. No test indicated a deleterious effect of methylmercury from the exposure levels received in this population. Four of the six measures showed better scores in the highest MeHg groups compared with lower groups for both prenatal and postnatal exposure. This result is likely due to the benefits of increased levels of fish in the diet, possibly because of increased consumption of omega-3-fatty acids. Serum from a subset of 49 of the children was sampled for polychlorinated biphenyl levels (PCBs). None of the samples had detectable levels (detection limit 0.2 ng/mL) for any of the 28 congeners assayed (from congener 28 to 206) indicating that was no concurrent (i.e, potentially confounding) exposure to PCBs in this population. The median level of total mercury for each of 25 species sampled was 0.004–0.75 ppm, with most medians in the range of 0.05–0.25 ppm, levels that are comparable to fish in the U.S. market. The authors conclude that this

most recent NOAEL of 6.8 ppm for the Seychelles cohort at 66 months of age strongly supports the findings at earlier ages, and that the benefits of eating fish outweigh the small risk of adverse effects from an increased exposure to methylmercury for this exposure pathway.

The differences in these studies highlight the importance of interpreting epidemiology results and, indeed, all study results on mercury toxicity within a fairly comprehensive context of the numerous factors that might affect the toxicokinetics and the amount absorbed (e.g., form of mercury, route of exposure, age, diet of population exposed, health status, other potential sources of exposure to mercury, dose duration, constancy of dose amount over time, etc.)

A route of exposure unique to children is breast milk. Both organic and inorganic mercury can move into breast milk from a nursing woman's body, and children will readily absorb this mercury. Oskarsson et al. (1996) assessed the total and inorganic mercury content in breast milk and blood in relation to fish consumption and amalgam fillings (an exposure source for older children). Total mercury concentrations were evaluated in breast milk, blood, and hair samples collected 6 weeks after delivery from 30 lactating Swedish women. In breast milk, about half of the total mercury was inorganic and half was methylmercury, whereas in blood only 26% was inorganic and 74% was methylmercury. That is because, unlike the placental barrier, which is crossed more easily by methylmercury than by inorganic mercury, inorganic mercury moves more easily into breast milk. Some researchers think that a carrier mediated process is involved (Sundberg et al 1998).

For the Swedish population in the study, Oskarsson et al. (1996) reports that there was an efficient transfer of inorganic mercury from blood to breast milk and that mercury from amalgam fillings was probably the main source of mercury in breast milk, while methylmercury levels in blood did not appear to be efficiently transferred to breast milk . Exposure of the infant to mercury in breast milk was calculated to range up to 0.3 μg/kg/day of which approximately one-half was inorganic mercury. This exposure corresponds to approximately one-half the tolerable daily intake of total mercury for adults recommended by the World Health organization. The authors concluded that efforts should be made to decrease total mercury burden in women of reproductive age Oskarsson et al. (1996).

The metabolism of mercury is relatively straightforward compared, for example, to pesticides or some organic solvents. No information was identified to indicate that metabolic pathways are different for children and adults, or that children have unique metabolites. Once absorbed, metallic and inorganic

mercury enter an oxidation-reduction cycle. Metallic mercury is oxidized to the divalent inorganic cation in the red blood cells and lungs of humans and animals. Evidence from animal studies suggests the liver as an additional site of oxidation. Absorbed divalent cation from exposure to mercuric mercury compounds can, in turn, be reduced to the metallic or monovalent form and released as exhaled metallic mercury vapor. In the presence of protein sulfhydryl groups, mercurous mercury (Hg^{+}) disproportionates to one divalent cation (Hg^{+2}) and one molecule at the zero oxidation state (Hg^{0}). The conversion of methylmercury or phenylmercury into divalent inorganic mercury can probably occur soon after absorption, also feeding into the oxidation-reduction pathway.

