chloride resulted in increased deaths in male mice ($B6C3F_1$ strain) at 0.69 mg Hg/kg/day but no increased mortality in females at up to 0.60 mg Hg/kg/day (Mitsumori et al. 1990).

The highest NOAEL values and all reliable LOAEL values for death for each species and duration category are recorded in Table 2-3 and plotted in Figure 2-3 for organic mercury.

2.2.2.2 Systemic Effects

Ingestion of mercury compounds has been associated with systemic toxicity in both humans and animals. As with inhalation exposure to metallic mercury vapor, the major target organs of toxicity following oral exposure to inorganic and organic mercury are the kidneys and the central nervous system, respectively. Available information is limited mainly to that concerning exposure to mercuric chloride and methyl-mercuric chloride. Oral exposure to mercury, especially the organic mercury form, has also been observed to result in adverse developmental effects in humans and experimental animals. A discussion of the differences in the toxicities of metallic mercury, inorganic compounds, and organic compounds of mercury is presented in Section 2.5. The systemic effects observed after oral exposure are discussed below.

The highest NOAEL values and all reliable LOAEL values for systemic effects for each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2 for inorganic mercury, and recorded in Table 2-3 and plotted in Figure 2-3 for organic mercury.

Respiratory Effects

Inorganic Mercury. Extremely limited information was located regarding respiratory effects in humans after oral exposure to inorganic forms of mercury. A 35-year-old man who swallowed an unknown amount of mercuric chloride had severe pulmonary edema and required artificial ventilation (Murphy et al. 1979). Fine rales were detected in a 19-month-old boy who swallowed powdered mercuric chloride (Samuels et al. 1982). A 50-year-old female who ingested 5 tablets of a Chinese medicine that contained an unspecified amount of mercurous chloride (Kang-Yum and Oransky 1992) experienced shortness of breath.

The only study located regarding respiratory effects in animals after oral exposure to inorganic mercury described forceful and labored breathing, bleeding from the nose, and other unspecified respiratory

difficulties in Long-Evans rats after dietary exposure to 2.2 mg Hg/kg/day as mercuric chloride for 3 months (Goldman and Blackburn 1979).

Organic Mercury. Limited information was located regarding respiratory effects in humans after oral exposure to organic mercury. Two boys who died after eating meat from a hog that had eaten seed treated with ethylmercuric chloride developed bronchopneumonia and edematous alveolitis, and required artificial ventilation (Cinca et al. 1979). Bronchopneumonia was also identified as the cause of death in four adults and one infant who died as the result of methylmercury poisoning in Iraq during 1972 (Al-Saleem and the Clinical Committee on Mercury Poisoning 1976). It is unclear whether these respiratory effects were the result of direct effects on the respiratory system or were secondary to other effects.

The only information located regarding respiratory effects in animals after oral exposure to organic mercury comes from a study in which rats were exposed to methylmercuric chloride in the diet for 2 years (Verschuuren et al. 1976). This study showed no treatment-related histopathological lesions in the lungs of exposed rats at 0.1 mg Hg/kg/day.

Cardiovascular Effects

Inorganic Mercury. Cardiovascular toxicity has been observed following ingestion of mercuric chloride and mercurous chloride in humans. 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. The only information located regarding cardiovascular effects in humans after ingestion of mercuric chloride comes from a case study of a 22-year-old who attempted suicide by ingesting approximately 20 mg Hg/kg as mercuric chloride (Chugh et al. 1978). An electrocardiogram showed no P wave, prolongation of the QRS segment, and a high T wave. The authors suggested that these cardiovascular effects were secondary to severe hyperkalemia.

Exposure of rats to 28 mg Hg/kg/day as mercuric chloride for 180 days in drinking water resulted in an increase in blood pressure, a decrease in cardiac contractility, and no effect on heart rate (Carmignani et al. 1992). The increase in blood pressure was attributed to a vasoconstrictor effect, and the decrease in contractility was attributed to the direct toxic effect of the mercury on the cardiac muscle. Slightly

different results were obtained following 350-day exposure of a different strain of rats to 7 mg Hg/kg/day as mercuric chloride in drinking water (Boscolo et al. 1989; Carmignani et al. 1989). In the chronic study, positive inotropic response, increased blood pressure and cardiac contractility, and decreased baroreceptor reflex sensitivity were observed. The investigators suggested that the mechanism for the cardiac effects in the chronic study involved the release of norepinephrine from presynaptic nerve terminals. Evidence of this release was provided by the fact that mercury administration reduced the cardiovascular response to bretylium (which blocks presynaptic release of the neurotransmitter norepinephrine) but not tyramine (which releases neurotransmitter from nerve terminals).

Organic Mercury. Electrocardiography in four family members who ate meat from a hog that had consumed seed treated with ethylmercuric chloride had 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.

A decrease in heart rate was observed in male rats given 2 gavage doses of 12 mg Hg/kg as methylmercuric chloride (Arito and Takahashi 1991). An increase in systolic blood pressure was observed in male rats after daily oral gavage doses of 0.4 mg Hg/kg/day as methylmercuric chloride for 3–4 weeks (Wakita 1987). This effect began approximately 60 days after initiation of exposure and persisted for at least 9 months. No treatment-related histopathological changes were observed in the hearts of rats exposed to 0.1 mg Hg/kg/day as methylmercuric chloride in the diet for up to 2 years (Verschuuren et al. 1976).

Gastrointestinal Effects

Inorganic Mercury. Ingestion of metallic mercury results in negligible absorption and little effect on the gastrointestinal tract. The two case histories identified are unusual in that the dose levels could be reasonably well quantified. The first case history reported ingestion of 15 mL (204 g) of metallic mercury by a 17-year-old male storekeeper who swallowed mercury from the pendulum of a clock (apparently out of curiosity rather than as a suicide attempt). On admission, and 24 hours later, he was symptom free, and

physical examination was normal. The patient complained of no gastrointestinal symptoms, and was treated with a mild laxative and bedrest (Wright et al. 1980).

In a second and massive incidence of ingestion, a 42-year-old man who had spent much of his life (since the age of 13) repairing instruments that contained mercury, intentionally ingested an estimated 220 mL (about 3,000 g) while repairing a sphygmomanometer (Lin and Lim 1993). Upon admission, the patient presented with significantly elevated mercury blood levels (103 µg/L, normal <10 µg/L) and urine levels (73µg/L, normal <20µg/L). In the previous 2 years he had developed mild hand tremors, forgetfulness, irritability, and fatigue. Only a mild abdominal discomfort and no hepatic complications were observed at admission. The neurological symptoms were attributed to the long occupational exposure to mercury and not to the recent acute exposure. The initial radiological examination showed a conglomeration of mercury globules in the fundus of the stomach and ascending colon, with fine metallic spots dispersed throughout the small intestine. Abdominal ultrasonography was normal. He was treated with immediate gastric lavage and cathartics. He also received D-penicillamine 1 g/day orally for 7 days. Seven days later, there were only spots of metallic mercury in the ascending colon. By 2 weeks, most of the mercury had been excreted in the feces and was measured at a total volume of 220 mL (this number was used to estimate the amount initially ingested). The authors reported that systemic absorption appeared low, based on the return to low levels of mercury in the urine and blood over the 10 days of monitoring following the exposure. A subsequent evaluation 6 months later revealed no further gastrointestinal involvement.

Ingestion of mercuric chloride is highly irritating to the tissues of the gastrointestinal tract. Blisters and ulcers on the lips and tongue and vomiting were observed in a 19-month-old boy who ingested an unknown amount of mercuric chloride powder (Samuels et al. 1982). Similarly ingestion of a lethal dose of mercuric chloride by a 35-year-old man resulted in vomiting, diarrhea, colicky abdominal pain, oropharyngeal pain, and ulceration and hemorrhages throughout the length of the gastrointestinal tract (Murphy et al. 1979). Ingestion by a woman of 30 mg Hg/kg as mercuric chloride resulted in severe abdominal pain, diarrhea, nausea, and vomiting (Afonso and deAlvarez 1960). Another report of an attempted suicide by a 22-year-old reported ulceration of the mouth and throat and bloody vomit after ingestion of approximately 20 mg Hg/kg (Chugh et al. 1978). Because of vomiting, the actual effective dose was unknown.

Reports of ingestion of mercurous chloride have not found similar caustic effects; however, a 50-year-old woman who ingested an unspecified amount of mercurous chloride in a Chinese medicine experienced nausea and vomiting (Kang-Yum and Oransky 1992). 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).

Inflammation and necrosis of the glandular stomach were observed in mice that were given oral doses of 59 mg Hg/kg as mercuric chloride 5 days a week for 2 weeks (NTP 1993). In a 2-year gavage study, an increased incidence of forestomach hyperplasia was observed in male rats exposed to 1.9 or 3.7 mg Hg/kg/day as mercuric chloride compared to the control group.

Organic Mercury. Case studies of individuals who were orally exposed to alkyl mercury compounds (unspecified form) reported diarrhea, tenesmus, irritation, and blisters in the upper gastrointestinal tract (Lundgren and Swensson 1949). Ingestion of meat from a hog that was fed seed treated with ethylmercuric chloride resulted in vomiting in two of the family members (Cinca et al. 1979). No quantitative data were available. Ingestion of flour made from seed grains that had been treated with ethylmercury *p*-toluene sulfonanilide also commonly resulted in abdominal pain and vomiting, diarrhea, or constipation (Jalili and Abbasi 1961).

Pfab et al. (1996) reported a case of a 44-year-old man who ingested 83 mg/kg Thiomersal in a suicide attempt (5 g/60 kg). Thiomersal is a widely used alkyl-aryl-organomercurial bactericide. The man developed gastritis, renal tubular failure, dermatitis, gingivitis, delirium, coma, polyneuropathy, and respiratory failure. Treatment was symptomatic plus gastric lavage and the oral chelation with dimercaptopropane sulfonate and dimercaptosuccinic acid. The patient's condition was at its worst on day 17; however, the patient recovered completely (after several months). Maximum mercury concentrations were: blood, 14 mg/L; serum, 1.7 mg/L; urine, 10.7 mg/L; and cerebrospinal fluid, 0.025 mg/L. Mercury concentration in blood declined with two velocities: first with a half-time of 2.2 days, then with a half-time of 40.5 days. The decline of mercury concentration in blood, urinary mercury excretion, and renal mercury clearance were not substantially influenced by chelation therapy.

Exposure of rats to phenylmercuric acetate for 2 years resulted in necrosis and ulceration of the cecum at doses as low as 4.2 mg Hg/kg/day in drinking water; no effect was observed at 1.7 mg Hg/kg/day in the feed (Fitzhugh et al. 1950; Solecki et al. 1991). Mice showed ulceration of the glandular stomach after 2 years of dietary exposure to methylmercuric chloride at 0.69 mg Hg/kg/day (Mitsumori et al. 1990). In contrast, no treatment-related histopathological lesions in the stomach or jejunum were observed in rats exposed via the diet to 0.1 mg Hg/kg/day as methylmercuric chloride (Verschuuren et al. 1976).

Hematological Effects

Inorganic Mercury. Information is limited regarding hematological effects in humans after ingestion of inorganic mercury. The only information located regarding hematological effects in humans was the report of anemia that developed (probably secondary to massive gastrointestinal hemorrhaging) in a 35-year-old man who ingested a lethal amount of mercuric chloride (Murphy et al. 1979). Bone marrow activity in the afflicted man was normal, but thrombocytopenia was also observed.

Groups of 10 female Sprague-Dawley rats were administered a single gavage dose of mercuric chloride at 7.4 or 9.2 mg Hg/kg in water and necropsied at 14 days postexposure. Blood samples were analyzed for hemoglobin concentration, hematocrit value, erythrocyte counts, total and differential leukocyte counts, and platelet counts. Serum was analyzed for sodium, potassium, inorganic phosphorus, total bilirubin, alkaline phosphatase, aspartate aminotransferase (AST), total protein, calcium, cholesterol, glucose, uric acid, and lactate dehydrogenase (LDH). There were no effects on body weight, and weights of other organs were not affected. Significant decreases in hemoglobin, erythrocytes, and hematocrit were also reported. There was a significant decrease in serum protein and calcium in the low-dose mercury group only. Mercury was found mainly in the kidneys (12.6 and 18.9 ppm at the low and high dose, respectively), but trace amounts were also detected in the liver, brain, and serum (Lecavalier et al. 1994).

No other studies were located regarding hematological effects in animals after oral exposure to inorganic mercury.

Organic Mercury. No studies were located regarding hematological effects in humans after oral exposure to organic mercury.

Rats that received phenylmercuric acetate in their drinking water for 2 years showed decreases in hemoglobin, hematocrit, and red blood cell counts at a dose of 4.2 mg Hg/kg/day (Solecki et al. 1991). The anemia observed in this study may have been secondary to blood loss associated with the ulcerative lesions in the large intestine seen at this dose (see Gastrointestinal Effects above). No treatment-related changes were observed in hematological parameters measured in rats (strain not specified) exposed via the diet for 2 years to 0.1 mg Hg/kg/day as methylmercuric chloride (Verschuuren et al. 1976).

Musculoskeletal Effects

Inorganic Mercury. A single case report was identified that found evidence of skeletal muscle degeneration (markedly elevated serum aldolase, LDH, and creatinine phosphokinase; and the presence of pigment granular casts and myoglobin in the urine) in a 22-year-old man who ingested 2 g of mercuric chloride in an attempt to commit suicide (Chugh et al. 1978). Several children who were treated with mercurous chloride for constipation, worms, or teething discomfort experienced muscle twitching or cramping in the legs and/or arms (Warkany and Hubbard 1953). The muscular effects were probably secondary to changes in electrolyte balance (i.e., potassium imbalance due to fluid loss or renal wasting).

No studies were located regarding musculoskeletal effects in animals after oral exposure to inorganic mercury.

Organic Mercury. Autopsy of one of two boys who died after eating meat from a hog that had consumed seed treated with ethylmercuric chloride showed muscle wasting (Cinca et al. 1979). This effect was probably secondary to neurotoxicity. Electromyography in the two surviving members of the family showed no abnormalities. Musculoskeletal effects observed in Iraqis poisoned by consuming flour made from grains treated with ethylmercury *p*-toluene sulfonanilide included deep skeletal pain and muscle twitching or fasciculations (Jalili and Abbasi 1961). It is likely that these effects were secondary to effects on the nervous system.

No treatment-related histopathological changes in skeletal muscle were observed in rats exposed via the diet for 2 years to 0.1 mg Hg/kg/day as methylmercuric chloride (Verschuuren et al. 1976).

Hepatic Effects

Inorganic Mercury. Limited information was located regarding hepatic effects in humans who ingested inorganic mercury. A 35-year-old man who ingested a lethal dose of mercuric chloride became jaundiced and exhibited elevated AST, alkaline phosphatase, LDH, and bilirubin (Murphy et al. 1979). An autopsy revealed an enlarged and softened liver. Hepatic enlargement was also observed in a 19-month-old boy who ingested an unknown amount of powdered mercuric chloride (Samuels et al. 1982).

Limited information was located regarding the hepatic effects of inorganic mercury in animals.

Groups of 10 female Sprague-Dawley rats were administered a single gavage dose of mercuric chloride at 7.4 or 9.2 mg Hg/kg in water and necropsied at 14 days postexposure. There were no effects on body or relative liver weights from mercuric chloride exposure. LDH activity was significantly decreased in animals exposed to $HgCl_2$ at both dose levels. Mercury was found mainly in the kidneys (12.6 and 18.9 ppm at the low and high dose, respectively), but trace amounts were also detected in the liver, brain, and serum (Lecavalier et al. 1994).

Two intermediate-duration studies in rats showed biochemical changes following ingestion of mercuric chloride (Jonker et al. 1993b; Rana and Boora 1992). Increases in hepatic lipid peroxidation and decreases in glutathione peroxidase were observed in rats orally exposed to an unspecified dose of mercuric chloride for 30 days (Rana and Boora 1992). In a 4-week range-finding study, groups of 5 rats per sex (10 per sex for controls) received diets containing mercuric chloride at 5, 10, or 20 mg Hg/kg/day in males and 5.5, 11.1, and 22.2 mg Hg/kg/day in females. Absolute liver weight decreased starting at the mid-dose group in males and in the high-dose group in females (Jonker et al. 1993b). The liver weight significantly increased in mice given 2.9 mg Hg/kg/day as mercuric chloride in the drinking water for 7 weeks; however, no histopathological changes were observed (Dieter et al. 1983). Male rats administered mercuric chloride by gavage for 2 years showed a slight increase in acute hepatic necrosis (11 of 50 versus 4 of 50 in controls); however, it is unclear whether this increase was statistically significant (NTP 1993).

Organic Mercury. Extremely limited information was also obtained regarding the hepatic effects of organic mercury exposure. An autopsy of four adults and four infants who died as the result of methyl-mercury poisoning in Iraq in 1972 reported fatty changes in the liver occurred in most cases (Al-Saleem and the Clinical Committee on Mercury Poisoning 1976). It is unclear whether these changes were the direct result of methylmercury on the liver or whether they were due to other causes. The prevalence of liver disease in a population from the Minamata area was not significantly increased when compared to unexposed controls (Futatsuka et al. 1992).

No treatment-related changes were observed in hepatic parameters measured in rats exposed via the diet to 0.1 mg Hg/kg/day as methylmercuric chloride (Verschuuren et al. 1976).

Renal Effects

Inorganic Mercury. The kidney appears to be the critical organ of toxicity for the ingestion of mercuric salts. Renal effects in humans have been observed following acute oral exposure to inorganic mercury. Acute renal failure has been observed in a number of case studies of mercuric chloride ingestion (Afonso and deAlvarez 1960; Murphy et al. 1979; Samuels et al. 1982). An 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 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 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 was 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 observed in a 22-year-old who attempted suicide by ingesting approximately 20 mg Hg/kg (Chugh et al. 1978). Myoglobin and pigmented casts were observed in the urine, and the authors suggested that these observations, in combination with a highly elevated level of serum creatine phosphokinase, indicated that rhabdomyolysis may have contributed to the renal failure.

Ingestion of mercurous chloride has also resulted in renal toxicity in humans. Decreased urinary output and edema were observed in a 60-year-old woman who ingested an unspecified amount of mercurous chloride in a Chinese medicine (Kang-Yum and Oransky 1992). Renal failure was a contributing factor in the death of this woman. Renal failure also developed in two female patients who chronically ingested a mercurous chloride-containing laxative (Davis et al. 1974).

Renal toxicity has been observed in Fischer 344 rats and B6C3F$_1$ mice following acute-, intermediate-, and chronic-duration exposures to mercuric chloride (Dieter et al. 1992; NTP 1993). In the 14-day study, male and female rats were exposed by gavage to 0.93–14.8 mg Hg/kg/day as mercuric chloride for 5 days a week. There was a significant increase in the absolute and relative kidney weights of males beginning at the 1.9-mg/kg/day dose level. An increased incidence of tubular necrosis was observed in rats exposed to at least 3.7 mg/kg/day; severity progressed with increasing dose levels. Increases in urinary levels of alkaline phosphatase, AST, and LDH were also observed at 3.7 mg Hg/kg/day; at 7.4 mg Hg/kg/day,

increased urinary γ-glutamyltransferase activity was also observed. Mice given a single gavage dose of 10 mg/Hg/kg as mercuric chloride showed minor renal tubular damage and rapid regeneration of the tubular epithelium (Nielsen et al. 1991). At 20 mg Hg/kg/day, the mice showed necrosis of the proximal tubules. Mice given gavage doses of mercuric chloride 5 days a week for 2 weeks showed an increase in absolute and relative kidney weights at 3.7 mg Hg/kg/day and acute renal necrosis at 59 mg Hg/kg/day (NTP 1993).

Groups of 10 female Sprague-Dawley rats were administered a single gavage dose of mercuric chloride at 7.4 or 9.2 mg Hg/kg in water and necropsied at 14 days postexposure. No effects on body weight or weights of other organs were found. Mercury was found mainly in the kidneys (12.6 and 18.9 ppm at the low and high doses, respectively), but trace amounts were also detected in the liver, brain, and serum. Mild-to-moderate morphological changes, consisting of protein casts, cellular casts, and interstitial sclerosis, were noted in the kidneys of $HgCl_2$-treated animals in both groups (Lecavalier et al. 1994).

In a 4-week range-finding study, groups of 5 rats per sex (10 per sex for controls) received diets containing mercuric chloride at 5, 10, or 20 mg Hg/kg/day for males and 5.5, 11.1, and 22.2 mg Hg/kg/day for females. Nephrosis and proteinaceous casts in the kidneys were observed in all groups (males and females) fed mercuric chloride. An increased number of epithelial cells in the urine was observed in males exposed at the low dose; however, this effect was not observed at higher dose levels and the authors noted that the effect could not be ascribed to treatment. The minimum-nephrotoxic-effect level (MNEL) and the no-nephrotoxic-effect level (NNEL) for mercuric chloride in feed were determined to be 8 mg Hg/kg/day in males and 8.9 mg Hg/kg/day in females and 1 mg Hg/kg/day in males and 1.1 mg Hg/kg/day in females, respectively (Jonker et al. 1993b). In a follow-up 4-week study, 10-week-old Wistar rats were fed mercuric chloride at the MNEL and NNEL. In males, the MNEL resulted in the presence of ketones in urine and an increase in the relative weight of kidneys. Effects observed in females in the MNEL group included decreased density of urine and increased absolute and relative kidney weights. Increased absolute and relative kidney weights were also seen in females at the NNEL. A few histopathological changes were found in the basophilic tubules in the outer cortex of the kidneys in 5 of 5 males and 1 of 5 females exposed to the MNEL (Jonker et al. 1993b).

Similarly, male mice receiving mercuric chloride in drinking water for 7 weeks showed slight degeneration of the tubular epithelial cells (nuclear swelling) at 2.9 mg Hg/kg/day and minimal renal nephropathy

(dilated tubules with either flattened eosinophilic epithelial cells or large cytomegalic cells with foamy cytoplasm) at 14.3 mg Hg/kg/day (Dieter et al. 1983).

In a 6-month exposure to 0.23–3.7 mg Hg/kg/day, a significant increase in severity of nephropathy (i.e., dilated tubules with hyaline casts, foci of tubular regeneration, and thickened tubular basement membrane) was observed in Fischer 344 rats exposed to 0.93 mg/kg/day of mercuric chloride compared to the controls (NTP 1993). The absolute and relative kidney weights were increased in males at 0.46 mg/kg/day. In $B6C3F_1$ mice, the incidence and severity of cytoplasmic vacuolation of renal tubule epithelium increased in males exposed to at least 3.7 mg Hg/kg/day as mercuric chloride for 6 months (NTP 1993). Administration of large doses of mercuric chloride (28 mg Hg/kg/day) in the drinking water for 6 months also resulted in focal degeneration of the tubular cells with decreased acid phosphatase in the lysosomes (indicative of the release of the lysosomal contents) (Carmignani et al. 1992). Notably, at this dose, renal glomerular changes were also evident. The glomeruli showed hypercellularity, and there was deposition of amorphous material in the mesangium; thickening of the basement membrane with IgM present was also observed.

When a strain of mice (SJL/N) sensitive to the immunotoxic effects of mercury was given mercuric chloride in the drinking water at 0.56 mg Hg/kg/day for 10 weeks, slight glomerular cell hyperplasia with granular IgG deposits in the renal mesangium and glomerular blood vessels were observed (Hultman and Enestrom 1992). No tubular necrosis was observed.

In a 2-year study, male rats gavaged with 1.9 or 3.7 mg Hg/kg/day as mercuric chloride 5 days a week exhibited an incidence of marked nephropathy (described as thickening of glomerular and tubular basement membranes and degeneration and atrophy of tubular epithelium) that was significantly greater in severity than in the control group (NTP 1993). In addition, the incidence of renal tubule hyperplasia was increased in the high-dose male rats. In the same study, the incidence and severity of nephropathy were significantly greater in male and female mice gavaged with 3.7 and 7.4 mg Hg/kg/day as mercuric chloride 5 days a week than in the controls. Administration of 7 mg Hg/kg/day as mercuric chloride to rats in the drinking water resulted in hydropic degeneration and desquamation of tubule cells (Carmignani et al. 1989). Electron microscopy showed lysosomal alterations in the proximal tubules and thickening of the basal membrane of the glomeruli.

Organic Mercury. Data on renal toxicity associated with ingestion of methylmercury in humans come from several case studies. An outbreak of ethylmercury fungicide-induced poisoning was reported by Jalili and Abbasi (1961). Affected individuals exhibited polyuria, polydypsia, and albuminuria. Two boys who ingested meat from a hog that had consumed seed treated with ethylmercuric chloride also had increased blood urea, urinary protein, and urinary sediment (Cinca et al. 1979); an autopsy revealed nephritis. A 13-month-old boy who ate porridge made from flour treated with an alkyl mercury compound (specific mercury compound not reported) experienced albuminuria, red and white cells, and casts in the urine (Engleson and Herner 1952). In autopsies carried out to evaluate the cause of death in 4 adults and 4 infants from the Iraqi epidemic of 1972, one case exhibited tubular degeneration in the kidneys (whether an adult or child was not specified) (Al-Saleem and the Clinical Committee on Mercury Poisoning 1976).

Organic mercury-induced nephrotoxicity has been demonstrated in rodents following acute-, intermediate-, and chronic-duration exposure. The usefulness of results from subchronic studies may be limited because the pathological changes observed were often not distinguished as primary or secondary effects (i.e., pathological changes secondary to induced shock). Nonetheless, they provide some useful indication of potential effects.

Administration of methylmercuric chloride to mice in a single gavage dose of 16 mg Hg/kg resulted in decreased renal function (decreased phenolsulfonphthalein excretion), increased plasma creatinine, and swelling of tubular epithelial cells, with exfoliation of the cells into the tubular lumen (Yasutake et al. 1991b). Although no effects were observed after a single gavage dose of 8 mg Hg/kg (Yasutake et al. 1991b), 5 daily gavage doses of 8 mg Hg/kg/day as methylmercuric chloride in rats resulted in vacuolization and tubular dilation in the proximal tubules with ongoing regeneration (Magos et al. 1985). Similar effects were observed after 5 doses of 8 mg Hg/kg/day as ethylmercuric chloride (Magos et al. 1985).

In an intermediate-duration study, histopathological changes were observed in the kidneys of female rats exposed to 0.86, 1.68, or 3.36 mg Hg/kg/day as methylmercury dicyanidiamide by gavage 5 days a week for 3–12 weeks (Magos and Butler 1972). The low-dose group exhibited large foci of basophilic tubular epithelial cells, desquamation, fibrosis, and inflammation in the renal cortex; however, no control group was used in the study (Magos and Butler 1972). A 12-week diet containing 0.08 mg Hg/kg/day as methyl-mercury caused ultrastructural changes (cytoplasmic masses containing ribosomes and bundles of smooth endoplasmic reticulum) in kidney proximal tubule cells of female rats, despite the normal appearance of the

glomeruli at the light microscopic level (Fowler 1972). The author concluded that these changes could be a result of metabolism to inorganic mercury and may account for proteinuria observed in exposed humans. Administration of methylmercuric chloride in the diet of mice for 26 weeks at a dose of 0.6 mg Hg/kg/day resulted in degeneration of the proximal tubules characterized by nuclear swelling and vacuolation of the cytoplasm (Hirano et al. 1986).

Rats fed daily doses of phenylmercuric acetate for up to 2 years exhibited slight-to-moderate renal damage (e.g., tubular dilatation, atrophy, granularity, fibrosis) (Fitzhugh et al. 1950). These effects were evident at doses (beginning at 0.02 mg Hg/kg/day) that were two orders of magnitude lower than those required to induce detectable effects in the mercuric acetate-treated rats (Fitzhugh et al. 1950). A NOAEL of 0.005 mg Hg/kg/day was determined. The authors concluded that some of the histological changes were present to some degree in the control animals, suggesting that low levels of mercury apparently hasten the normal degenerative processes of the kidneys (see Inorganic Mercury above). Problems in this study limit its usefulness in determining effect levels. Increased severity of renal nephrosis was also observed in another study in which rats were given 0.4 mg Hg/kg/day as phenylmercuric acetate in the drinking water for 2 years (Solecki et al. 1991). Lower doses in this study were not tested. Mice given methylmercuric chloride in the diet at a dose of 0.13 mg Hg/kg/day showed epithelial cell degeneration and interstitial fibrosis, with ongoing regeneration of the tubules present (Mitsumori et al. 1990); no effect was observed at 0.03 mg Hg/kg/day. Similar effects were seen in mice given methylmercuric chloride in the diet for 2 years at a dose of 0.11 mg Hg/kg/day (Hirano et al. 1986). Rats given methylmercuric chloride in the diet for 2 years at a dose of 0.1 mg Hg/kg/day had increased kidney weights and decreased enzymes (alkaline phosphatase, ATPase, NADH- and NADPH-oxidoreductase, and AMPase) in the proximal convoluted tubules (Verschuuren et al. 1976). However, histopathological examination revealed no treatment-related lesions.

A 2-year study conducted with mercuric acetate in the feed of rats showed an increased severity of renal damage at doses of mercury as low as 2 mg Hg/kg/day (Fitzhugh et al. 1950). Rats initially showed hypertrophy and dilation of the proximal convoluted tubules. At this stage, eosinophilia, rounding, and granular degeneration of the epithelial cells were observed. Occasionally basophilic cytoplasm and sloughing of the cells were observed. As the lesion progressed, tubular dilation increased, and hyaline casts appeared within the tubules; fibrosis and inflammation were observed. Finally, tubules appeared as cysts, and extensive fibrosis and glomerular changes were observed. However, this study was limited because

group sizes were small, survival data were not reported, and a considerable number of early deaths from pneumonia were noted.

Endocrine Effects

Inorganic Mercury. No studies were located regarding endocrine effects in humans after oral exposure to inorganic mercury.

Several studies have reported effects on the thyroid after acute- or intermediate-duration exposure to mercuric chloride. An increase in iodine release from the thyroid was observed following gavage administration of 7.4 mg Hg/kg/day as mercuric chloride to rats for 6 days (Goldman and Blackburn 1979). Serum levels of thyroid hormones (triiodothyronine and/or thyroxine) in mice decreased after administration of 6 mg Hg/kg/day as mercuric chloride or mercuric sulfide for 10 days by gavage (Sin et al. 1990). Similar effects were observed after 4 weeks of dosing with mercuric sulfide (Sin and The 1992). Administration by gavage of 5.3 mg Hg/kg/day as mercuric chloride to rats for 40 days resulted in increased thyroid weight, thyroidal iodine uptake, and protein-bound iodine in the serum (Goldman and Blackburn 1979). Decreased triiodothyronine and monoiodotyrosine were also observed. Dietary exposure of rats to 2.2 mg Hg/kg/day as mercuric chloride for 3 months resulted in decreased thyroidal iodine uptake, release, and turnover (Goldman and Blackburn 1979). Adrenocortical function was evaluated in male rats exposed to 0, 9, 18, or 36 mg Hg/kg/day as mercuric chloride in drinking water for 60–180 days (Agrawal and Chansouria 1989). A significant increase in adrenal and plasma corticosterone levels in all dose groups was observed after 120 days of exposure. After 180 days of exposure, corticosterone levels had returned to control values. The relative adrenal gland weight was significantly increased for all exposed groups compared to control values.

In a 4-week range-finding study, groups of 5 rats per sex (10 per sex for controls) received diets containing mercuric chloride at 5, 10, or 20 mg Hg/kg/day in males and 5.5, 11.1, and 22.2 mg Hg/kg/day in females. The high dose resulted in an increased relative adrenal weight in males and a decreased absolute adrenal weight in females (Jonker et al. 1993b)

Organic Mercury. No studies were located regarding endocrine effects in humans or animals after oral exposure to organic mercury.

Dermal Effects

Inorganic Mercury. Limited information was located regarding dermal effects of inorganic mercury in humans. 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. Kang-Yum and Oransky (1992) reported hives in a woman who ingested a Chinese medicine containing an unspecified amount of mercurous chloride, which suggests an allergic response to the medicine.

