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Organic mercury compounds: human exposure and its relevance to public health

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Humans may be exposed to organic forms of mercury by either inhalation, oral, or dermal routes, and the effects of such exposure depend upon both the type of mercury to which exposed and the magnitude of the exposure. In general, the effects of exposure to organic mercury are primarily neurologic, while a host of other organ systems may also be involved, including gastrointestinal, respiratory, hepatic, immune, dermal, and renal. While the primary source of exposure to organic mercury for most populations is the consumption of methylmercury-contaminated fish and shellfish, there are a number of other organomercurials to which humans might be exposed. The antibacterial and antifungal properties of organomercurials have resulted in their long use as topical disinfectants (thimerosal and merbromin) and preservatives in medical preparations (thimerosal) and grain products (both methyl and ethyl mercurials). Phenylmercury has been used in the past in paints, and dialkyl mercurials are still used in some industrial processes and in the calibration of certain analytical laboratory equipment. The effects of exposure to different organic mercurials by different routes of exposure are summarized in this article. *Toxicology and Industrial Health* 2002; **18:** 109–160.

Key words: *ethylmercury; mercury; methylmercury; organomercurials; phenylmercury; thimerosal*

Introduction

Mercury is a naturally occurring element in the earth's crust. Over geological time, it has been distributed throughout the environment by natural processes, such as volcanic activity, fires, movement of rivers, lakes, and streams, oceanic upwelling, and biological processes. Since the advent of the industrial revolution over 200 years ago, however, anthropogenic sources have become a significant contributor to the environmental distribution of mercury and its compounds.

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In the environment, elemental mercury can combine with chlorine, sulfur, phosphorous, and other elements to form inorganic compounds. Primarily through the action of micro-organisms, inorganic mercury can be combined with carbon to form organic mercury compounds, of which methylmercury is the most abundant. In surface waters, it is rapidly accumulated by aquatic organisms, where it biomagnifies as it ascends the food chain.

In addition to methylmercury, there are a number of other organomercurials to which humans might be exposed. The antibacterial and antifungal properties of organomercurials have resulted in their long use as topical disinfectants (thimerosal and

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merbromin) and preservatives in medical preparations (thimerosal) and grain products (both methyl and ethyl mercurials). Phenylmercury has been used in the past in paints, and dialkyl mercurials are still used in some industrial processes and in the calibration of certain analytical laboratory equipment.

In this paper, we summarize what is known about the behavior of some of these organic forms of mercury in humans and their potential impact on public health.

Human exposure

Humans may be exposed to organic forms of mercury by either inhalation, oral, or dermal routes. The primary route of entry depends on the form of organomercurial and the nature of the exposure. Ingestion is the most common route of entry into the human body for alkyl mercury compounds, but the use of some of these compounds in medical practice [e.g., thimerosal ($C_9H_9HgNaO_2S$) as a preservative in certain vaccines] adds parenteral administration to known routes of human exposure. Some aryl mercurials, particularly phenylmercury, may present exposure through either the oral route or via inhalation of elemental mercury as the mercurial volatilizes. Dialkyl forms of mercury, which fortunately have extremely limited usage (all occupational), are rapidly and substantially absorbed by all routes of exposure.

The primary source of exposure to organic mercury for most populations is the consumption of methylmercury-contaminated fish and shellfish. The body burden of methylmercury in fish is typically higher in older fish and in fish that are higher up in the food chain. Carnivorous species of fish at the top of their food chain (e.g., freshwater pike and marine sharks and swordfish) may have mercury tissue concentrations as much as 10000–100000 times the concentration in their ambient waters (Callahan *et al.*, 1979; EPA, 1984; WHO, 1990; 1991).

In aquatic mammals, mercury concentrations in the tissues of predator species increase as you ascend the food chain/web. Weihe *et al.* (1996) reported that muscle tissue of pilot whales caught around the Faroe Islands contains an average mercury concentration of 3.3 ppm, about half of which is methylmercury. Among terrestrial mammals, those that consume fish or other mammals typically have higher body burdens of mercury than do vegetarian species. The highest concentrations of mercury are found in the liver and kidney, with successively smaller amounts being sequestered in the muscle and brain. Fur is also a major indicator of methylmercury exposure, as mercury is redistributed from other organs over time (Aulerich *et al.*, 1974; Wobeser and Swift, 1976; Wobeser *et al.*, 1976).

The US Food and Drug Administration (FDA) has estimated that, on average, the intake rate for total mercury (both inorganic and organic) is between 50 and 100 nanograms (ng)/kg body weight per day (equivalent to 0.05–0.1 νg/kg per day or 3.5–7.0 νg/day for a 70-kg adult). This is based on the FDA total diet study of 1982–84 (Gunderson, 1988). Approximately 80–90% of the mercury in the FDA estimate would be expected to be in the form of methylmercury. A separate estimate of the average intake of methylmercury alone, based on a survey of fish eaters and average levels of methylmercury in fish, places the average intake of methylmercury at 36 ng/kg per day (equivalent to 0.036 νg/kg per day or 2.52 νg/day for a 70-kg adult), with a 99% upper bound estimate at 243 ng/kg per day (equivalent to 0.243 νg/kg per day or 17 νg/day for a 70-kg adult; Clarkson, 1990).

Forms of organic mercury to which the general public is fortunately not significantly exposed are the dialkyl mercurials, diethylmercury and dimethylmercury. Diethylmercury has been used in small amounts in certain industrial processes for over 100 years (ATSDR, 1999). Dimethylmercury is primarily used in the calibration of laboratory equipment, as a reagent, and in the manufacture of other chemicals. It is used in some chemistry laboratories as a reference material in nuclear magnetic resonance spectroscopy, which has resulted in one lethal exposure through extremely small dermal contact (Siegler *et al.*, 1999). Quite obviously, dimethylmercury is extremely toxic and, unlike other forms of mercury, is almost immediately and entirely absorbed through the intact skin. Further, the vapor pressure of dimethylmercury (58.8 at 23.7°C) is such that small quantities that

are spilled will evaporate rapidly. Toribara *et al.* (1997) estimate that a cubic meter of saturated air could hold more than 600 g of mercury. Dimethylmercury can cause delayed permanent brain damage and death with little or no warning during exposure. As there is a paucity of toxicity and other information concerning this particular chemical, extreme caution should be exercised by those using dimethylmercury or other dialkyl mercury compounds.