A number of good physiologically based pharmacokinetic models are currently available for mercury, including some that address developmental toxicity and maternal/fetal transfer. Two models were constructed based upon data from the kinetics of methylmercury in rats. Farris et al. (1993) developed a PBPK model that simulates the long-term disposition of methylmercury and its primary biotransformation product, mercuric mercury, in the male Sprague-Dawley rat following a single oral nontoxic exposure. Gray (1995) developed a PBPK model that simulates the kinetics of methylmercury in the pregnant rat and fetus. The Gray model was developed to provide fetal and maternal organ methylmercury concentration-time profiles for any maternal dosing regimen. Sundberg et al. (1998) fitted a three compartment model to the elimination kinetics of methylmercury and inorganic mercury transfer to milk in lactating and nonlactating mice. Luecke et al. (1997) developed a model based on human physiology but extended to simulate animal data that depict internal disposition of two chemicals (singly or in combination) during pregnancy in the mother and the embryo/fetus. Leroux et al. (1996) developed a biologically based-dose-response model to describe the dynamics of organogenesis, based on the branching process models of cell kinetics. Gearhart et al. (1995) developed a PBPK model to coherently describe methylmercury pharmacokinetics in a variety of species (adult rat, monkey, and human), and to predict fetal levels of methylmercury from an i*n utero* exposure.

No information was identified on biomarkers of exposure for children. Mercury levels in hair, urine, and blood are the standard measures of exposure. There are biomarkers for developmental effects that are unique to specific ages and stages of development throughout the child's developmental process. Developing the best measures for evaluation of cognitive functions is an area of intense debate and on-going research.

Concerning interactions with other chemicals, there is an ongoing debate about the value of fish in the diet versus the risk from increased exposure to methylmercury that may be in the fish. One recent study reported a beneficial effect from increased fish consumption even though mercury body burdens were increased to some extent (Davidson et al. 1998). One possible factor in the fish that could improve health is omega 3-fatty acid. Children and adults both benefit from a healthy diet, but there may more emphasis on the benefits to growing children. Other interactions for mercury include the effect of various substances on its gastrointestinal absorption (e.g., iron, zinc) or possibly protective effects from prevention or repair of mercury related oxidative damage (e.g., interactions with selenium as an antioxidant). No information was identified that specifically addresses differences in these interactions for children compared to adults.

The methods used to reduce peak absorption and to reduce body burdens in exposed adults (i.e., chelation therapy) are also used for exposures in children.

No information was identified on parental exposures affecting children in areas of parental germ cells or germ line mutations. The topic of exposure pathways for mercury via nursing or pregnant women who have been exposed is of main concern and has been addressed earlier in this section.

2.7 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biological systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s), that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biological half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed

to hazardous substances that are commonly found in body tissues and fluids (essential mineral nutrients [e.g., copper, zinc, and selenium]). Biomarkers of exposure to mercury are discussed in Section 2.7.1.

Biomarkers of effect are defined as any measurable biochemical, physiological, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathological changes in female genital epithelial cells), as well as physiological signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but they can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by mercury are discussed in Section 2.7.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a pre-existing disease that results in an increase in the absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.8 (Populations That Are Unusually Susceptible).

2.7.1 Biomarkers Used to Identify or Quantify Exposure to Mercury

Blood and urine mercury concentrations are commonly used as biomarkers of exposure to mercury. Hair has been used as a biomarker of exposure to methylmercury. Occupational studies show that recent mercury exposure is reflected in blood and urine (Naleway et al. 1991; WHO 1991). However, at low exposure levels (<0.05 mg Hg/m^3), correlation to blood or urine mercury levels is low (Lindstedt et al. 1979). Blood levels of mercury peak sharply during and soon after short-term exposures, indicating that measurements should be made soon after exposure (Cherian et al. 1978). The specific time frame at which measurements become less reliable has not been determined. Workers exposed for a chronic duration, however, may have a high body burden of mercury, therefore, mercury levels would probably still be elevated in the urine and blood for a long period of time after cessation of exposure (Lindstedt et al. 1979). The following discussion of blood and urine mercury levels generally refers to measurements taken immediately or within a few days following the last exposure.

The mean total mercury levels in whole blood and urine of the general population are approximately 1–8 µg/L and 4–5 µg/L, respectively (Gerhardsson and Brune 1989; WHO 1990). Recently, the International Commission on Occupational Health (ICOH) and the International Union of Pure and Applied Chemistry (IUPAC) Commission on Toxicology determined that a mean value of 2 µg/L was the background blood level of mercury in persons who do not eat fish (Nordberg et al. 1992). These blood and urine levels are "background" in the sense that they represent the average levels in blood in the general population and are not associated with a particular source for mercury. However, the intra- and inter-individual differences in these biomarkers are substantial, possibly due to dental amalgams (urine) and ingestion of contaminated fish (blood) (Verschoor et al. 1988; WHO 1991). Long-term consumption of fish is the source of nearly all of the methylmercury measured in the general population, and individuals in communities with high fish consumption rates have been shown to have blood levels of 200 µg/L, with daily intake of 200 µg mercury (WHO 1990). However, acute inhalation exposure to low levels of metallic mercury resulted in much lower levels in the blood (0.028 and 0.18 µg/100 mL) and urine (from 94 to >438 µg/L) (Kanluen and Gottlieb 1991; Rowens et al. 1991).