No studies were located regarding dermal effects in animals after oral exposure to inorganic mercury.

Organic Mercury. Only a few studies were identified regarding dermal effects of organic mercury, however, the case history concerning dimethylmercury exposure is a very important alert to the hazards of this organomercurial.

Blayney et al. (1997) originally reported the fatal case of a dimethylmercury exposure after a dermal exposure to an extremely small amount of material. The case history was subsequently detailed by Nierenberg et al. (1998). The exposure occurred to a 48-year-old female chemistry professor who was admitted to the hospital 5 months (154 days) after, as best as can be determined, she inadvertently spilled several drops (estimated at 0.4–0.5 mL; about 1,500 mg) of dimethylmercury from the tip of her pipette onto the back of her disposable latex gloves. The spill was cleaned and the gloves disposed of. Hair analysis on a long strand of hair revealed that after a brief lag time, mercury content rose rapidly to almost 1,100 ppm (normal level, <0.26 ppm; toxic level, >50 ppm), and then slowly declined with a half-life of 74.6 days. These results support the occurrence of one or several episodes of exposure, and are consistent with laboratory notebook accounts of a single accidental exposure. Testing of family members, laboratory coworkers, and laboratory surfaces failed to reveal any unsuspected mercury spills or other cases of toxic blood or urinary mercury levels. Permeation tests subsequently performed on disposable latex gloves similar to those the patient had worn at the time of the lone exposure revealed that dimethylmercury penetrates such gloves rapidly and completely, with penetration occurring in 15 seconds or less and perhaps instantly. Polyvinyl chloride gloves were equally permeable to dimethylmercury. Five days prior to

admission, the patient developed a progressive deterioration in balance, gait, and speech. During the previous 2 months, she had experienced brief episodes (spaced weeks apart) of nausea, diarrhea, and abdominal discomfort; and had lost 6.8 kg (15 lb). Medical examination revealed moderate upper-extremity dysmetria, dystaxic handwriting, a widely based gait, and "mild scanning speech." Routine laboratory test results were normal. Computed tomography (CT) and magnetic resonance imaging (MRI) of the head were normal except for the incidental finding of a probable meningioma, 1 cm in diameter. The cerebrospinal fluid was clear, with a protein concentration of 42 mg/dL and no cells. A preliminary laboratory report indicated that the whole-blood mercury concentration was more than 1,000 µg/L (normal range, 1–8 µg/L; toxic level, >200 µg/L). Chelation therapy with oral succimer (10 mg/kg orally every 8 hours) was begun on day 168 after exposure. Whole blood concentrations rose to 4,000 µg/L after one day of chelation, and urinary mercury levels were 234 µg/L (normal range, 1–5 µg/L; toxic level, >50 µg/L). Despite the initial success of chelation therapy, administration of vitamin E, and a blood exchange transfusion, at 176 days postexposure, the patient became comatose. Further aggressive general support and chelation therapy failed, life support ws removed (following the patient's advance directive), and the patient died 298 days post exposure. Autopsy results revealed diffusely thin cortex of the cerebral hemispheres (to 3 mm), and extensive gliosis of the visual cortex around the calcarine fissure and the superior surface of the superior temporal gyri. The cerebellum showed diffuse atrophy of both vermal and hemispheric folia. Microscope evaluation revealed extensive neuronal loss and gliosis bilaterally within the primary visual and auditory cortices, with milder loss of neurons and gliosis in the motor and sensory cortices. There was widespread loss of cerebellar granular-cell neurons, Purkinje cells, and basket-cell neurons, with evidence of loss of parallel fibers in the molecular layer. Bergmann's gliosis was well developed and widespread.

In the only other organic mercury studies identified for dermal exposures, a study of a large group of people who consumed methylmercury-contaminated bread over a 1- to 3-month period showed a dose-related history of rashes (Al-Mufti et al. 1976). These may also have been allergic responses. 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). Also, Iraqis who consumed flour made from grain treated with ethylmercury *p*-toluene sulfonanilide exhibited skin lesions consisting of pruritus on the palms, soles, and genitalia (Jalili and Abbasi 1961). In severe cases, exfoliative dermatitis of the hands and feet was also observed.

The only information located regarding dermal effects in animals after oral exposure to organic mercury comes from a study in which rats were exposed to methylmercuric chloride in the diet for 2 years (Verschuuren et al. 1976). No treatment-related lesions were observed upon histopathological examination of the skin of rats exposed to 0.1 mg Hg/kg/day.

Ocular Effects

Inorganic Mercury. No information was located regarding ocular effects in humans from ingestion of inorganic mercury.

No studies were located regarding ocular effects in animals after oral exposure to inorganic mercury.

Organic Mercury. No information was located regarding ocular effects in humans from ingestion of organic mercury. While visual effects result from methylmercury exposure, they are cortical in origin (see neurotoxicity below).

The only report of ocular effects in animals after oral exposure to organic mercury comes from a study in which rats were exposed to methylmercuric chloride via the diet for 2 years (Verschuuren et al. 1976). No treatment-related lesions were observed upon histopathological examination of the eyes of rats exposed to 0.1 mg Hg/kg/day. As in humans, the visual effects resulting from methylmercury exposure in primates are considered to be centrally mediated (Rice and Gilbert 1982, 1990).

Body Weight Effects

Inorganic Mercury. No information was located regarding body weight effects in humans from ingestion of inorganic mercury.

A single dose of mercuric chloride administered to female Sprague-Dawley rats (10/group) at 7.4 or 9.2 mg Hg/kg in water resulted in no effects on body weight at 14 days postexposure (Lecavalier et al. 1994). However, a number of animal studies have reported decreases in body weight or body weight gain after ingestion of mercuric chloride (Chang and Hartmann 1972a; Dieter et al. 1992; NTP 1993). After a 4-week exposure to mercuric chloride in the food, male Wistar rats had a 21% body weight decrease at 10 mg Hg/kg/day, and female Wistar rats had a 27% decrease in body weight at 22.2 mg Hg/kg/day. No

significant loss was observed at the next-lower-dose groups of 5 and 11.1 mg Hg/kg/day in males and females, respectively (Jonker et al. 1993b).

Doses of 14.8 mg Hg/kg/day administered to rats 5 days a week for 2 weeks resulted in a 10% decrease in male body weight gain (NTP 1993). Much lower doses produced decreases in body weight gain when administered over longer periods. In rats, decreases in body weight gain of approximately 10% were observed with doses of 0.93 mg Hg/kg as mercuric chloride when administered by gavage 5 days a week for 6 months (NTP 1993). Mice were less sensitive, showing no effect at 7.4 mg Hg/kg/day and a 26% decrease in body weight gain at 14.8 mg Hg/kg/day in the same study (NTP 1993).

Organic Mercury. No information was located regarding body weight effects in humans from ingestion of organic mercury.

A number of animal studies have reported decreases in body weight or body weight gain after ingestion of methyl or phenyl mercury. A 20–25% decrease in body weight gain in male and female rats was observed after 5 gavage doses of 8 mg Hg/kg/day as methylmercuric chloride or ethylmercuric chloride (Magos et al. 1985). In intermediate-duration studies with methylmercury, biologically significant decreases in body weight gain have been observed in rats after exposure to doses as low as 0.8 mg Hg/kg/day for 6 weeks (Chang and Hartmann 1972a) and in mice after exposure to 1 mg Hg/kg/day for 60 days (Berthoud et al. 1976). No effect on female body weight gain was observed after dietary exposure to 0.195 mg Hg/kg/day as methylmercuric chloride for 14 weeks (Lindstrom et al. 1991). A 2-year exposure to 0.4 mg Hg/kg/day as phenylmercuric acetate in the feed resulted in a 10% decrease in body weight gain in rats (Solecki et al. 1991). Gavage administration of methylmercuric chloride to rats for 2 days at 12 mg Hg/kg/day resulted in a persistent decrease in the body temperature of the rats (Arito and Takahashi 1991).

Other Systemic Effects

Inorganic Mercury. Several children who were treated with mercurous chloride contained in powders or tablets for constipation, worms, or teething discomfort exhibited low-grade or intermittent fevers (Warkany and Hubbard 1953).

No studies were located on other systemic effects in animals after oral exposure to inorganic mercury.

Organic Mercury. No studies were located regarding other systemic effects in humans or animals after oral exposure to organic mercury.

2.2.2.3 Immunological and Lymphoreticular Effects

Inorganic Mercury. No studies were located regarding immunological or lymphoreticular effects in humans after oral exposure to inorganic mercury.

The immune response to mercury exposure is complex, depending in part on the dose of mercury and the genetic characteristics of the exposed population (see Section 2.4). Administration of 14.8 mg Hg/kg/day as mercuric chloride to $B6C3F_1$ mice 5 days a week for 2 weeks resulted in a decrease in thymus weight (NTP 1993), suggesting immune suppression. However, a 2-week exposure to 0.7 mg Hg/kg/day as mercuric chloride in the drinking water resulted in an increase in the lymphoproliferative response after stimulation with T-cell mitogens in a strain of mice particularly sensitive to the autoimmune effects of mercury (SJL/N) (Hultman and Johansson 1991). In contrast, a similar exposure of a strain of mice (DBA/2) not predisposed to the autoimmune effects of mercury showed no increase in lymphocyte proliferation.

A significant suppression of the lymphoproliferative response to T-cell mitogens, concanavalin A, and phytohemagglutinin was observed in male $B6C3F_1$ mice administered 2.9 or 14.3 mg Hg/kg/day as mercuric chloride in drinking water for 7 weeks (Dieter et al. 1983). A significant decrease in the weight of the thymus and spleen and a decrease in antibody response were also exhibited at 14.3 mg Hg/kg/day. An increase in B-cell-mediated lymphoproliferation was, however, observed at both 2.9 and 14.3 mg Hg/kg/day. No immunological effects were observed at the lowest dose of 0.57 mg Hg/kg/day. When SJL/N mice were administered mercuric chloride in the drinking water for 10 weeks, an increase in circulating antinucleolar antibodies was observed at 0.28 mg Hg/kg/day, and deposition of granular IgG deposits was observed in the renal mesangium and glomerular blood vessels at 0.56 mg Hg/kg/day (Hultman and Enestrom 1992).

In rats, immune deposits have been observed in the basement membrane of the intestines and kidneys following gavage exposure to 2.2 mg Hg/kg/day as mercuric chloride twice weekly for 2 months, although no functional changes were evident in these tissues (Andres 1984). The observation of these deposits suggests that autoimmunity to specific components of these tissues has developed.

The highest NOAEL values and all reliable LOAEL values for immunological and lymphoreticular effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2 for inorganic mercury.

Organic Mercury. No studies were located regarding immunological effects in humans after oral exposure to organic mercury.

In BALB/c mice administered a diet containing 0.5 mg Hg/kg/day as methylmercury for 12 weeks, the thymus weight and cell number decreased by 22 and 50%, respectively, compared to the control group (Ilback 1991). The natural killer cell activity was reduced by 44 and 75% in the spleen and blood, respectively. However, the lymphoproliferative response in the spleen increased at this dose of mercury.

The LOAEL value for immunological and lymphoreticular effects in mice for intermediate-duration oral exposure to organic mercury is recorded in Table 2-3 and plotted in Figure 2-3.

2.2.2.4 Neurological Effects

Inorganic Mercury. The oral absorption of metallic mercury is negligible, and even massive doses have not resulted in neurological effects. The wo case histories identified are unusual in that the dose levels could be reasonably well quantified. The first case history reported ingestion of 15 mL (204 g) of metallic mercury by a 17-year-old male storekeeper who swallowed mercury from the pendulum of a clock (apparently out of curiosity rather than as a suicide attempt). On admission, and 24 hours later, he was symptom free, and physical examination was normal. The patient complained of no gastrointestinal symptoms, and was treated with a mild laxative and bed rest. The results of serial daily urine mercury estimates were normal (all less than 15 µg) and did not suggest significant absorption. The radiological investigation illustrated a characteristic pattern of finely divided globules of mercury in the gastrointestinal tract (Wright et al. 1980).

The second and massive incidence of ingestion involved a 42-year-old man who had spent much of his life (since the age of 13) repairing instruments that contained mercury. He intentionally ingested an estimated 220 mL (or about 3,000 g) while repairing a sphygmomanometer (Lin and Lim 1993). Upon admission, the patient presented with significantly elevated mercury blood levels (103 µg/L, normal <10 µg/L) and urine levels (73 µg/L, <20 µg/L). In the previous 2 years he had developed mild hand tremors,

forgetfulness, irritability, and fatigue. The occupational exposures made it difficult to determine any additional neurological effects from the acute exposure. There was no history of peripheral neuropathy, vertigo, insomnia, or muscular weakness. Neuropsychiatric and psychology evaluations indicated poor concentration and a defect in recent memory. EEG results indicated diffuse cortical dysfunction predominantly on the left hemisphere. He was treated with immediate gastric lavage and cathartics. He also received D-penicillamine 1 g/day orally for 7 days. Blood and urine mercury levels obtained 3 days after chelating therapy were 116.9 and 22.9µg/L, respectively. By 2 weeks postexposure, most of the mercury had been excreted in the feces and was measured at a total volume of 220 mL (this number was used to estimate the amount initially ingested). The patient was lost to follow-up, but returned to the hospital 6 months later (for glycemic control), at which time examination revealed a lessening of his hand tremors.

Most case studies of neurotoxicity in humans induced by oral exposure to inorganic mercury salts have reported neurotoxic effects as the result of ingestion of therapeutic agents that contain mercurous chloride (e.g., teething powders, ointments, and laxatives). Several children treated with tablets or powders containing mercurous chloride exhibited irritability, fretfulness, sleeplessness, weakness, photophobia, muscle twitching, hyperactive or hypoactive tendon reflexes, and/or confusion (Warkany and Hubbard 1953). 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). Davis et al. (1974) reported that two women developed dementia and irritability due to chronic ingestion of a tablet laxative that contained 120 mg of USP-grade mercurous chloride (0.72 mg Hg/kg/day, assuming an average body weight of 70 kg). One woman had taken 2 tablets daily for 25 years, and the other woman took 2 tablets daily for 6 years. Both patients died from inorganic mercury poisoning, and at autopsy, low brain weight and volume and a reduced number of nerve cells in the cerebellum were seen. Light microscopic analysis revealed granules of mercury within neuronal cytoplasm. Electron microscopy revealed mercury deposits in some neurons.

In addition, neurotoxicity has been observed after ingestion of lethal doses of mercuric chloride. Blurred vision and diplopia were reported by a 35-year-old man who ingested a lethal dose of mercuric chloride (Murphy et al. 1979). Prior to death, the man experienced repeated seizures. An autopsy revealed abscesses on the occipital lobe and cerebellum.

Acute- and intermediate-duration studies describing neurotoxic effects in animals following exposure to inorganic mercury salts are limited. A study was conducted by Chang and Hartmann (1972b) in which mercuric chloride was administered both by gavage and subcutaneously. Evidence of disruption of the blood-brain barrier (i.e., leakage of dye into the brain tissue) was observed 12 hours after a single dose of 0.74 mg Hg/kg as mercuric chloride in rats (Chang and Hartmann 1972b). These investigators also administered 0.74 mg Hg/kg/day as mercuric chloride to rats for up to 11 weeks. Within 2 weeks, there were coagulative or lucid changes in cerebellar granule cells and fragmentation, vacuolation, and cytoplasmic lesions in the neurons of dorsal root ganglia. Neurological disturbances consisted of severe ataxia and sensory loss, with an accompanying loss in body weight. No conclusions regarding the oral neurotoxicity of mercuric chloride can be drawn from the results of this study because the discussion of the results observed in the study did not clearly differentiate whether the effects were observed as the result of oral or subcutaneous exposure. It is expected that mercuric chloride administered subcutaneously would be much more toxic than that administered orally because of the very poor absorption of inorganic forms of mercury from the gastrointestinal tract.

Dietary exposure of rats to 2.2 mg Hg/kg/day as mercuric chloride for 3 months resulted in inactivity and abnormal gait (Goldman and Blackburn 1979). However, it is unclear whether the effects observed in this study were the direct result of effects on the nervous system, or whether they may have been secondary to other toxic effects. No evidence of neurotoxicity (clinical signs of neurotoxicity and optic and peripheral nerve structure) was seen in mice administered 0.74 or 2.2 mg Hg/kg/day as mercuric chloride in the drinking water for 110 days (Ganser and Kirschner 1985). The investigators increased the dose administered to the low-dosed animals to 7.4–14.8 mg Hg/kg/day for an additional 400 days; however, still no neurotoxic effects were observed. Similarly, no histopathological evidence of brain lesions was observed in rats receiving gavage doses of mercuric chloride as high as 3.7 mg Hg/kg/day 5 days a week for up to 2 years or in mice receiving gavage doses as high as 7.4 mg Hg/kg/day 5 days a week for up to 2 years (NTP 1993).

The highest NOAEL values and all reliable LOAEL values for neurotoxic effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2 for inorganic mercury.

Organic Mercury. Most of the information concerning neurotoxicity in humans following oral exposure to organic mercury comes from reports describing the effects of ingesting contaminated fish or fungicide-treated grains (or meat from animals fed such grains). Information about doses at which the effects

occurred is frequently limited because of difficulties in retracing prior exposure and uncertainties in estimating dose levels based on assumed food intake and contamination levels.

Although isolated instances of alkyl mercury poisoning have been reported (Cinca et al. 1979; Engleson and Herner 1952), the epidemic poisonings in Japan and Iraq focused attention on the neurotoxicity of these compounds. The first reported widespread outbreak of neurological disorders associated with the ingestion of methylmercury-contaminated fish occurred in the Minamata area of Japan (Kutsuna 1968). The neurological syndrome was characterized by a long list of symptoms including prickling, tingling sensation in the extremities (paresthesia); impaired peripheral vision, hearing, taste, and smell; slurred speech; unsteadiness of gait and limbs; muscle weakness; irritability; memory loss; depression; and sleeping difficulties (Kutsuna 1968; Tsubaki and Takahashi 1986). Elevated concentrations of methylmercury were observed in the hair and brains of victims (see Section 2.5). Epidemics of similar neurological disorders were reported in Iraq in 1956 and 1960 (Bakir et al. 1973; Jalili and Abbasi 1961) as the result of eating flour made from seed grain treated with ethylmercury *p*-toluene sulfonanilide. Affected individuals had an inability to walk, cerebellar ataxia, speech difficulties, paraplegia, spasticity, abnormal reflexes, restriction of visual fields or blindness, tremors, paresthesia, insomnia, confusion, hallucinations, excitement, and loss of consciousness. In the winter of 1971–1972 in Iraq, more than 6,530 patients required hospitalization and 459 deaths occurred, usually due to central nervous system damage, after the ingestion of contaminated bread prepared from wheat and other cereals treated with a methylmercury fungicide (Bakir et al. 1973).

Al-Mufti et al. (1976) attempted to correlate symptoms of the poisoning incident with an estimate of methylmercury intake based on average levels found in grain and self-reported estimates of the number of loaves ingested. A number of assumptions were made in the estimates, and there were logistical constraints in surveying the widely spread rural population in Iraq. Moreover, only a total mercury intake was derived and compared with the results of a clinical evaluation and a survey for symptoms. Nonetheless, interesting and useful results were reported based on the 2,147 people surveyed. The mean period of exposure for the Iraqi population exposed to contaminated bread was 32 days, with some people consuming the bread for as long as 3 months. A mean of 121 loaves per person was eaten; the maximum was 480 loaves. Based on the mean number of loaves, the total intake of methylmercury was estimated at between 80 mg and 250 mg. However, those who had consumed the most loaves may have ingested up to 1,000 mg of methylmercury over a 3-month period. Of those with symptoms of alkylmercury poisoning at the time of the survey (October 1972–May 1973), 80% had eaten more than 100 loaves. Of the 75 people who had reported eating more than 200 loaves, 53 (71%) presented with some evidence of poisoning. The incidence rate for

poisoning was estimated at 271 per 1,000; this includes a mortality of 59 per 1,000, a severe disability rate of 32 per 1,000, a rate of mild or moderate disability of 41 per 1,000; and a rate for those with only a subjective evidence of poisoning of 138 per 1,000. Based on estimates of total intake, dose-related increases were observed in the incidence and severity of paresthesia, astereognosis (loss of the ability to judge the form of an object by touch), persistent pain in the limbs, persistent headaches, difficulty walking, difficulty using the arms, and changes in speech, sight, and hearing. The most commonly observed symptom was paresthesia, most frequently involving the extremities but also on the trunk and the circumoral region. Difficulty walking and a feeling of weakness were the next most common symptoms. The total estimated intake in total milligrams associated with the four categories (no evidence of poisoning, subjective evidence, mild to moderate evidence, and severe symptoms) is as follows for all ages combined (number of persons in parentheses): 95 mg (n=59), 141 mg (n=131), 160 mg (n=35), 173 mg (n=22). This dose range is small for such dramatically different health states, and does not widen when the data are evaluated by age group. Interestingly, the total intake associated with severity of symptoms decreases on a mg/kg body weight basis with increasing age in contrast with what would be expected if children were more susceptible. For example, intakes (mg/kg over the total exposure period) associated with severe symptoms are as follows for the age groups 5–9 years, 10–14 years, and 15 years and older, respectively: 7.8 mg/kg (n=9), 4 mg/kg (n=7), and 3.6 mg/kg (n=6). Comparable numbers are for the mild/moderate symptoms and the subjective symptoms (shown): 6 mg/kg (n=19), 3.4 mg/kg (n=20), and 2.4 mg/kg (n=92). It is possible that child sensitivity may not be as large a factor when exposures reach the levels experienced in Iraq.

Neurotoxic effects seen in the Minamata (Japan) and Iraqi poisonings have been associated with neuronal degeneration and glial proliferation in the cortical and cerebellar gray matter and basal ganglia (Al-Saleem and the Clinical Committee on Mercury Poisoning 1976), and derangement of basic developmental processes such as neuronal migration (Choi et al. 1978; Matsumoto et al. 1965) and neuronal cell division (Sager et al. 1983). In the brain, Purkinje, basket, and stellate cells were severely affected. Granule cells were variably affected. Sural nerves removed from two women with neurotoxicity associated with the Minamata incident also showed evidence of peripheral nerve degeneration and regeneration (Miyakawa et al. 1976).

Similar effects have been observed in persons ingesting meat contaminated with ethylmercuric chloride (Cinca et al. 1979). Neurotoxic signs observed in two boys who ultimately died as the result of the exposure included gait disturbance, ataxia, dysarthria, dysphagia, aphonia, hyperreactive tendon reflexes,

hypotonia, spasticity, mydriasis, horizontal nystagmus, agitation, and coma. Electroencephalography showed decreased alpha activity and increased slow-wave activity. Autopsy showed nerve cell loss and glial proliferation in the cerebral cortex (calcarine cortex, midbrain, bulbar reticular formation), demyelination, granule cell loss in the cerebellum, and motor neuron loss in the ventral horns of the spinal cord. Neurotoxic signs in the surviving family members were generally similar (ataxia, gait impairment, spasticity, drowsiness, intentional tremor, agitation, hypoesthesia in the limbs, speech difficulties, and visual disturbances); all but the narrowing of the visual fields resolved after termination of exposure.

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. The inorganic mercury levels in different regions of the brain of the 29-year-old patient ranged from 82 to 100% of the total mercury present. 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.

LeBel et al. (1996) studied early nervous system dysfunction in Amazonian populations exposed to low levels of methylmercury. A preliminary study was undertaken in two villages on the Tapajos River, an effluent of the Amazon, situated over 200 km downstream from the methylmercury extraction areas. The study population included 29 young adults $35 years (14 women and 15 men) randomly chosen from a previous survey. Hair analyses were conducted with cold vapor atomic fluorescence spectrophotometry. Total hair Hg (THg) varied between 5.6 µg/g and 38.4 µg/g, with MeHg levels from 72.2% to 93.3% of

the THg. A quantitative behavioral neurophysiological test battery, designed for use under standard conditions in an area without electricity and for persons with minimal formal education was administered to all participants. The results of visual testing showed that although all participants had good near and far visual acuity, color discrimination capacity (Lanthony D-15 desaturated panel) decreased with increasing THg ($F=4.1$; $p=0.05$); near visual contrast sensitivity profiles (Vistech 6000) and peripheral visual field profiles (Goldman Perimetry with Targets I and V) were reduced for those with the highest levels of THg. For the women, manual dexterity (Santa Ana, Helsinki version) decreased with increasing THg ($F=16.7$; $p<0.01$); this was not the case for the men. Although the women showed a tendency towards reduced grip strength, muscular fatigue did not vary with THg for either sex. The authors claim that this study demonstrates that it is possible, using a sensitive test battery, to detect alterations in nervous system functions, consistent with knowledge of Hg toxicity, at levels below the currently recognized threshold of 50 μg/g THg.

Mental retardation has not generally been reported as a neurotoxic effect of alkyl mercurial exposure in adults. However, a 9-month-old infant who received porridge made from alkyl mercury-contaminated grains for approximately 4 months lost the ability to crawl or walk and exhibited persistent mental retardation (Engleson and Herner 1952). These effects are similar to those seen in infants born to mothers who consumed methylmercury-contaminated food during pregnancy (see Section 2.2.2.6), suggesting that in addition to the prenatal period, infancy may also be a susceptible period for the development of these types of effects.

Studies in experimental animals also indicate that organic mercury is a potent neurotoxicant. Adult female monkeys *(Macaca fascicularis)* were exposed to methylmercury (0.050 mg Hg/kg/day) in apple juice by mouth for 6, 12, or 18 months, or 12 months followed by 6 months without exposure (clearance group). A fifth group of monkeys was administered mercuric chloride (0.200 mg Hg/kg/day) by constant-rate intravenous infusion through an in-dwelling catheter for 3 months. Controls were housed and handled with the exposed monkeys, but were not administered mercury. The number of neurons, astrocytes, reactive glia, oligodendrocytes, endothelia, and pericytes in the cortex of the calcarine sulcus was estimated by use of the optical volume fractionator stereology technique. Reactive glia showed a significant increase in number for every treatment group, increasing 72% in the 6-month, 152% in the 12-month, and 120% in the 18-month methylmercury-exposed groups, and the number of reactive glia in the clearance group remained elevated (89%). In the mercuric chloride group, there was a 165% increase in the number of reactive glia. Neurons, astrocytes, oligodendrocytes, endothelia, and pericytes showed no significant change in number in

any exposure group. The methylmercury-treated monkeys (all groups) appeared normal in terms of cage behavior throughout the entire exposure period, supporting the conclusion that there was no significant loss in the neuron population. Examination of tissue samples did not reveal any apparent degradation in the structure of neurons or chronic changes in the glial cells (e.g., the appearance of hypertrophic astrocytes), which are commonly observed following exposure to high levels of mercury. No apparent dilation of the perivascular spaces was observed. The average volume of the cortex of the calcarine sulcus did not differ significantly from controls for any methylmercury-treated group. The methylmercury-clearance group had low levels of methylmercury present in tissues; however, the level of inorganic mercury was also elevated. The astrocytes and microglia in the methylmercury group contained the largest deposits of inorganic mercury. Comparing the results of the methylmercury and inorganic mercury groups suggests that inorganic mercury may be responsible for the increase in reactive glia (Charleston et al. 1994).

Charleston et al. (1996) studied the effects of long-term subclinical exposure to methylmercury on the number of neurons, oligodendrocytes, astrocytes, microglia, endothelial cells and pericytes within the thalamus from the left side of the brain of the monkey *Macaca fascicularis.* These parameters were determined by use of the Optical Volume Fractionator stereological method. The accumulated burden of inorganic mercury (IHg) within these same cell types has been determined by autometallographic methods. Four groups of female monkeys (n=4-5) were exposed to 50 µg Hg/kg/day methylmercury in apple juice for 6, 12, or 18 months, or 12 months followed by 6 months without exposure (clearance group). One control animal each was sacrificed with the 6- and 12-month exposure groups, and two additional animals were sacrificed at the end of the experiment. All monkeys appeared normal—no changes in behavior or motor skills were observed. Hematological function (white blood cell count and differentiation, erythrocyte count, hemoglobin, hematocrit, and red cell indices) and blood chemistry (urea nitrogen, creatine, bilirubin, albumin, total protein, alkaline phosphatase, and electrolytes) were normal. No weight loss was observed. Neurons, oligodendrocytes, endothelia, and pericytes did not show a significant change in cell number for any exposure group. Astrocyte cell number exhibited a significant decline for both the 6-month (44.6%) and clearance exposure groups (37.2%); decreased astrocyte counts were also observed in the other exposure groups, but these were not significant. The microglia, in contrast, showed a significant increase in the 18-month (228%) and clearance exposure groups (162%). Results from mercury speciation and quantification analysis of contralateral matched samples from the thalamus of the right side of the brain from these same monkeys indicated that methylmercury concentrations plateaued at around 12 months exposure, whereas the inorganic levels, presumably derived from demethylation of methylmercury, continued to increase throughout all exposure durations. Autometallographic determination of the

distribution of IHg by cell type indicates that both the astrocytes and microglia contain substantially elevated IHg deposits relative to all other cell types. The data suggest that the inorganic mercury present in the brain, accumulating after long-term subclinical methylmercury exposure, may be a proximate toxic form of mercury responsible for the changes within the astrocyte and microglial populations.

Rice (1996a) evaluated delayed neurotoxicity produced by methylmercury in monkeys treated with methylmercury from birth to 7 years of age. When these monkeys reached 13 years of age, individuals began exhibiting clumsiness not present previously. Further exploration revealed that treated monkeys required more time to retrieve treats than did nonexposed monkeys and displayed abnormalities on a clinical assessment of sense of touch in hands and feet, despite the fact that clinical examinations performed routinely during the period of dosing had not yielded abnormal results. Another group of monkeys, dosed from *in utero* to 4 years of age, also took longer to retrieve treats when assessed years after cessation of exposure. These observations were pursued in both groups of monkeys by objective assessment of somatosensory function in the hands: both groups of monkeys exhibited impaired vibration sensitivity. The results suggest that a delayed neurotoxicity occurred when these monkeys reached middle age. The author notes persons with Minamata disease also have symptoms of delayed neurotoxicity. The results from a study of more than 1,100 Minamata patients over the age of 40 indicated a difficulty in performing daily activities that increased as a function of age compared to matched controls. Methylmercury may represent the only environmental toxicant for which there is good evidence for delayed neurotoxicity observable many years after cessation of exposure.

Rice (1996b) further compares the sensory and cognitive effects of developmental methylmercury exposure in monkeys to the effects in rodents. Developmental exposure to methylmercury in the Macaque monkey produced impairment of function in the visual, auditory, and somatosensory systems. In addition, delayed neurotoxicity was observed in monkeys years after cessation of dosing, manifested as overall clumsiness and slowness in reaching for objects. The effects of developmental methylmercury exposure on cognitive function in monkeys are more equivocal; both positive and negative results have been obtained, with no obvious pattern with regard to possible domains of impairment. Prenatal methylmercury exposure in rodents produced retarded development and impairment of motor function, while the evidence for cognitive impairment is less consistent. Derivation of reference doses based on these data from monkeys and rodents is remarkably congruent, and is virtually identical to values derived from evidence for developmental impairment in humans. Research needs include determination of neurotoxic effects at lower body burdens

in the monkey, including dose-effect data, and a more systematic exploration of the pattern of behavioral deficits in both primates and rodents.