Background and general population exposures to organomercurials

Diet

Based on the FDA total diet study of 1982–84 (Gunderson, 1988), the FDA estimated the average intake for total mercury (both inorganic and organic) to be between 50 and 100 ng/kg per day. Based on the more recent 1989–90 FDA total diet study, the estimated intake of total mercury is 27–60 ng/kg per day (Cramer, 1994). An estimated 86% of the mercury in the total diet study is derived from fish (Tollefson and Cordle, 1986). A separate estimate of the average intake of methylmercury alone, based on a survey of fish eaters and average levels of methylmercury in fish, places the average intake of methylmercury at 36 ng/kg per day, with a 99% upper bound at 243 ng/kg per day (Clarkson, 1990).

Inhalation

Alkyl, dialkyl, and aryl mercurials are all sources of inhalation exposure to mercury. Alkyl and dialkyl mercurials volatilize as the organic compounds, whereas phenylmercury volatilizes as metallic mercury vapor. Once volatilization has occurred, all forms are readily absorbed through the lungs into the blood, and are systemically distributed via the general circulation.

Parenteral

Various forms of mercury have been used in medical practice for decades because of its antimicrobial and antifungal properties. The over-the-counter organomercurial products Merthiolate (thimerosal) and Mercurochrome (merbromin) have long been widely used as topical disinfectants for the treatment of childhood cuts, scrapes, and abrasions; and thimerosal has been used since the 1930s as a preservative to protect open multidose vials of many vaccines. These common uses all provide some degree of direct entry into the blood for distribution throughout the body. The topical applications also allow for some (probably minimal) inhalation of the active organomercurial component as well, depending upon the location of application on the body.

Health effects attributable to organomercurials

Most of what we know about the effects of prolonged high-level exposure to alkyl mercurial compounds comes from massive poisoning incidents in Japan and Iraq, and to a lesser extent from acute individual poisoning episodes or suicide attempts. Death resulting from organic mercury ingestion has been well documented following outbreaks of poisoning after consumption of methylmercury-contaminated fish in Minamata, Japan (Tsubaki and Takahashi, 1986) and consumption of grains contaminated with methylmercury and ethylmercury in Iraq (Bakir *et al.*, 1973; Al-Saleem and the Clinical Committee on Mercury Poisoning, 1976). A case-control study comparing the cause of death for patients with Minamata disease with the cause of death in unexposed persons showed that in deaths prior to 1970, Minamata patients had significantly increased noninflammatory diseases of the nervous system compared with unexposed persons (Tamashiro *et al.*, 1984). Pneumonia and nonischemic heart disease were reported as prominent secondary causes of death in the exposed group. For those patients who died between 1970 and 1980, Minamata disease was reported as the primary cause of death. Nonischemic heart disease correlated with the incidence of Minamata disease, and noninflammatory central nervous system disease was a prominent secondary cause of death among this group. More recent prospective epidemiologic studies of seafood-eating populations in the Seychelles (Davidson *et al.*, 1995; 1998) and Faroe Islands (Grandjean *et al.*, 1997; 1998) have focused on the investigation of more subtle neurobehavioral or neuropsychological effects in children exposed *in utero*.

Organic mercury compounds can produce effects on the nervous system, kidneys, liver, and other

organs in vertebrates. However, specific levels of exposure necessary to produce adverse effects in mammals vary among species. In general, smaller animals (e.g., minks and monkeys) are more susceptible to mercury toxicity than are larger mammals (e.g., seals and mule deer) (EPA, 1997). This may be due to a number of factors, including differences in pharmacokinetics and higher per unit of body weight food consumption necessary to maintain physiologic homeostasis in smaller animals.

Also, the form of mercury to which an animal is exposed is an important factor in the absorption, metabolism, distribution, excretion, and toxicity of the mercury (Goyer, 1993; ATSDR, 1999). For example, methylmercury, which is far more readily absorbed through the intestine, has considerably greater toxicity per equivalent dose than does inorganic mercury (Aulerich *et al.*, 1974). In the case of pharmacokinetics, ethylmercury, which might be expected to have a similar half-life to its alkyl sister methylmercury, has a much shorter half-life than methylmercury (Pichichero *et al.*, 2002). Further, ingestion of large amounts of mercurial salts would produce its most profound effect on the kidney (Zalups and Lash, 1994), while ingestion of organic forms of mercury would primarily affect the nervous system.

Lethality

Human

Cinca *et al.* (1979) reported the death of four family members who had ingested pork from a hog fed grain contaminated with ethylmercuric chloride. Bronchopheumonia was reported in four adults and one infant who ingested bread made from grain contaminated with alklymercurials in Iraq during a mercury poisoning outbreak in 1972 (Al-Saleem and the Clinical Committee on Mercury Poisoning, 1976).

Intramuscular injection of thimerosal has been reported to have resulted in the death of three children and two adults (Axton, 1972). In this case, six individuals were administered repeated doses of the antibiotic chloramphenicol, which contained thimerosal as a preservative. In this most unfortunate instance, a manufacturing error had resulted in each vial containing 510 mg of thimerosal, instead of the intended 0.51 mg per vial. As a result, total thimerosal dosage ranged from 90 to 330 mg/kg. Signs and symptoms of thimerosal poisoning included necrosis at the site of injection; pyrexia beginning four days after injection, renal tubular failure, with proteinuria, glycosuria, and anuria; liver failure (in two patients); delirium; and paraplegia and coma 11–16 days after injections. All five patients died in a comatose state 22–30 days following injections. A fourth child administered a total dose of 150 mg thimerosal/kg body weight survived following surgical removal of the contaminated injection site tissue.