Urine mercury measurement is reliable and simple, and it provides rapid identification of individuals with elevated mercury levels (Naleway et al. 1991). It is a more appropriate marker of inorganic mercury, because organic mercury represents only a small fraction of urinary mercury. Yoshida (1985) found that urinary mercury levels were better correlated with exposure than were blood inorganic mercury concentrations in workers exposed to metallic mercury vapor.

Several studies have reported a correlation between mercury in blood and urine; however, results vary, and it is not known whether the ratio between concentrations in urine and blood remains constant at different exposure levels (Lindstedt et al. 1979; Roels et al. 1987; Smith et al. 1970). Significant correlations between occupational exposure to mercury vapor and mercury levels in the blood and urine of 642 workers in 21 chloralkali facilities were reported by Smith et al. (1970). According to the investigators, an air concentration (8-hour TWA) of 0.1 mg/m^3 was associated with blood levels of 6 µg/100 mL and urine levels of 220 (not corrected for specific gravity), 200, or 260 µg/L (corrected to specific gravities of 1.018 or 1.024, respectively). It is likely that current worker exposure is significantly less than this study indicates, because practices such as requiring showers after workshifts and cleaning work clothes after use have been implemented since 1970, when the Smith study was conducted. Another group of investigators, Henderson et al. (1974), found the concentrations reported in Smith et al. (1970) to be 2–10 times higher than those found 2 years later. As suggested by Roels et al. (1982), the actual mercury absorption by

workers exposed to the same air concentration may vary; therefore, researchers should report urine mercury levels together with estimated exposure concentrations to address the issue of variance between individuals.

Studies assessing mercury vapor exposure have suggested various ratios relating the concentration of mercury in the air (in μg/m^3) to the levels of mercury in the urine (in μg/L). Such estimates include 1:1 (Bell et al. 1973), 1:1.22 (Roels et al. 1987), and 1:2.5 (Lindstedt et al. 1979; Rosenman et al. 1986). Urinary metallic mercury levels ranging from 0.05 to 1.7 μg/L were detected in the urine of workers exposed to mercury vapor (>0.1 mg/m^3); this elemental mercury represented <1% of the inorganic mercury content of the urine (Yoshida and Yamamura 1982). With increased exposure to mercury vapor (0.47–0.67 mg/m^3), the amount of elemental mercury in the urine increased. A "rough" correlation between levels of metallic mercury vapor in air and mercury levels in blood and urine was established by Rosenman et al. (1986). They associated levels of 50 μg/100 mL in blood and 250 μg/L in urine with a mercury level in air of approximately 0.1 mg/m^3 (8-hour TWA), and 28 μg/100 mL in blood and 100 μg/L in urine with a TWA of 0.05 mg/m^3. Roels et al. (1987) found a correlation between daily mercury vapor exposure and blood or urine mercury levels in 10 workers employed for at least 1 year at an alkaline battery plant. The mercury levels in the air and the pre- or post-workshift levels of blood and urinary mercury correlated well (r=0.79–0.86 [blood] and r=0.70–0.80 [urine]). Based on a ratio of 1:0.045:1.22 (mercury in air:blood mercury:urinary mercury), Roels et al. (1987) concluded that exposure to 0.05 mg/m^3 mercury vapor would result in a blood mercury of 2.26 μg/100 mL and a urinary mercury of 61 μg/g creatinine. This correlation differed from that reported by Rosenman et al. (1986), possibly because fewer subjects were evaluated and determination of mercury vapor concentration by Roels et al. (1987) was based on air sampling collection during 5 consecutive days at 10 different workplaces.

Expired air samples have been considered as possible biomarkers of exposure for mercury. Following inhalation of metallic mercury vapor, some of the mercury may be eliminated in the expired air, but excretion from this pathway is negligible 5–7 days after exposure (Cherian et al. 1978; Hursh et al. 1976). Thus, expired air as a measure of mercury exposure can only be used soon after short-term exposure to mercury vapor. There is no information on the amount of mercury in expired air following long-term exposure to mercury.