Gilbert et al. (1996) used fixed interval/fixed ratio performance in adult monkeys to evaluate effects from exposure *in utero* to methylmercury. The fixed interval/fixed ratio (FI/FR) schedule is considered to be a sensitive indicator of neurotoxicity. In the present study, monkeys (*Macaca fascicularis*) were exposed *in utero* to methylmercury. Maternal doses of methylmercury of 0, 50, 70, or 90 µg/kg/day (in apple juice) (n=11, 9, 2, and 2, respectively) resulted in infant blood mercury levels at birth ranging from 1.04 to 2.45 ppm. Monkeys were tested on a multiple FI/FR schedule of reinforcement at 8–10 years of age. Four FI/FR cycles were run per session. Pause time and run rate were calculated for FI and FR components, as well as FI quarter-life and local FI response rates. Methylmercury treatment and sex effects were investigated by fitting a linear orthogonal polynomial regression to each monkey's profile across sessions and performing two-way ANOVAs on the resulting linear and intercept terms. Results from all treated monkeys were combined and compared to the control group. There were no treatment-related effects on either the fixed interval (FI) or fixed ratio (FR) component for pause time or run rate. Analysis of the quarter-life revealed a significant treatment by sex effect as well as a main effect for sex. Post hoc t-tests revealed a significant difference in quarter-life of treated male and female monkeys and a marginal difference between treated and control males. The FI run rate of the male monkeys was significantly greater than that of the female monkeys whereas the FR run rate of the males was marginally greater. These results indicate that there may be a differential effect of methylmercury on male and female monkeys, which could be interpreted as an effect on temporal discrimination. The authors concluded that adult monkeys exposed to *in utero* methylmercury exhibited very limited sex-related effects on the FI/FR intermittent schedule of reinforcement.

Typical neurotoxic signs observed in rats exposed to methylmercury include muscle spasms, gait disturbances, flailing, and hindlimb crossing (Fuyuta et al. 1978; Inouye and Murakami 1975; Magos et al. 1980, 1985). These effects have been observed after acute-duration gavage dosing with methylmercury concentrations at doses as low as 4 mg Hg/kg/day for 8 days (Inouye and Murakami 1975) and may not be observed until several days after cessation of dosing (Inouye and Murakami 1975; Magos et al. 1985). Histopathological examination of the nervous systems of affected rats has shown degeneration of cerebellar granule cells and dorsal root ganglia (Magos et al. 1980, 1985) and degenerative changes in peripheral nerves (Fehling et al. 1975; Miyakawa et al. 1974, 1976). Comparison of the effects of 5 doses of 8 mg Hg/kg/day as ethyl- or methylmercury showed dorsal root damage as well as flailing and hindlimb

crossing after exposure to both chemicals, but only methylmercury caused substantial cerebellar damage (Magos et al. 1985). Additional changes in rats exposed to methylmercury have also been observed. Rats exposed to a single gavage dose of 19.9 mg Hg/kg as methylmercuric chloride were found to have statistically significant differences in open-field tests, such as decreases in standing upright, area traversed, and activity, compared to controls. However, no accompanying histopathological changes were observed (Post et al. 1973). The exposed animals were also lethargic and ataxic initially, but symptoms disappeared within 2–3 hours. Changes in the phases of sleep were also observed in rats given 2 doses of 4 mg Hg/kg/day as methylmercuric chloride (Arito and Takahashi 1991). Paradoxical sleep was decreased and slow-wave sleep was increased. At a higher dose (12 mg Hg/kg/day for 2 days), circadian sleep patterns were also disrupted. Administration of a single dose of methylmercuric chloride (0.8 mg Hg/kg) produced blood-brain barrier dysfunction in rats (Chang and Hartmann 1972b) similar to that reported for inorganic mercury as discussed previously. In rabbits given 5.5 mg Hg/kg as methylmercuric acetate for 1–4 days, widespread neuronal degenerative changes (in cervical ganglion cells, cerebellum, and cerebral cortex) have been observed without accompanying behavioral changes (Jacobs et al. 1977).

Longer-duration studies in animals have shown qualitatively similar effects, but generally at lower daily doses with increasing durations of exposure. Rats given a dose of 10 mg Hg/kg as methylmercuric chloride once every 3 days for 15 days showed degeneration in the cerebellum with flailing and hind leg crossing (Leyshon and Morgan 1991). Rats given a TWA dose of 2.1 mg Hg/kg/day as methylmercury iodide or 2.4 mg Hg/kg/day as methylmercury nitrate by oral gavage for 29 days became weak and severely ataxic and developed paralysis of the hind legs (Hunter et al. 1940). Severe degeneration of peripheral nerves, posterior spinal roots, and trigeminal nerves were reported. Severe degenerative changes were also observed in the dorsal root fibers of rats given 1.6 mg Hg/kg/day as methylmercuric chloride for 8 weeks (Yip and Chang 1981). Similarly, ataxia (beginning the second week of exposure) and cerebellar edema and necrosis occurred in rats after 7 weeks of exposure by gavage to 1.68 mg Hg/kg as methylmercury dicyanidiamide for 5 days a week (Magos and Butler 1972). When rats were administered 0.8 mg Hg/kg/day as methylmercuric chloride by gavage for up to 11 weeks, effects similar to those reported for mercuric chloride (e.g., neuronal degeneration of the cerebellum and dorsal root ganglia and neurotoxic clinical signs) were seen but with increased severity (Chang and Hartmann 1972a).

Mice have shown comparable effects at similar doses. Mice exposed to 1.9 or 9.5 mg Hg/kg/day as methylmercury in the drinking water for 28 weeks exhibited degeneration of Purkinje cells and loss of granular cells in the cerebellum (MacDonald and Harbison 1977). At the higher of these doses, hind limb

paralysis was observed as early as 8 days, whereas at 1.9 mg Hg/kg/day, decreases in motor activity and hind limb paralysis did not develop until 24 weeks of exposure. Interestingly, cerebellar lesions were observed at 1.9 mg Hg/kg/day as early as 8 days after the start of dosing. Neuronal degeneration and microgliosis were observed in the corpus striatum, cerebral cortex, thalamus, and hypothalamus, accompanied by hind leg weakness, in mice administered 1 or 4 mg Hg/kg/day as methylmercuric chloride by gavage for 60 days (Berthoud et al. 1976). Similarly, a marked neurotoxic disturbance (not further identified) was reported in mice that received 3.1 mg Hg/kg/day as methylmercuric chloride in the diet for 26 weeks (Mitsumori et al. 1981). No effects of this type were observed in this study at 1.6 mg Hg/kg/day, but it is unknown whether more subtle neurological effects may have been missed, as the intent of this study was not to identify neurotoxic effects of methylmercury.

Some studies suggest that cats and monkeys are more sensitive to the neurotoxic effects of organic mercury than rodents. Cats fed tuna contaminated with methylmercury at doses equivalent to 0.015 mg Hg/kg/day for 11 months, starting when the cats were kittens, displayed degenerative changes in the cerebellum and the cerebral cortex (Chang et al. 1974). However, only 3 of 16 animals exhibited incoordination and weakness. Similarly, cats given gavage doses of methylmercuric chloride as low as 0.25 mg Hg/kg/day for 44–243 days displayed degenerative lesions in the granule and Purkinje cells of the cerebral cortex and/or cerebellum and degenerative changes in the white matter, but no manifestations of neurotoxicity (ataxia, loss of righting reflex, visual and sensory impairments) were observed until 0.5 mg Hg/kg/day was given (Khera et al. 1974). Neonatal monkeys given 0.5 mg Hg/kg/day as methylmercuric chloride in infant formula for 28–29 days exhibited stumbling and falling prior to termination of exposure (Willes et al. 1978). Despite the termination of exposure, abnormalities in the several reflexes; blindness; abnormal behavior consisting of shrieking, crying, and temper tantrums; and coma developed. Histopathological analyses showed diffuse degeneration in the cerebral cortex (especially the calcarine, insular, pre-, and postcentral gyri, and occipital lobe), cerebellum, basal ganglia, thalamus, amygdala, and lateral geniculate nuclei. Macaque monkeys exposed to methylmercuric chloride in biscuits exhibited tremors and visual field impairment (Evans et al. 1977). These effects were observed in animals that were first administered 4–5 priming doses of 1 mg Hg/kg at 5-day intervals (no toxicity observed), followed by "maintenance" doses of 0.5–0.6 mg Hg/kg once a week for 87–256 days. Squirrel monkeys developed similar symptoms after receiving a single priming dose of 1 or 2 mg Hg/kg as methylmercuric chloride by gavage, followed 77 days later by maintenance doses of 0.2 mg Hg/kg once a week for 90–270 days (Evans et al. 1977). The doses were adjusted to maintain steady-state blood mercury levels in the range of 1–4 ppm. No tremors or convulsions were observed in female monkeys (*Macaca fascicularis*) during a 150-day exposure

to methylmercury chloride at 0.04 mg Hg/kg/day (Petruccioli and Turillazzi 1991). However, beginning at 177–395 days after exposure to methylmercuric hydroxide at 0.08 mg Hg/kg/day, 6 of 7 female monkeys (*Macaca fascicularis*) exhibited slight tremors and decreased sucking responses, followed by claw-like grasp, gross motor incoordination, and apparent blindness (Burbacher et al. 1984, 1988). These effects were also observed in one animal from each of the lower-dose groups (0.04 and 0.06 mg Hg/kg/day) (Burbacher et al. 1988).

Miyama et al. (1983) attempted to correlate electrophysiological changes with "early" neurological signs in rats during dietary exposure to methylmercuric chloride for an unspecified period of time. They observed the following sequence in the onset of electrophysiological-somatic signs: fall in compound action potential > decrease in sensory nerve conduction velocity > tail rotation > weight loss. However, varying doses of selenium were co-administered with the methylmercury, complicating the interpretation of these results.

Evidence for a neurochemical component of methylmercury-induced toxicity following intermediate-duration exposures has been reported (Concas et al. 1983; Sharma et al. 1982; Tsuzuki 1981). A depression in the synthesis of the neurotransmitter, dopamine (whole-brain levels), was observed in the absence of clinical signs of neurotoxicity in rats fed doses as low as 0.8 mg Hg/kg/day as methylmercuric chloride once every 3 days for 15 days (Sharma et al. 1982). An increased number (but not an affinity) of benzodiazepine receptor binding sites and a decreased content of cyclic guanosine monophosphate (cGMP) were observed in the cerebellar cortex of rats administered 3.92 mg Hg/kg/day as methylmercuric chloride in the drinking water for 20 days (Concas et al. 1983). Activities of several enzymes associated with central neurotransmitter metabolism in the cerebellum (e.g., acetylcholinesterase, tryptophan hydroxylase, monoamine oxidase, catechol-*o*-methyltransferase) were depressed in rats administered 3.2 mg Hg/kg/day as methylmercury by gavage for 50 days (Tsuzuki 1981). These findings suggest that an alteration in neurotransmission may be one mechanism of action for mercury-induced neurotoxicity. However, the observed effects on the neurotransmitters may be secondary to other effects on the nervous system.

The chronic neurotoxic effects of methylmercury in animals are similar to those seen after intermediate exposure. Mice administered methylmercuric chloride in the diet for 2 years at approximately 0.6 mg Hg/kg/day showed posterior paralysis and sensory neuropathy, characterized by cerebral and cerebellar necrosis, as well as degeneration of spinal dorsal nerve roots and the sciatic nerve (Hirano et al. 1986; Mitsumori et al. 1990). Cats fed contaminated fish or contaminated fish and methylmercury at doses

as low as 0.046 mg Hg/kg/day for 2 years exhibited neurobehavioral toxic signs, including mild impairment of motor activity and diminished sensitivity to pain (Charbonneau et al. 1976). These effects began after 60 weeks of exposure but did not progress during the remainder of the 2 years of exposure. At higher doses of 0.074 and 0.18 mg Hg/kg/day, ataxia, alterations in gait, motor incoordination, muscle weakness, changes in temperament, and convulsions were also observed. Histopathological analyses showed neuronal degeneration in the motor, sensory, auditory, and occipital cortices and cerebellar granule cell degeneration. Five monkeys fed 0.05 mg Hg/kg/day as methylmercuric chloride from birth until the age of 3–4 years displayed impaired spatial vision at that time (Rice and Gilbert 1982). Continued dosing until 6.5–7 years of age resulted in clumsiness, decreased fine motor performance, and insensitivity to touch when tested at 13 years of age (Rice 1989c). Impaired high-frequency hearing was also displayed by these monkeys when tested at 14 years of age (Rice and Gilbert 1992). It is noteworthy that a wide range of neurotoxic symptoms (motor, visual, and auditory) were observed in a species similar to humans several years after dosing had ceased. No clinical signs or histopathological evidence of neurotoxicity was observed in rats that received 0.1 mg Hg/kg/day as methylmercuric chloride in the diet for 2 years (Verschuuren et al. 1976).

Deficiencies in many of the studies make it difficult to fully evaluate the quality of the data reported. General problems include the following: (1) many details of experimental protocols were omitted, thereby prohibiting an evaluation of the study's adequacy; (2) very often, only one dose was used, so an analysis of any possible dose-response relationships was not possible, and the possibility that certain observed effects were not compound-related cannot be excluded; (3) control data often were not presented; and (4) the results were frequently described in subjective terms, and no attempt was made to quantitate the data. Despite these limitations, animal studies do provide irrefutable evidence that the central and peripheral nervous systems are target organs for organic mercury-induced toxicity.

In summary, methylmercury is neurotoxic to humans and to several species of experimental animals following acute, intermediate, and chronic oral exposure. The major effects that are seen across the studies include motor disturbances, such as ataxia and tremors, as well as signs of sensory dysfunction, such as impaired vision. The predominant neuropathological feature is degenerative changes in the cerebellum, which is likely to be the mechanism involved in many of the motor dysfunctions. In humans, disruptions of higher functions have also been noted, as evidenced by depression and irritability.

The highest NOAEL values and all reliable LOAEL values for neurotoxic effects in each species and duration category are listed in Table 2-3 and plotted in Figure 2-3 for organic mercury.

2.2.2.5 Reproductive Effects

Inorganic Mercury. In an attempt to terminate her pregnancy, a 31-year-old woman ingested 30 mg Hg/kg as mercuric chloride in week 10 of her pregnancy (Afonso and deAlvarez 1960). Despite gastric lavage and treatment with dicapmerol, 13 days after exposure vaginal bleeding and uterine cramps occurred, followed by spontaneous abortion of the fetus and placenta. It was inconclusive whether the abortion was directly due to the mercury exposure.

Organic Mercury. No studies were located regarding reproductive effects in humans following oral exposure to organic mercury.

Abortions and decreased mean litter size are the predominant reproductive effects in different species of animals following oral exposure to organic mercury. Groups of 30 pregnant Fischer 344 rats were orally administered 10, 20, or 30 mg/kg as methylmercuric chloride dissolved in saline on Gd 7. Controls were given saline only (n=30). Maternal body weight gain and deaths were monitored. On Gd 20, the dams were laparotomized under ether anesthesia, and the fetuses were removed. Surviving fetuses were examined for gross toxic effects, sex, and weight; half were stained for skeletal examination. Mercury levels in maternal and fetal organs were measured. The LD_{50} of methylmercuric chloride for fetuses was calculated. Maternal body weights were decreased for 2 days in rats given 10 mg/kg, for 6 days in rats given 20 mg/kg, and were continuously decreased for rats given 30 mg/kg methylmercuric chloride. Survival rates of fetuses were 19.2, 41.4, and 91.1% less than controls for the 10, 20, and 30 mg/kg methylmercuric chloride groups, respectively. Implantation sites in the 3 groups decreased by 5.9, 13.7, and 22.5%, respectively, compared with controls. Preimplantation losses in the 3 groups were 17.2, 24.8, and 30.1%, respectively; postimplantation losses were 16.7, 34.1, and 88.9%, respectively. The reduction of litter weight was greatly enhanced with increasing methylmercuric chloride doses (32.3, 67.0, and 89.2%, respectively), presumably due to postimplantation loss, which already increased at high treatment levels. The LD_{50} of methylmercuric chloride for fetuses was determined to be 16.5 mg/kg. Mercury content in maternal organs was highest in the kidneys, followed by blood, spleen, liver, and brain, while in fetal organs it was highest in the liver (Lee and Han 1995).

Pregnant hamsters that received a single oral gavage dose of mercuric acetate on Gd 8 showed an increase in the incidence of resorptions at doses as low as 22 mg Hg/kg (Gale 1974). The incidence of resorptions was 35% at 22 mg Hg/kg, and increases were observed in a dose-related manner (53% at 32 mg Hg/kg, 68% at 47 mg Hg/kg, and 99% at 63 mg Hg/kg).

In a study by Khera (1973), after 5–7 days of oral gavage doses of 1, 2.5, or 5 mg Hg/kg/day as methylmercuric chloride, male rats were mated to unexposed female rats. A dose-related reduction of mean litter size was attributed to preimplantation losses from incompatibility of sperm-to-implantation events after mercury treatment of the parent male rat. At doses of 2 mg Hg/kg/day as methylmercury by gavage during Gd 6–9, pregnant Sprague-Dawley rats showed no differences in maternal body weight gain before parturition or in the body weights of the offspring (Fredriksson et al. 1996).

In male mice, no reduction in the incidence of fertile matings was observed after administration of 5–7 oral doses of up to 5 mg Hg/kg/day as methylmercuric chloride (Khera 1973). There was a significant dose-related decrease in the number of pups born per litter in mice receiving oral doses of 3, 5, or 10 mg Hg/kg administered on Gd 8 as methylmercuric hydroxide (Hughes and Annau 1976). Effects were not observed at 2 mg Hg/kg/day. Similarly, female mice administered 20 mg Hg/kg/day as methylmercuric chloride by gavage on Gd 10 had increased resorptions, decreased live fetuses per litter, and decreased numbers of fetuses per litter (Fuyuta et al. 1978). After guinea pigs were exposed to 11.5 mg Hg/kg as methylmercuric chloride by gavage on Gd 21, 28, 35, 42, or 49, half of the litters were aborted 4–6 days after treatment (Inouye and Kajiwara 1988). An increased rate of reproductive failure due to decreased conceptions and increased early abortions and stillbirths occurred in female monkeys exposed to 0.06 or 0.08 mg Hg/kg/day as methylmercury for 4 months (Burbacher et al. 1988). The menstrual cycle length was not affected at these dose levels. Reproductive effects were not observed in monkeys exposed to 0.04 mg/kg/day for the same duration.

Testicular functions were studied in monkeys *(M. fascicularis)* exposed to 0.025 or 0.035 mg Hg/kg/day as methylmercury by gavage for 20 weeks (Mohamed et al. 1987). The mean percentage of motile spermatozoa and the mean sperm speed were significantly decreased for both treatment groups compared to controls. Morphological examination of semen smears indicated an increased incidence of tail defects (primarily bent and kinked tails) in both exposed groups. No histopathological effects were evident on the testes. The study was limited because there were only three animals in each exposure group.

Testicular effects were also observed after chronic-duration exposure to methylmercuric chloride. Tubular atrophy of the testes was observed in mice ingesting 0.69 mg Hg/kg/day in their feed for up to 2 years (Mitsumori et al. 1990). Decreased spermatogenesis was observed in mice receiving 0.73 mg Hg/kg/day in the diet (Hirano et al. 1986). No adverse effects on the testes were observed in these studies at 0.14–0.15 mg Hg/kg/day. Similarly, no adverse effects were observed in the testes, prostate, ovaries, or uterus of rats exposed through the diet to 0.1 mg Hg/kg/day as methylmercuric chloride for 2 years (Verschuuren et al. 1976).

The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species and duration category are recorded in Table 2-3 and plotted in Figure 2-3 for organic mercury.

2.2.2.6 Developmental Effects

Inorganic Mercury. No studies were located regarding developmental effects in humans or animals following oral exposure to inorganic mercury.

Organic Mercury. When grains treated with fungicides containing mercury have been accidentally consumed or when fish with high levels of methylmercury have been eaten, epidemics of human mercury poisonings have occurred with high incidences of developmental toxicity. These episodes, as well as case reports from isolated incidences of maternal consumption of organic forms of mercury during pregnancy, have provided evidence that the developing nervous system of the fetus is highly sensitive to mercury toxicity. 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). In the 1955 mercury poisoning outbreak in Minamata, Japan, severe brain damage was described in 22 infants whose mothers had ingested fish contaminated with methylmercury during pregnancy (Harada 1978). The types of nervous system effects described in the Minamata outbreak included mental retardation; retention of primitive reflexes; cerebellar symptoms;

dysarthria; hyperkinesia; hypersalivation; atrophy and hypoplasia of the cerebral cortex, corpus callosum, and granule cell layer of the cerebellum; dysmyelination of the pyramidal tracts; and an abnormal neuronal cytoarchitecture. It has been suggested that the widespread damage involved derangement of basic developmental processes, such as neuronal migration (Choi et al. 1978; Matsumoto et al. 1965) and neuronal cell division (Sager et al. 1983).

Large-scale poisonings also occurred in Iraq in 1956 and 1960 (Bakir et al. 1973). Thirty-one pregnant women were victims of poisoning; 14 women died from ingesting wheat flour from seeds treated with ethylmercury *p*-toluene sulfonanilide (Bakir et al. 1973). Infants were born with blood mercury concentrations of 250 µg/100 mL and suffered severe brain damage. Similar cases of severe brain damage resulting from prenatal exposure to methylmercury were reported in an outbreak of methylmercury poisoning in Iraq occurring in 1971–1972 (Amin-Zaki et al. 1974). Attempts to correlate symptoms with exposure levels have shown that a dose-response relationship exists between the severity of the neurological symptoms in offspring and the maternal intake of methylmercury (as determined using analysis of hair for mercury content) (Cox et al. 1989; Marsh et al. 1980, 1981, 1987). Delays in walking and talking were more often associated with lower peak hair levels during pregnancy than were mental retardation and seizures (Marsh et al. 1981, 1987). These studies showed that the most severely affected children had been exposed to methylmercury during the second trimester of pregnancy. Male offspring were more severely affected than female offspring. Neurological abnormalities have also been observed among offspring of Cree Indians in Quebec, Canada, exposed to methylmercury in fish (McKeown-Eyssen et al. 1983).

A significant correlation was observed between male offspring with abnormal muscle tone or reflexes and maternal prenatal exposure (as determined using hair levels). An analysis of peak hair mercury levels during pregnancy in mothers exposed during the 1971–1972 outbreak in Iraq has led to an estimated population threshold of 10 ppm (highest value during pregnancy, for total mercury in hair) associated with delays in the onset of walking in infants (Cox et al. 1989). However, this estimated threshold for the Iraqi population depends heavily on the assumed background frequency for abnormal onset of walking time, as well as the threshold chosen to define onset of walking as abnormal. Furthermore, most of the positive responses (i.e., reported delays in onset of walking or talking) were observed for maternal hair levels above about 60 ppm. Only 3 of 24 children with positive responses were born to mothers with hair levels below 59 ppm. The peak total mercury hair levels during pregnancy for the mothers of those 3 children were 14, 18, and 38 ppm (WHO 1990). A maternal exposure level of 0.0012 mg/kg/day, corresponding to a hair

level of 14 ppm, was estimated for the Iraqi women using a simple, one-compartment pharmacokinetic model (see Section 2.4).

Davidson et al. (1995b) studied the effects of prenatal methylmercury exposure from a diet high in fish on developmental milestones in children living in the Republic of Seychelles (i.e., the Seychelles Child Development Study (SCDS). In this double blind study, children were evaluated with the Bayley Scales of Infant Development (BSID) at 19 months of age (n=738). The 19-month cohort represented 94% of the initially enrolled pairs. The cohort was evaluated again at 29 months (n=736) with the BSID and the Bayley Infant Behavior Record. Mercury exposure was determined by cold vapor atomic absorption analysis of maternal hair segments during pregnancy. The 29-month cohort represented approximately 50% of all live births in the year 1989. This particular study population was carefully selected based on the following reasons: (1) they regularly consume a high quantity and variety of ocean fish; (2) pre-study mercury concentration in maternal hair was in the appropriate range (<5 to >45 ppm) to study low-level exposure; (3) there is no local industry for pollution, and the Seychelles location is 1,000 miles from any continent or large population center; (4) the Seychellois population is highly literate, cooperative, and has minimal immigration; and (5) the Seychellois constitute a generally healthy population, with low maternal alcohol and tobacco use. The association between maternal hair mercury concentrations and neurodevelopmental outcomes at 19 and 29 months of age was examined by multiple regression analysis with adjustment for confounding variables. 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 Indexes at both 19 and 29 months were comparable to the mean performance of U.S. children. The mean BSID Psychomotor Scale Indexes at 19 and 29 months were 2 standard deviation units above U.S. norms, but consistent with previous findings of motoric precocity in children reared in African countries. No effect of mercury was detected on BSID scores at either age. On the Bayley Infant Behavior Record, activity level in boys, but not girls, decreased with increasing mercury exposure. The only subjective observation correlated with prenatal mercury exposure was a slight decrease in activity level in boys (but not girls) as determined by the Bayley Infant Behavior Record.

The overall study cohort was broken down into sub-groups based upon maternal hair mercury concentration as follows: $3 ppm (n=164), 4–6 ppm (n=215), 7–9 ppm (n=161), 10–12 ppm (n=97), and

>12 ppm (n=99). No significant or remarkable effect on the activity of the respective groups of children was observed outside the highest concentration group (i.e., maternal hair concentrations >12 ppm). When boys were examined separately, there appeared to be a trend toward decreased activity with increasing mercury concentrations, most visible above the group median value of 5.9 ppm. The mercury effect was highly significant in males (p=0.0004), but it was not statistically significant (p=0.87) in females. The activity level scores for males decreases 1/10 point (on a 9-point scale) for every ppm of mercury in maternal hair. While the activity score for the overall cohort was comparable to the mode of 5 for U.S. children, those children born to mothers with hair mercury levels of 20 ppm, males scored >1 point below the U.S. mode value. Scores of females remained at the comparable value for U.S. children, regardless of the magnitude of maternal hair mercury level. When the subjective activity scores for male and female children are evaluated collectively, no significant/remarkable decrease in activity is apparent outside the >12 ppm maternal hair concentration group. The affect on activity level in boys is not considered an adverse effect, and the 5.9 ppm level is categorized as a NOAEL. Since the children had been exposed *in utero*, they represent the most sensitive subpopulation.

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 11.0 (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. The 5.8 ppm NOAEL of this study is thus considerably below the one derived from the dose-response analysis of the data for the Iraqi methymercury poisonings (10 ppm).

The SCDS cohort continues to be monitored and evaluated for developmental effects. In an analysis of the latest round of outcome measures for children at age 66 months (n=708), Davidson et al. (1998) report no

adverse developmental effects associated with prenatal and postnatal exposure to methylmercury in fish at the levels experienced in this cohort. The actual exposure is reflected in a mean maternal hair level of 6.8 ppm for the prenatal exposure (SD=4.5, n=711, range, 0.5-26.7) and in a mean children's hair level of 6.5 ppm (SD=3.3, n=708, range, 0.9-25.8) 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. The authors conclude that 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 (PCB) levels. 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 SCDS 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.

Weihe et al. (1996) began a long-term evaluation of the health implications for people living in the Faroe Islands who are exposed to heavy metals and polychlorinated biphenyls (PCBs) from the consumption of fish and pilot whales. A birth cohort of 1,000 children was examined at approximately 7 years of age for neurobehavioral dysfunctions associated with prenatal exposure to mercury and PCB. Preliminary analyses of the data show that several neurobehavioral tests are associated with mercury exposure parameters. With emphasis on prenatal exposures to PCB, another cohort was generated during 1994–1995, and this cohort will be followed closely during the next years. In the Faroe Islands, marine food constitutes a considerable part of the diet. In addition to fish, both meat and blubber from pilot whales are included in the diet. Muscle tissue of pilot whales caught in the Faroe Islands contains an average mercury concentration of 3.3 µg/g (16 nmol/g), about half of which is methylmercury. In some years an evenly distributed annual catch of pilot whales would make the average dietary intake of mercury close to more than the Provisional

Temporary Weekly Intake of 0.3 mg recommended by WHO. In 1 of 8 consecutive births, the mercury concentration in maternal hair exceeded a limit of 10 µg/g, a level where neurobehavioral dysfunction in the child may occur. The maximum level was 39.1 µg/g. Mercury concentrations in umbilical cord blood showed a similar distribution with a maximum of 351 µg/L. The large variation in mercury exposure is associated with differences in the frequency of whale dinners. The average PCB concentration in pilot whale blubber is very high (about 30 µg/g). With an estimated daily consumption of 7 g of blubber, the average daily PCB intake could therefore exceed 200 µg (i.e., close to the Acceptable Daily Intake). In Scandinavia, the average daily PCB intake is about 15–20 µg.

In the continuation of this work, Grandjean et al. (1997b, 1998) studied 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 estimated from mercury concentrations in cord blood and maternal hair. At approximately 7 years of age, 917 of the children underwent detailed neurobehavioral examination. Neuropsychological tests included 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). 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. Mercury in cord blood, maternal hair (at parturition), child hair at 12 months, and child hair at 7 years of age were measured. The geometric average mercury concentrations were 22.9, 4.27, 1.12, and 2.99 µg/g, respectively. Mercury concentrations in cord blood were most closely associated with the concentrations in maternal hair at parturition and less so with children's hair at 12 months and 7 years. 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. The authors state that these associations remain after adjustment for covariates and after exclusion of children with maternal hair mercury concentrations above 10 µg/g (50 nmol/g). They further conclude that the effects on brain function associated with prenatal methylmercury exposure appear diverse, with early dysfunction in the Faroe Island population detectable at exposure levels currently considered to be safe.

In animals, there is evidence of developmental effects following oral exposure to organic mercury during gestation, lactation, and/or postweaning. Increases in several parameters indicative of developmental toxicity have been observed. Not all studies have examined neurological end points, but developmental neurotoxicity has been observed at very low exposure levels.

Methylmercuric chloride administered via gavage to pregnant rats at 8 mg Hg/kg on Gd 10 resulted in increased skeletal variations (incomplete fusion of the sternebrae) (Fuyuta et al. 1979). At higher doses, decreased fetal weight and increased malformations (cleft palate) were observed. Administration of lower doses of methylmercury (4 mg Hg/kg/day) for a longer duration of gestation (Gd 7–9 or 6–14) resulted in an increased incidence of rat fetuses with incomplete ossification or calcification (Nolen et al. 1972). The incidence of skeletal variations at 0.2 mg/kg/day was not significantly different from controls. Methylmercuric chloride administered to pregnant rats (n=10) via gavage at 2 mg Hg/kg/day throughout gestation (Gd 0–20) resulted in increased numbers of malformed fetuses (Inouye and Murakami 1975). The most common malformations were generalized edema and brain lesions. When methylmercuric chloride was administration to pregnant rats at 4 mg Hg/kg/day during Gd 7–14, there was a decreased fetal weight and an increased number of total malformations, hydrocephalus, and wavy ribs (Fuyuta et al. 1978). At 6 mg Hg/kg/day, increased resorptions, fetal deaths, cleft palate, generalized edema, brain lesions, absence of vertebral centra, and defects of the sternum were observed. Skeletal variations seen at 6 mg Hg/kg/day included absence of one or more sternebrae, bipartite sternebrae, and bilobed vertebral centra. Administration of a single dose of 24 mg Hg/kg as methylmercuric chloride to pregnant rats during Gd 6–12 resulted in decreased fetal weights and increased malformations (Inouye and Murakami 1975). The incidence of malformations (hydrocephalus, cleft palate, micrognathia, microglossia, generalized edema, subcutaneous bleeding, and hydronephrosis and hypoplasia of the kidneys) increased with later treatments (after Gd 7). Hydrocephalic brains had lesions in the brain mantle, corpus callosum, caudate putamen, and primordial cerebellum. Brains without hydrocephalus had lesions in similar brain areas, as well as dilation of the third ventricle and partial ablation of the ependymal lining.