Accidental ingestion of thimerosal by an 18-month-old baby resulted in the death of the child (FDA, 1983; Rohyans *et al.*, 1984). The source of the thimerosal was an aqueous solution used to irrigate an infected ear, but which accidentally flowed via a tympanostomy into the nasopharynx of the infant and was ingested. The total dose of thimerosal over six weeks of treatment was 127 mg/kg. At six weeks, the patient manifested neurologic symptoms, including ataxia, stupor, and coma. The patient remained comatose until death at 140 days.

Hay *et al.* (1963) reported the case of a 29-year-old male who became ill seven weeks after his first day of organomercurial exposure. At the time of hospital admission, he had weakness of the legs, ataxia, dysarthria, and bilateral deafness. He died 25 weeks following his initial exposure, and just 18 weeks after falling ill. Post-mortem blood mercury was estimated to be >15 νg/dL.

Of all the organomercurials, dialkyl mercury compounds are by far the most lethal. In a fatal case of acute dimethylmercury dermal exposure, dysmetria, dystaxic handwriting, wide-based gait, and impaired speech were observed (Nierenberg *et al.*, 1998; Siegler *et al.*, 1999). Similar symptomatology was reported in two fatal occupational exposures to diethylmercury about 100 years ago.

Experimental mammals

A single oral dose of methylmercuric chloride at 16 mg Hg/kg resulted in the death of four of six male mice (Yasutake *et al.*, 1991). Twenty-six weeks of dietary exposure to methylmercuric chloride resulted in increased mortality in both male and female mice at 3.1 mg Hg/kg per day (Mitsumori *et al.*, 1981). Chronic (104 weeks) dietary exposure of mice to methylmercuric chloride resulted in in-

creased deaths among males given 0.69 mg Hg/kg per day, but no increase in mortality among females at up to 0.60 mg Hg/kg per day (Mitsumori *et al.*, 1990).

Respiratory effects

Human

Dyspnea, respiratory depression, and frequent mucus obstruction were observed in a farmer who had treated grain with phenylmercuric acetate for several seasons (Brown, 1954). An autopsy revealed purulent bronchopneumonia. In a suicide attempt in which a 44-year-old male ingested 5 g of thimerosal, the patient required mechanical ventilation for three days as the result of a severe ascending sensorimotor peripheral neuropathy (Pfab *et al.*, 1996).

In a fatal case of oral exposure, 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).

Experimental mammals

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 two years (Verschuuren *et al.*, 1976). This study showed no treatment-related histopathologic lesions in the lungs of exposed rats at 0.1 mg Hg/kg per day.

Cardiovascular effects

Human

Electrocardiography in four family members who ate meat from a hog that had consumed seed treated with ethylmercuric chloride revealed abnormal heart rhythms, S-T segment depression, and T-wave inversion (Cinca *et al.*, 1979). Death in the two children of 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 (with occasional bradycardia and ectopic ventricular beats), and electrocardiograms showing prolongation of the Q-T interval, depression of the S-T segment, and T-wave inversion.

An epidemiologic study of 3235 men, aged 42–60 years, in eastern Finland suggested a relationship between high dietary intake of freshwater fish containing mercury residues and the risk of acute myocardial infarction and death from cardiovascular disease (Salonen *et al.*, 1995). A second report concerning the same study cohort (Salonen *et al.*, 2000) indicated that men with hair mercury concentrations >2.81 ppm were associated with accelerated progression of carotid atherosclerosis. These reports, however, are in contrast with the results of other prospective epidemiologic studies, which have indicated an association between reduced mortality from coronary heart disease and high fish intake (Kromhout *et al.*, 1985; Shekelle *et al.*, 1985; Norell *et al.*, 1986; Dolecek and Granditis, 1991; Shekelle and Stamler, 1993).

Sorensen *et al.* (1999) examined the blood pressure, heart rate, and heart rate variability among over 900 children prenatally exposed to methylmercury through episodic maternal consumption of methylmercury-contaminated pilot whale meat. After adjustment for body weight, diastolic and systolic blood pressure increased by 13.9 mmHg and 14.6 mmHg, respectively, when umbilical cord concentrations increased from 1 to 10 νg/L. No further increase was seen above this level. Sorensen *et al.* (1999) stated that their findings suggest that prenatal exposure to methylmercury may affect the development of cardiovascular homeostasis. The attribution of the results exclusively to methylmercury exposure is contestable, however, since the mothers of the study children were also episodically exposed to relatively high levels of polychlorinated biphenyls (PCBs) during pregnancy.

To investigate the putative link between mercury (of which fish is the most common dietary source) and the risk of cardiovascular disease, Guallar *et al.* (2002) conducted a study of 684 subjects with a first diagnosis of myocardial infarction (MI). Those

patients comprised residents of eight European countries and Israel. A control group of 724 men was selected from the same populations. As a basis of mercury exposure, toenail clippings were obtained from the study participants and analyzed for mercury. After adjustment for docosahexaenoic acid (DHA) in adipose tissue, it was determined that the mercury levels in the MI patients were 15% higher than those in controls. In contrast, DHA levels, after adjustment for the mercury level, were inversely associated with the risk of MI.

To investigate the effect of fish consumption and long chain omega-3 polyunsaturated fatty acid (PUFA) intake on the risk of stroke, He *et al.* (2002) followed 43 671 men (aged 40–75) over a period of 12 years. The participants, all free of cardiovascular disease at the time of enrollment, completed a detailed 'semiquantitative' food frequency questionnaire to determine dietary practices. During the next 12 years, a total of 608 strokes were documented among the initial population. Of those strokes, 377 were ischemic, 106 were hemorrhagic, and 125 were unclassified in nature. This study found that ischemic stroke was significantly lower among men who consumed fish one to three times a month. Fish consumption beyond that level, however, did not result in further risk reduction. No significant associations were found between fish or long chain omega-3 PUFA intake and risk of hemorrhagic stroke.

To examine whether an association exists between high dietary intake of mercury from fish consumption and the risk of coronary heart disease, Yoshizawa *et al.* (2002) investigated the association between mercury in toenails and the risk of heart disease among male health professionals (aged 40–75 years) with no previous history of cardiovascular disease or cancer. During a five-year follow-up of the 33 737 enrollees, 470 cases of coronary heart disease were reported. These cases included nonfatal MI, coronary artery surgery, and fatal coronary heart disease. In this study, it was found that mercury level was significantly correlated with fish consumption. In addition, the mean mercury level was found to be higher in dentists than in nondentists. However, after controlling for age, smoking, and other risk factors for coronary heart disease, the mercury level was determined not to be significantly associated with the risk of coronary heart disease.