Nonoccupational exposure to mercury includes the use of mercury-containing products and consumption of mercury-contaminated food. Urine samples from young women using skin-lightening creams containing

5–10% mercuric ammonium chloride had a mean mercury concentration of 109 µg/L, compared to 6 µg/L for urine samples from women who had discontinued use and to 2 µg/L for women who had never used the creams (Barr et al. 1973). Increased urinary excretion and blood levels of mercury were observed in volunteers who used phenylmercuric borate solutions or lozenges intended for the treatment of mouth or throat infections (Lauwerys et al. 1977). Swedes consuming fish contaminated with 0.3–7 mg Hg/kg (0.3-7 ppm) had blood cell levels of total mercury ranging from 8 to 390 ng/g (Skerfving 1974). Long-term exposure to methylmercury at 4 µg Hg/kg/day was associated with a mercury level in blood cells of approximately 300 ng/g (Skerfving 1974). The steady-state concentration of methylmercury in blood may be related to daily intake in the following equation (Task Group on Metal Accumulation 1973; WHO 1990):

$$C = \frac{f \cdot d}{b \cdot V} \cdot \frac{A_D \cdot A_B \cdot d}{b \cdot V}$$

Where:

C = concentration in blood
f = fraction of the daily intake taken up by the blood
d = daily dietary intake
b = elimination constant
A_D = percent of mercury intake in diet that is absorbed
A_B = percent of the absorbed amount that enters the blood
V = volume of blood in the body

Hair is a biomarker of long-term exposure to methylmercury. Once mercury is incorporated into hair, it remains unchanged (Clarkson et al. 1973; Nielsen and Andersen 1991a, 1991b). A number of studies have examined the level of mercury in hair relative to the amount of fish consumed (see Table 2-10) (Airey 1983b; Haxton et al. 1979; Oskarsson et al. 1990; Sherlock et al. 1982). A fairly strong correlation has been demonstrated by these studies between the amount of fish consumed, the level of mercury in the fish, and the level of mercury in hair. Furthermore, the relationship between hair levels and blood levels has been well studied (see Table 2-9) (Amin Zaki et al. 1976; Den Tonkelaar et al. 1974; Haxton et al. 1979; Kershaw et al. 1980; Phelps et al. 1980; Sherlock et al. 1982; Skerfving 1974; Soria et al. 1992).

A number of studies report that hair mercury levels correlate with total intake levels and with organ-specific levels of mercury. Suzuki et al. (1993) analyzed 46 human autopsies in Tokyo, Japan and reported that hair mercury levels were highly significantly correlated with organ Hg levels in the cerebrum, cerebellum, heart, spleen, liver, kidney cortex, and kidney medulla, when the total mercury or methyl mercury value in the organ was compared with the hair total mercury or organic mercury, respectively.

When the inorganic mercury value was tested, significant correlations remained, with weaker coefficients in all the organs but the spleen. Stepwise multiple regression analysis indicated that hair organic mercury value was the major correlating variable for the organ total mercury or organ methyl mercury value in all the organs. With respect to the organ inorganic mercury value, the hair organic mercury value was the major correlate for the cerebrum and kidney (both cortex and medulla), the hair inorganic mercury value was the major variable for the cerebellum and heart, and the hair phosphorous and hair organic mercury were the major variables for the liver. No explanatory variable existed for the spleen. Auxiliary correlating variables accounted for the organ total mercury and inorganic mercury levels, among which the hair selenium value was conspicuous and with negative regression coefficients.

Nakagawa (1995) analyzed total mercury in hair samples from 365 volunteers in Tokyo, and reported higher mercury levels in those who preferred fish in their diet, compared to those who preferred other foods (preference choices were fish, fish and meat, meat, and vegetables). The mean hair mercury levels were 4 ppm in men who preferred fish and 2.7 ppm in women who preferred fish. The lowest hair mercury levels were seen in men and women who preferred vegetables, 2.27 and 1.31 ppm, respectively. The mean hair level for the whole group was 2.23 ppm (median 1.98).