Groups of 30 pregnant Fischer 344 rats were orally administered 10, 20, or 30 mg/kg methylmercuric chloride dissolved in saline on Gd 7. Controls were given saline only (n=30). Maternal body weight gain and deaths were monitored. Maternal body weights were decreased for 2 days in rats given 10 mg/kg as methylmercuric chloride and for 6 days in rats given 20 mg/kg and were continuously decreased for those given 30 mg/kg. Maternal death rates were 6.7, 16.7, and 30% in the 10, 20, and 30 mg/kg methylmercuric chloride dose groups; no control dams died. Survival rates of fetuses were 19.2, 41.4, and 91.1%

less than controls for the 10, 20, and 30 mg/kg methylmercuric chloride groups, respectively. The LD_{50} of methylmercuric chloride for fetuses was determined to be 16.5 mg/kg. The backbones of fetuses were severely curved at the high-dose level; mean fetal body lengths were reduced by 9.6, 21.7, and 48.8% in the 10, 20, and 30 mg/kg methylmercuric chloride groups, respectively, as compared to controls. Mercury content in maternal organs was highest in kidneys, followed by blood, spleen, liver, and brain, while in fetal organs it was highest in liver. Fetal liver and brain contained more mercury than maternal liver and brain; however, fetal kidneys retained less mercury than maternal kidneys. The fetal ossification center was not completely formed in sternebrae, particularly in the fifth and second bones, pelvic bones, and pectoral phalanges of fetuses in rats treated with 30 mg/kg methylmercuric chloride. The ossified lengths of skeletal bone stained with alizarin red S were developed least in the fifth sternebrae, metacarpals in the pectoral girdle, and ischium in the pelvic girdle, and were severely retarded in development as position of the ribs goes from the sixth bone (center) to the first and 13th bone (each edge) (Lee and Han 1995).

Four groups of 12 pregnant Sprague-Dawley rats were exposed to methylmercury or elemental mercury alone or in combination as follows: one group was administered 2 mg/kg/day methylmercury via gavage during Gd 6–9; another was exposed by inhalation to 1.8 mg/m^3 metallic mercury (elemental Hg) vapor for 1.5 hours per day during Gd 14–19; a third was exposed to both methylmercury by gavage (2 mg/kg/day, Gd 6–9) and elemental Hg vapor by inhalation (1.8 mg/m^3, Gd 14–19) (methylmercury + elemental Hg); a fourth group was given combined vehicle administration for each of the 2 treatments (control). The inhalation regimen corresponded to an approximate dose of 0.1 mg Hg/kg/day. Maternal body weights were monitored. At postpartum day 3, each litter was reduced to 4 male offspring. Body weight, pinna unfolding, tooth eruption, and eye opening were monitored. Testing of behavioral function was performed between 4 and 5 months of age and included spontaneous motor activity, spatial learning in a circular bath, and instrumental maze learning for food reward. Surface righting reflex and negative geotaxis were measured before weaning. There were no differences between any of the groups in maternal body weight gain or in body weight, pinna unfolding, tooth eruption, surface righting reflex, and negative geotaxis in offspring. Offspring of dams exposed to elemental mercury showed hyperactivity in the spontaneous motor activity test chambers over all three of the following parameters: locomotion, rearing, and total activity. This effect was potentiated in the animals of the methylmercury + elemental mercury group. In the swim maze test, the methylmercury + elemental mercury and elemental mercury groups evidenced longer latencies to reach a submerged platform, which they had learned to mount the day before, compared to either the control or methylmercury groups. In the modified, enclosed radial-arm maze, both the methyl-mercury + elemental Hg and elemental Hg groups showed more ambulations and rearings in the activity

test prior to the learning test. During the learning trial, the same groups (i.e., methylmercury + elemental mercury and elemental mercury) showed longer latencies and made more errors in acquiring all eight pellets. Generally, the results indicate that co-exposure to methylmercury, which by itself did not alter these functions at the dose given in this study, served to significantly enhance the effects of prenatal exposure to elemental mercury (i.e., alterations to both spontaneous and learned behaviors). Brain mercury concentrations in offspring were 1 ng/g w/w in the controls, 4 ng/g in the methylmercury group, 5 ng/g in the elemental mercury group, and 12 ng/g in the methylmercury + elemental mercury group (Fredriksson et al. 1996).

Pregnant Sprague-Dawley rats were treated by gavage with a single oral dose of 8 mg/kg of methylmercury chloride or saline on Gd 15. Within 24 hours after birth, litters were reduced to 6 pups per litter. Pups were weighed weekly and weaned 21 days after birth. Offspring of control and treated rats were killed at 14, 21, and 60 days of age. The binding characteristics of muscarinic receptors labeled in cortical membrane preparation by [3H]-L-quinuclidinyl benzilate were studied, and the mercury level in the same brain area was assessed. Total mercury content in cortical tissues was determined at 21 and 60 days of age. Furthermore, the performance in passive avoidance tasks was evaluated in 10 rats from each group at 8 weeks of age. No differences in mortality or weight gain were observed in methylmercury-exposed pups compared to controls. At 21 days of age, the level of mercury in the cortex was about 30 times higher in exposed rats than in controls (190.2 ng/g w/w versus 6.4 ng/g); at 60 days, mercury levels did not differ significantly (7.4 versus 5 ng/g, respectively). Perinatal exposure to methylmercury significantly reduced the maximum number of muscarinic receptors (Bmax) in the brain of 14-day-old (53%) and 21-day-old (21.3%) rats, while there was no notable difference in 60-day-old rats. This phenomenon seems to be strictly related to the presence of mercury in the cortex since it disappeared with the normalization of mercury levels in the brain. Despite the recovery of muscarinic receptor densities in methylmercury-exposed rats at 8 weeks of age, the avoidance latency was reduced in passive avoidance tests, indicating learning and memory deficits in these animals (Zanoli et al. 1994).

Similar effects were observed in mice exposed to organic mercury. Methylmercuric chloride administered orally by gavage to mice at 5 mg Hg/kg/day during Gd 6–17 resulted in 100% stillbirths or neonatal deaths and the failure of 6 of 9 dams to deliver, with no apparent maternal toxicity (Khera and Tabacova 1973). At lower doses (2 and 4 mg Hg/kg/day) for a shorter duration during gestation (days 6–13), no increase in deaths or resorptions was observed, but increases in malformations, skeletal variations, and delays in ossification were observed (Fuyuta et al. 1978). A higher dose of methylmercuric chloride (16 mg Hg/kg)

administered to mice by gavage on either Gd 10 or 12 resulted in decreased fetal weight, cleft palate, and dilation of the renal pelvis (Yasuda et al. 1985).

Thuvander et al. (1996) evaluated the immunomodulation of methylmercury from perinatal exposure in mice. Offspring from Balb/c mice were exposed to methylmercuric chloride in the diet. Dams (16.0±0.5g) were exposed to 0 (n=72), 0.5 (n=27), or 5 (n=37) mg Hg/kg for 10 weeks prior to mating, and during gestation and lactation. Pups were exposed to mercury until day 15 of lactation; thereafter, the pups were given control milk and control diet. Samples for mercury analysis were collected from the pups on days 22 and 50, and for immunological studies on days 10, 22, and 50. Immunological parameters included numbers of splenocytes and thymocytes, proportions of lymphocyte subpopulations within the thymus, the proliferative response of splenocytes to the B-cell mitogen LPS, NK-cell activity of splenocytes, and the primary antibody response to a viral antigen. Eight pups (n=8NS) were taken from at least three different litters for the immune function analysis.

No disturbances in the behavior of dams or pups were observed for any of the dose groups. All dams gave birth to normal sized litters (8–10 animals/litter). The high dose dams did have a small (4%) but significant increase in body weight (weeks 4, 5, 9 $p<0.05$, week 8 $p<0.01$). The exposure resulted in significantly increased total Hg concentrations in whole blood of offspring on day 22 and 50 from the 5 mg Hg/kg group (170 and 22 ng Hg/g blood in 5 mg/kg dose group compared to 7 and 5 ng Hg/g in control, respectively), and of offspring from the 0.5 mg Hg/kg group on day 22 (24 ng Hg/g compared to 7 ng Hg/g in control). On day 50, blood mercury levels in the 0.5 mg Hg/kg group had decreased to 11 ng Hg/g compared to 5 ng Hg/g in controls. Pups showed a decreased body weight (8%) in the 5 mg/kg group at 10 days of age. Significantly increased numbers of splenocytes were found only in offspring from the 0.5 mg Hg/kg group at 10 and 22 days, and increased number of thymocytes in the 0.5 mg/kg group at 22 days. Flow cytometry analysis of thymocytes revealed increased numbers and altered proportions of lymphocyte subpopulations within the thymus in offspring from both of the exposed groups at 22 days. The only sign of immunosuppression was a decrease in the proportion of CD4+ thymocytes at 10 days, but this was seen in both mercury groups so was probably not related to a decrease in body weight. The proliferative response of splenocytes to the B-cell mitogen LPS was increased in offspring from dams exposed to 5 mg Hg/kg, and the primary antibody response to a viral antigen was stimulated in pups from dams exposed to 0.5 mg Hg/kg. No significant differences were observed in the NK-cell activity of splenocytes except for a transient increase in activity at 22 days in the 5 mg/kg group at one of the two effector-to-target-cell ratios tested. The present results indicate that placental and lactational transfer of

low dose mercury affects thymocyte development and stimulates certain mitogen- or antigen-induced lymphocyte activities in mice. The authors note that these results, in the context of other studies where methylmercury was observed to have suppressive effects, suggests that methylmercury enhances immune function within a narrow dose range. The blood levels of mercury in the present study are close to the levels found in fish-eating populations. The authors note that the clinical relevance of slight stimulation of some immune functions is unknown, but the induction of autoimmunity by methylmercury can not be excluded.

Groups of guinea pigs exposed to a single dose of 11.5 mg Hg/kg as methylmercuric chloride at various times during gestation (66–69 days) showed differences in the manifestation of developmental neurotoxicity, depending on the period of development when exposure occurred (Inouye and Kajiwara 1988). Primarily developmental disturbances of the brain (e.g., smaller brains, dilated lateral ventricles, reduced size of hippocampus and nucleus caudate-putamen) occurred with exposures at 3, 4, or 5 weeks of pregnancy. Exposure during a later pregnancy stage (6 or 7 weeks) produced widespread focal degeneration of neurons in the neocortical region of fetal brains. In hamsters, methylmercuric chloride administered as a single dose of 8 mg on Gd 10 or of 1.6 mg Hg/kg/day on Gd 10–15 resulted in degeneration of cerebellar neurons in neonates (Reuhl et al. 1981a). Examination of offspring 275–300 days after birth showed similar degeneration (Reuhl et al. 1981b). It was not reported whether these histopathological changes correlated with behavioral changes.

Functional disturbances have also been observed following exposure to methylmercuric chloride during gestation. A single dose of 16 mg Hg/kg as methylmercuric chloride administered on Gd 13, 14, 15, 16, or 17 resulted in decreased spontaneous locomotor activity at 5 weeks of age, decreased righting response, abnormal tail position during walking, flexion, and crossing of the hindlimbs (Inouye et al. 1985). Histopathological examination of these animals showed dilated lateral ventricles, decreased caudate putamen, and a slightly simplified cerebellar pattern. Neonates in this study were cross-fostered within 24 hours after birth to prevent intake of mercury through the milk. The offspring of mice receiving 3, 5, or 10 mg Hg/kg/day as methylmercuric hydroxide on day 8 of gestation exhibited a decreased number of avoidances, an increased number of escapes, and an increased trials to reach the criterion on a 2-way avoidance task (Hughes and Annau 1976). No effects were present in the 2 mg Hg/kg dose group. Offspring of rats exposed to 4 mg Hg/kg/day as methylmercuric chloride on Gd 6–9 showed impaired swimming behavior, increased passiveness, and an increased startle response (Stoltenburg-Didinger and Markwort 1990). At 0.4 mg Hg/kg/day, the offspring showed an increased startle response, but at 0.04 mg

Hg/kg/day, no effects were observed. Exposure to 6.4 mg Hg/kg as methylmercuric chloride on Gd 15 resulted in decreases in spontaneous locomotor activity, increased sensitivity to pentylenetetrazol-induced convulsions, and a transient increase in γ-aminobutyric acid (GABA) and benzodiazepine receptors (Guidetti et al. 1992). Using the same exposure paradigm, shorter avoidance latency was observed in 14-, 21-, and 61-day-old rats (Cagiano et al. 1990). Glutamate receptor binding affinity and dopamine receptor number were also significantly affected in the brains of these offspring. Thus, multiple neurotransmitter systems may participate in the neurological effects observed.

A sensitive test for neurological effects of gestational exposure to methylmercury is operant behavioral performance (i.e., rewarded responses to total lever presses). Bornhausen et al. (1980) reported a significant reduction in operant behavioral performance in 4-month-old rat offspring exposed to methylmercuric chloride at 0.008 mg Hg/kg/day on Gd 6–9. A dose of 0.004 mg Hg/kg/day did not alter the behavioral performance of the offspring. No other studies have confirmed this result to date.

Pregnant hamsters received single oral gavage doses of 2.5–63 mg Hg/kg as mercuric acetate on Gd 8 (Gale 1974). Decreased crown-rump length was observed at 5 mg Hg/kg, although this effect did not increase linearly with the dose level. The incidence of resorptions increased at 22 mg Hg/kg and occurred in a dose-related manner. Other effects that occurred at higher dose levels included growth-retarded or edematous embryos. No significant developmental effects were evident at 2.5 mg Hg/kg.

Developmental neurotoxicity and changes in tissues, including the liver and immune system, have been observed in studies in which exposure occurred prior to gestation and/or was continued after gestation for intermediate durations. Retarded behavioral maturation (swimming behavior, righting reflexes) and learning disability (maze learning) were demonstrated in rat offspring receiving a diet of 0.1 mg Hg/kg/day (unspecified forms of mercury) in a contaminated fish diet from Gd 1 to postnatal day 42 (Olson and Boush 1975). Decreased performance in a paradigm intended to assess tactile-kinesthetic function (use of too much force) was observed in offspring of rats exposed to 0.08 mg Hg/kg/day as methylmercuric chloride for 2 weeks prior to mating through weaning (Elsner 1991). No morphological changes were observed in the brains of the offspring of maternal rats given 0.195 mg Hg/kg/day as methylmercuric chloride for 14 weeks prior to mating through postpartum day 50 (Lindstrom et al. 1991). However, norepinephrine levels in the cerebellum of offspring were significantly increased. Methylmercuric chloride at doses of 0.25 mg Hg/kg/day administered beginning several weeks prior to gestation resulted in an increase in the incidence of unilateral or bilateral ocular lesions in the neonates, associated with histological changes in the

Harderian, exorbital lachrymal, and parotid salivary glands (Khera and Tabacova 1973). No effects occurred at the lower dose of 0.05 mg Hg/kg/day. Fetal liver injury at the ultrastructural level (e.g., decreased mitochondrial volume density, enzyme activity, and protein synthesis in fetal hepatocytes) was reported after chronic exposure to low doses of 0.7–1.4 mg Hg/kg/day as methylmercury in the drinking water of rats for 1 month before mating and up to the end of pregnancy (Fowler and Woods 1977). The developing immune system was affected in newborn Sprague-Dawley rats exposed to 0.5 mg Hg/kg/day as methylmercury through the placenta and/or milk (Ilback et al. 1991). Results showed that exposure caused increased thymus lymphocyte activity in offspring exposed during gestation and lactation, while decreased spleen lymphocyte activities were observed in offspring exposed during lactation only. Cell-mediated cytotoxicity was decreased by 41% ($p<0.01$) in offspring exposed during gestation and lactation.

In chronic-duration studies, impaired visual function has been reported. Impaired visual recognition memory was reported for 50-to-60-day-old monkeys born to mothers that received 0.04 or 0.06 mg Hg/kg/day as methylmercury in apple juice for an average of 168 or 747 days prior to mating (Gunderson et al. 1988). In this study, neonates were separated from their mothers at birth to prevent intake of mercury while nursing. Impaired spatial visual function was observed in another study in which infant monkeys were exposed to 0.01, 0.025, or 0.05 mg Hg/kg/day as methylmercuric chloride throughout gestation, followed by gavage doses 5 days a week until 4–4.5 years of age (Rice and Gilbert 1990). The study was limited, however, because only 1–5 animals were tested at each dose level. Furthermore, two of the high-dose animals were unavailable for testing as a result of overt mercury intoxication, and thus the two most affected animals were eliminated. Slight changes in temporal discrimination were also observed in these monkeys at 2–3 years of age (Rice 1992). However, no LOAEL can be determined for this effect because results from monkeys at the mid- and high doses were pooled.

The highest NOAEL values and all reliable LOAEL values for developmental effects in each species and duration category are recorded in Table 2-3 and plotted in Figure 2-3 for organic mercury.

2.2.2.7 Genotoxic Effects

Several studies were located regarding genotoxic effects in humans after oral exposure to organic mercury. A positive correlation between blood mercury levels and structural and numerical chromosome aberrations was found in the lymphocytes of 23 people who consumed mercury-contaminated fish (Skerfving et al. 1974). However, several factors preclude acceptance of these findings as valid. With respect to the

increased incidence of structural aberrations, smokers were not identified, it was unclear whether chromatid and chromosome gaps were excluded from the evaluation, and significant effects were obtained only from lymphocyte cultures initiated several days after collection. The more reliable approach of initiating cultures on the day of collection did not yield significant results. Similarly, the evidence of aneuploidy is suspect. Considering the age of the subjects (54–89 years in the control group and 47–84 years in the exposure group), the average incidence of aneuploidy in the control (1.8%) and exposed (2.8%) groups was lower than would be expected, according to results indicating that aneuploidy in humans increases with age (Cimino et al. 1986). Skerfving et al. (1970) also reported that a significant ($p<0.05$) correlation was found between mercury concentrations and chromosome breaks in the lymphocytes of 9 subjects who had consumed fish contaminated with methylmercury. For reasons similar to those listed for the evaluation of the report by Skerfving et al. (1974), there is not yet a scientific basis to support an association between consumption of fish containing high methylmercury and clastogenesis in human lymphocytes. In addition, one of the test subjects was regularly medicated with isoproterenol, a known clastogen for mammalian cells. Although an increased incidence of sister chromatid exchange was reported in humans who ate mercury-contaminated seal meat (Wulf et al. 1986), data on smoking and consumption of other heavy metals (lead and cadmium) were not provided. Therefore, the possible relevance of the increase in sister chromatid exchanges (SCEs) cannot be determined. A statistical correlation between micronucleus frequency in peripheral blood lymphocytes and total mercury concentration in blood ($p=0.00041$), as well as between micronucleus frequency and age ($p=0.017$), was found in a population of fishers who had eaten mercury contaminated seafood (Franchi et al. 1994).

A single oral gavage administration of mercuric chloride to male Swiss albino mice (5 per group) at doses of 2.2, 4.4, or 8.9 mg Hg/kg induced a dose-related increase in the frequency of chromosome aberrations and the percentage of aberrant cells in the bone marrow (Ghosh et al. 1991). Chromatid breaks were the most common aberration. There was no clear evidence of unscheduled DNA synthesis (UDS) in lymphocytes harvested from male and female cats (3 per group) chronically exposed (39 months) to dietary concentrations of 0.0084, 0.020, or 0.046 mg Hg/kg/day as methylmercury (Miller et al. 1979). In a parallel study, significant increases in nuclear abnormalities were scored in bone marrow cells collected from the three treatment groups (5–8 cats per group); the response, however, was not dose-related. Signs of compound toxicity (slight neurological impairment and minimal central nervous system pathology) were seen in the high-dose group, but these animals yielded the lowest number of abnormal chromosome figures.

Other genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

Inorganic Mercury. No studies were located regarding cancer in humans after oral exposure to inorganic mercury.

Results of a 2-year National Toxicology Project (NTP 1993) study indicated that mercuric chloride may induce tumors in rats. Fischer 344 rats (60 per sex per group) received 0, 1.9, or 3.7 mg Hg/kg/day as mercuric chloride by gavage for 2 years. There were increases in the incidence of forestomach squamous cell papillomas and an increase in the incidence of thyroid follicular cell carcinomas in males in the 3.7 mg/kg group (NTP 1993). In $B6C3F_1$ mice exposed to 0, 3.7, or 7.4 mg Hg/kg/day as mercuric chloride, renal tubule tumors were evident in 3 of the 49 high-dose males (NTP 1993), but the incidence of these tumors was not significantly increased. There was no evidence of carcinogenicity in the exposed female mice. The cancer effect level (CEL) from this study is recorded in Table 2-2 and is plotted in Figure 2-2.

Organic Mercury. No studies were located regarding cancer in humans following oral exposure to organic mercury.

Significant increases in renal tumors have been observed in rodents exposed either to methylmercuric chloride or phenylmercuric acetate. Dietary exposure of both ICR and $B6C3F_1$ mice for 2 years has resulted in significant increases in renal epithelial cell tumors (Hirano et al. 1986; Mitsumori et al. 1981, 1990). At the highest dose of 0.69 mg Hg/kg/day (dose levels 0, 0.03, 0.14, 0.69), only male $B6C3F_1$ mice (n=60M,60F) showed significant increases in the incidence of renal epithelial cell adenomas and carcinomas (Mitsumori et al. 1990). No tumors were observed in the females $B6C3F_1$ mice exposed to up to 0.6 mg Hg/kg/day. The high dose in males and females also resulted in chronic nephropathy and regeneration of the proximal tubules (more severe in males). At 0.73 mg Hg/kg/day, male ICR mice showed significant increases in the incidence of epithelial cell adenocarcinomas (Hirano et al. 1986). Similar effects were observed in the ICR male mice at the highest dose of 1.6 mg Hg/kg/day (Mitsumori et al. 1981). No increase in tumor incidence was observed in rats exposed via the diet for 2 years to methyl-mercuric chloride at doses as high as 0.1 mg Hg/kg/day (Verschuuren et al. 1976).

Exposure of male Wistar rats to phenylmercuric acetate in the drinking water at 4.2 mg Hg/kg/day for 2 years resulted in a significant increase in renal cell adenomas (Solecki et al. 1991). However, this report

is limited because the assay was not intended as a carcinogenicity assay, and too few animals were used (20 per dose) to adequately assess the carcinogenicity of the phenylmercuric acetate.

No tumors or precancerous lesions were reported in rats administered 0.04–66.0 mg Hg/kg/day as phenylmercuric acetate in the diet for 2 years (Fitzhugh et al. 1950). As discussed above for mercuric acetate, no conclusions can be drawn from this study because of its limitations.

In a 2-year oral chronic-duration feeding study, no tumors or precancerous lesions were noted in rats administered mercuric acetate in the diet at doses of 0.2–66 mg Hg/kg/day (Fitzhugh et al. 1950); no conclusions could be derived on the carcinogenicity of mercuric acetate. The study was limited because the group sizes were small (10–12 rats per group); survival data were not reported; a considerable but unspecified number of rats reportedly died from pneumonia, which reduced the sensitivity of the study to detect a carcinogenic response; and only limited histopathological analyses were performed.

The CELs from these studies are recorded in Table 2-3 and plotted in Figure 2-3.

2.2.3 Dermal Exposure

Occupational exposure to both inorganic and organic mercury compounds may result in dermal as well as inhalation exposure to these chemicals. The results reported in Section 2.2.1 regarding the effects associated with occupational exposure to mercury-containing chemicals will not be repeated here. The studies discussed below concern reports in which dermal exposure was expected to be the primary route of exposure.

2.2.3.1 Death

Inorganic Mercury. A case study reported that a 27-year-old woman died 4 days after inserting an 8.75-g tablet of mercuric chloride (93 mg Hg/kg assuming 70-kg weight) into her vagina (Millar 1916). Another case study described the death of a man who had been receiving treatment for a wound with daily applications for approximately 2 months of a Chinese medicine containing mercurous chloride (Kang-Yum and Oransky 1992). The patient was reported to have died from renal failure.

An early study conducted by Schamberg et al. (1918) reported death in rabbits after an ointment containing 50% mercury was "rubbed" into the skin for 5 minutes; however, inadequate experimental methodology and an absence of study details prevent a determination of the amount of mercury involved.

Organic Mercury. No studies were located regarding death in humans or animals after dermal exposure to organic mercury.

2.2.3.2 Systemic Effects

No studies were located regarding respiratory, hematological, musculoskeletal, or hepatic effects in humans or animals after dermal exposure to inorganic or organic mercury.

Cardiovascular Effects

Inorganic Mercury. 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 (Warkany and Hubbard 1953).

No studies were located regarding cardiovascular effects in animals after dermal exposure to inorganic mercury.

Organic Mercury. No studies were located regarding cardiovascular effects in humans or animals after dermal exposure to organic mercury.

Gastrointestinal Effects

Inorganic Mercury. Patients who were hypersensitive to mercury (indicated by positive patch tests) developed stomatitis at the sites of contact with amalgam fillings (Veien 1990). The contact stomatitis faded when amalgam fillings were removed but persisted in a patient who chose to leave them in place. Abdominal pain, nausea, vomiting, and black stools were seen in a man who had been receiving treatment for a wound with daily applications for about 2 months of a Chinese medicine containing mercurous chloride (Kang-Yum and Oransky 1992). Anorexia was reported in a child who had been treated with an ammoniated mercury-containing ointment (Warkany and Hubbard 1953). Extensive necrosis, swelling, and

ulceration in the intestinal mucosa, vomiting, and diarrhea occurred in a woman who inserted a mercuric chloride tablet into her vagina (Millar 1916).

No studies were located regarding gastrointestinal effects in animals following dermal exposure to inorganic mercury.

Organic Mercury. No studies were located regarding gastrointestinal effects in humans or animals after dermal exposure to organic mercury.

Renal Effects

Inorganic Mercury. Congested medulla; pale and swollen cortex; and extensive necrosis, degeneration, and calcification of tubular epithelium were reported in the kidneys of a 27-year-old woman after inserting an 8.75-g tablet of mercuric chloride (93 mg Hg/kg assuming 70-kg weight) into her vagina (Millar 1916). Decreased renal output and renal failure were reported in a man who had been receiving daily applications for 2 months of a Chinese medicine containing mercurous chloride (Kang-Yum and Oransky 1992). A woman who used a depigmenting cream containing mercuric ammonium chloride for approximately 18 years developed an impaired renal function (Dyall-Smith and Scurry 1990). Similarly, a man who used an ointment containing ammoniated mercury for psoriasis for more than 10 years developed a nephrotic syndrome with severe edema (Williams and Bridge 1958). A study of young African women who used skin lightening creams containing ammoniated mercuric chloride for 1–36 months (average, 13 months) showed a nephrotic syndrome among a large portion of the women (Barr et al. 1972). The syndrome was characterized by elevated urinary protein, edema, decreased serum albumin, alpha-1-globulin, beta-globulin, and gamma globulin and increased alpha-2-globulin. Remission was observed in 77% of those who discontinued use of the creams.

No studies were located regarding renal effects in animals after dermal exposure to inorganic mercury.

Organic Mercury. No studies were located regarding renal effects in humans or animals after dermal exposure to organic mercury.

Endocrine Effects

No studies were located regarding endocrine effects in humans or animals after dermal exposure to inorganic or organic mercury.

Organic Mercury. No studies were located regarding endocrine effects in humans or animals after dermal exposure to organic mercury.

Dermal Effects

Inorganic Mercury. Contact dermatitis caused by acute, longer-term, or occupational inorganic mercury exposure has been described in a number of case reports (Bagley et al. 1987; Biro and Klein 1967; Faria and Freitas 1992; Goh and Ng 1988; Handley et al. 1993; Kanerva et al. 1993; Nordlind and Liden 1992; Pambor and Timmel 1989; Skoglund and Egelrud 1991; Veien 1990). Patch tests conducted in many of the cases show some cross-reactivity between various inorganic and organic forms of mercury (Faria and Freitas 1992; Handley et al. 1993; Kanerva et al. 1993; Pambor and Timmel 1989; Veien 1990). In these studies, dermal exposure occurred as a result of the breakage of mercury-containing thermometers or sphygmomanometers, dental amalgams containing elemental mercury, inoculation with vaccines containing merthiolate preservatives, or mercuric sulfide in tattoos. One report of contact dermatitis caused by a mercuric sulfide-containing tattoo suggested that the reaction was not to mercuric sulfide itself but to a mercury derivative that was formed in the skin (Biro and Klein 1967).

Excluding reports of contact dermatitis, limited information was obtained regarding the dermal effects of inorganic mercury. Application of an ammoniated mercury ointment to the skin of children or exposure to diapers that had been rinsed in a mercuric chloride-containing solution resulted in itching, flushing, swelling, and/or desquamation of the palms of the hands and soles of the feet (Warkany and Hubbard 1953). In addition, rashes, conjunctivitis, and/or excessive perspiration were observed. These dermal 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).

Application of a 1% solution of ammoniated mercuric chloride to the skin resulted in only minor irritation in 2 of 11 exposed subjects (Kawahara et al. 1993). After 18 years of using a mercury-containing cream, a

patient exhibited blue-black pigmentation in a perifollicular distribution on the chin and glabella (Dyall-Smith and Scurry 1990). A skin biopsy revealed black nonrefractile granules in the cytoplasm of macrophages in the papillary dermis and around the upper part of hair follicles. A boy who broke a thermometer in his mouth developed a mass consisting of hyperplasia of the epidermis, necrosis, and ulceration (Sau et al. 1991). This effect may have resulted from a combined effect of the physical injury and the mercury metal.

No studies were located regarding dermal effects in animals after dermal exposure to inorganic mercury.

Organic Mercury. Case report studies suggest that dermal exposure to methylmercury or phenylmercury in humans can cause rashes and blisters on the skin (Hunter et al. 1940; Morris 1960). A 33-year-old male worker exposed to methylmercury nitrate dust for 2 years developed burns and blisters on his forearm (Hunter et al. 1940). These effects healed within 9 days. Sensitivity to phenylmercuric salts is shown by individuals who developed itchy, pruritic, papular eruptions or rashes on their skin following acute dermal exposure (Morris 1960). A 54-year-old woman with a family history of atopy was found to display erythema (at 30 minutes postexposure) and urticaria (at 60 minutes) when treated topically with a 0.01% solution of phenylmercuric acetate (Torresani et al. 1993). This positive reaction was associated with aggravation of facial edema and an attack of bronchospasm. The woman, who was a farmer, was believed to have been previously exposed to phenylmercuric acetate during contact with pesticides and herbicides used on farm crops.

No studies were located regarding dermal effects in animals following dermal exposure to organic mercury.

Ocular Effects. No studies were located regarding ocular effects in humans or animals after dermal exposure to inorganic or organic mercury.

Body Weight Effects. No studies were located regarding body weight effects in humans or animals after dermal exposure to inorganic or organic mercury.

2.2.3.3 Immunological and Lymphoreticular Effects

Inorganic Mercury. As indicated above, contact dermatitis may develop as a result of acute or occupational exposure to inorganic mercury (Anneroth et al. 1992; Bagley et al. 1987; Biro and Klein

1967; Faria and Freitas 1992; Goh and Ng 1988; Nordlind and Liden 1992; Pambor and Timmel 1989; Skoglund and Egelrud 1991; Veien 1990). Patch tests conducted in many of the cases show some cross-reactivity between various inorganic and organic forms of mercury (Faria and Freitas 1992; Pambor and Timmel 1989; Veien 1990). In these studies, dermal exposure occurred as a result of the breakage of mercury-containing thermometers or sphygmomanometers, dental amalgams containing elemental mercury, or mercuric sulfide in tattoos. One report of contact dermatitis caused by mercuric sulfide in a tattoo suggested that the reaction was not to mercuric sulfide itself but to a mercury derivative that was formed in the skin (Biro and Klein 1967).

No studies were located regarding immunological or lymphoreticular effects in animals following dermal exposure to inorganic mercury.

Organic Mercury. No studies were located regarding immunological or lymphoreticular effects in humans or animals after dermal exposure to organic mercury.

2.2.3.4 Neurological Effects

Inorganic Mercury. DeBont et al. (1986) described a 4-month-old boy who had signs of acrodynia accompanied by coma, paralysis of one side of the body, generalized muscle stiffness, and muscular tremors 12 days after he was treated with yellow mercuric oxide ointment for eczema. Topical application of a depigmenting cream containing 17.5% mercuric ammonium chloride for 18 years resulted in mild tremors, anxiety, depression, and paranoid delusions in a 42-year-old woman (Dyall-Smith and Scurry 1990). Children who were treated with an ointment containing ammoniated mercury or who were exposed to diapers that had been rinsed in a mercuric chloride-containing solution experienced irritability, fretfulness, and sleeplessness (Warkany and Hubbard 1953).

No studies were located regarding neurological effects in animals after dermal exposure to inorganic mercury.