Bradycardia and/or death due to cardiac arrhythmias have been reported following intravenous injection of the organomercurials salygran and mercupurin as diuretics (Barker *et al.*, 1942; Brown *et al.*, 1942). Barker *et al.* (1942) reported tachycardia, fibrillation, cyanosis, cardiac standstill, and death within 3–30 minutes after infusion, while Brown *et al.* (1942) observed bradycardia, cyanosis, and seizure activity in one nonfatal case. However, serious pre-existing cardiac, renal, and other diseases were present in all cases. DeGraff and Nadler (1942) concluded that the deaths seen following injection of those mercurial diuretics were likely due to fluid and electrolyte imbalances, diuresis-induced digitalis toxicity, or were idiosyncratic.

Experimental mammals

A decrease in heart rate was observed in male rats given two 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 per day as methylmercuric chloride for three to four weeks (Wakita, 1987). This effect began approximately 60 days after initiation of exposure and persisted for at least nine months.

Gastrointestinal effects

Human

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). 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). Nausea and diarrhea lasting three months was reported in an adult who chronically ingested merthiolate (thimerosal) (Nascimento *et al.*, 1990).

Experimental mammals

Exposure of rats to phenylmercuric acetate in drinking water for two years resulted in necrosis

and ulceration of the cecum at dosages as low as 4.2 mg Hg/kg per day; no effect was observed at 1.7 mg Hg/kg per day when presented in the feed (Fitzhugh *et al.*, 1950; Solecki *et al.*, 1991). Mice showed ulceration of the glandular stomach after two years of dietary exposure to methylmercuric chloride at 0.69 mg Hg/kg per day (Mitsumori *et al.*, 1990). In contrast, no treatment-related histopathologic lesions in the stomach or jejunum were observed in rats exposed via the diet to 0.1 mg Hg/kg per day as methylmercuric chloride (Verschuuren *et al.*, 1976).

Methylmercury has been shown to hyperpolarize then-depolarized Purkinje cell membranes and block synaptically activated local responses (Yuan and Atchison, 1999). Further, methylmercury switched the pattern of repetitive firing of rat Purkinje cells generated spontaneously, or by depolarizing current injection at Purkinje cell soma, from predominantly Na^+-dependent, fast somatic spikes to predominantly Ca^{2+}-dependent, low amplitude, slow dendritic spike bursts. Yuan and Atchison (1999) concluded that methylmercury causes a complex pattern of effects on cerebellar synaptic transmission, with apparent actions on both neuronal excitability and chemical synaptic transmission.

Neurologic effects

Human

Organomercurials can produce a variety of neurologic effects by all routes of exposure. Although other isolated instances of alkyl mercury poisoning have been reported (Engleson and Herner, 1952; Cinca *et al.*, 1979), epidemic poisonings in Japan and Iraq demonstrated conclusively the neurotoxicity of these compounds. The first reported widespread outbreak of neurologic disorders associated with methylmercury involved the ingestion of contaminated fish in the Minamata area of Japan (Kutsuna, 1968). The neurologic syndrome observed in this poisoning incident was characterized by a long list of symptoms, including paresthesia (prickling, tingling sensation in the extremities); 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.

Epidemics of neurologic disorders similar to that described above were reported in Iraq in 1956 and 1960 (Jalili and Abbasi, 1961; Bakir *et al.*, 1973) as the result of eating flour made from seed grain treated with ethylmercury *p*-toluene sulfonanilide. Effects observed in affected individuals included 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 another incident following the ingestion of contaminated bread prepared from wheat and other cereals treated with a methylmercury fungicide in 1971–72, more than 6000 patients required hospitalization and more than 500 deaths occurred, usually due to central nervous system failure. Attempts to correlate symptoms with an estimate of methylmercury intake in the 1971–72 epidemic (based on average levels found in grain and self-reported estimates of number of loaves ingested) indicated that no effects were observed in persons consuming a total of 3.6 mg Hg/kg at ages 5–9 years, 2.8 mg Hg/kg at ages 10–14 years, or 1.7 mg Hg/kg at ages 15 years and above (consumption values reflect total intake and not daily intake) (Al-Mufti *et al.*, 1976). Using 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, vision, and hearing.

Autopsy results from several persons who died following ingestion of the contaminated seed grain in Iraq showed 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). Granule cells in the brain were variably affected, and Purkinje, basket, and stellate cells were severely affected. Sural nerves removed from two women with neurotoxicity associated with the Minamata incident also showed evidence of peripheral nerve degeneration (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, intention 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, USA, family, which included a pregnant mother, a 20-year-old female, a 13-year-old male, and an 8-year-old female, experienced severe, delayed neurologic effects following ingestion of pork from hogs fed seed grain that had been treated with a methylmercury fungicide (Davis *et al.*, 1994). Several months after the exposures, the children developed symptoms of neurologic dysfunction. The neonate, who was exposed as a fetus through maternal consumption of the contaminated pork, showed signs of central nervous system disorder from birth. This child developed quadriplegia, blindness, severe mental retardation, choreoathetosis, and seizures, and died at the age of 21. The 13-year-old and the 20-year-old eventually developed, over a course of years, cortical blindness or constricted visual fields, diminished hand proprioception, choreoathetosis, and attentional deficits. Magnetic resonance imaging (MRI) examination of these two revealed residual brain damage in the calcarine cortices, parietal cortices, and cerebellum. The 8-year-old (at the time of exposure) eventually died of aspiration pneumonia (with a superimposed Klebsiella bronchopneumonia and sepsis) at age 29; at autopsy, his brain 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.