Drasch et al. (1997) assayed tissue samples of 150 human cadavers (75 males, 75 females) from a "normal" European (German) population, i.e., there were no occupational or higher than average exposures to metals found in any of the biographies of the deceased. The objective was to evaluate the validity of blood, urine, hair, and muscle as biomarkers for internal burdens of mercury, lead, and cadmium in the general population. All individuals died suddenly and not as a result of chronic ailments. Age ranged from 16 to 93 years, and every decade was represented by approximately 10 males and 10 females. Tissues sampled included kidney cortex, liver, cerebral cortex, cerebellum, petrous portion of the temporal bone, (pars petrosis ossis temporalis), pelvic bone (spina iliaca anterior-superior), muscle (musculus gluteus), blood (heart blood), urine, and hair (scalp-hair). Statistically significant rank correlations between biomarker levels and tissues were observed but with large confidence intervals for the regressions. The authors conclude that specific biomarkers relative to each metal are useful in estimating body burdens and trends in groups, but are not useful for determining the body burden (and therefore the health risks) in individuals. A notable exception was, that in comparison to a generally poor correlation of cadmium, lead, and mercury between hair and tissue, there was a strong correlation between mercury in hair and mercury in brain (cerebrum and cerebellum). The authors state that this may be due to the high lipophilicity of elemental and short-chain alkyl mercury compounds. As seen in other studies comparing European to

Japanese hair mercury levels, the hair levels reported by Nakagawa (1995) of 2–4 ppm for a Japanese population are 10–20 times higher than levels observed in the Drasch et al. (1997) study (median, 0.247 μg/g in hair; range, 0.43-2.5 μg/g).

Other studies have confirmed a good correlation between hair mercury and brain mercury levels. In a study on the Seychelles Islands cohort, Cernichiari et al. (1995b) compared maternal hair levels, maternal blood levels, fetal blood levels, and fetal brain levels. Autopsy brains were obtained from infants dying from a variety of causes. The concentrations of total mercury in six major regions of the brain were highly correlated with maternal hair levels. This correlation was confirmed by a sequence of comparisons among the four measures. Maternal hair correlated to maternal blood (r=0.82) and infant brain level (r=0.6–0.8). Maternal blood correlated to infant blood (r=0.65); and infant blood correlated to infant brain (r=0.4–0.8).

There are potential confounding factors and other factors to consider when assessing mercury exposure based upon mercury hair levels. Mercury may be deposited to hair from the air when significant sources of mercury are present in the air or when certain hair treatments are used (Hac and Krechniak 1993; WHO 1991). Potential sources of external mercury exposure should, therefore, be evaluated as part of an exposure assessment. Some studies also report a sex related difference in mercury tissue levels. Nielson et al. (1994) observed a significant sex-related differences in the toxicokinetics of methylmercury in mice following administration of a single radiolabeled dose. Drasch et al. (1997) reported that mercury levels in all tissues assayed in their human cadaver study had higher levels compared to male tissues. The difference was significant for the kidney (median female kidney mercury level=92.0 ng/g, males=40.8 ng/g; p=0.002). In blood and urine there was a similar trend. In contrast, the authors report that mercury hair levels in females were significantly lower than in males (median females=205 ng/g, males 285 ng/g; p=0.02). Nakagawa (1995) also report higher mean mercury hair levels in males (2.98 μg/g) compared with females (2.02 μg/g) in a Japanese population. Further research is, therefore, needed to characterize potential sex related difference in the toxicokinetics of mercury under different exposure scenarios.

Eide and Wesenberg (1993) studied mercury concentrations in various organs and tissues in rats exposed to mercury vapor for approximately 2 months and proposed that human deciduous teeth may be useful indicators of chronic mercury exposure, as well as indicators of mercury uptake in organs such as the kidneys and the brain.

Other potential biomarkers of exposure include renal dysfunction parameters, neurological effects, and increased urinary porphyrins, and are discussed below in Section 2.7.2.

2.7.2 Biomarkers Used to Characterize Effects Caused by Mercury

Several potential biomarkers of effect for mercury have been evaluated, usually for neurological and renal dysfunction. Many of these toxic effects have been correlated with blood and urine levels (see Table 2-13). However, most indicators are nonspecific and may have resulted from other influences. As discussed in Section 2.2, many studies have examined the relationship between urine mercury levels and specific renal and neurological effects. Renal dysfunction has been studied extensively as a potential sensitive measure of mercury exposure. Signs of renal dysfunction at mercury air concentration of 0.1 mg/m^3 were reported by Stewart et al. (1977). Case reports have associated the therapeutic use of inorganic mercury salts with the occurrence of nephrotic syndrome (Kazantzis et al. 1962).