Organic Mercury. No studies were located regarding neurological effects in humans or animals after dermal exposure to organic mercury.

No studies were located regarding the following effects in humans or animals after dermal exposure to inorganic or organic mercury:

2.2.3.5 Reproductive Effects

2.2.3.6 Developmental Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

No studies were located regarding cancer in humans or animals after dermal exposure to inorganic or organic mercury.

2.3 TOXICOKINETICS

Absorption is high (approximately 70–80%) for inhaled metallic mercury vapor, and negligible for oral exposure to liquid metallic mercury. Absorption of inorganic mercuric salts may range from 2 to 38% depending upon the form and test conditions. Oral absorption of organic mercury is nearly complete, but respiratory absorption data are lacking, particularly for the alkyl mercurials.

The distribution data for metallic, inorganic, and organic mercury are consistent in identifying the kidney as the organ with the highest mercury bioaccumulation. Because of its high lipophilicity, metallic mercury can also be transferred readily through the placenta and blood-brain barrier. The oxidation of metallic mercury to inorganic divalent cation in the brain can result in retention in the brain. Inorganic mercury compounds can reach most organs; however, their low lipophilicity reduces their ability to penetrate barriers to and accumulate in the brain and fetus. The distribution of methylmercury is similar to that of metallic mercury; a relatively large amount of mercury can accumulate in the brain and fetus (compared to inorganic mercury) because of its ability to penetrate the blood-brain and placental barriers and its conversion in the brain and fetus to the inorganic divalent cation.

Metallic mercury can be oxidized to inorganic divalent mercury by the hydrogen peroxidase-catalase pathway, which is present in most tissues. The inorganic divalent cation can, in turn, be reduced to

metallic mercury. The mercurous ion is unstable in the presence of sulfhydryl groups, and undergoes disproportionation into one atom of metallic mercury and one ion of mercuric mercury. As with metallic mercury, organic mercury can also be converted to inorganic divalent mercury; however, the extent of conversion is less than with metallic mercury.

Following exposure to metallic mercury, the elimination of mercury can occur via the urine, feces, and expired air. Following exposure to inorganic mercury (mercuric), mercury is eliminated in the urine and feces. Organic mercury compounds are excreted predominantly via the feces in humans. In animals, methylmercury is excreted in the feces, and phenylmercury compounds are initially excreted in the feces and then in the urine. Organic mercury compounds are excreted predominantly in the inorganic form. Both inorganic mercury and methylmercury are excreted in breast milk.

Absorption of metallic mercury vapor is believed to occur by rapid diffusion through the lungs. Oral absorption of inorganic mercuric mercury compounds may also involve rapid diffusion through the gastro-intestinal tract. The mechanism for oral absorption of mercurous mercury compounds is not known. Oral absorption of organic mercury is believed to depend on the ability of the organic mercury molecule to bind to molecules such as cysteine. The mechanism of action of inorganic and organic mercury compounds may involve the affinity of these chemicals for sulfhydryl or thiol groups of proteins and other biological compounds.

2.3.1 Absorption

Absorption following inhalation of metallic mercury vapors is relatively high. Absorption following acute oral exposure to metallic mercury is negligible in both humans and animals. Methyl- and phenylmercury compounds are absorbed much more readily than inorganic mercury. Animal studies suggest oral absorption of both organic and inorganic mercury may be influenced by age and diet. Limited information was located regarding dermal absorption of inorganic or organic mercury compounds in humans or animals.

2.3.1.1 Inhalation Exposure

Metallic and Inorganic Mercury. There are limited quantitative data on the absorption of metallic mercury vapor by humans after inhalation exposure, although it is the most common route of inorganic mercury uptake. Metallic mercury is highly lipophilic, and absorption of the inhaled vapor, followed by rapid diffusion across the alveolar membranes of the lungs into the blood, has been reported to be substantial. Exposure to 0.1–0.2 mg/m^3 elemental mercury vapor resulted in approximately 74–80% of inhaled elemental mercury vapor being retained in human tissues (Hursh et al. 1976; Teisinger and Fiserova-Bergerova 1965). Indirect evidence of rapid absorption was provided by elevated mercury levels found in red blood cells, plasma, and excreta of 5 volunteers who inhaled radiolabeled mercury for 14–24 minutes (Cherian et al. 1978). Elevated blood levels of mercury were also observed in humans following a brief occupational exposure (3 days) to less than 0.1 mg/m^3 metallic mercury vapor (Barregard et al. 1992).

Recently, Sandborgh-Englund et al. (1998) evaluated the absorption, blood levels, and excretion of mercury in humans after a single dose of mercury vapor. Nine healthy volunteers (2M, 7F) were exposed to 400 µg Hg/m^3 mercury vapor in air (median 399 µg Hg/m^3; range, 365–430 µg Hg/m^3) for 15 minutes. This dose corresponded to 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. During the first 3 days after exposure 7.5–12% of the absorbed dose was lost by exhalation, with the median half-time of Hg in expired breath being 2 days. In blood and plasma, a rapid absorption phase of Hg was seen, followed by a biexponential decline of the curves in both media. A substantial interindividual variation was observed in the area under the concentration-time curves of Hg in blood and plasma. In plasma, the median half-time of the second phase was 10 days. About 1.0% of the absorbed Hg was excreted via the urine during the first 3 days after exposure whereas the estimated amount excreted during the 30 days ranged from 8 to 40%. In order to evaluate the chronic exposure to mercury from dental amalgam in the general population, the daily Hg dose from the 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 of amalgam fillings.

There are few reports regarding the respiratory absorption of elemental and inorganic mercury compounds in animals. Elevated levels of mercury were detected in blood and tissues of pregnant or nursing guinea pigs after short-term exposure (2–2.5 hours) to metallic mercury vapors (6–10 mg/m^3) (Yoshida et al.

1990, 1992). Following repeated exposure (5 weeks) of rats to mercury vapor (1 mg/m^3), high levels were detected in the blood and brain (Warfvinge et al. 1992). The absorption of inorganic divalent mercury has not been measured, but it is estimated to be approximately 40% in dogs (Morrow et al. 1964).

Organic Mercury. No studies were located regarding absorption in humans or animals after inhalation exposure to compounds of phenyl- or methylmercury. However, indirect evidence indicates organic mercury can be absorbed readily through the lungs. Following inhalation of ^{203}Hg-labeled dimethyl-mercury, radioactivity was excreted within 6 hours, followed by a slower excretion phase (Ostlund 1969).

2.3.1.2 Oral Exposure

Metallic and Inorganic Mercury. Few studies in humans were located regarding absorption of ingested metallic or inorganic mercury. For metallic mercury, ingesting small amounts such as contained in a standard thermometer (about 0.1 mL or about 1 g) does not produce symptoms of intoxication (Wright et al. 1980). Reports of ingestion of substantial amounts of elemental mercury indicate that absorption is negligible (Sue 1994; Wright et al. 1980). Two case histories were identified on acute effects of relatively large ingestions of metallic mercury. The first case history was described an ingestion of 15 mL (204 g) of metallic mercury by a 17-year-old male storekeeper who swallowed mercury from the pendulum of a clock (apparently out of curiosity rather than as a suicide attempt). On admission, and 24 hours later, he was symptom free, and physical examination was normal. The patient had no gastrointestinal symptoms, and was treated with a mild laxative and bed rest. The results of serial daily urine mercury estimates were normal (all less than 15 µg), and did not suggest significant absorption. The radiological investigation illustrated a characteristic pattern of finely divided globules of mercury in the gastrointestinal tract (Wright et al. 1980).

A second and massive incidence of ingestion involved a 42-year-old man who had spent much of his life (since the age of 13) repairing instruments that contained mercury. He intentionally ingested an estimated 220 mL (about 3,000 g) of mercury (Lin and Lim 1993). Upon admission, the patient presented with significantly elevated mercury blood levels (103 µg/L, normal <10 µg/L) and urine levels (73 µg/L, normal <20 µg/L). It is not known how much the occupational exposure had contributed to these levels. The patient was treated with immediate gastric lavage and cathartics. He also received D-penicillamine 1 g/day orally for 7 days. Blood and urine mercury levels obtained 3 days after chelation therapy were 116.9 and 22.9 µg/L, respectively. By 2 weeks postexposure, most of the mercury had been excreted in the feces and

was measured at a total volume of 220 mL (this number was used to estimate the amount initially ingested). The patient was lost to follow-up for 6 months, but at 10 months following the incident, blood mercury had decreased to 1 µg/L and urine mercury to µg/L.

Approximately 15% of a trace dose of mercuric nitrate in an aqueous solution or bound to calf liver protein was absorbed by the gastrointestinal tract of humans (Rahola et al. 1973). The mercurous ion demonstrated limited absorption. No information was located regarding the percentage of absorption of mercuric chloride by the gastrointestinal tract of humans. However, an extremely high serum inorganic mercury concentration (116.5 nmol/mL) was found in a woman who ingested a potentially lethal dose of powdered mercuric chloride (13.8 mg Hg/kg) (Suzuki et al. 1992). Similarly, no information was located regarding the percentage of absorption of mercuric sulfide by the gastrointestinal tract in humans. However, elevated mercury was detected in the urine of two subjects who ingested an unspecified amount of mercuric sulfide (Yeoh et al. 1989).

A number of animal studies indicate absorption of inorganic mercury in the 10–30% range. In earlier studies, absorption rate was reported as low. Only 1–2% of an orally administered dose of mercuric chloride was absorbed in mice (Clarkson 1971). In rats, using whole-body retention data, estimated mercuric chloride absorptions of 3–4, 8.5, and 6.5% were calculated for single oral doses of 0.2–12.5, 17.5, and 20 mg/kg, respectively (Piotrowski et al. 1992). More recent studies using whole-body retention data, however, indicate absorption of 20–25% calculated from single oral doses of 0.2–20 mg Hg/kg as mercuric chloride in mice. Comparison was made of retention data after oral and intraperitoneal dosing, taking excretion and intestinal reabsorption into account (Nielsen and Andersen 1990). In a subsequent study, the whole-body retention of mercury after mercuric chloride administration was observed to initially decline rapidly, indicating incomplete intestinal absorption (Nielsen and Andersen 1992). Mercury was rapidly cleared from the gastrointestinal tract (to <30% of the initial dose within 2 days), and relative carcass retention increased throughout the experimental period, reaching levels around 40% of initial whole-body retention. Blood levels of mercury were closely correlated to whole-body retention of mercury during the first 3 days after administration of mercuric chloride (1 mg Hg/kg). After the initial 3 days, the amount of mercury in the blood declined more rapidly than the whole-body burden.

Morcillo and Santamaria (1995) report absorption of 30–40% for radiolabeled mercuric chloride when administered in drinking water at 5, 50, and 500 µM Hg for 8 weeks to male rats. The percentage of total

mercury excreted by the fecal route was significantly lower in the 500 compared to the 5 and 50 μM Hg group.

The rate of oral absorption of mercuric mercury compounds in rats is dependent on several factors (e.g., intestinal pH, compound dissociation) (Endo et al. 1990). Age and diet also can influence the extent of absorption in mice (Kostial et al. 1978). One-week-old suckling mice absorbed 38% of the orally administered mercuric chloride, whereas adult mice absorbed only 1% of the dose in standard diets. When the adult mice received a milk diet instead of the standard diet, absorption increased to 7% of the administered dose (Kostial et al. 1978).

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 mercury as 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. Furthermore, the relative bioavailability of mercuric sulfide in humans has not been examined.

Organic Mercury. Organic mercury compounds are more readily absorbed by the oral route than inorganic mercury compounds. Based on retention and excretion studies in humans, approximately 95% of an oral tracer dose of aqueous methylmercuric nitrate was absorbed (Aberg et al. 1969). Absorption of mercury was also reported in studies in which volunteers received doses of methylmercury bound to protein (Miettinen 1973) or ate bread contaminated with a fungicide that contained methylmercury (Al-Shahristani et al. 1976); however, no quantitative data regarding the percentage of absorption were available.

In vitro evidence suggests that organic mercury is also readily absorbed in the gastrointestinal tract and that methylmercuric chloride is absorbed to a greater extent than phenylmercuric chloride (Endo et al. 1989). Complexing of methylmercury with nonprotein sulfhydryls also may play a role in intestinal absorption and reabsorption (Urano et al. 1990). Phenylmercuric salt in the diet was completely absorbed in mice (Clarkson 1972a) and readily absorbed in rats following long-term oral administration (Fitzhugh et al. 1950). Absorption was nearly complete within 6 hours after female cynomolgus monkeys were given 0.5 mg Hg/kg as methylmercuric chloride by gavage (Rice 1989b). Following a single oral exposure

(1 mg/kg) of methylmercuric chloride, the level of mercury in the blood of mice declined slowly. At day 14 post-dosing, the blood level was still around 25% of the value at day 1 (Nielsen 1992). Blood levels of mercury were closely correlated to whole-body retention of mercury during the first 3 days after administration of methylmercuric chloride (1 mg Hg/kg) (Nielsen and Andersen 1992). However, at later times after administration, the amount of mercury in the blood declined more rapidly than the whole-body burden. The gastrointestinal retention of mercury slowly decreased in mice given organic mercury. This phenomenon is probably the result of biliary excretion and reabsorption of mercury (Nielsen and Andersen 1992).

Bioavailability of methylmercury in food. Measurements of absorption and toxicity have generally been made using aqueous solutions of methylmercury. The absorption and bioavailability of methylmercury in food, specifically fish and bread, may be affected by dietary components. Potential confounders that may affect bioavailability of methylmercury are dietary phytate and other dietary fibrous materials found in bread and the complexation of methylmercury with selenium in fish.

Dietary fiber and phytate. Dietary fiber and phytate are known as potential inhibitors of the absorption of divalent cations; however, the literature regarding the effect of dietary fiber and phytate on the bioavailability of minerals is contradictory. Data by Yannai and Sachs (1993) indicate that phytate does not affect methylmercury absorption. Yannai and Sachs (1993) compared the absorption by rats of mercury found intrinsically in experimental fish meal with and without added phytate and found no significant differences in the absorption of Hg (93±5%) between 2 experimental fish meal diets (containing 1.4 µmol Hg/kg diet), with or without added sodium phytate. The authors speculated that phytate might be preferentially bound to zinc, iron, and copper, which were present at much higher concentrations in the diet.

In another experiment by Yannai and Sachs (1993), the absorption of mercury was reduced when rats were fed a mercury-contaminated corn diet and corn silage meal. Mercury was incorporated intrinsically into the corn diet using radioactive isotopes (^{203}Hg) infused by capillary action into the stalks of developing corn plants, which then incorporated trace amounts of isotopes into developing kernels. The corn silage meal was from a crop grown in the vicinity of an industrial zone and contained elevated amounts of mercury. Reduced absorptions of 48 and 51% were found for the corn silage and corn diet experiments, respectively.

The reduced bioavailability of the plant food diet compared with the animal-based diet (fish meal) may be due to the presence of indigestible fibrous materials present in plants. Another factor that might affect absorption is the form of mercury (^{203}Hg and methylmercury in the corn and fish meal diets, respectively). The experiments by Yannai and Sachs (1993) are different from other absorption experiments because mercury was intrinsic to the fish, grain, or silage, while in other studies mercury is simply mixed with the experimental diet, usually as mercury salts. In the Iraqi epidemic, methylmercury fungicide was applied extrinsically to wheat that was made into bread. However, no studies were located that measured the absorption of methylmercury when mixed with grain. It is also not known whether the putative component(s) of grain affecting bioavailability are the same in corn and wheat.

Interaction with selenium in diet. The co-administration of methylmercury and selenium is known to depress methylmercury toxicity (Cuvin-Aralar and Furness 1991; Imura and Naganuma 1991). Furthermore, the level of selenium in human hair has been found to negatively correlate with the level of mercury in brain tissue (Suzuki et al. 1993). Methylmercury forms a bismethylmercury selenide complex. Selenium in foods (especially fish) may also complex with methylmercury and, therefore, may potentially reduce the bioavailability of methylmercury. The available data indicate that neither methylmercury uptake nor bioavailability is affected by its presence in fish. Experimental studies on the metabolism of methyl-mercury in humans following oral ingestion 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 added to the homogenate, of methylmercury 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 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 2 highest dose groups (0.074 and 0.176 mg Hg), at 100 weeks of exposure no significant differences were seen in total mercury concentrations in 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 the nervous system tissue or other tissues (renal cortex, renal medulla, liver, spleen, adrenal, bladder, atria, ventricle, ovaries, testes, muscle) between the 2 highest dose groups receiving the dose as methylmercuric chloride or as contaminated fish at the same dose level.

2.3.1.3 Dermal Exposure

Metallic and Inorganic Mercury. Hursh et al. (1989) conclude that dermal absorption of mercury vapor poses a very minor occupational hazard compared to inhalation exposure. They measured dermal absorption of radiolabeled metallic mercury vapor in five human volunteers, using arm skin as representing the whole body skin. About half of the mercury taken up was shed by desquamation of epidermal cells during the following several weeks. The remainder was slowly and diffusely released into the general circulation in contrast to the rapid release and more focal release from the lungs. When absorption for the total skin area (as represented by the forearm skin) was compared with the inhalation route for the same ambient concentration, the dermal route absorbed was estimated at 2.6% of the amount absorbed by the lung.

There was no information found on the dermal absorption of liquid metallic mercury, but unless the skin surface was damaged or the contaminated surface was occluded, it would not be expected to be high (i.e., in light of the very low absorption rate from the gastrointestinal tract). On the other hand, sloughing from the gastrointestinal tract may account for the low rate of absorption.

Indirect evidence of dermal absorption is provided by clinical case studies in which mercury intoxication was reported in individuals following dermal application of ointments that contained inorganic mercury salts (Bourgeois et al. 1986; DeBont et al. 1986).

Absorption of mercurous salts in animals can occur through the skin (Schamberg et al. 1918); however, no quantitative data are available. The rate of absorption for mercuric chloride was not evaluated in any study. However, skin biopsies taken from 2 to 96 hours after application of a 0.1% solution of mercuric chloride showed electron-dense deposits, tentatively identified as mercury, in the cells in the dermis, indicating that mercuric chloride could penetrate the outer layer of the skin (Silberberg et al. 1969).

Organic Mercury. No information was identified for absorption of methylmercury via dermal absorption. There is extremely important hazard assessment information on the dermal absorption of dialkylmercurials. A case history indicates nearly complete absorption of dimethylmercury through the skin resulting in a highly toxic exposure pathway. The exposure occurred to a 48-year-old female chemistry professor who was admitted to the hospital 5 months (154 days) after she inadvertently spilled several drops (estimated at 0.4–0.5 mL) of dimethylmercury from the tip of her pipette onto the back of her disposable latex gloves

(Blayney et al. 1997; Nierenberg et al. 1998). The spill was cleaned and the gloves disposed of. Hair analysis on a long strand of hair revealed that, after a brief lag time, mercury content rose rapidly to almost 1,100 ppm (normal level <0.26 ppm, toxic level >50 ppm), and then slowly declined, with a half-life of 74.6 days. These results support the occurrence of one or several episodes of exposure, and are consistent with laboratory notebook accounts of a single accidental exposure. Testing of family members, laboratory coworkers, and laboratory surfaces also failed to reveal any unsuspected mercury spills or other cases of toxic blood or urinary mercury levels. Permeation tests subsequently performed on disposable latex gloves similar to those the patient had worn at the time of the lone exposure revealed that dimethylmercury penetrates such gloves rapidly and completely, with penetration occurring in 15 seconds or less and perhaps instantly. Severe neurotoxicity developed 5 months postexposure and the patient died 9 months postexposure. The mercury content of hair, blood, and urine were monitored from 5 months postexposure (i.e., following admission of the patient to the hospital) until the patient died. Based on the half-lives and kinetics of mercury in the body, the hair and blood levels were used to estimate the total body burden and the amount of the initial acute dermal dose. They determined that a dose of 0.44 mL of liquid Dimethylmercury (about 1,344 mg), if completely absorbed, would have been sufficient to have produced the levels observed in the patient. This amount is in good agreement with the patient's account and the laboratory records on the amount spilled. Some inhalation exposure, however, could also have occurred during the cleanup of the spill, so this finding needs additional confirmation.

Infants exposed to diapers that had been treated with a phenylmercury fungicide exhibited higher urinary levels of mercury than unexposed infants (Gotelli et al. 1985). In rats, dermal absorption of phenylmercuric acetate from the vaginal tract was 75% of the dose within 8 hours after administration (Laug and Kunze 1949).

2.3.1.4 Other Routes of Exposure

There is some information on the subcutaneous injection of metallic mercury. Schwarz et al. (1996) describe a case history of a female nurse who accidentally plunged a mercury thermometer into her left hand while shaking it. Radiographic imaging revealed that some liquid metallic mercury had infiltrated into the soft tissues of her palm (amount unspecified). The diffusely distributed mercury could not be removed surgically. No immediate follow-up mercury levels in blood or urine were reported. A slightly elevated blood mercury concentration (15 µg/L, toxic level >50) was reported 2 years after this event, which then

declined (no reason provided). Other sources of mercury could have caused the increase, so little can be concluded about how much of the subcutaneous liquid mercury entered the systemic circulation.

In a much more informative case history, a 19-year-old man had injected mercury subcutaneously (Bradberry et al. 1996). Blood and urine mercury concentrations were followed for 6 years after presentation. Hematological and biochemical profiles were normal. Histological results indicated a chronic inflammatory reaction with granuloma formation, secondary to the globular mercury. A postoperative X-ray of the elbow indicated persistent subcutaneous mercury particles. Apart from the initial local discomfort, the patient remained asymptomatic and clinical examination revealed no abnormality up to 6 years postsurgery. No systemic features of mercury poisoning were evident. Blood mercury levels declined from 60 to 70 µg/L at 1 year postoperation to 10 µg/L at 6 years. Serial sampling results of total mercury in 24 urine collections indicated peaks up to 1.2 mg during the first year postoperation, which declined to 59 µg/L at 6 years. The elevated blood and urine levels indicate some systemic absorption. The effects of the surgery on migration of mercury from the subcutaneous tissue to the systemic circulation are not known.

2.3.2 Distribution

In humans, metallic mercury is distributed throughout the body following inhalation exposure. It can readily cross the blood-brain and placental barriers because of its high lipophilicity. After oxidation to mercuric mercury, it accumulates primarily in the kidneys. Inorganic divalent mercury compounds similarly reach all organs; however, the extent of accumulation in the brain and fetus is lower than metallic mercury because of the lower lipophilicity of inorganic mercury compounds. Organic mercury compounds distribute throughout the body following oral exposure and have the highest accumulation in the kidneys. As with metallic mercury, the ability of methyl- and phenyl mercury compounds to cross the blood-brain and placental barriers allows distribution, and subsequent accumulation, in the brain and fetus.

2.3.2.1 Inhalation Exposure

Metallic Mercury. The lipophilic nature of metallic mercury results in its distribution throughout the body. Metallic mercury in solution in the body is highly lipophilic, thereby allowing it to cross blood-brain and placental barriers with ease (Clarkson 1989). Mercury distributes to all tissues and reaches peak levels within 24 hours, except in the brain where peak levels are achieved within 2–3 days (Hursh et al. 1976).

The longest retention of mercury after inhalation of mercury vapor occurs in the brain (Takahata et al. 1970). Japanese workers who died 10 years after their last exposure to metallic mercury vapors still had high residual levels of mercury in their brains (Takahata et al. 1970). Autopsies of 3 dentists revealed 0.945–2.110 mg Hg/kg in the renal cortex, compared to 0.021–0.810 mg Hg/kg for unexposed controls (Nylander et al. 1989).

In volunteers who inhaled a tracer dose of metallic mercury vapor for 20 minutes, approximately 2% of the absorbed dose was deposited per liter of whole blood after the initial distribution was complete (Cherian et al. 1978). Uptake into the red blood cells was complete after 2 hours, but plasma uptake was not complete until after 24 hours. Mercury concentration in red blood cells was twice that measured in the plasma. This ratio persisted for at least 6 days after exposure. However, the ratios of 1–2 have been reported for metallic mercury vapor (Miettinen 1973).

Exposure of rats to mercury vapor (10–100 µg/m^3) for 6 hours a day, 5 days a week from the 4th through 11th weeks of life resulted in measurable amounts of mercury in the blood, hair, teeth, kidneys, brain, lungs, liver, spleen, and tongue, with the kidney cortex having the highest mercury concentration (Eide and Wesenberg 1993). Further, tissue concentrations were positively and significantly correlated with exposure concentrations. In this study, the rat molars were found to have the highest correlation coefficient with measured kidney mercury values, leading to a suggestion by the authors that human deciduous teeth may be useful indicators of chronic mercury exposure and of the mercury uptake by the kidneys and cerebrum (Eide and Wesenberg 1993). In another study, a 4-hour exposure of mice to metallic mercury vapor produced the highest mercury retention in the brain compared to other organs (Berlin et al. 1966). Exposure of mice to metallic mercury vapor (8 mg/m^3, for 6 hours a day for 10 days) resulted in higher mercury levels in the gray than in the white brain matter (Cassano et al. 1966, 1969). Exposure of rats to 1 mg/m^3 metallic mercury vapor for 24 hours a day every day for 5 weeks or 6 hours a day, 3 days a week for 5 weeks resulted in mean mercury brain concentrations of 5.03 and 0.71 µg/g, respectively (Warfvinge et al. 1992). Mercury was found primarily in the neocortex, basal nuclei, and the cerebellar Purkinje cells.

Mercury also accumulates in several cell types populating the dorsal root ganglia (Schionning et al. 1991). After 12–14 hours of exposure of rats to a relatively small amount of metallic mercury vapor (0.55 mg/m^3), accumulation of mercury was observed within all cell types examined (ganglion cells, satellite cells, fibroblasts, and macrophages). Mercury has also been detected in dorsal root neurons and satellite cells of

primates exposed for one year to mercury through amalgams in dental fillings or the maxillary bone (Danscher et al. 1990).

The kidney is the major organ of mercury deposition after inhalation exposure to metallic mercury vapor. Mercury concentrations in the kidneys are orders of magnitude higher than in other tissues (Rothstein and Hayes 1964). Monkeys exposed for one year to metallic mercury vapor from amalgam in dental fillings accumulated mercury in the spinal ganglia, anterior pituitary, adrenal, medulla, liver, kidneys, lungs, and intestinal lymph glands (Danscher et al. 1990). The largest deposits of mercury were found in the kidneys (2.5–5.2 ppm), specifically in the proximal tubule cells.

The kidney contains metallothionein, a metal-binding protein that is also found in fetal and maternal livers and other organs. In the kidneys, the production of metallothionein is stimulated by exposure to mercury. The increased levels of metallothionein increase the amount of mercuric ion binding in the kidneys (Cherian and Clarkson 1976; Piotrowski et al. 1973). Three classes of sulfhydryl groups have been identified in the kidneys, with metallothionein having the greatest affinity for mercury (Clarkson and Magos 1966). Low molecular-weight complexes of mercury have been identified in the urine, suggesting that they may exist in the kidneys and contribute to the kidneys' accumulation of mercury (Piotrowski et al. 1973).

Metallothionein exists in higher concentration in the fetal liver than in the maternal liver of rats. Exposure to mercury in the pregnant dam results in the binding of mercury to metallothionein in fetal liver initially, followed by a redistribution to other organs (Yoshida et al. 1990). Metallothionein and mercury levels were elevated in the kidneys of guinea pig neonates exposed to 6–10 mg/m^3 mercury vapor (Piotrowski et al. 1973).

After exposure to mercury vapor, mercury is distributed throughout the body in different chemical and physical states. Metallic mercury dissolves in the blood upon inhalation, and some remains unchanged (Magos 1967). Metallic mercury in the blood is oxidized to its divalent form in the red blood cells (Halbach and Clarkson 1978). The divalent cation exists as a diffusible or nondiffusible form. The nondiffusible form is mercuric ions that bind to protein and are held in high-molecular weight complexes, existing in equilibrium with the diffusible form.

In the plasma, the mercuric ion is predominantly nondiffusible and binds to albumin and globulins (Berlin and Gibson 1963; Cember et al. 1968; Clarkson et al. 1961). Following mercuric salt administration,

levels of mercuric ions in the plasma are similar to levels of mercuric ions in the red blood cells. Binding of mercury also occurs in tissues, and retention varies, with the brain retaining mercury the longest.

The influence of age on mercury distribution following exposure to metallic mercury was evaluated in neonatal (12 hours old) and adult guinea pigs exposed to 8 or 10 mg Hg/m^3 vapor for 120 minutes (Yoshida et al. 1989). The mercury concentrations were 28, 58, and 64% higher in the brain, lungs, and heart, respectively, of the neonates compared to the mothers. However, the mercury level in the kidneys was approximately 50% lower in the neonates. The lower uptake of mercury in the kidneys of neonates may be due to the functional immaturity of the kidneys at parturition. The higher levels in other highly perfused tissues suggest that mercury accumulation in organs is dependent on how easily metallic mercury can reach the tissues from blood. Similar findings were reported by Jugo (1976) who found higher mercury concentrations in the liver, blood, and brain, but lower concentrations in the kidneys of 2-week-old rats compared to similar tissues in 21-week-old rats. These results also suggest that infants may accumulate mercury more readily after acute exposure and, therefore, may be more likely to exhibit neurotoxicity from mercury vapors.

The extent of mercury accumulation with aging was studied in mice maintained under normal care conditions in a conventional rodent colony without exposure to known mercury sources other than background concentrations normally found in food, water, and air (Massie et al. 1993). There was no significant change in the total amount of mercury in the organs (lungs, heart, brain, and liver) from male C57BL/6J mice ranging in age from 133 to 904 days. However, the ratios of mercury levels in the brain to mercury levels in the liver and kidneys were found to increase significantly and dramatically with age. The increase with aging in the brain-to-liver and brain-to-kidneys ratios suggests that mercury removal from the brain may be less efficient in some older organisms.

Metallic mercury vapor easily penetrates the placental barrier and accumulates in fetal tissues. The high lipophilicity of metallic mercury favors its penetration across the barrier. The uptake of mercury appears to increase during the later gestation period in mice, as indicated by increased mercury accumulation in the fetus after exposure to metallic mercury (Dencker et al. 1983). Guinea pig fetuses that were exposed to 6–13 mg/m^3 mercury vapor during late gestation had elevated mercury concentrations in the liver, while the levels in other tissues were only slightly increased relative to controls (Yoshida et al. 1990). Newborn guinea pigs that were nursed by their mothers, who had been and exposed to mercury vapor (6–9 mg/m^3) for 120 minutes immediately after parturition, had the highest mercury concentrations in the kidneys,

followed by the liver and lungs (Yoshida et al. 1992). In the brain and whole blood, mercury concentrations were slightly elevated compared to nonexposed controls. Levels of mercury in the fetus were approximately 4 times higher after exposure to metallic mercury vapor than after mercuric chloride administration for mice and 10–40 times higher for rats (Clarkson et al. 1972). The transport of the mercuric ions is limited at the placental barrier by the presence of high-affinity binding sites (Dencker et al. 1983).

Inorganic Mercury. No studies were located regarding the distribution of inorganic mercury in humans or animals following inhalation exposure to inorganic mercury compounds.

Organic Mercury. No studies were located regarding the distribution of organic mercury in humans or animals following inhalation exposure to organic mercury compounds.

2.3.2.2 Oral Exposure

Metallic and Inorganic Mercury. Data on the distribution of ingested elemental mercury were not located, and data on the ingestion of inorganic mercury are limited. The metallic mercury that is absorbed from an oral exposure is expected to resemble many aspects of the distribution of mercuric salts because metallic mercury is oxidized to mercuric ion in biological fluids, and the resulting distribution reflects that of the mercuric ion. Unlike elemental mercury, however, the amount of divalent mercury that crosses the blood-brain and placental barriers is much lower because of its lower lipid solubility (Clarkson 1989).

In some studies there is a combined exposure to both organic and inorganic 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. 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. The results of a regression analysis for mercury in hair, blood, and milk indicated 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).