Hunter *et al.* (1940) reported numbness and tingling of limbs, unsteadiness in gait, difficulty in performing fine movements (e.g., buttoning a shirt), irritability, and constricted visual fields in four men following acute inhalation of dust containing methylmercury. Two years later, the men had not fully recovered from their symptoms. Signs of cerebellar involvement, including ataxia, loss of co-ordination, and tremor, have been reported following ingestion of food contaminated with ethylmercury or methylmercury (Nagi and Yassin, 1974; Bakir *et al.*, 1980; McKeown-Eyssen *et al.*, 1983; Zhang, 1984; Davis *et al.*, 1994). Ataxia, stupor, and coma were observed in an 18-month-old who had been administered a total dose of 127 mg thimerosal/kg body weight over a six-week period. The dosing was the accidental result of irrigation of an infected ear with a thimerosal-containing solution, which inadvertently drained into the throat and was ingested (Rohyans *et al.*, 1984).

Similar symptoms have been observed following inhalation of vapors from phenylmercury ammonium acetate (O'Carroll *et al.*, 1995). Hyper-reflexia, muscle rigidity and/or myoclonic, jerky, and choreoathetotic movements were observed in individuals who had ingested food contaminated with methylmercury (Skerfving and Vostel, 1972; Bakir *et al.*, 1980; Davis *et al.*, 1994), as well as in an adult following long-term ingestion of thimerosal (Nascimento *et al.*, 1990).

In another incident, a 9-month-old infant who received porridge made from alkyl mercurial-contaminated grains for approximately four 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, suggesting that infancy may also be a susceptible period for alkyl mercurial neurotoxicity. Mental retardation has not generally been reported as a neurotoxic effect of the alkyl mercurials in adults.

Chronic ingestion of merthiolate by an adult for an unknown duration (cumulative dose fatal) resulted in agitation and other neurologic effects (Nascimento *et al.*, 1990). Following a suicide attempt using thimerosal (5 g), a 44-year-old male developed an ascending peripheral axonal sensor-

imotor polyneuropathy six days following ingestion (Pfab *et al.*, 1996).

In a fatal case of acute dimethylmercury dermal exposure, dysmetria, dystaxic handwriting, wide-based gait, and impaired speech were observed (Nierenberg *et al.*, 1998). Similar symptomatology was reported in two fatal occupational exposures to diethylmercury about 100 years ago.

Organomercurials have also been shown to cause signs of psychological dysfunction, including anxiety, agitation, irrational fears, and impulsiveness, in addition to headache (O'Carroll *et al.*, 1995). In the aforementioned suicide attempt using thimerosal, the 44-year-old man developed delirium before lapsing into a coma 11 days after ingestion of 5 g of thimerosal (Pfab *et al.*, 1996).

Two occupational case studies report severe neurotoxicity in individuals working in the production of ethylmercury. Hay *et al.* (1963) reported the case of a 29-year-old male who became ill seven weeks after his first day of exposure. At the time of hospital admission, he had weakness of the legs, ataxia, dysarthria, and bilateral deafness. He died 25 weeks following his initial exposure. Autopsy revealed severe brain atrophy in the calcarine cortex, suggesting constriction of the visual field, or perhaps even blindness. Post-mortem blood mercury was estimated to be >15 νg/dL.

In the second study, a 50-year-old worker developed paresthesia, 60–70% constriction of the visual field, and dysarthria, in addition to symptoms typically associated with mercury vapor exposure (Schmidt and Harzmann, 1970). During the subsequent 18 years, there was no change in the visual field. Blood levels were not provided.

Grandjean *et al.* (1999b) also reported decrements in performance on neuropsychological tests of motor function, attention, and visuospatial performance in Amazonian children exposed to methylmercury from fish consumption in the Amazon River Basin. However, lack of prenatal exposure data, individual exposure history, maternal exposure history, compromised health/nutritional status, and history of exposure to other neurotoxic substances render the meaning of these data unclear.

In another study, also of limited use in determining specific causal relationships, Harada *et al.* (1999) found an average total mercury hair level of 48.3 ppm in 14 highly exposed individuals in the Lake Victoria area of Tanzania. The highest hair levels were 953 ppm among six gold miners, 416 ppm among four fishermen and their families, and 474 ppm among four Mwanza people. These high exposure individuals were identified in a total study population of 150 gold miners, 103 fishermen and their families, and 19 residents of Mwanza city. Aside from the 14 most highly exposed individuals, all mean hair total mercury levels were below 10 ppm. Among the 150 miners, there were 14 cases of 'a mild form of inorganic mercury poisoning,' with symptoms including polyneuropathy, neurasthenia, or tremors. There was reportedly a low ratio of methylmercury to total mercury in hair samples, which is in contrast to what would be expected if the exposures were exclusively or primarily due to dietary organic mercury intake. Factors that might contribute to this disparity would be direct contact of the scalp hair with airborne, waterborne, or other mercury emanating directly from mining/amalgamation operations or from the home use of mercury-containing products. Some of the subjects in this study who had high total mercury hair levels habitually used toilet soap that contained 'much' mercury (Harada *et al.*, 1999).

Jones (1999) determined that the average daily intake of methylmercury (coming mainly from fish) that may cause demonstrable health effects in the most sensitive individual is 300 νg/day, equivalent to 4.2 νg/kg per day.

Courdier *et al.* (2002) conducted neurological and neuropsychological examinations of children from three French Guyana communities known to have variable levels of methylmercury exposures resulting from gold mining activities. Three respective exposure groups were studied: a high exposure group consisted of 156 children from the Upper Maroni; a median exposure group comprised of 69 children from Camopi; and a low exposure group consisting of Awala on the Atlantic coast. Exposure to methylmercury was determined by total mercury in the hair of children and their respective mothers. The geometric mean hair mercury concentration in the high exposure group was 12.7 νg/g (ppm). After adjustment for potential confounders, Courdier *et al.* (2002) found a dose-dependent association between maternal hair mercury and increased deep tendon reflexes, poorer leg co-ordination,

and decreased visuospatial organization, as measured by the Stanford-Binet Copying score. For the Stanford-Binet test, the frequency of rotation errors was found to be high in the five to six years of age group and increased with mercury exposure. These associations were reported to be stronger among boys. The authors of this study point out, however, that the interpretation their results is limited by the cross-sectional design of the study. In addition to the comments by Courdier *et al.* (2002), it also possible that the observed effects were in part due to concurrent exposure to elemental and inorganic mercury compounds from gold mining operations, which would not be reflected in hair mercury concentrations.