Several different biomarkers have been evaluated for assessing renal damage; however, renal parameters are interdependent (Verschoor et al. 1988). Furthermore, these markers are not specific for mercury exposure and may be a consequence of other concurrent chemical exposures. Markers for renal toxicity may indicate decreased function, cytotoxicity, or biochemical changes (Cardenas et al. 1993). Biomarkers for decreased function include increases in urinary proteins and elevation of serum creatinine or β_2-microglobulin. Biomarkers for renal cytotoxicity include increases in urinary excretion of antigens and enzymes located within renal tissues. Biomarkers for biochemical changes occurring within the kidneys include eicosanoids, fibronectin, kallikrein activity, and glycosaminoglycans in urine. Glomerular changes resulting from mercury exposure have predominantly been reported as increases in high-molecular weight proteinuria (Buchet et al. 1980; Kazantzis et al. 1962; Stonard et al. 1983; Tubbs et al. 1982). Renal tubular changes in workers exposed to mercury include increased urinary excretion of *N*-acetyl-β-D-glucosaminidase (NAG), β-galactosidase, and retinol binding protein (Barregard et al. 1988; Langworth et al. 1992b; Rosenman et al. 1986). Elevated urinary NAG levels occurred with urinary mercury levels of 100–250 µg/L in a study population of mixed ethnic background (Rosenman et al. 1986), with urinary levels of 35 µg/g creatinine in chloralkali workers (Barregard et al. 1988), with urinary mercury levels >25 µg/g creatinine in chloralkali workers (Langworth et al. 1992b), and with urinary mercury levels >50 µg/g creatinine in another group of chloralkali workers (Cardenas et al. 1993). NAG levels were not affected in chloralkali workers with urinary mercury levels of 15 µg/g creatinine (Piikivi and Ruokonen 1989). No significant increase of proteinuria, albuminuria, and other indicators of renal

Table 2-13. Health Effects Associated with Mercury Levels in Human Blood and Urine

Parameter	Normal levels found in tissue	Observed effect levels	Effect	Reference
Blood (whole)	<0.5–2 µ/100 mL			Iyengar et al. 1978
		<1–>10 µg/100 mL	Increased tremors	Verbeck et al. 1986
		>1.5 µg/100 mL	Disturbances in tests on verbal intelligence and memory. No effect level for proteinuria	Piikivi et al. 1984
		1.6 µg/100 mL	No effect level for proteinuria	Lauwreys et al. 1983
		1–2 µg/100 mL	Increased prevalence of abnormal psychomotor scores	Roels et al. 1982
		12 µg/100 mL	Increased tremors. Impaired eye-hand coordination	Smith et al. 1970
		>3 µg/100 mL	(Estimated threshold level): Increased urinary excretion of β-galactosidase and high molecular weight proteins	Buchet et al. 1980
Urine	0.43–11.4			Iyengar et al. 1978
		2–472 µg/g creatinine	Decreased delta-aminolevulinic acid dehydratase and cholinesterase activity. Increased urine coproporphyrin levels	Wade et ad. 1969
		3–272 µg/g creatinine	Increased anti-laminin antibodies (implicated in the etiology of autoimmune glomerulo-nephritis)	Lauwreys et al. 1983
		50–100 µg/g creatinine	Increased tremors. Impaired eye-hand coordination	Smith et al. 1970
		50 µg/g creatinine	(Estimated threshold level): Increased urinary excretion of β-galactosidase and high molecular weight proteins	Buchet et al. 1980

Table 2-13. Health Effects Associated with Mercury Levels in Human Blood and Urine (continued)