Inorganic Mercury. The liver and kidneys of mice had the highest mercury levels 14 days after exposure to a single oral dose of 0.2–20 mg ^{203}Hg/kg as mercuric chloride (Nielsen and Andersen 1990). The brain has substantially lower mercury levels; however, retention was longest in this tissue. Sin et al. (1983) report that the kidneys also had the highest mercury levels following repeated oral exposure of mice to mercuric chloride (4–5 mg Hg/kg) for 2–8 weeks. Mercuric sulfide did not accumulate in the tissues of mice to any significant extent following exposure to low levels of mercuric sulfide (4–5 mg Hg/kg) for 2–8 weeks (Sin et al. 1983). However, the mercury content in the liver and kidneys of mice treated with higher doses of mercuric sulfide (. 8–200 mg Hg/kg/day) for 7 days was significantly increased compared to the controls (Yeoh et al. 1986, 1989). Mice fed mercuric sulfide (86 mg Hg/kg/day) for 1 week exhibited a 21-fold increase in the kidneys' mercury content ($p<0.001$) and an 8.6-fold increase in the liver content compared to controls (Yeoh et al. 1989). Moderate renal effects, with a corresponding mercury concentration of 50 µg/g in the kidneys, were seen in rats exposed to mercuric nitrate (Fitzhugh et al. 1950).

Mercury can accumulate in human hair following oral exposure to mercuric chloride (Suzuki et al. 1992). Hair mercury levels, determined using segmental hair analysis, can be used to monitor exposure to mercury and may leave a historical record of exposure or uptake. In hair cut 41 days after mercuric mercury ingestion (13.8 mg/kg), a sharp peak (40 nmol/g [8 µg/g]) was found in the 1 cm segment closest to the scalp, while the levels were #5 nmol/g in all other segments. Ninety-five days after ingestion, the peak of inorganic mercury shifted to the 2–3 cm segment, while 160 days after ingestion the peak shifted to the 3–4 cm segment. During this time, the height of the peak decreased. An estimated biological half-life of inorganic mercury in hair was 57.8 days. Inorganic mercury in hair had different patterns of longitudinal variation from that of organic mercury.

Organic Mercury. Distribution of organic mercury compounds in humans and animals is similar to that of metallic mercury. Methylmercury distributes readily to all tissues, including the brain and fetus, after absorption from the gastrointestinal tract. The uniform tissue distribution is due to methylmercury's ability to cross diffusion barriers and penetrate all membranes without difficulty (Aberg et al. 1969; Miettinen 1973). Thus, tissue concentrations tend to remain constant relative to blood levels. About 90% of the

methylmercury in blood is found in the red blood cells (Kershaw et al. 1980). The mean mercury concentrations in red blood cells were 27.5 ng/g and 20.4 ng/g in males and females, respectively, exposed to mercury, primarily from mercury-contaminated fish (Sakamoto et al. 1991). Because of this uniform distribution in tissues, blood levels are a good indicator of tissue concentrations independent of dose (Nordberg 1976).

Although distribution is generally uniform, the highest levels of organic mercury are found in the kidneys (Nielsen and Andersen 1991b; Rice 1989b; Ryan et al. 1991). After a single oral dose of 0.04, 0.2, 1, or 5 mg Hg/kg as methylmercuric chloride administered to mice, mercury was retained mostly in the kidneys and liver at 14 days postexposure (Nielsen and Andersen 1991a). The deposition of mercury in the carcass was about 70%, with retention primarily in the skin, hair, and muscles and to a lower degree in the fat and bones (Nielsen and Andersen 1991b). More than 200 days after cynomolgus monkeys were given 0.025 and 0.05 mg Hg/kg/day as methylmercuric chloride in apple juice for about 2 years, the kidneys contained 10.18–27.89 ppm mercury in the cortex and 1.12–10.11 ppm in the medulla, compared to <2 ppm in the other tissues measured (Rice 1989b).

Demethylation of methylmercury to inorganic mercury is species-, tissue-, dose-, and time-dependent. The demethylated inorganic mercury accumulates in the kidney and liver. Suda et al. (1991) evaluated the transformation of methylmercury to inorganic mercury by phagocytic cells. The liver and kidneys are also potential sites of biotransformation (Lind et al. 1988; Magos et al. 1976; Norseth and Clarkson 1970).

The distribution of mercury in the brain has been studied in humans following oral absorption of organic mercury. It is suggested by Aschner and Aschner (1990) that, following acute exposure to methylmercury, most of the total mercury in the brain is represented by organic mercury; however, after chronic exposure, most of the mercury in the brain is inorganic mercury. An explanation for these findings is that organic mercury is converted into inorganic mercury in the brain. After chronic methylmercury exposure in monkeys, estimated half-lives were considerably longer in brain than in blood, also possibly due to conversion of methylmercury to a form that is highly bound to brain tissue (Rice 1989b).

The autopsy of a man whose first symptoms of methylmercury poisoning occurred 26 years earlier revealed that the highest mercury levels (0.62–1.19 µg Hg/g) were in the gyrus of the cerebral cortex, cerebellum, pallidum, and occipital pole of the brain (Takeuchi et al. 1989). Furthermore, total mercury levels (0.02–1.19 µg/g) were much higher than methylmercury levels (approximately <0.01 µg/g) in the brain.

This finding supports the assumption by Suda et al. (1989) that ingested methylmercury is dealkylated to inorganic mercury in the brain.

Monkeys were fed 0.05 or 0.09 mg Hg/kg/day as methylmercury, containing 5% impurity of inorganic mercury, for 0.5–1.5 years (Lind et al. 1988). The low-dosed monkeys were found to have 10–33% of the total mercury present in the inorganic form in brain cortices, while the high-dosed monkeys had 90% in the inorganic form. Demethylation of methylmercury in the brain, as well as in other organs, including the kidneys and liver, is believed to contribute substantially to the high concentration of inorganic mercury in the brain. Following oral exposure to methylmercuric chloride, regional distribution of total mercury in the brain of monkeys was observed; the highest levels were in the thalamus and hypothalamus (Rice 1989b).

In contrast, in the brain of 21-day-old neonatal rats that had been previously exposed to a gavage dose of 6.4 mg Hg/kg as methylmercury chloride *in utero*, the cerebellum had the highest mercury concentrations and the brainstem had the lowest (Braghiroli et al. 1990). By 60 days of age, concentrations in the brain reached normal values, with an estimated half-life of approximately 37 days (Braghiroli et al. 1990). Therefore, age can affect regional distribution in the brain of animals.

Massie et al. (1993) reported no significant change in the total amount of mercury in the organs (lung, heart, kidney, brain, and liver) of male C57BL/6J mice ranging in age from 133 to 904 days of age maintained under conventional conditions with no known source of mercury exposure other than background concentrations. The ratio of mercury in the brain to that in the liver or to that in the kidney was significantly increased with age, indicating that older mice are less able to maintain a low brain-to-liver ratio of mercury regardless of the total body content of mercury.

In a study of organs from sledge dogs fed methylmercury-laden meat and organs from predatory marine animals (Hansen and Danscher 1995), the highest concentration of total mercury was found in the mesenterial lymph nodes, followed by liver and kidneys, indicating that the lymphatic system may play an important role in the transport of mercury to target organs. The tissue concentrations of mercury observed in this study were found to be age-related, and the results suggest that demethylation takes place in all organs, except the skeletal muscle. Demethylation of methylmercury was found to be lower in the brain than in other organs (Hansen and Danscher 1995).

Mercury accumulates in hair following exposure to methylmercury in humans and mice (Grandjean et al.

1992; Nielsen and Andersen 1991a, 1991b; Soria et al. 1992; Suzuki et al. 1992). Hair mercury levels, determined using segmental hair analysis, can be used to monitor exposure to mercury and may leave a historical record of exposure or uptake (Phelps et al. 1980; Suzuki et al. 1992). The concentration of mercury in the hair is considered proportional to the concentration of mercury in the blood. Correlations can be drawn to determine blood concentrations of mercury relative to its concentration in the hair (see the discussion of the methylmercury MRL in Section 2.5). Mercury concentrations in maternal hair were significantly correlated with cord blood levels of mercury in pregnant women who had frequently ingested whale meat throughout pregnancy (Grandjean et al. 1992). The frequent ingestion of whale meat dinners during pregnancy and, to a lesser degree, the frequent consumption of fish, as well as increased parity or age, were associated with high mercury concentrations in cord blood and hair. The incorporation of mercury into hair is irreversible; the loss of hair mercury occurs as the result of hair loss (Nielsen and Andersen 1991b).

As with metallic mercury, methylmercury can readily traverse the placental barrier. In humans with no known exposure to mercury, blood mercury levels increased with advancing gestation such that the mean blood mercury level on admission for delivery (1.15 ppb) was significantly higher than that of the first prenatal visit (0.79 ppb) (Kuntz et al. 1982). Cord blood levels were similar to maternal blood values in labor and postpartum. Concentrations of methylmercury in the fetal blood are slightly higher than in the maternal blood (Inouye and Kajiwara 1988; Kuhnert et al. 1981). Following an oral dose of methylmercuric chloride during gestation, accumulation of mercury was much greater in the fetal kidneys than in the maternal kidneys of guinea pigs (Inouye and Kajiwara 1988). Mercury levels in the liver were slightly higher in the fetus compared to the dam when exposed to organic mercury at late gestation but were similar at early gestation. Distribution of mercury in the maternal and fetal brains was uneven, with the highest concentrations in the neopallium, diencephalon, and mesencephalon and the lowest in the rhombencephalon. Exposure at later gestational weeks resulted in higher concentrations for both maternal and fetal brains (Inouye and Kajiwara 1988).

Methylmercury may also be secreted in mother's milk (Bakir et al. 1973). Following intravenous dosing of methylmercuric chloride (1.6 mg Hg/kg) to pregnant mice on one of days 9–17 of pregnancy, methylmercury was readily transferred to the fetuses from the mothers more predominantly at the later gestational stage (Inouye and Kajiwara 1990). The placental transfer of methylmercury was more efficient compared to the lactational transfer in rats exposed to methylmercury in the diet during 11 weeks prior to mating, during gestation, and during lactation (Sundberg and Oskarsson 1992). A higher concentration of mercury

in the brain in relation to the blood mercury concentration was found after exposure *in utero* compared to exposure in milk. Mercury was present as methylmercury in the blood of the offspring exposed only during gestation, indicating little or no demethylation during the first 15 days after birth. However, inorganic mercury was present in the blood of offspring exposed only through milk, probably resulting from demethylation of methylmercury in the dam and transport of inorganic mercury to the sucklings through milk.

In animal studies, mercury transfer to and distribution in offspring depends on the form administered to the dam. Yoshida et al. (1994) administered either mercury chloride or methylmercury at 1 mg Hg/kg body weight to maternal guinea pigs (Hartley strain) via intraperitoneal injection 12 hours after parturition. Exposure of the offspring was studied on days 3, 5, and 10 postpartum. Concentrations of mercury were lower in the milk than in maternal plasma regardless of the form of administered mercury, but total milk mercury was higher in the dams given mercury chloride. While the ratio of methylmercury to total mercury decreased in plasma from dams, it did not decrease in the milk. Regardless of the form of mercury given to the dams, the highest concentration of mercury in the offspring was found in the kidney, followed by the liver and the brain. Brain mercury, however, was significantly higher in the offspring of methylmercury-treated dams. Mercury levels in major organs of the offspring peaked at 5 days from mercury-chloride-treated dams and at 10 days from methylmercury-treated dams.

Tissue distribution of phenylmercury is initially similar to methylmercury. One week after administration, the distribution pattern resembles that seen after administration of inorganic compounds (Nordberg 1976). Once in the blood, phenylmercury distributes to a greater extent into the red blood cells than the plasma. Phenylmercury also predominantly distributes to the liver (Berlin 1963). It is less permeable to the placental and blood-brain barriers than methylmercury (Yamaguchi and Nunotani 1974). Phenylmercury also accumulates in the fur of rats but to a lesser extent than detected with methylmercury exposure (Gage 1964).

2.3.2.3 Dermal Exposure

No information was identified for distribution of metallic, inorganic, or methylmercury via dermal absorption. A case history for a dermal absorption of dimethylmercury (see Section 2.3.1.3) does provide some information on distribution (Blayney et al. 1997; Nierenberg et al. 1998). A 48-year-old female absorbed approximately 0.4–0.5 mL of dimethylmercury (about 1,500 mg) through the skin on the dorsal

side of her hand. A preliminary laboratory report at 5 months after exposure indicated that the whole-blood mercury concentration was more than 1,000 µg/L (normal range, 1–8 µg/L; toxic level, >200 µg/L). Chelation therapy with oral succimer (10 mg/kg orally every 8 hours) was begun on day 168 after exposure. Whole blood concentrations rose to 4,000 µg/L after one day of chelation, and urinary mercury levels were 234 µg/L (normal range, 1–5 µg/L; toxic level, >50 µg/L). Chelation therapy continued up to the time of the patients death 298 days postexposure, with blood mercury level falling to around 200 µg/L. Metal analysis of the patient's tissues revealed extremely high levels of mercury in the frontal lobe and visual cortex (average value, 3.1 µg/g [3,100 ppb]), liver (20.1 µg/g), and kidney cortex (34.8 µg/g). The mercury content in the brain was approximately 6 times that of the whole blood at the time of death, and was much higher than levels in the brains of nonmercury exposed patients (2–50 ppb).

2.3.2.4 Other Routes of Exposure

Strain and sex differences were observed in renal mercury accumulation 4 hours after a subcutaneous methylmercuric chloride injection (1 µmol/kg) to 5 strains (BALB/cA, C57BL/6N, CBA/JN, C3H/HeN, and ICR) of male mice and 3 strains (BALB/cA, C57BL/6N, and ICR) of female mice (Tanaka et al. 1991). Mercury was distributed to the kidneys, brain, heart, lungs, liver, spleen, carcass, plasma, and red blood cells of all mice tested. Strain and sex differences were found in renal mercury content. In three strains (ICR, BALB/cA, and C57BL/6N), males showed higher renal mercury levels than females.

Differences in tissue concentrations in different inbred mice strains were evaluated by Griem et al. (1997). Female mice from five different strains (C57BL/6, B10.D2, B10.S, A.SW, and DBA/2) received 3 weekly subcutaneous injections of 0.5 mg Hg/kg body weight for up to 12 weeks. Except for the thymus, in which mercury concentrations continued to increase, steady state levels were obtained in blood and liver after 4 weeks and in spleen and kidney after 8 weeks. In the closely related strains C57BL/6, B10.D2, and B10.S, which differ only or primarily at the major histocompatibility complex, mercury concentrations in blood and liver were about 2-fold lower and renal concentrations were from 3- to 5-fold lower than measured in A.SW, and DBA/2 strains. Mercury concentrations in the spleen of C57BL/6, B10.D2, B10.S mice were significantly higher than in the spleen of A.SW, and DBA/2 mice. The higher concentration of Hg in this immune system organ concentration of C57BL/6, B10.D2, B10.S correlates with the increased susceptibility of these strains to a mercury chloride-induced systemic autoimmune syndrome. The strains with lower splenic mercury are more resistant.

Treatment of mice with ethanol results in increased accumulation of mercury in the fetus (Khayat and Dencker 1982). The concurrent generation of NADPH during the oxidation of alcohol enhances the reduction of mercuric ion to metallic mercury, making it more favorable for permeating the placenta. Mercuric chloride's limited ability to cross the placental barrier was also demonstrated in an intravenous study using mice (Inouye and Kajiwara 1990). Following intravenous dosing of mercuric chloride (1.4 mg/kg) to pregnant mice on 1 day between days 9 and 17 of pregnancy, mercuric chloride was transferred inefficiently to the fetus, being blocked almost completely by the fetal membrane. The mercury accumulated in the placenta and yolk sac but not in the amnion or fetal body (Inouye and Kajiwara 1990). A histochemical study demonstrated that mercuric mercury (Hg^{+2}) was blocked in the proximal wall of the yolk sac.

2.3.3 Metabolism

The available evidence indicates that the metabolism of all forms of mercury is similar for humans and animals. 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.

Metallic and Inorganic Mercury. Metallic mercury vapor is inhaled through the lungs and rapidly enters the bloodstream. The dissolved vapor can undergo rapid oxidation, primarily in the red blood cells, to its inorganic divalent form by the hydrogen peroxide-catalase pathway (Clarkson 1989; Halbach and Clarkson 1978). It is believed that the rate of oxidation is dependent on: (1) concentration of catalase in the tissue; (2) endogenous production of hydrogen peroxide; and (3) availability of mercury vapor at the oxidation site (Magos et al. 1978). In red blood cells *in vivo*, hydrogen peroxide production is probably a rate-determining step because Nielsen-Kudsk (1973) found that stimulation of hydrogen peroxide production in red cells increased the uptake of mercury vapors in red blood cells. After a low dose, the total mercury content in the blood is proportionately higher than (to the administered dose) after a high dose, indicating that a higher proportion of the lower dose is oxidized (Magos et al. 1989). The hydrogen peroxide-catalase

pathway in red cells may become saturated at higher dose levels (Magos et al. 1989). This oxidation pathway of metallic mercury can be inhibited by ethanol since ethanol is a competitive substrate for the hydrogen peroxide catalase and, consequently, can block mercury uptake by red blood cells (Nielsen-Kudsk 1973).

The oxidation of metallic mercury may also occur in the brain, liver (adult and fetal) (Magos et al. 1978), lungs (Hursh et al. 1980), and probably all other tissues to some degree (Clarkson 1989). In rat liver homogenates, hydrogen peroxide catalase is the predominant oxidative pathway in tissues. Its capacity is very high. Unlike oxidation in red cells, the rate-limiting step in *in vitro* oxidation in the liver is dependent on the rate of mercury delivery to the enzyme (Magos et al. 1978). Unoxidized metallic mercury can still reach the brain because the oxidation of metallic mercury is a slow process compared with the circulation time from the lungs to the brain (Magos 1967). In the brain, unoxidized metallic mercury can be oxidized and become trapped in the brain because it is more difficult for the divalent form to cross the barrier. Autoradiographic studies suggest that mercury oxidation also occurs in the placenta and fetus (Dencker et al. 1983), although the extent of oxidation is not known. The rate of distribution of metallic mercury to the brain and fetus is probably nonlinear because the rate of oxidation in red cells is nonlinear (i.e., can become saturated at higher doses) (Magos et al. 1989).

There is evidence to suggest that the divalent inorganic mercury cation is reduced by mammalian tissue to metallic mercury after its oxidation. Rats and mice pretreated parenterally with mercuric chloride exhale metallic mercury vapor (Clarkson and Rothstein 1964; Dunn et al. 1981a). Liver and kidney homogenates in animals also release mercury vapor after exposure to mercuric chloride. The amount of mercury released increases upon treatment with ethanol (Dunn et al. 1981b). This increase suggests that glutathione reductase is responsible for mercuric ion reduction (Williams et al. 1982). Oxidation of alcohol to acetaldehyde stimulates NADPH production, which is required for mercuric ion reduction. However, alcohol is primarily oxidized in the liver, and this location is not consistent with the increases in metallic mercury vapor released from the kidney homogenates (Dunn et al. 1981b).

Organic Mercury. Once absorbed, methylmercury can apparently be converted into inorganic mercury in tissues, specifically the divalent cation (Hg^{+2}) (Dunn and Clarkson 1980). Several investigators have reported high levels of inorganic mercury in tissues (Magos and Butler 1972; WHO 1990) and feces after methylmercury exposure (Turner et al. 1975). Rat liver microsomes can degrade methylmercury into inorganic mercury. Inorganic mercury production from methylmercury paralleled the hydroxyl radical

production (Suda and Hirayama 1992). The promotion and inhibition of the hydroxyl radical formation and the hydroxyl radical scavenger, affected inorganic mercury production. These results suggest that hydroxyl radicals produced from microsomes may play a predominant role in alkyl mercury degradation. NADPH-cytochrome P-450 reductase is known to be responsible for hydroxyl radical production in liver microsomes. Alkyl mercury degradation varied in proportion to the enzyme activities and hydroxyl radical production. These results suggest that hydroxyl radicals produced by cytochrome P-450 reductase might be the primary reactive species that induces alkyl mercury degradation. *In vitro* studies using a peroxidase-hydrogen peroxide-halide system indicated that besides the hydroxyl radical, hypochlorous acid (HOCl) scavengers are also capable of degrading methylmercury (Suda and Takahashi 1992). Also, metallic mercury exhaled in mice dosed with methylmercury was dependent on the level of inorganic mercury present in the tissue (Dunn and Clarkson 1980). The cation then enters the oxidation-reduction cycle, and metabolism occurs as discussed previously under Inorganic Mercury.

A small amount of an oral dose of methylmercuric chloride can also be converted into inorganic mercury in the intestinal flora (Nakamura et al. 1977; Rowland et al. 1980). However, inorganic mercury is poorly absorbed across the intestinal wall and, therefore, most of it is excreted.

Phenylmercury also rapidly metabolizes to inorganic mercury (Nordberg 1976). The metabolism of phenyl-mercury involves hydroxylation of the benzene ring to an unstable metabolite that spontaneously releases inorganic mercury. Consequently, its tissue disposition following initial metabolism resembles that seen after the administration of inorganic salts (Gage 1973).

Studies in mice indicate that toxicity from exposure to dimethylmercury is the result of metabolic conversion of dimethylmercury to methylmercury, and that dimethylmercury does not enter the brain until it has been metabolized to methylmercury, which occurs over the first several days following absorption (Ostland 1969). Nierenberg et al. (1998) report the results of an analyses of mercury content in the hair of a 48-year-old female who died subsequent to an acute exposure to dimethylmercury. The results are consistent with the kinetic profiles for methylmercury, and support the hypothesis of a rapid conversion of dimethylmercury to a methylmercury metabolite.

2.3.4 Elimination and Excretion

Elimination of metallic mercury occurs through the urine, feces, and expired air, while inorganic mercury is excreted in the urine and feces in humans. Animal data on excretion are limited but indicate that excretion is species and dose dependent. The feces are a major elimination route for inorganic mercury compounds, but high acute doses increase the percentage of excretion via the urine. Excretion of organic mercury is predominantly thought to occur through the fecal (biliary) route in humans. In animals, phenylmercury is excreted initially though the bile and then shifts to urine, whereas methylmercury is primarily excreted in the bile and then the feces. Age is a factor in the elimination of mercury in rats following inorganic and organic mercury exposure, with younger rats demonstrating significantly higher retention than older rats. Both inorganic and organic mercury compounds can be excreted in breast milk. There are no data suggesting that the route of exposure affects the ultimate elimination of inorganic and organic mercury that is absorbed into the body.

Metallic and Inorganic Mercury. The urine and feces are the main excretory pathways of metallic and inorganic mercury in humans, with a body burden half-life of approximately 1–2 months (Clarkson 1989). In a study of former chloralkali workers exposed to metallic mercury vapor for 2–18 years (median, 5 years), Sallsten et al. (1995) found that the elimination of mercury in urine was well characterized by a one-compartment model, which estimated a half-life of 55 days. There was a tendency toward longer half-lives with shorter duration exposures than with long-term exposure, when uptake and elimination have reached a steady state. This might be due to the induction of a higher metabolic rate after a longer exposure time, but there is no experimental evidence to support such an effect (Sallsten et al. 1995). For high-level exposure to inorganic divalent mercury, the urine is probably the major elimination route, with a half-life similar to that of metallic mercury (Clarkson 1989). An elimination half-life from urine was estimated to be 25.9 days following an acute exposure to a high level of mercuric chloride (13.8 mg/kg) (Suzuki et al. 1992). Exhalation in the lungs and secretion in saliva, bile, and sweat may also contribute a small portion to the excretion process (Joselow et al. 1968b; Lovejoy et al. 1974). After an acute mercury exposure in humans, urinary excretion accounts for 13% of the total body burden. After long-term exposure, urinary excretion increases to 58%. Humans inhaling mercury vapor for less than an hour expired approximately 7% of the retained dose of mercury (Cherian et al. 1978; Hursh et al. 1976). The half-life for this elimination pathway was 14–25 hours; therefore, excretion through expired air is negligible 5–7 days after exposure (Cherian et al. 1978). Using a two-compartment model, elimination half-lives in the urine of workers exposed for 20–45 hours to >0.1 mg/m^3 metallic mercury vapor were

estimated to be 28 and 41 days for a fast and slow phase, respectively (Barregard et al. 1992). Mercury is excreted in the urine following oral exposure to mercuric sulfide (0.5 mg Hg/kg) (Yeoh et al. 1989).

The overall elimination rate of inorganic mercury from the body is the same as the rate of elimination from the kidneys, where most of the body burden is localized (see Table 2-4). Inorganic mercury is also readily cleared from the lung. Elimination from the blood and the brain is thought to be a biphasic process with an initial rapid phase in which the decline in the body burden is associated with high levels of mercury being cleared from tissues, followed by a slower phase of mercury clearance from the same tissues (Takahata et al. 1970). An even longer terminal-elimination phase is also possible because of persistent accumulation of mercury, primarily in the brain (Takahata et al. 1970). Following a single oral dose of divalent mercury in 10 volunteers, 85% of the ^{203}Hg activity was excreted within 4–5 days, predominantly in the feces (Rahola et al. 1973).

Following acute mercury vapor intoxication of two humans, it was found that, despite chelation therapy with multiple chelators (2,3-dimercaptopropanol [BAL] followed by 2,3-dimercaptosuccoinic acid [DMSA]), relatively high concentrations of mercury remained in the plasma for a very long time (Houeto et al. 1994). The authors suggested that this could be explained by the progressive release of mercury from red blood cells and tissues after oxidation. In a group of chloralkali workers exposed to metallic mercury vapor for 1–24 years (median, 10 years), a decrease in the mercury concentration (following temporary discontinuation of exposure) in whole blood, plasma, and erythrocytes was found to be best characterized by a two-compartment model (Sallsten et al. 1993). Using a two-compartment model, half-lives were estimated, respectively, to be 3.8 and 45 days for the fast and slow phase in whole blood; plasma, 2 and 36 days in plasma, and 3.6 and 16 days in erythrocytes. The half-lives for the slow phases in whole blood and plasma were longer, and the relative fractions of the slow phases were higher (about 50%) after long-term exposures than after brief exposures (Sallsten et al. 1993).

Workers exposed to vapors of 0.016–0.68 mg Hg/m^3 had detectable levels of mercury in the urine (>2 µg Hg/L) (Stopford et al. 1978). Metallic mercury accounted for <1% of the total mercury in the urine. The rapid appearance of metallic mercury in the urine is probably due to mercury filtered directly from the blood through the glomerulus, whereas mercuric ions found in the urine are attributable to the mercury taken up by the kidneys prior to excretion. Therefore, urinary metallic mercury provides a relative index for blood levels of metallic mercury, and urinary mercuric ions provide a relative index for kidney levels of inorganic mercury. Three different forms of mercury have been identified in the urine from

Table 2-4. Half-lives of Inorganic Mercury in Humans

Tissue	Half-life	Phase	Reference
Lung	2 days	Early phase	Berlin et al. 1969a
Brain	20 days	Biphasic	Hursh et al. 1976
Blood	3.3 days	Early phase	Cherian et al. 1978
Plasma	3.3 days	Early phase	Cherian et al. 1978
Blood	2.4 days	Early phase	Clarkson 1978
Blood	15 days	Late phase	Clarkson 1978
Blood	28 days	Late phase	Rahola et al. 1973
Whole body	60 days		Rahola et al. 1973
Whole body	60 days		Hursh et al. 1976
Kidney	60 days		Hursh et al. 1976

Compiled from: Bakir et al. 1973; Cox et al. 1989; Kershaw et al. 1980; Miettenen et al. 1971; Sherlock et al. 1984

workers occupationally exposed to mercury: a metallic form, a mercuric-cysteine complex that is reducible, and a large complex in which the mercury can only be released by organic destruction (Henderson et al. 1974).

Data are limited on elimination of metallic and inorganic mercury in animals. Initial excretion of mercury is predominantly in the fecal matter following inhalation of metallic mercury vapor, but as mercury concentrations increase in the kidneys, urinary excretion increases (Rothstein and Hayes 1964). After inhalation, approximately 10–20% of the total excreted metallic mercury is by exhalation (Rothstein and Hayes 1964). Mercury is excreted in the urine of mice exposed orally to mercuric sulfide (. 8–200 mg Hg/kg) (Yeoh et al. 1986, 1989). The amount of mercury in the urine of the treated group was 4.5–15-fold greater than the control levels. Urinary rates of mercury excretion were 1.6–2.2 ng/hour. Neonatal rats (1, 8, and 15 days old) eliminated mercury slower than older rats (22 and 29 days old) given mercuric chloride subcutaneously (Daston et al. 1986).

Inorganic mercury is also excreted in breast milk (Yoshida et al. 1992). Newborn guinea pigs were exposed to inorganic mercury in breast milk from mothers exposed to mercury vapor (6–9 mg/m^3) for 120 minutes after parturition (Yoshida et al. 1992). Mercury concentrations in breast milk were slightly lower than plasma mercury concentrations of the maternal guinea pigs over the observation period. Ratios of milk to plasma were 0.24–0.44 on day 3, 0.45–0.46 on day 5, and 0.46–0.66 on day 10. The decrease in the mercury concentration in breast milk with time was slower than that in maternal plasma. The distribution of mercury to organs in the suckling neonates indicated that they were exposed to the inorganic rather than to elemental mercury.

Sundberg et al. (1998) studied the elimination of radiolabeled inorganic mercury in lactating and nonlactating mice exposed to mercuric chloride via a single intravenous injection at 0.5 mg Hg/kg body weight. A three-compartment pharmacokinetic model was used to fit the data. The study was designed to provide additional information on the speciation of mercury in breast milk and the differences between methylmercury and inorganic mercury migration into milk. Unlike placenta, where methylmercury moves more easily across the placental border than inorganic mercury, inorganic mercury is more readily eliminated in milk than methylmercury. For inorganic mercury, no significant differences were observed between lactating and nonlactating mice for plasma clearance (43.3 and 44.4 mL/hour/kg, respectively) and volume of
distribution (4,950 and 3,780 mL/kg). The terminal half-lives of inorganic mercury in plasma were 297 hours for lactating, and 162 hours for nonlactating mice. The milk-to-plasma concentration ratio

for inorganic mercury varied between 0.1 and 3.6, with a mean of 0.64 at plasma levels below 300 ng Hg/g (in the linear region of the relationship) and a mean of 0.17 at higher plasma mercury levels. In contrast, the values for the methylmercury kinetic parameters were significantly higher in lactating than nonlactating mice: plasma clearance (93.5 and 47.1 mL/hour/kg, respectively) and volume of distribution (18,500 and 9,400 mL/kg, respectively). The terminal half-life of methylmercury in plasma was 170 hours for lactating and 158 hours for nonlactating mice. The milk-to- plasma concentration ratios for total mercury after methylmercury administration were lower than those seen with inorganic mercury, and varied between 0.1 and 0.7 with a mean of 0.20. The nearly five-fold higher peak value for plasma to blood mercury levels observed for inorganic mercury reflects the more efficient migration of inorganic mercury from blood to milk compared with that for methylmercury. Mercury concentrations in milk also decreased more quickly for inorganic (terminal half-life of 107 hours) than for methylmercury (constant levels throughout the 9-day follow-up period postexposure). The authors hypothesize that the nonlinear relationship between mercury in milk and plasma following inorganic mercury administration suggests that inorganic mercury enters the mammary gland via a carrier-mediated transport system that is saturated at high plasma levels of inorganic mercury. The results suggest that the physiological changes during lactation alter the pharmacokinetics for methylmercury in mice, but not for inorganic mercury.

Organic Mercury. The fecal (biliary) pathway is the predominant excretory route for methylmercury, with less than one-third of the total mercury excretion occurring through the urine, following oral and inhalation exposure (Norseth and Clarkson 1970). In humans, nearly all of the total mercury in the feces after organic mercury administration is in the inorganic form. The conversion of methylmercury to inorganic mercury is a major step that is dependent on the duration of exposure and/or the duration after cessation of exposure.