It is known that organic mercury produces degenerative changes in the dorsal root ganglia in dorsal nerve roots in rats (Schionning and Danscher, 1999). At the ultrastructural level, deposition has been shown to occur within lysosomes of target glial cells. Madsen *et al.* (2002) conducted a retrospective study of a cohort of 537303 children, of which 440655 had received the MMR vaccine containing thimerosal (with ethylmercury) as a preservative. The children were all born in Denmark between January 1991 and December 1998. This study identified 316 children with a diagnosis of autistic disorder and another 422 with a diagnosis of autistic-spectrum disorder (based on psychiatric diagnoses listed on the Danish Psychiatric Central Register). After adjustment for potential confounders, no association between age at the time of vaccination, the time since vaccination, or the date of vaccination and the development of autistic disorder.

Experimental mammals

Studies using experimental animals also indicate that organic mercury is a potent neurotoxicant. The neurotoxicity of organomercurials in experimental animals is manifested as functional, behavioral, and morphologic changes, as well as alterations in brain neurochemistry (ATSDR, 1999). Effects span the spectrum of neurotransmitters and include affects on cholinergic transmission at the neuromuscular junction (Eldefrawi *et al.*, 1977; Sager *et al.*, 1982), changes in gamma-aminobutyric acid (GABA) receptor activity and number (Concas *et al.*, 1983; Arakawa *et al.*, 1991), and changes in the activities of enzymes involved in cholinergic, adrenergic, dopaminergic, and serotonergic synthesis and/or catabolism (Tsuzuki, 1981; Sharma *et al.*, 1982).

In adult female *Macaca fascicularis* administered methylmercury (50 νg Hg/kg body weight per day) orally in apple juice for six, 12, 18, or 12 months followed by six months without exposure (clearance group), reactive glia were significantly increased in number for every treatment group (72% in the six-month, 152% in the 12-month, and 120% in the 18-month groups). Examination of tissue samples revealed no degradation in the structure of neurons nor chronic changes in glial cells commonly observed following exposure to high levels of mercury (Charleston *et al.*, 1994).

Rice and Hayward (1999) assessed the visual function during adulthood in monkeys exposed to 50 νg MeHg/kg per day from birth to seven years, as well as in monkeys exposed to 10, 25, or 50 νg MeHg/kg per day throughout gestation, and up to four years of age. Age-related decrements were observed on both spatial and temporal visual function. Treatment-related effects were observed in the monkeys exposed to methylmercury *in utero* and post-natally during the first assessment period, but not during aging. Four of 10 methylmercury-treated monkeys exhibited slight constriction of visual fields at the second assessment that had not been present earlier. These results were determined to support previous findings of evidence of delayed neurotoxicity in the somatosensory and auditory systems following methylmercury exposure.

Schionning and Danscher (1999) used an autometallographic silver-enhancement technique to trace inorganic mercury bound to sulfide or selenide in sections of dorsal root ganglia and dorsal nerve roots taken from rats treated with 2 mg organic Hg/kg body weight for 19 days. In the dorsal roots, inorganic mercury–sulfide/selenide complexes were observed in only a few macrophages. At the ultrastructural level, however, such mercury complexes were observed within lysosomes of target cells. The authors concluded that the inorganic mercury complexes were located primarily within glial cells, and that the pattern of deposition was the same as that seen in morphological changes in rats intoxicated with organomercurials (Schionning and Danscher, 1999).

Typical neurotoxic signs observed in rats exposed to methylmercury include muscle spasms, gait disturbances, flailing, and hindlimb crossing (Inouye and Murakami, 1975; Fuyuta *et al.*, 1978; 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 per day for eight days (Inouye and Murakami, 1975), but 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 degenerative changes in cerebellar granule cells, dorsal root ganglia (Magos *et al.*, 1980, 1985), and peripheral nerves (Miyakawa *et al.*, 1974; 1976; Fehling *et al.*, 1975). Comparison of the effects of five daily doses of 8 mg Hg/kg per day as ethylmercury or methylmercury showed dorsal root damage, as well as flailing and hindlimb crossing after exposure to both chemicals; however, only methylmercury caused substantial cerebellar damage (Magos *et al.*, 1985). Similarly, rats given a dose of 10 mg Hg/kg as methylmercuric chloride once every three days for 15 days showed degeneration in the cerebellum with flailing and hindleg crossing (Leyshon and Morgan, 1991). Rats given a time-weighted average dose of 2.1 mg Hg/kg per day as methylmercury iodide or 2.4 mg Hg/kg per day as methylmercury nitrate by oral gavage for 29 days became weak, severely ataxic, and developed paralysis of the hindlegs (Hunter *et al.*, 1940). Severe degeneration of peripheral nerves, posterior spinal roots, and trigeminal nerves was reported.

Administration of a single oral dose of methylmercuric chloride (0.8 mg Hg/kg) produced blood–brain barrier dysfunction in rats (Chang and Hartmann, 1972a). In rabbits given 5.5 mg Hg/kg per day as methylmercuric acetate for one to four days, widespread neuronal degenerative changes (in cervical ganglion cells, cerebellum, and cerebral cortex) have been observed without accompanying behavioral changes (Jacobs *et al.*, 1977). Severe degenerative changes were also observed in the dorsal root fibers of rats given 1.6 mg Hg/kg per day as methylmercuric chloride for eight weeks (Yip and Chang, 1981). Similarly, ataxia (beginning the second week of exposure) and cerebellar edema and necrosis occurred in rats gavaged with 1.68 mg Hg/kg per day as methylmercury dicyanidiamide for five days/week for seven weeks (Magos and Butler, 1972). When rats were administered 0.8 mg Hg/kg per 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, 1972b). Mice have shown comparable effects at similar doses (Berthoud *et al.*, 1976; MacDonald and Harbison, 1977; Mitsumori *et al.*, 1981).