Parameter	Normal levels found in tissue	Observed effect levels	Effect	Reference
Urine (cont.)		56 µg/g creatinine	No effect level for proteinuria	Lauwreys et al. 1983
		7–1,101 µg/24 hours	Abnormal memory tests; decreased tibial nerve velocity; increased median nerve latency in both motor and sensory nerves	Vroom and Greer 1972
		0–510 µg/L	Short-term memory loss	Smith et al. 1983
		5–1,000 µg/L	Increased tremor frequency and reaction time; impaired eye-hand coordination	Miller et al. 1975
		<10–>1,000 µg/L	Increased tremors	Verbeck et al. 1986
		20–450 µg/L	Increased motor and sensory nerve latency	Levine et al. 1982
		>56 µg/L	Disturbances in tests on verbal intelligence and memory	Piikivi et al. 1984
		100–250 µg/L	Increased acetyl β-D-glucosaminidase (NAG) enzyme levels in urine	Rosenman et al. 1986
		>200 µg/L	Increased tremors; impaired eye-hand coordination	Williamson et al. 1982
		300–1,400 µg/L	Nephrotic syndrome; albuminuria; hypercholesterolemia	Kazantis et al. 1962

dysfunction was evident in 62 mercury workers with average blood mercury levels of 1.6 µg/100 mL (range, 0.25–7.56 µg/100 mL) and average urine mercury levels of 56 µg/g creatinine (range, 3–272 µg/g creatinine) (Lauwerys et al. 1983). Another renal parameter evaluated is β-microglobulin, which has a normal range of 0.004–0.37 mg/L (Naleway et al. 1991). No statistically significant relationship was found between urinary β-microglobulin levels and elevated urinary mercury concentrations (Ehrenberg et al. 1991; Naleway et al. 1991). Examination of a wide range of biomarkers for renal toxicity in a group of chloralkali workers identified several other changes at low urinary mercury levels (Cardenas et al. 1993). Workers with urinary mercury levels in the range of 5–50 µg/g creatinine showed statistically significant increases in urinary Tamm-Horsfall glycoprotein (localized in the epithelial cells of the convoluted tubules) and decreases in urinary prostaglandins E2 and F2α. In workers with >50 µg/g creatinine, increased NAG, tubular brush border antigens, alkaline phosphatase, thromboxane B2, and glycosaminoglycans were also observed. Urinary porphyrins, which are intermediates in the biosynthesis of heme, may be another potential biomarker of effect for mercury exposure. A correlation was observed between urinary mercury and urinary coproporphyrin (Wada et al. 1969). Correlations were also observed for decreases in δ-aminolevulinic acid-dehydratase and cholinesterase activity with increases in urinary mercury. Porphyrins are considered a nonspecific measure of effect because they are influenced by other metal exposures. Woods et al. (1991) present data suggesting that there is a specific urinary porphyrin profile that may serve as a biomarker of mercury accumulation in the kidneys during prolonged inorganic and organic mercury exposure. A urinary porphyrin pattern, characterized by elevated coproporphyrin, pentacarboxyl porphyrin, and precoproporphyrin, for methylmercury hydroxide exposure was observed in mice for up to 30 weeks. This profile is observed at variable dose levels, as well as up to at least 40 weeks after cessation of exposure. The time course of the profile during prolonged treatment is closely associated with divalent inorganic mercury (Hg^{+2}), suggesting that the effects are mediated by this cation because it inhibits the heme pathway (Woods et al. 1991). Specificity may be a problem unless the porphyrin levels are analyzed at the same time as urinary mercury measurements.

The neurophysiological and neuropsychological health effects of mercury have been extensively studied in occupationally exposed individuals in an effort to monitor body levels and to determine a threshold value below which these effects are unlikely to occur. As with other biomarkers of effect, neurological changes induced by mercury may resemble exposure to other chemicals that can cause damage to the brain.

Case studies have associated exposure to mercury vapor with neurological effects (e.g., tremors, insomnia, shyness, emotional instability, decreased motor function and muscle reflexes, headaches, and abnormal

EEGs) (Davis et al. 1974; Jaffe et al. 1983; McFarland and Reigel 1978). Some studies have examined the relationship between nerve function and mercury levels in blood, urine, and tissue. Tissue levels of mercury have also been found to correlate with impaired nerve function. Among 23 dentists with mercury levels greater than 20 µg/g (measured in wrist tissue), 30% exhibited reduced nerve conduction velocity when compared with dentists with tissue levels of mercury below 20 µg/g (Shapiro et al. 1982). The decrease in nerve conduction velocity was observed in both sensory and motor nerves.