In rats and nonhuman primates, methylmercury is secreted in the bile and can be reabsorbed in the intestine (Berlin et al. 1975; Norseth and Clarkson 1971; Urano et al. 1990). It is believed that methylmercury is complexed to nonprotein sulfhydryl compounds in the bile and reabsorbed in this form by a transport system (Ballatori and Clarkson 1982; Urano et al. 1990). In guinea pigs, hamsters, and monkeys, methyl-mercury, but not inorganic mercury, is extensively reabsorbed from the gall bladder, providing evidence for the biliary-hepatic recycling of this metal (Dutczak et al. 1991). The biliary-hepatic cycle probably contributes to the long biological half-life and toxicity of methylmercury. However, methylmercury can be converted into its inorganic form in the gastrointestinal lumen by intestinal flora (Nakamura et al. 1977;

Rowland et al. 1980), thus decreasing reabsorption and increasing the rate of fecal excretion (Berlin et al. 1975).

During the first few days after intravenous dosing, phenylmercury compounds are also eliminated primarily in the feces as a result of biliary secretion and its concentration in the gastrointestinal tract (mucosa and lumen) (Berlin and Ullberg 1963). The initial urinary excretion of phenylmercury represents primarily the parent compound (Gage 1964). Several days after exposure, however, elimination is primarily in the urine, which contains predominantly inorganic mercury (Gotelli et al. 1985).

Clearance half-times are longer with methylmercury than with inorganic compounds (see Table 2-5). Elimination of methylmercury compounds generally follows first-order kinetics because excretion is directly proportional to body burden and independent of the route of administration (oral or intraperitoneal) (Nielsen and Andersen 1991a). Furthermore, duration of exposure may affect the excretion process of mercury. A two-compartment model was established by Rice et al. (1989) for a single oral dose study in monkeys because of the appearance of an initial rapid elimination phase followed by a slower elimination phase. However, following repeated dosing for 2 years, a one-compartment model was considered a more reasonable fit for the data. Therefore, it was concluded that the average steady-state blood levels of mercury after chronic-duration exposure should not be estimated on the basis of short-term exposure data.

Elimination rates for methylmercury vary with species, dose, sex, and strain (Nielsen 1992). There is also evidence of sex-related differences in the elimination of methylmercury in humans (Miettinen 1973). The direction of the sex-related difference may differ for the fast and slow components of methylmercury elimination, with males excreting faster during the fast component and females excreting faster during the slow component. The net difference in elimination rates at time points distant from exposure indicates that females excrete methylmercury slightly faster than males. This net difference is seen in whole-body biological half-time derived by combining both fast and slow elimination components (Miettinen 1973). Clear sex-related differences were not reported for these volunteers for time points soon after exposure. In contrast, male mice excreted methylmercury much faster than females did for the first 14 days (i.e., primarily the fast component) (Nielsen 1992). Significant sex-related differences in elimination were also observed in rats dosed at 56 days of age (Thomas et al. 1982). As is apparently the case in humans, the difference was measured in the slow component only, with males excreting slightly slower than females. It should be noted that an insignificant difference in elimination was measured for the fast component of

Table 2-5. Elimination Constants for Methylmercury Measured in Blood and Hair

Reference	Clearance half-time in days	Elimination constant[a]	Number of subjects
Cox et al. 1989	48 (hair, women)	0.014	55
Miettenen et al. 1971	53 (blood)	0.013	15
Kershaw et al. 1980	52 (blood)	0.013	7
Sherlock et al 1984	51 (blood, women)	0.0136	6
Sherlock et al 1984	49 (blood, men)	0.0142	14
Bakir et al. 1973	65[b] (blood)	0.015	16

[a]Conversions between reported values for elimination constant and clearance half-time are based on the equation $T_{1/2}$= ln2/b, where $T_{1/2}$ is clearance half-time and b is elimination constant (WHO 1976).
[b]At least two of these individuals were young males (ages 6 and 10). The sex of the other 14 individuals was not reported.

Compiled from: Bakir et al. 1973; Cox et al. 1989; Kershaw et al. 1980; Miettenen et al. 1971; Sherlock et al. 1984

excretion in the rats, with males excreting slightly faster than females. Interestingly, a sex-difference elimination rate was not observed in rats dosed at 24 days or younger (Thomas et al. 1982).

The rate of mercury excretion was also slower in younger animals (7 or 15 days) than in older animals (24 and 56 days) (Thomas et al. 1982). This age-dependent difference in the rate of mercury excretion may reflect differences in the sites of mercury deposition (i.e., hair, red blood cells, skin). In neonatal rats, the excretion of methylmercury is longer than in adult rats because of the inability of the neonatal liver to secrete the toxicant into the bile. Therefore, the immaturity of the transport system in neonatal rats affects the elimination of mercury.

Methylmercury is also excreted in the breast milk of rats, humans, and guinea pigs (Sundberg and Oskarsson 1992; Yoshida et al. 1992). In pups exposed only through milk, approximately 80% of the total mercury in blood was present as methylmercury. Because suckling animals have a limited ability to demethylate methylmercury, the inorganic mercury present in the blood of the offspring probably originated from inorganic mercury in the milk. Since the dams were exposed only to methylmercury in their diet, some demethylation occurred in the dams, followed by the transport of the inorganic mercury to the sucklings via milk.

Sundberg et al. (1998) studied the elimination of radiolabeled methylmercury in lactating and nonlactating mice exposed to methylmercuric chloride via a single intravenous injection at 0.5 mg Hg/kg body weight. A comparison of the results for methylmercury with results for inorganic mercury is discussed in the section above on elimination of "Inorganic Mercury." A three compartment pharmacokinetic model was used to fit the data. The values for the methylmercury kinetic parameters were significantly higher in lactating than nonlactating mice: plasma clearance (93.5 and 47.1 mL/hour/kg, respectively) and volume of distribution (18,500 and 9,400 mL/kg, respectively). The terminal half-life of methylmercury in plasma was 170 hours for lactating and 158 hours for nonlactating mice. The milk-to- plasma concentration ratios for total mercury after methylmercury administration were lower than those seen with inorganic mercury, and varied between 0.1 and 0.7, with a mean of 0.20. Mercury concentrations in milk were constant throughout the 9-day follow-up period postexposure. The results indicate that physiological changes during lactation alter the pharmacokinetics for methylmercury in mice.

2.3.5 Physiologically based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Krishnan 1994). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the

model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 2-4 shows a conceptualized representation of a PBPK model.

PBPK models for mercury exist, and the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

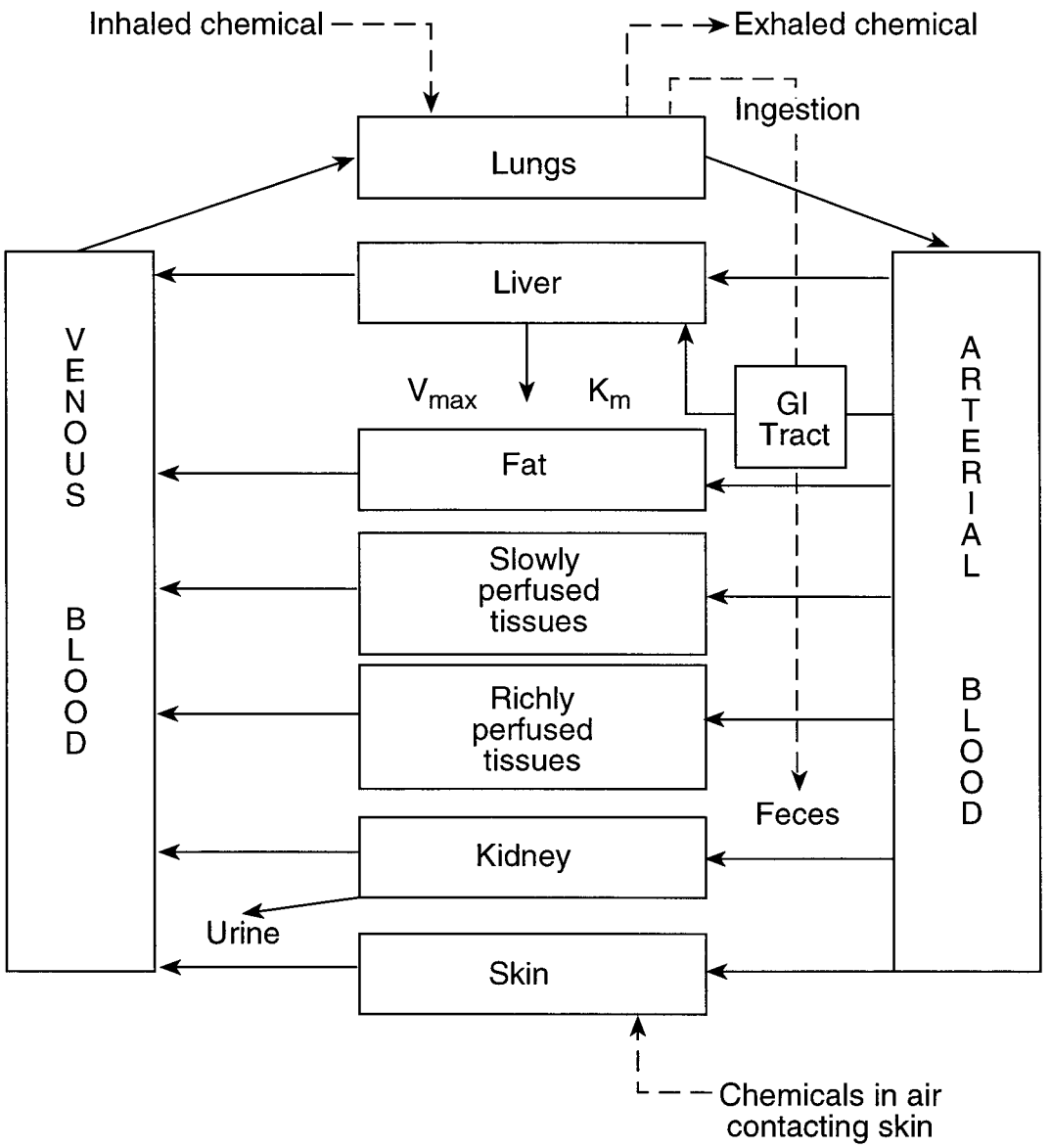
2.3.5.1 Summary of PBPK Models

Two physiologically based pharmacokinetic models have been developed recently that model 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. These model provide useful insight into the key physiological processes that determine the distribution and fate of mercury in the body, but neither model is currently being used in human risk assessment.

2.3.5.2 Mercury PBPK Model Comparison

Both the Farris et al. (1993) and the Gray (1995) PBPK models address the kinetics of methylmercury in rats. Both models provide useful insights into important physiological processes determining methyl-mercury distribution and changes in tissue concentrations. Also, both studies suggest further work to enhance the utility and accuracy of the models The Farris et al. model dealt more effectively with the conversion of methylmercury to mercuric mercury, while the Gray model specifically addressed fetal tissue concentrations as a function of maternal exposures and the extrapolation from short-term to continuous

Figure 2-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

dosing. The latter is of direct relevance to methylmercury risk assessments currently based on human studies of short-term exposures, while the general public exposure is more typically continuous. Neither model ran simulations nor validated against data for other species (including human). Nor did the models address high-to-low dose extrapolations or different routes of exposure.

2.3.5.3 Discussion of Models

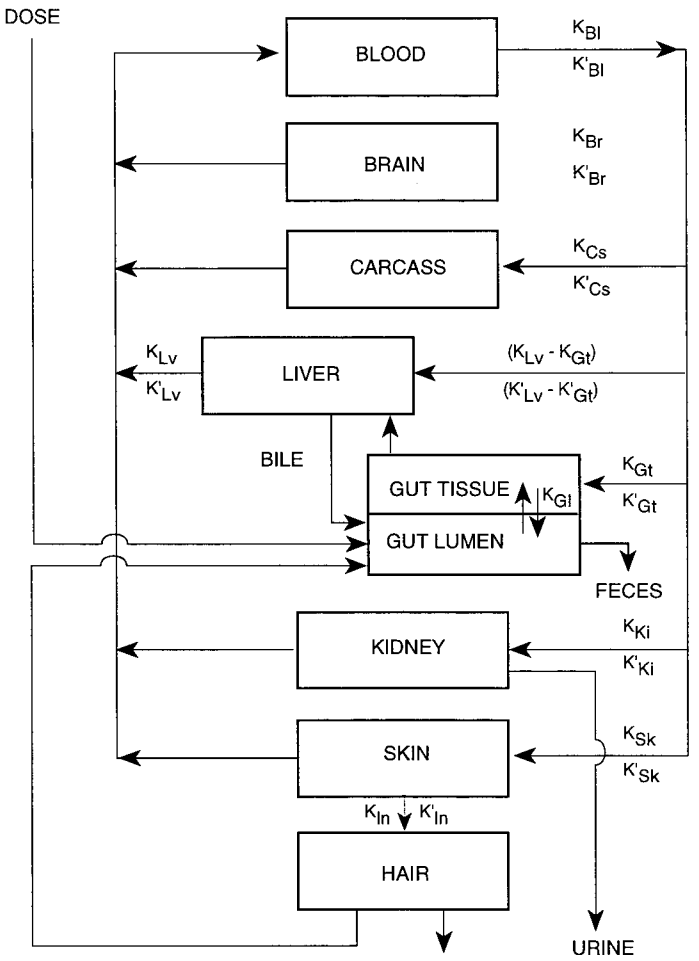
The Farris et al. Model for Methylmercury. The Farris et al. (1993) model is a physiologically based model that simulates the long-term disposition of methylmercury and its primary biotransformation product, mercuric mercury, in growing mammals following a single nontoxic oral dose of the parent compound. The test animal used to develop and validate the model was the male Sprague-Dawley rat. A tracer dose was used in the validation studies to preclude the possibility that the results would be biased by toxic or saturation effects. The model incorporates a number of features, including a time-dependent compartment for volume changes (i.e., the rats grew from 300 to 500 g in body weight over the 98-day time course of the validation study), compartment volume-dependent clearances, and the recycling of mercury from ingestion of hair by rats during grooming.

Risk assessment. The Farris et al. model has not been used in human risk assessment. The authors, however, suggest that the model would be useful in developing a better understanding of species differences and in predicting the affects of altered biochemical or physiological states on methylmercury pharmaco-kinetics. For example, the authors suggest that the model can be adapted to simulate data for neonatal animals or humans that are known to secrete glutathione poorly. It could also help elucidate the mercury kinetics for animals that have altered bile flow or that have nonabsorbable sulfhydryl-containing resins.

Description of the model. The Farris et al. model consists of nine lumped compartments, each of which represent a major site of mercury accumulation, elimination, or effect in mammals. The compartment labeled “carcass” is a residual compartment and consists of all tissues and organs not specifically represented by the other eight compartments in the model. A flow diagram of the model is shown in Figure 2-5. The interdepartmental mass transport parameters used in the model are shown in Table 2-6.

Methylmercury transport between all compartments except brain and hair is modeled as plasma flow limited (i.e., plasma levels rapidly equilibrate with erythrocytes). Mercuric mercury transport parameters

Figure 2-5. Compartmental Flow Diagram for a Pharmacokinetic Model of Methylmercury in the Growing Rat



The symbol K represents intercompartmental mass transport parameters. The subscripts Bl, Br, Cs, Gl, Gt, In, Ki, Lv, and Sk denote blood brain, carcass, gut lumen, gut tissue, integument, kidney, liver, and skin, respectively. Primed symbols designate inorganic mercury.

Source: Farris et al. 1993

Table 2-6. Intercompartmental Mass Transport Parameters Used to Model Methylmercury and Mercuric Mercury Pharmacokinetics in Rats

	Parameter	
Transport processes	Methylmercury	Inorganic mercury
Blood to brain K_{br}, K'_{Br}	3.4×10^{-5} [a,b]	3.2×10^{-5} [a,b]
Blood to carcass K_{Cs}, K'_{Cs}	7.7×10^{-4} [a,c]	3.1×10^{-4} [a,d]
Blood to liver $(L_{Lv} - K_{Gt})$, $(K'_{Lv} - K'_{Gt})$	9.1×10^{-4} [a,c,e]	9.1×10^{-2} [a,f]
Blood to kidney K_{Ki}, K'_{Ki}	2.0×10^{-2} [a,c]	8.4×10^{-3} [a,b]
Blood to skin K_{Sk}, K'_{Ki}	1.0×10^{-3} [a,c]	4.2×10^{-4} [a,d]
Blood to GI tissue G_{GT}, K'_{Gt}	8.6×10^{-3} [a,c]	3.5×10^{-3} [a,d]
GI tissue to lumen K_{Gi}, K'_{Gi}	1.0×10^{-1} [g]	2.0×10^{-4} [h]
Skin to hair K_{In}, K'_{In}	1.2×10^{-4} [j,i]	1.2×10^{-4} [b,i]

[a] Units are mL blood/min g tissue
[b] Estimated from experimental data
[c] Value is plasma flow rate to tissue divided by 100. Plasma flow rate is calculated from blood flow rate (Bonaccorsi et al.1978) assuming a hematocrit of 0.374
[d] Value is plasma flow rate to tissue divided by 250. Plasma flow rate is calculated as described in footnote[c].
[e] Based on flow via hepatic artery
[f] Hepatic artery plasma flow
[g] Value is based on assumption that transport from gut tissue represents about 79% of total methyl mercury transport to gut lumen. Units are mL tissue/min/g tissue.
[h] Value represents the average rate of GI mucosal cell exfoliation. Estimated from Bertalanffy (1960).
[i] Units are mL skin/min/g skin.

the carcass, gastrointestinal tissue, skin, and kidneys are assumed to follow a common mechanism and are based on the empirically estimated parameter for the kidneys. Transport of both organic and inorganic mercury to brain and hair compartments is assumed to be limited by the blood-brain barrier and the rate of hair growth. Recycled mercury from ingested hair during grooming was assumed available for reabsorption from the gut lumen at 100% for methylmercury and 10% for inorganic mercury.

The authors make the assumption that all of the inorganic mercury resulting from the demethylation of methylmercury is mercuric mercury. Farris et al. (1993) note that the precise site of demethylation is unknown, although the body's tissues and the lumen of the gastrointestinal tract seem most likely. For convenience, however, they modeled demethylation entirely in the liver compartment. Bidirectional and symmetric transport of methylmercury between the gut tissue and lumen is assumed and modeled accordingly. Biliary secretion of both methylmercury and inorganic mercury are modeled as undergoing low-molecular weight nonprotein sulfhydryl (NPSH) secretion d-dependent transport. Methylmercury secreted into the gut lumen, either from biliary secretion or from the gut tissue, is modeled as being readily reabsorbed. In line with previous studies, the model sets a value of 10% for resorption of inorganic mercury secreted into the lumen from bile or from exfoliation of the gastrointestinal mucosal cells.

The assumptions in the model were incorporated into a series of mass-balance differential equations that account for the changes in the amount of methylmercury and mercuric mercury in each compartment. The entire equation set was solved numerically using Gear's method for stiff differential equations (Gear 1971). The initial mercury dose was administered at 100% methylmercury, administered as a bolus to the gut lumen compartment. The mass transport parameters listed in Table 2-6 were multiplied by the time-dependent compartment volumes to give the mass transport parameters used in the model equations.

Validation of the model. The Farris et al. model simulations were compared to an extensive set of data collected by the authors on the metabolism and distribution of an orally dosed bolus of radiolabeled methyl-mercury in male Sprague-Dawley rats. In a distribution study, tissue samples were collected on days 3, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, and 98 post-dosing. In a metabolism study with the same dosing regimen, whole body counts and 24-hour feces and urine samples were collected daily for 15 days post-dosing, and then twice weekly.

The model simulations were in close agreement with the observed results from the distribution and metabolism studies. Physiological processes that were highlighted by the results and the discrepancies that

did occur include the probable active transport into the brain (versus passive diffusion) of a methylmercury-cysteine complex, the bidirectional transport of methylmercury between the gut lumen and gut tissue as a more important determinant of methylmercury fecal excretion than biliary secretion, the importance for the determination of methylmercury half-life in rats of the recycling of mercury from ingested hair, and the need for better estimates of the rate constants for the demethylation of methylmercury in order to adapt the model to other species.

No human data were presented to validate the model, and validation was not performed for other routes or duration of mercury exposure.

Target tissues. The target tissues for this model included the blood, liver, gut, kidneys, and brain.

Species extrapolation. The model was developed and validated using the male Sprague-Dawley rat. No other species were tested and data from other species were not used to validate the model. The authors, however, suggest that this model would prove useful in developing better rate constants or other important determinants of species differences (for example, demethylation rates, which differ based on differences in gut flora and tissue enzyme levels).

High-low dose extrapolations. Only the single nontoxic dose was evaluated. No data were presented to evaluate the utility of the model for high-to-low dose extrapolations.

Interroute extrapolation. Only the single oral dose was evaluated. No data were presented to evaluate the validity of the model in extrapolating from an oral to an inhalation or dermal dose. No compartment was included for the lungs. Although a skin compartment was included in the model, absorption from a dermal application of methylmercury was not addressed.

The Gray Model for Methylmercury.

The Gray (1995) PBPK model 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.

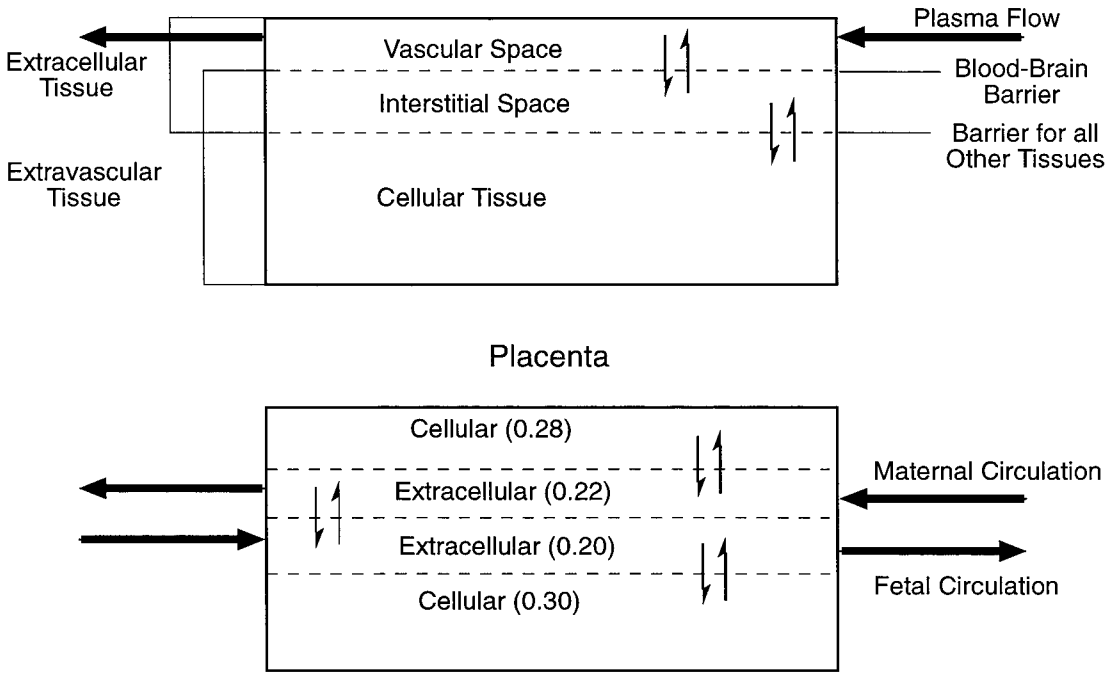
Risk assessment. The Gray model has not been used in human risk assessment. The author, however, suggests that the model would be useful to incorporate rat developmental toxicity data into the assessment of methylmercury risk. Specifically, the author suggests the model be used to convert the short-term exposure data from studies presently being used in risk assessments into continuous-exposure scenarios, which are more typical of the general public's likely exposure pattern.

Description of the model. The Gray model is a membrane-limited PBPK model for methylmercury developed using experimental data from the literature. The model parameters include constants for linear binding, membrane transfer, biliary transport, and gut reabsorption; and physiological parameters for tissue cellular and extracellular volumes and plasma flow rates. Mass balance equations were developed that describe the transport to all organ systems important to the distribution or toxicity of methylmercury to the pregnant rat or fetus. Mass balance equations were solved using an Advanced Continuous Simulation Language (ACSL) program developed by Mitchell and Gauthier Associates.

The compartments and barriers to methylmercury transport in the tissue compartments and placenta are shown in Figure 2-6. The cell membrane is assumed to be the barrier for methylmercury transport for all tissues except the brain and placenta. The barrier to methylmercury transport to the brain is the endothelial cell wall of the cerebral vascular system (the blood-brain barrier). The placenta is modeled as four compartments, with separate transfer constants for placental barrier and placental tissue transport. There is a tissue compartment for both the maternal and fetal sides of the placenta.

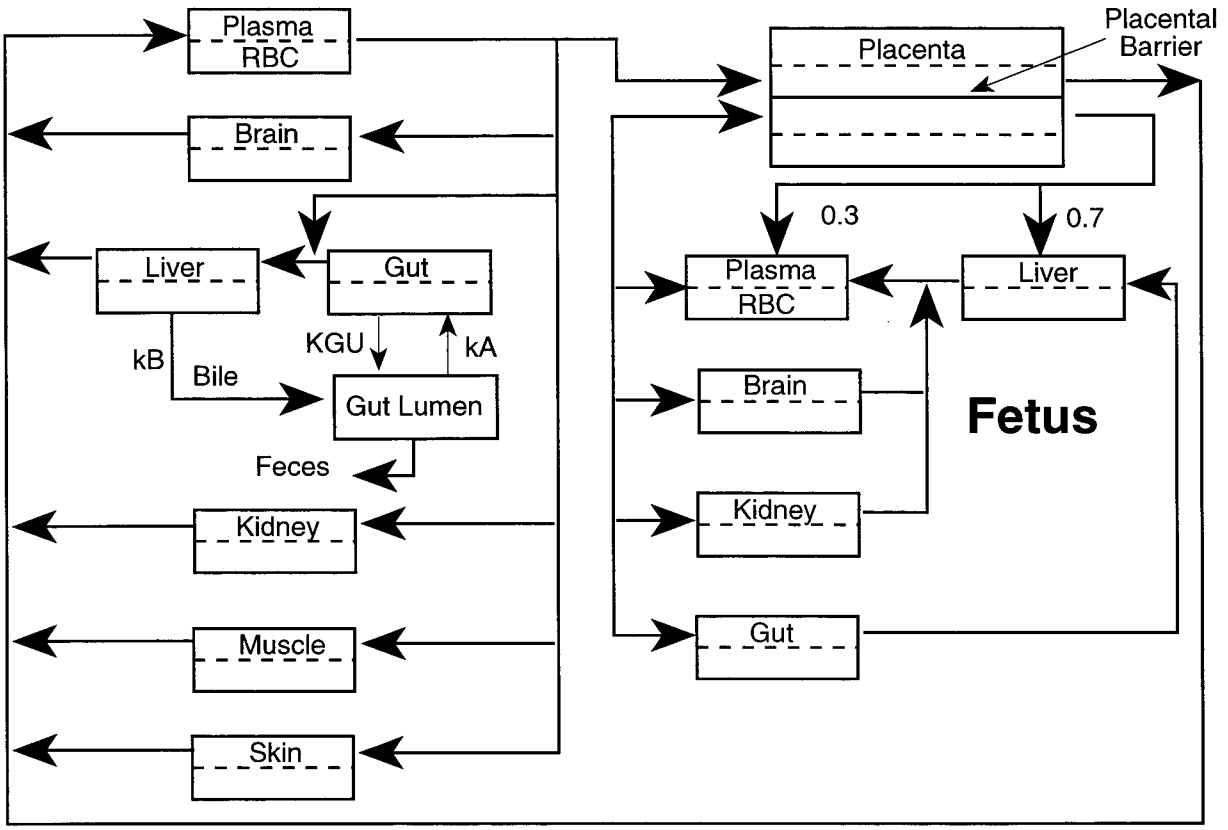
The flow chart shown in Figure 2-7 illustrates the transport pathways among the 8 compartments of the pregnant rat, the 5 compartments of the fetus, and the placental interface. The linear binding, membrane transfer transport, and secretion/reabsorption constants used in the Gray model are shown in Tables 2-7 and 2-8. The linear binding constants were estimated directly from *in vivo* tissue distribution studies using the ratio of tissue to plasma concentrations at pseudoequilibrium. They represent the degree to which methyl-mercury binds to intracellular sites. Because the skin (which includes the outer layers of hair and the pelt) contained excreted methylmercury that does not exchange with plasma, the linear binding constant for a typical organ (in this case the liver) was used as the constant for skin. No experimental data were available for fetal red blood cell (RBC) binding, so the author made the assumption that the fetal RBC binding constant would be equal to the maternal RBC binding constant. The conversion of methylmercury into mercuric mercury in the gut is not explicitly calculated in the Gray model; instead, the calculated

Figure 2-6. PBPK Model for Mercury in the Pregnant Rat



Source: Gray 1995

Figure 2-7. Model for Mercury Transport in the Pregnant Rat and Fetus



Source: Gray 1995

Table 2-7. Linear Binding and Membrane Transfer Constants

Tissue	Linear binding[a]	Membrane transfer (min^{-1})
Maternal		
Liver	14.2	0.56[b]
Kidney	164	1.55[b]
Brain	11.1	0.02[b]
Red blood cells	302	1.0[c]
Muscle	14.5	0.063[d]
Skin	14.2	0.506
Fetal		
Placenta	102[c]	0.064[c]
Placental barrier	—	0.33[f]
Liver	54.9	0.506
Kidney	21.3	1.55
Brain	23.7	0.028
Red blood cells	302	0.08[f]

[a] Wannag (1976)
[b] Thomas et al. (1986)
[c] Hirayama (9185)
[d] Berlin (1963)
[e] Mansour et al. (1974)
[f] Fit to Wannag (1976) data

Table 2-8. Secretion/Reabsorption Constants

Rate	Constant
Biliary secretion rate kB (excretion rate[a] /liver MeHg mass[b])	0.0021 min^{-1}
Gut resorption rate kA (resorption rate[a] /excretion rate x gut)	0.0026 min^{-1}
Gut volume[c]	24.4 mL
Fecal flow rate[c]	0.069 mL/min
Excretion rate[a] (average over 10 days)	8.68×10^{-6} mg/min
Reabsorption rate (average over 10 days)	7.99×10^{-6} mg/min

[a] Norseth and Clarkson 1971
[b] Wannag 1976
[c] Harrison and Gibaldi 1977

reabsorption rate of secreted or shed methylmercury in the gut implicitly accounts for the amount converted (i.e., the amount of demethylated mercury that subsequently would not be reabsorbed).

Published data were used directly or to estimate values for the maternal and fetal extracellular space, maternal plasma volume and flow expansion during pregnancy, and maternal and fetal organ volumes and plasma flow.

The model was run with a single intravenous bolus dose of 1 mg/kg at various times during a 22-day rat gestation period and compared with previously published (different author) maternal and fetal organ concentrations. The model was also run with a daily dosing for 98 days, ending on Gd 20, to simulate a typical human dietary exposure pattern for a frequent consumer of methylmercury-contaminated food.

Validation of the model. The Gray model simulations were validated against published values in the literature for mercury concentrations in maternal and fetal rat tissue from a variety of dosing patterns over the 22-day rat gestation period. Model-derived estimates of methylmercury half-life in red blood cells of 14.8 days for the rat were consistent with published values from 14 to 16 days. Consistent values were also obtained for the timing of the peak mercury concentration in the brain. Model estimates were in agreement with published values for most tissue mercury concentrations for dosing at various times, with percent differences generally <25%. Model estimates of maternal kidney methylmercury concentrations were consistently below published values, possibly due to an underestimate of the inorganic fraction of mercuric mercury in the kidneys.

The model results for a total fetal methylmercury concentration of 0.79% 24 hours after maternal methyl-mercury dosing on Gd 19 compare favorably with published values of 0.6 and 0.88% for administered doses on Gd 19 and 20, respectively.