Cats and monkeys appear to be more sensitive than rodents and have shown signs of neurotoxicity at approximately 10-fold lower doses (0.05 mg Hg/kg per day) following long-term exposure to methylmercuric chloride (Charbonneau *et al.*, 1976; Rice, 1989; Rice and Gilbert, 1982; 1992). Cats fed tuna contaminated with methylmercury at doses equivalent to 0.015 mg/kg per day for 11 months, starting when the cats were kittens, displayed degenerative changes in the cerebellum and the cerebral cortex (Chang *et al.*, 1974). Similarly, cats given gavage doses of methylmercuric chloride as low as 0.25 mg Hg/kg per day for 44–243 days displayed degenerative lesions in the granule and Purkinje cells of the cerebral cortex and/or cerebellum, as well as degenerative changes in the white matter (Khera *et al.*, 1974).

Neonatal monkeys given 0.5 mg Hg/kg per 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 cessation of exposure, abnormalities in several reflexes, blindness, abnormal behavior (shrieking, crying, and temper tantrums), and coma developed. Histopathologic analyses showed diffuse degeneration in the cerebral cortex (especially the calcarine sulcus, the insula, the precentral and postcentral gyri, and the 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 four to five priming doses of 1 mg Hg/kg at five-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 (doses adjusted to maintain steady state blood mercury levels in the range of 1–4 ppm) (Evans *et al.*, 1977). Similarly, 177–395 days after beginning of exposure, slight tremors and decreased sucking responses, followed by claw-like grasp, gross motor inco-ordination, and apparent blindness, were observed in four of seven female monkeys administered 0.08 mg Hg/kg per day as methylmercuric hydroxide daily for four months (Burbacher *et al.*, 1984, 1988). These effects were also displayed in one animal for each of the lower dose groups (0.04 and 0.06 mg/kg per day groups). The animal at 0.04 mg Hg/kg per day that showed neurotoxicity had chicken pox, which may have accelerated the appearance of the effects in this animal. No tremors or convulsions were observed in female monkeys receiving daily doses of 0.04 mg Hg/kg for 150 days (Petruccioli and Turillazzi, 1991).

Burbacher *et al.* (1999) assessed visual and auditory functions in 15-year-old monkeys (*Macaca fascicularis*) that had been exposed to methylmercury *in utero* through maternal dosages of 0, 50, 70, or 90 vg/kg per day. At approximately 15 years of age, the monkeys were administered spatial visual contrast sensitivity and auditory pure tone detection tasks, suggesting that *in utero* exposure to methylmercury may have long-term effects on visual contrast sensitivity thresholds. In contrast, preliminary results from the auditory task testing suggested that auditory thresholds were not affected.

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 per day as methylmercuric chloride once every three days for 15 days (Sharma *et al.*, 1982). An increased number (but not affinity) of benzodiazepine receptor binding sites and decreased content of cyclic guanosine monophosphate (cGMP) were observed in the cerebellar cortex of rats administered 3.92 mg Hg/kg per 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 per day as methylmercury by gavage for 50 days (Tsuzuki, 1981).

Mice administered methylmercuric chloride in the diet for two years at approximately 0.6 mg MeHg/kg per day showed posterior paralysis and sensory neuropathy, characterized by cerebral and cerebellar necrosis and degeneration of spinal dorsal nerve roots and sciatic nerve (Hirano *et al.*, 1986; Mitsumori *et al.*, 1990). Cats fed doses as low as 0.046 mg MeHg/kg per day for two years exhibited neurobehavioral toxic signs including mild impairment of motor activity and diminished sensitivity to pain (Charbonneau *et al.*, 1976), beginning after 60 weeks of exposure. At higher doses, ataxia, alterations in gait, motor inco-ordination, muscle weakness, changes in temperament, and convulsions were also observed. Histopathologic analyses showed neuronal degeneration in the motor, sensory, auditory, and occipital cortices and cerebellar granule cell degeneration. Five monkeys fed 0.05 mg MeHg/kg per 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, 1989). Impaired high-frequency hearing was also displayed by these monkeys when tested at 14 years of age (Rice and Gilbert, 1992). No clinical signs or histopathologic evidence of neurotoxicity was observed in rats that received 0.1 mg MeHg/kg per day in the diet for two years (Verschuuren *et al.*, 1976).

The upregulation of muscarinic acetylcholine (ACh) receptors in selected brain regions has been suggested following prolonged low-level ingestion of methylmercury by rats. Exposure to concentrations of 0.5 or 2.0 MeHg/kg per day in drinking water for 16 days significantly increased the density of muscarinic ACh receptors in the hippocampus and cerebellum, while not affecting the affinity of the receptors. These effects were delayed in onset and were preceded by a marked increase in the

density of similar receptors on lymphocytes (Coccini *et al.*, 2000).

The effects of 40 νM, 400 νM, or 4 mM methylmercury on the dopaminergic system of the rat striatum in conscious, freely moving animals were studied by Faro *et al.* (2000). All doses increased dopamine (DA) release (907±31%, 2324±156%, and 9032±70%, for low, middle, and high dose concentrations, respectively). High-dose exposure also caused significant decreases in extracellular levels of the DA metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillinic acid (AV). The authors attributed these effects to the methylmercury-stimulated DA release or decreased DA intraneuronal degradation, or both.

Developmental/reproductive effects

Human

Epidemics of human mercury poisonings, occurring when grains treated with fungicides containing mercury have been accidentally consumed or when fish with high levels of methylmercury have been eaten, have been associated with widespread occurrence of developmental toxicity on several occasions. 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 was ingested by a pregnant woman. At birth, the neonate appeared normal, but the infant later displayed signs of brain damage, manifested as mental retardation, inco-ordination, and inability to move (Engleson and Herner, 1952). In another incident, a 40-year-old woman, who was three months pregnant, consumed methylmercury-contaminated meat for an unspecified period. Subsequently, the woman delivered a male infant with elevated urinary mercury levels. At three months of age, the infant was hypotonic, irritable, and exhibited myoclonic seizures. At six years of age, the child displayed severe neurologic impairment (e.g., blindness, myoclonic seizures, neuromuscular weakness, inability to speak) (Snyder and Seelinger, 1976).