A dose-response relationship has also been reported for the association between paresthesia and blood mercury concentrations in an Iraqi population exposed to methylmercury. At a blood mercury level of 24 µg/100 mL 65 days after cessation of exposure, the incidence of paresthesia caused by methylmercury rose significantly (Clarkson et al. 1976). Below this concentration, any incidence of paresthesia was assumed to be related to other causes, according to the investigators. As a result of the reported blood mercury half-life of 65 days in this population, the maximum blood mercury concentration was likely to have been 48 µg/100 mL at the end of the exposure. Some evidence of paresthesia, sensory impairment, general ataxia, and visual field effects in exposed Swedes was reported; however, no significant increases in occurrence were found in Swedes with high levels of mercury in blood cells (82–1,100 ng/g) as compared to Swedes with lower blood cell mercury levels (12–75 ng/g) (Skerfving 1974). The study did not include a matched control group.

Many possible biomarkers of effect for mercury exposure have been correlated with urinary mercury levels. Workers exposed to elemental mercury vapor with urinary mercury excretion levels ranging from 7 to 1,101 µg/day exhibited significantly reduced tibial nerve velocity and increased median nerve latency in both motor and sensory nerves as compared with controls (Vroom and Greer 1972). Prolonged motor and sensory nerve latency was also associated with urine mercury levels ranging from 20 to 450 µg/L in 18 male workers exposed to elemental mercury vapor at a mercury cell chlorine plant (Levine et al. 1982). Urine mercury levels exceeding 200 µg/L have been reported to be associated with tremors and poor eye-hand coordination (Williamson et al. 1982). Twelve workers chronically exposed to elemental mercury vapor had urinary mercury levels ranging from <10 to 670 µg/L. A significant relationship between urine mercury and hand steadiness was reported. Increased tremor frequency, increased reaction time, and reduced eye-hand coordination were observed as urine mercury levels increased from 5 to 1,000 µg/L in 77 exposed individuals (Miller et al. 1975). A weak but significant quantitative relationship between urine mercury levels and finger tremors was elucidated by Verberk et al. (1986). The relationship between acceleration of finger tremors and excretion of mercury in the urine of 20 workers exposed to metallic

mercury was expressed by the equation 10 log (acceleration)=G0.888 + 0.0059 (urine mercury) (r=0.39, p<0.05, n=20). Tremors have also been reported in 567 workers from chloralkali production facilities whose blood mercury levels ranged from <1 to >10 µg/100 mL and whose urine mercury levels ranged from <10 to >1,000 µg/L. Increased tremors and reduced eye-hand coordination were associated with blood mercury levels of 1–2 µg/100 mL and urine mercury levels of 50–100 µg/g creatinine (Smith et al. 1970). Cavalleri et al. (1995) have suggested that exposure to elemental mercury vapors at levels producing urine mercury concentrations >50 µg/g creatinine can cause a dose-related loss of color vision.

An association between urine mercury levels and performance on memory tests and verbal intelligence tests has been established. Abnormal results on memory tests were reported for 9 workers exposed to mercury in the production of thermometers; urinary mercury excretion levels were 7–1,101 µg/24 hours (Vroom and Greer 1972). The short-term memory span of 26 workers was examined by Smith et al. (1983) and found to decrease with increasing urine mercury levels. The range of mercury found in the urine of these workers was 0–510 µg/L. A significant linear relationship was reported between subjects' 50% memory threshold spans and 12-month urinary mercury concentrations. Disturbances on tests of verbal intelligence and memory were more frequent among individuals with mercury blood levels above 1.5 µg/100 mL and mercury urine levels above 56 µg/L in 36 male chloralkali workers (Piikivi et al. 1984).

Potential biomarkers for the autoimmune effects of mercury include measurement of antiglomerular basement membrane antibodies, anti-DNA antibodies, serum IgE complexes, and total IgE (Cardenas et al. 1993). Elevated IgE, antiglomerular basement membrane antibodies, and anti-DNA antibodies have been observed in a few persons with exposure to mercury from dental amalgams (Anneroth et al. 1992). Other individuals have also been shown to have elevated anti-DNA or antiglomerular basement membrane antibodies (Cardenas et al. 1993; Langworth et al. 1992b).

Recent data regarding the action of low-level mercury exposure on receptors and signal transduction pathways in peripheral lymphocytes suggest potential applications of certain surrogate markers in mechanistic studies of neurotoxicity and, possibly, in assessing early biochemical effects of neurotoxicants in humans (Manzo et al. 1995). Additional biomarkers for effects on the immune, renal, hepatic, and neurological systems are presented in the CDC/ATSDR (1990) and OTA (1990) reports. See Section 2.2 for a more detailed discussion of the effects caused by mercury.