No human data were presented to validate the model, and validation was not performed for other routes of mercury exposure.

Target tissues. The target tissues for this model included the blood, liver, gut, kidneys, and brain.

Species extrapolation. The model validated the use of published data for the rat. No other species were tested, and data from other species were not used to validate the model. The author, however,

suggests that generally good agreement between the model simulated results and the published values indicate that the model accurately reflects the underlying biological processes and that scaling factors for species-to-species extrapolations should be considered.

High-low dose extrapolations. No data were presented to evaluate the utility of the model for high-to-low dose extrapolations. A continuous exposure was simulated, but it was not validated against published data.

Interroute extrapolation. Only the intravenous route of exposure was evaluated. No data were presented to evaluate the validity of the model in extrapolating to an oral, inhalation, or dermal route of exposure. No compartment was included in the model for the lungs. Although a skin compartment was included in the model, absorption from a dermal application of methylmercury was not addressed.

2.4 MECHANISMS OF ACTION

2.4.1 Pharmacokinetic Mechanisms

The absorption of metallic mercury through the lungs is by rapid diffusion. It is suggested that oral absorption of inorganic mercury compounds depends on their dissociation in the intestinal tract. In several cases, the underlying mechanism for the toxic effects of mercury has been attributed to the high affinity of mercury for protein-containing sulfhydryl or thiol groups.

The mechanism of absorption for metallic mercury vapors is rapid diffusion across alveolar membranes (Berlin et al. 1969; Clarkson 1989). Mercury distribution in the brains of mercury-sensitive SJL/N mice exposed for 10 weeks (5 days per week) to relatively high concentrations (0.5–1.0 mg/m^3) of mercury vapor was found to be affected by the magnitude of exposure (Warfvinge 1995). In animals exposed to 0.5 mg/m^3 for 19 hours a day or 1 mg/m^3 for 3 hours a day, mercury was found in almost the entire brain, whereas in those exposed to 0.3 mg/m^3 for 6 hours a day, mercury was primarily found in the neocortical layer V, the white matter, the thalamus, and the brain stem. In mice exposed to 1 mg/m^3 for just 1.5 hours a day, the white matter and brain stem were the targets for mercury accumulation. These findings in mice were generally in agreement with brain distribution patterns observed in mercury-sensitive rats (Schionning et al. 1991; Warfvinge et al. 1992), except that the white matter was not found to be a target for mercury accumulation.

Oral absorption of metallic mercury is low, possibly because of an *in vivo* conversion to divalent mercury and subsequent binding to sulfhydral groups, or possibly because of poor absorption of the elemental form. For inorganic mercuric compounds, the low absorption in the lungs is probably due to the deposition of particles in the upper respiratory system that should be cleared rapidly (Friberg and Nordberg 1973). Solubility and other chemical properties may also be factors in the absorption. The mechanism for intestinal absorption of inorganic mercuric mercury may also involve the process of diffusion, and the absorption rate is proportional to the concentration of mercury in the lumen of the intestines (Piotrowski et al. 1992). The extent of transport of inorganic mercury across the intestinal tract may depend on its solubility (Friberg and Nordberg 1973) or on how easily the compounds dissociate in the lumen (Endo et al. 1990). Absorption of mercurous compounds is less likely, probably because of solubility (Friberg and Nordberg 1973) or its conversion into the divalent cation in the gastrointestinal tract.

The divalent cation exists in both a nondiffusible form (tissues) and a diffusible form (blood) (Halbach and Clarkson 1978; Magos 1967) (see Section 2.3.2). The mechanism for the distribution of mercury and its compounds probably depends on the extent of uptake of the diffusible forms into different tissues or on the mercury-binding to protein-binding sites (sulfhydryl groups) in red cells and plasma proteins (Clarkson 1972b).

Mechanisms for the toxic effects of inorganic and organic mercury are believed to be similar. It has been suggested that the relative toxicities of the different forms of mercury (e.g., metallic, monovalent, and divalent cations and methyl- and phenylmercury compounds) are related, in part, to its differential accumulation in sensitive tissues. This theory is supported by the observation that mercury rapidly accumulates in the kidneys and specific areas of the central nervous system (Rothstein and Hayes 1960; Somjen et al. 1973).

The accumulation of methylmercury and inorganic mercury in the brain of female monkeys *(Macaca fascicularis)* was studied by Vahter et al. (1994). In this study, animals received oral doses of 50 µg/kg/day for either 6, 12, or 18 months. In normal-weight monkeys (2.4–4.1 kg), a steady-state blood concentration for total mercury was attained in approximately 4 months. The elimination half-life in the blood was found to be 26 days. Accumulation in the brain appeared to be biphasic, with an elimination half-life of 35 days for brain methylmercury in those monkeys exposed for 12 months. The elimination half-life of inorganic mercury, on the other hand, was reported be on the order of years. It was also found that inorganic mercury accounted for approximately 9% of the total brain mercury at 6–12 months, 18% at

18 months, and 74% 6 months after termination of exposure. The authors stated that the presence of inorganic mercury in the brain was thought to be the result of demethylation of methylmercury in the brain. In heavier monkeys, there was a limited distribution of mercury in the fat. A finding of higher brain concentrations in the heavy monkeys than in those of normal weight was probably due to higher blood mercury levels and a higher brain-to-blood distribution ratio. *In vivo* methylation of inorganic mercury, on the other hand, was not shown to occur in occupationally exposed workers (Barregard et al. 1994a, 1994b), contrary to the findings of previous *in vitro* studies.

Distribution of organic mercury is believed to involve complexes with proteins in the body. Methylmercury associates with water-soluble molecules (e.g., proteins) or thiol-containing amino acids because of the high affinity of the methylmercuric cation (CH_3Hg^+) for the sulfhydryl groups (SH-) (Aschner and Aschner 1990). Complexes of methylmercury with cysteine or glutathione have been identified in blood, liver, and bile (Aschner and Aschner 1990). The transport of methylmercury to the brain after subcutaneous injection appears to be closely linked to thiol-containing amino acids (Aschner and Clarkson 1988). The methyl-mercury cation can bind to the thiol group of the amino acid cysteine, forming a complex in which the valence bonds link the mercury atom to adjacent iron and sulfur atoms at an 180E angle, creating a chemical structure similar to that of the essential amino acid methionine (Clarkson 1995). In such a manner, methyl-mercury 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). The uptake of methylmercury by the brain is inhibited by the presence of other amino acids such as leucine, methionine, phenylalanine, and other large neutral amino acids (Clarkson 1995).

The mechanism by which methylmercury crosses the blood-brain barrier has also been examined in the rat using a rapid carotid infusion technique (Kerper et al. 1992). The results of this study also showed that methylmercury may enter the brain as a cysteine complex. The uptake of Me_20_3Hg complexed with either L- or D-cysteine was measured as a function of Me_20_3Hg-cysteine concentration in the injection solution. There was a faster rate of uptake of Me_20_3Hg-L-cysteine as compared to the D-cysteine complex. The nonlinearity of Me_20_3Hg-L-cysteine uptake with the increasing concentration suggests that transport of this complex is saturable, while the D-cysteine complex is taken up by simple diffusion. The mechanism for the distribution in the brain of inorganic mercury (resulting from the demethylation of organic mercury) is not well understood.

Strain and sex differences in renal mercury content in mice are attributable, in part, to differences in tissue glutathione content and to differences in renal γ-glutamyltranspeptidase activity, which is controlled, at least in part, by testosterone (Tanaka et al. 1991, 1992). The correlation of hepatic glutathione (or plasma glutathione) with the rate of renal uptake of methylmercury suggests that methylmercury is transported to the kidneys as a glutathione complex (Tanaka et al. 1991). In addition to strain and sex differences in renal mercury content, it has also been demonstrated using mice (133–904 days old) that the ratio of mercury in the brain to that in the liver and the kidneys increased significantly with age (Massie et al. 1993).

In a study of the absorption of inorganic mercury by the rat jejunum, Foulkes and Bergman (1993) found that while tissue mercury could not be rigorously separated into membrane-bound and intracellular compartments (as can the heavy metal cadmium), its uptake into the jejunum includes a relatively temperature-insensitive and rapid influx into a pool readily accessible to suitable extracellular chelators. A separate, slower and more temperature-sensitive component, however, leads to the filling of a relatively chelation-resistant compartment. Nonspecific membrane properties, such as surface charge or membrane fluidity, might account for mucosal mercury uptake (Foulkes and Bergman 1993).

2.4.2 Mechanisms of Toxicity

High-affinity binding of the divalent mercuric ion to thiol or sulfhydryl groups of proteins is believed to be a major mechanism for the biological activity of mercury (Clarkson 1972a; Hughes 1957; Passow et al. 1961). Because proteins containing sulfhydryl groups occur in both extracellular and intracellular membranes and organelles, and because most sulfhydryl groups play an integral part in the structure or function of most proteins, the precise target(s) for mercury is not easily determined, if indeed there is a specific target. Possibilities include the inactivation of various enzymes, structural proteins, or transport processes (Bulger 1986); or alteration of cell membrane permeability by the formation of mercaptides (Sahaphong and Trump 1971). Binding may also occur to other sites (e.g., amine, carboxyl groups) that are less favored than sulfhydryl groups. A variety of mercury-induced alterations are being investigated, including increased oxidative stress, disruption of microtubule formation, increased permeability of the blood-brain barrier, disruption of protein synthesis, disruption of DNA replication and DNA polymerase activity, impairment of synaptic transmission, membrane disruption, impairment of the immune response, and disruption in calcium homeostasis. These alterations may be acting singly or in combination.

Mercury has been shown to affect hepatic microsomal enzyme activity (Alexidis et al. 1994). Intraperitoneal administration of mercuric acetate (6.2 µmol/kg/day) once daily for 6 days or once as a single dose of 15.68 µmol/kg resulted in an increase in kidney weight and a significant decrease in total cytochrome P-450 content. The single 15.68 µmol/kg injection resulted in the reduction of both microsomal protein level and P-450 content, possibly resulting from the generation of free radicals during the Hg^{++} intoxication process.

Through alterations in intracellular thiol status, mercury can promote oxidative stress, lipid peroxidation, mitochondrial dysfunction, and changes in heme metabolism (Zalups and Lash 1994). $HgCl_2$ has been shown to cause depolarization of the mitochondrial inner membrane, with a consequent increase in the formation of H_2O_2 (Lund et al. 1993). These events are coupled with a Hg^{++}-mediated glutathione depletion and pyridine nucleotide oxidation, creating an oxidant stress condition characterized by increased susceptibility of the mitochondrial membrane to iron-dependent lipid peroxidation. Lund et al. (1993) further postulated that mercury-induced alterations in mitochondrial calcium homeostasis may exacerbate Hg^{++}-induced oxidative stress in kidney cells. As a result of oxidative damage to the kidneys, numerous biochemical changes may occur, including the excretion of excess porphyrins in the urine (porphyrinuria). In a study of the mechanism of porphyrinogen oxidation by mercuric chloride, Miller and Woods (1993) found that mercury-thiol complexes possess redox activity, which promotes the oxidation of porphyrinogen and possibly other biomolecules.

The steps between thiol binding and cellular dysfunction or damage have not been completely elucidated, but several theories exist. Conner and Fowler (1993) have suggested that following entry of the mercuric or methylmercuric ion into the proximal tubular epithelial cell by transport across either the brush-border or basolateral membrane, mercury interacts with thiol-containing compounds, principally glutathione and metallothionein. This interaction initially produces alterations in membrane permeability to calcium ions and inhibition of mitochondrial function. Through unknown signaling mechanisms, mercury subsequently induces the synthesis of glutathione, various glutathione-dependent enzymes, metallothionein, and several stress proteins (Conner and Fowler 1993). In the kidneys, epithelial cell damage is believed to occur as the result of enhanced free radical formation and lipid peroxidation (Gstraunthaler et al. 1983). Treatment with mercury results in depletion of cellular defense mechanisms against oxidative damage such as glutathione, superoxide dismutase, catalase, and glutathione peroxidase (Gstraunthaler et al. 1983). Further, enhancement of glutathione peroxidase has been observed in mercury-treated rats in direct relationship with kidney mercury content (Guillermina and Elias 1995), but inhibition of renal redox cycle

enzymes *in vivo* did not appear to be a significant determinant of the increased lipid peroxidation observed during $HgCl_2$-induced nephrotoxicity. The selenium-dependent form of glutathione peroxidase is highly sensitive to inhibition by mercury, and it has been proposed that mercury interactions with selenium in the epithelial cells limit the amount of selenium available for this enzyme (Nielsen et al. 1991). Depletion of mitochondrial glutathione and increases in mitochondrial hydrogen peroxide at the inner mitochondrial membrane (Lund et al. 1991) may contribute to acceleration of the turnover of potassium and magnesium observed at this membrane (Humes and Weinberg 1983). Acute renal failure resulting from mercury exposure has been proposed to result from decreased renal reabsorption of sodium and chloride in the proximal tubules and increased concentrations of these ions at the macula densa (Barnes et al. 1980). This increase in ions at the macula densa, in turn, results in the local release of renin, vasoconstriction of the afferent arteriole, and filtration failure. These authors based this hypothesis on the observation that saline pretreatment of rats prior to mercuric chloride treatment did not prevent the proximal tubular damage but did prevent the acute renal failure. The saline pretreatment was suggested to have depleted the glomerular renin and thereby prevented the cascade of events occurring after accumulation of sodium and chloride ions at the glomerular macula densa (Barnes et al. 1980). A pivotal role for extracellular glutathione and membrane-bound γ-glutamyltransferase has also been identified in the renal incorporation, toxicity, and excretion of inorganic mercury ($HgCl_2$) in rats (Ceaurriz et al. 1994).

A similar mechanism for the promotion of neuronal degeneration by mercury has been proposed (Sarafian and Verity 1991). Increases in the formation of reactive oxygen species in several brain areas have been observed following intraperitoneal administration of methylmercuric chloride to rodents (Ali et al. 1992; LeBel et al. 1990, 1992). A dissociation between increases in lipid peroxidation and cytotoxicity has been demonstrated by showing inhibition of the lipid peroxidation with α-tocopherol without blocking the cytotoxicity (Verity and Sarafian 1991). These authors were able to show partial protection against the cytotoxicity with ethylene glycol tetra-acetate (EGTA), suggesting that increases in intracellular calcium may play a role in the cytotoxicity. They ultimately concluded that a synergistic interaction occurred between changes in intracellular calcium homeostasis and intracellular thiol status, culminating in lipoperoxidation, activation of Ca2+-dependent proteolysis, endonuclease activation, and phospholipid hydrolysis (Verity and Sarafian 1991). It has been suggested that neurons are highly sensitive to mercury either because of their low endogenous glutathione content or their inefficient glutathione redox activity. Inhibition of protein synthesis has been reported in neurons from rats exposed to methylmercury (Syversen 1977). However, it is unknown whether this inhibition is secondary to neuronal cytotoxicity.

At the functional level, both mercuric chloride and methylmercury have been shown to induce a slow inward current in patch-clamped dorsal root ganglion cells (Arakawa et al. 1991). The current does not appear to be mediated by either the sodium or calcium channels, but it may be activated by increases in intracellular calcium. Such slow inward currents suppress voltage- and neurotransmitter-activated currents. Studies of the effects of inorganic mercury, methylmercury, and phenylmercuric acetate on synaptic transmission in rat hippocampal slices (Yuan and Atchison 1994) revealed that the mechanisms that underlie the effects of various mercurials on central synaptic transmission differ with respect to the sites of action, the potency, and the reversibility of the effect. Inorganic mercury (Hg^{++}) appeared to act primarily on the postsynaptic neuronal membrane, whereas the action of methylmercury and phenylmercuric acetate was at both the pre- and postsynaptic sites but primarily on the postsynaptic membranes. Yuan and Atchison (1994) suggested that these differences may result, in part, from the differences in lipophilicity among the different mercurials studied. Differences in lipophilicity were also implicated by Roed and Herlofson (1994) as playing a role in the different effects produced by methylmercuric chloride and mercuric chloride. Roed and Herlofson (1994) suggested that the high lipid solubility of methylmercuric chloride may divert that organomercurial to the myelin of the nerve, where it very efficiently inhibits neuronal excitability. Further, they suggested that mercuric chloride probably causes inhibitory activity by binding to sulfhydryl groups in transport proteins that convey the messenger function of intracellular Ca^{++}. This, in turn, leads to both inhibition of muscle contraction and enhancement of $HgCl_2$-induced neuronal inhibition. The authors further suggest that $HgCl_2$ inhibits an internal Ca^{++} signal necessary for choline re-uptake and acetylcholine resynthesis.

Gallagher and Lee (1980) evaluated the similarity of inorganic and organic mercury toxicity to nervous tissue by injecting equimolar concentrations of both mercuric chloride and methylmercuric acetate directly into the cerebrum of rats, thereby circumventing systemic metabolic conversion pathways. The lesions induced by mercuric chloride were expected to have been much greater after the mercuric chloride injection, since this process circumvents the necessity for biotransformation. However, the lesions were only slightly larger than those seen after methylmercury injection, suggesting that there is a mechanism for organic mercury neurotoxicity that does not involve conversion into inorganic mercury. This suggestion is supported by the findings of Magos et al. (1985) who failed to establish a correlation between neuronal, cytoplasmic, mercuric ions and neuronal degeneration, or clinical evidence of neurotoxicity. These results do not, however, preclude the possibility that intracellular transport of mercuric mercury may be limited, and the limitations on transport may determine the effects observed.

Recent data from an *in vitro* study suggest that mercuric mercury may be more effective than methylmercury in some paradigms. Using patch-clamped dorsal root neurons, Arakawa et al. (1991) showed augmentation of the GABA-activated chloride current at extremely low mercuric chloride concentrations (0.1 μM), while a 1,000-fold higher concentration of methylmercury showed no such effect. The correlation between these effects observed *in vitro* and what may be occurring *in vivo*, however, is not known.

The experimental data concerning the mechanism of action of methylmercury on the developing nervous system indicate that effects on the microtubules and amino acid transport are disrupted in neuronal cells before overt signs of intoxication are observed. Vogel et al. (1985) demonstrated the potent inhibitory effects of methylmercury on microtubule assembly at ratios stoichiometric with the tubulin dimer. The effects were thought to be mediated through MeHg binding to free sulfhydryl groups on both ends and on the surface of microtubules, which would provide multiple classes of binding sites for MeHg. In subsequent *in vitro* studies, Vogel et al. (1989) identified a single high affinity class of binding sites on tubulin for methylmercury with 15 sites. The authors report that MeHg binds to tubulin stoichiometrically within microtubules, and does not induce microtubule disassembly at this low binding ratio. Free subunits of tubulin, however, will act as uncompetitive inhibitors for MeHg binding to the polymer, and MeHg binding to the multiple sites in the free dimer blocks subsequent assembly. In contrast, the stoichiometric polymer surface binding sites for MeHg in microtubules apparently do not interfere with subsequent polymerization. Mitotic inhibition from damage to microtubulin and binding to tubulin has also been reported by Sager et al. (1983).

Comparison of the effects of mercury on structural elements and enzyme activities (Vignani et al. 1992) suggests that effects on cytoskeletal elements may be observed at lower concentrations than on enzyme activities. 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. The toxic effects of methylmercury on the developing nervous system may also be due to deranged neuronal cell migration (Choi et al. 1978; Matsumoto et al. 1965). Examination of the brains of two infants who died following *in utero* exposure to methylmercury revealed an abnormal pattern in the

organization and a distorted alignment of neurons in the cerebral cortex. Exposures first occurred during the critical period of neuronal migration (from gestation week 7 into the third trimester) in the fetus. Both could result from a direct effect of mercury on microtubule proteins. Cell division and cell migration both require intact microtubules for normal functioning and, therefore, have been suggested as primary targets for methylmercury disruption in the developing nervous system. It is hypothesized by Aschner and Clarkson (1988) that the uptake of methylmercury through the blood-brain barrier in developing and mature animals is closely linked to amino acid transport and metabolism because of the infusion of L-cysteine enhanced ^{203}Hg uptake. The enhanced transport in the fetus may be a result of the immaturity of the transport systems in the blood-brain barrier or of possible physical immaturity of the barrier itself. Methylmercury has also been shown to increase intracellular Ca^{++} and inositol phosphate levels (Sarafian 1993). The observed stimulation of protein phosphorylation in rat cerebral neuronal culture was believed to be the result of elevation of intracellular second messengers (Ca^{++}, inositol phosphate) rather than to a direct interaction between methylmercury and protein kinase enzymes. This observation was considered to suggest a specific interference with neuronal signal transduction.

The mercuric ion is also an extremely potent inhibitor of microtubule polymerization, both *in vivo* and *in vitro* (Duhr et al. 1993). Duhr and his colleagues further reported that the ability of Hg^{++} to inhibit microtubule polymerization or to disrupt already formed microtubules not only cannot be prevented by binding with the chelating agents EDTA and EGTA, but that the binding of these two potent chelators potentiates the Hg^{++}-induced inhibition of tubulin polymerization by disrupting the interaction of GTP with the E-site of brain beta-tubulin, an obligatory step in the polymerization of tubulin.

Mercury has been shown to inhibit a variety of enzymes in the nervous system. The effects of mercuric chloride and methylmercuric chloride on the activity of protein kinase C in rat brain homogenate were studied by Rajanna et al. (1995). In this study, it was found that both forms of mercury inhibited protein kinase C activity in a dose-dependent manner at micromolar concentrations, with methylmercury being a more potent inhibitor than $HgCl_2$. Mercuric chloride has also been shown to cause the inhibition and ultrastructural localization of cerebral alkaline phosphatase (Albrecht et al. 1994) following a single intraperitoneal injection of 6 mg $HgCl_2$/kg body weight. The observed inhibition and subsequent translocation of alkaline phosphatase activity from the luminal to abluminal site and the accompanying ultrastructural alterations were reported to be typical of the formation of "leaky" microvessels known to be associated with damage to the blood-brain barrier. Mercuric chloride has also been demonstrated to block the uptake of [3H]-histamine by cultured rat astroglial cells and brain endothelial cells (Huszti and Balogh

1995). This effect was seen at mercury concentrations as low as 1 µM, and the inhibition was greater in astroglial cells than in the cerebral endothelial cells. At a concentration of 100 µM, however, $HgCl_2$ caused the stimulation of histamine uptake, which was greater in the cerebral endothelial than in the astroglial cells. The mechanisms of these dose-dependent effects were considered to be different, with the inhibition of histamine uptake associated with the loss of the transmembrane Na+ and/or K+ gradient and the stimulation of histamine uptake by the higher mercury concentration being possibly related to a direct effect on the histamine transporter.

Sekowski et al. (1997) used an intact human cell multiprotein complex (which they call a DNA synthesome) to evaluate the effects of mercuric chloride on DNA synthesome-mediated *in vitro* DNA replication and DNA synthesis. The authors state that the DNA synthesome has the advantage of providing the highly ordered environment in which DNA replication occurs while allowing more precise identification of the mechanism or site of effects than possible from the use of whole cells. The results showed that DNA replication and DNA polymerase activity, as well as DNA replication fidelity of the human cell synthesome, were specifically inhibited by mercuric ion at physiologically attainable concentrations. The results suggest that mercuric ions (at concentrations above 10 µM) actively inhibit the elongation stage of DNA replication.

It has been shown that Hg^{++} promotes dose-dependent toxic effects on heart muscle through actions on the sarcolemma, the sarcoplasmic reticulum, and contractile proteins (Oliveira et al. 1994). In this study, inorganic mercury ($HgCl_2$) was shown to have a dose-dependent effect on rat papillary muscle, with a concentration of 1 µM causing a small increase in the force of isometric contraction. Concentrations of 2.5, 5, and 10 µM produced a dose-dependent decrease in contractile force. The rate of force development, however, was effected differently, increasing at 1 and 2.5 µM Hg^{++} but decreasing to control levels at 5 and 10 µM concentrations. Oliveira et al. (1994) suggested that this response was due to an observed progressive reduction in the time to peak tension with increasing mercury concentrations, an effect they attributed to the binding of mercuric ions to SH groups inducing Ca^{++} release from the sarcoplasmic reticulum, the activity of which itself was depressed by mercury in a dose-dependent fashion. Further, tetanic tension did not change during treatment with 1 µM Hg^{++} but decreased with 5 µM, suggesting a toxic effect on the contractile proteins only at high Hg^{++} concentrations (Oliveira et al. 1994).

The molecular events leading to activation of the autoimmune response in susceptible individuals have yet to be fully elucidated. However, chemical modification of major histocompatibility complex (MHC) class

II molecules or modification of self peptides, T-cell receptors, or cell-surface adhesion molecules has been suggested (Mathieson 1992). The immune suppressive effect of mercury has been examined in human B-cells (Shenker et al. 1993). This study showed inhibition of B-cell proliferation, expression of surface antigens, and synthesis of IgG and IgM by both methylmercury and mercuric mercury. These chemicals caused a sustained elevation of intracellular calcium. Based on concurrent degenerative changes in the nucleus (hyperchromaticity, nuclear fragmentation, and condensation of nucleoplasm) in the presence of sustained membrane integrity, the author suggested that the increase in intracellular calcium was initiating apoptic changes in the B-cells, ultimately resulting in decreased viability.

The glomerulopathy produced by exposure of Brown-Norway rats to mercuric chloride has been related to the presence of antilaminin antibodies (Icard et al. 1993). Kosuda et al. (1993) suggest that both genetic background and immune regulatory networks (possibly acting through T-lymphocytes of the RT6 subset) may play an important role in the expression of autoimmunity after exposure to mercury. A strain (Brown-Norway) of rats known to be susceptible to mercury-induced production of autoantibodies to certain renal antigens (e.g., laminin) and autoimmune glomerulonephritis was compared to a nonsusceptible strain (Lewis). Different responses to subcutaneous injections of mercuric chloride regarding $RT6^+$ T-lymphocytes (a subpopulation of lymphocytes considered to have possible immunoregulatory properties) were observed. While a relative decrease in $RT6^+$ T-cells occurring with the development of renal autoantigen autoimmune responses was observed in the mercury-treated Brown-Norway rats, the Lewis rats did not develop renal autoimmunity and were found to have undergone significant change in the $RT6^+$-to-$RT6^+$ T-lymphocyte ratio. When Brown-Norway-Lewis F_1 hybrid rats were similarly dosed, effects similar to those in the Brown-Norway strain were seen, with the autoimmune responses to kidney antigens occurring concomitantly with a change in RT6 population proportionally in favor of T-lymphocytes that do not express the RT6 phenotype. Kosuda et al. (1993) proposed that there are both endogenous and exogenous components of mercury-induced autoimmunity. The endogenous (a genetically determined) component includes T-cell receptors, the major histocompatibility complex, and an immuno-regulatory network based upon a rather delicate balance between helper and suppressor (e.g., the $RT6^+$ T-lymphocytes) cells; whereas the exogenous component is represented by an environmental factor (e.g., mercury) capable of altering the balance within the immunoregulatory network. The manifestation of autoimmunity requires the presence and interaction of both of these components. In a similar study, Castedo et al. (1993) found that mercuric chloride induced $CD4^+$ autoreactive T-cells proliferate in the presence of class II^+ cells in susceptible Brown-Norway rats as well as in resistant Lewis rats. However, while those cells were believed to collaborate with B-cells in Brown-Norway rats to produce

autoantibodies, in Lewis rats they apparently initiate a suppressor circuit involving antiergotypic $CD8^+$ suppressor cells.

In Brown-Norway rats given 5 subcutaneous 1 mg/kg injections of mercuric chloride over a 10-day period, tissue injury (including vasculitis) was seen within 24 hours of the first injection (Qasim et al. 1995). The rapid onset of tissue injury suggests that cells other than T-cells may be involved in the primary induction of vasculitis typically seen as a response to mercuric chloride in this species. It is possible that this injury occurs through a direct action of $HgCl_2$ on neutrophils or through activation of mast cells, resulting in the release of TNF and IL8, which promote chemotaxis and activation of neutrophils. However, the changes in the Th2-like (CD4+CD45) T-cell subsets seen in this study were considered to provide support for the hypothesis that a rise in T helper cells drives the observed autoimmune syndrome, providing B-cell help, which leads to polyclonal activation and production of a range of antibodies.

Jiang and Moller (1995) found that mercuric chloride induced increased DNA synthesis *in vitro* (peak activity between days 4 and 6) in lymphocytes from several mouse strains and suggested a crucial role for helper T-cells in $HgCl_2$-induced immunotoxicity. The results of this study indicated that: (1) mercuric chloride activated CD4+ and CD8+ T-cells *(in vitro)* in a manner analogous to a specific antigen-driven response; (2) activation was dependent upon the presence of accessory cells; and (3) helper T-cells were induced to divide and transform in responder organ cells. This led Jiang and Moller (1995) to hypothesize that mercury binds to molecules on the antigen-presenting cell (APC) and transforms molecules on these cells to superantigens capable of activating T-cells with a particular set of antigen-binding receptors. In this manner, mercury could induce an internal activation of the immune system, which would in turn result in a variety of symptoms in predisposed individuals.

Both mercuric chloride (1 μM) and methylmercury (2 μM) have been shown to increase intracellular Ca^{++} concentrations in splenic lymphocytes in a concentration-dependent manner (Tang et al. 1993). The time course for the effect was, however, different for the two mercurials. In the case of methylmercury, the increase in intracellular Ca^{++} was rapid and the increased level was sustained over time, whereas the Ca^{++} rise caused by $HgCl_2$ was slower. While the effects of those mercurials did not appear to be associated with alterations of membrane integrity, both $HgCl_2$ and methylmercury did appear to cause membrane damage when the incubation time was extended. This study also found that methylmercury and mercuric chloride appear to exert their effects on internal lymphocyte Ca^{++} levels in different ways. Methylmercury increases intracellular Ca^{++} by both an apparent increase in the permeability of the membrane to

extracellular Ca^{++} and the mobilization of Ca^{++} from intracellular stores (perhaps the endoplasmic reticulum and mitochondria), whereas $HgCl_2$ causes only an increased influx of extracellular Ca^{++}.

2.4.3 Animal-to-Human Extrapolations

Mechanisms for the end toxic effects of inorganic and organic mercury are believed to be similar, and the differences in parent compound toxicity result from difference in the kinetics and metabolism of the parent compound. Animal models generally reflect the toxic events observed in humans (i.e., neurological for methylmercury toxicity and the kidneys for inorganic mercury); however, there are species and strain differences in response to mercury exposure. Prenatal exposures in animals result in neurological damage to the more sensitive developing fetus as is the case in humans. The observed inter- and intraspecies differences in the type and severity of the toxic response to mercury may result from differences in the absorption, distribution, transformation, and end tissue concentration of the parent mercury compound. For example, C57BL/6, B10.D2, B10.S inbred mice accumulated higher concentrations of mercury in the spleen than A.SW, and DBA/2 strains, subjected to the same dosage regimen. The higher concentration of splenic mercury in C57BL/6, B10.D2, B10.S correlated with the increased susceptibility of these strains to a mercury chloride-induced systemic autoimmune syndrome. The lower splenic mercury in A.SW, and DBA/2 strains resulted in more resistance to an autoimmune response (Griem et al. 1997).

A better understanding of certain physiological and biochemical processes affecting mercury kinetics may help explain these species differences. Specific processes that appear likely determinants include differences in demethylation rates affecting methylmercury fecal secretion, reabsorption, and membrane transport (Farris et al. 1993); differences in tissue glutathione content and renal γ-glutamyltranspeptidase activity (Tanaka et al. 1991, 1992), differences in antioxidative status (Miller and Woods 1993), differences in plasma cysteine concentrations compared with other thiol-containing amino acids (Aschner and Clarkson 1988; Clarkson 1995), and differences in factors that could affect gut lumenal uptake (Foulkes and Bergman 1993; Urano et al. 1990). Better controls and reporting of dietary factors, volume and timing of doses, and housing conditions would assist in the comparisons of effects among species and strains.

Further development of PBPK/PBPD models will assist in addressing these differences and in extrapolating animal data to support risk assessments for mercury exposure in humans.