In a 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 neurologic effects described in the Minamata outbreak included mental retardation, retention of primitive reflexes, cerebellar symptoms, dysarthria, hyperkinesia, hypersalivation, dysmyelination of the pyramidal tracts, an abnormal neuronal cytoarchitecture, and atrophy and hypoplasia of the cerebral cortex, corpus callosum, and granule cell layer of the cerebellum. The widespread damage has been suggested to involve derangement of basic developmental processes, such as neuronal migration (Matsumoto *et al.*, 1965; Choi *et al.*, 1978) 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 poisoned through ingestion of wheat flour made from seeds treated with ethylmercury *p*-toluene sulfonanilide; 14 of these women died as a result of the poisoning (Bakir *et al.*, 1973). Infants of the surviving women were born with blood mercury concentrations of 250 νg/100 dL 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 that occurred in 1971 and 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 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 associated with lower peak hair levels during pregnancy than were mental retardation and seizures (Marsh *et al.*, 1981, 1987). Male offspring were more severely affected than female offspring, and the most severely affected children had been exposed to methylmercury during the second trimester of pregnancy. This finding cannot, however, be interpreted to mean that the second trimester is the most sensitive period of development for low-dose methylmercury exposure. At present, a single most critical period/trimester in

fetal development cannot be determined (Risher *et al.*, 1999).

Neurologic abnormalities have also been observed among the offspring of Cree Indians in Quebec, 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). Recent analysis of peak maternal hair mercury levels during pregnancy in the 1971–72 outbreak in Iraq led to an estimated population threshold of 10 ppm (highest value for total maternal hair mercury during pregnancy) associated with delays in onset of walking (Cox *et al.*, 1989). Only three 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 three children were 14, 18, and 38 ppm (WHO, 1990). A maternal exposure level of 0.0012 mg/kg per day, corresponding to the hair level of 14 ppm, was estimated for the Iraqi women using a simple, one-compartment pharmacokinetic model.

Results of two outstanding longitudinal epidemiologic studies of fish-consuming populations in the Seychelles and Faroe Islands have been published in recent years: Davidson *et al.* (1995; 1998) and Grandjean *et al.* (1997; 1998; 2001). These studies examine a combined population of almost 2000 mother–infant pairs, and together constitute one of the richest sources of human data concerning multigeneration exposure and the most sensitive subgroups of any environmental substance examined to date.

The Seychelles Child Development Study (SCDS), in which over 700 mother–infant pairs have, to date, been followed and tested from parturition through 66 months of age (Davidson *et al.*, 1998), was conducted as a double-blind study in which maternal hair mercury was used as the index of fetal exposure. The Seychellois regularly consume a high quantity and variety of ocean fish, with 12 fish meals per week constituting a typical methylmercury exposure scenario. The median total mercury concentration in 350 fish sampled from 25 species consumed by the Seychellois was < 1 ppm (range 0.004–0.75 ppm), comparable with the mercury concentration in commercially obtainable fish in the United States and many other countries.

In this study, developing fetuses were exposed *in utero* through maternal fish ingestion both before and during pregnancy (Davidson *et al.*, 1998). Neonates continued to be exposed to maternal mercury during breastfeeding (i.e., some mercury is secreted in breast milk), and methylmercury exposure from the solid diet began after the gradual post-weaning shift to a fish diet. The children of exposed mothers were evaluated at 6, 19, 29, and 66 months of age using a broad battery of neurobehavioral and neurodevelopmental tests. The standardized neurobehavioral test battery used in the 66-month Seychelles study was designed to assess multiple developmental domains (Davidson *et al.*, 1998). The results of the testing through 66 months of age revealed no evidence of adverse effects attributable to chronic ingestion of low levels of methylmercury in fish (Davidson *et al.*, 1995; 1998). In the 66-month study cohort, the mean maternal hair level of total mercury during pregnancy was 6.8 ppm (range 0.5–26.7 ppm, $n = 711$) and the mean child hair level at the 66-month testing interval was 6.5 ppm (range 0.9–25.8 ppm, $n = 708$).

A follow-up evaluation of the Seychelles data from another perspective was reported by Davidson *et al.* (1999). In this study, secondary analyses were conducted to determine if effect modification from social and environmental factors was affecting the associations between methylmercury and the observed outcomes. Using maternal hair mercury concentrations representative of methylmercury exposure during pregnancy (median value of 5.9 ppm), children in the main cohort in the SCDS were evaluated at 6.5 months ($n = 740$) for visual recognition memory and visual attention using the Fagan Infant Test, and at 19 ($n = 738$) and 29 months ($n = 736$) using the Bayley Scales of Infant Development (BSID). Multiple regression analysis was used in this study to evaluate interactions between methylmercury and caregiver intelligence, home environment, and family income. Davidson and his colleagues reported that no effect modification was found for preferential looking or visual attention behavior at 6.5 months, or for the BSID Psychomotor Scale at 29 or 19 months. Likewise, no effect modification was found for activity level

at 29 months, as measured by the BSID Infant Behavior Record. While a slight, but statistically significant, correlation between methylmercury and both caregiver intelligence and family income for the BSID Mental Scale was seen at 19 months, no such correlation was found in the 29-month test cohort. The authors concluded that the data revealed no consistent, major effect modification by environmental or social factors. Further, the small degree of effect modification observed for social factors and caregiver intelligence at 19 months is consistent with the enhanced performance seen in this cohort at 66 months.

In a supporting publication, Davidson *et al.* (2000) reported the results of an evaluation of 87 nine-year-olds from a pilot cohort in the Seychelles. This study found no adverse association between maternal methylmercury exposure and any developmental outcome measure. In contrast to what might be expected, the results of the Boston Naming Test and of two tests of visual motor coordination revealed an enhanced performance in males with increasing prenatal exposure to methylmercury. A secondary analysis that included both prenatal and postnatal exposure to methylmercury supported the findings of the 66-month SCDS testing.

A recently published study by Myers *et al.* (2003) reported the results of the testing of the nine-year cohort in the Seychelles Child Development Study. Testing was conducted on 643 children (83% of the original cohort of 770), whose mothers had been exposed throughout pregnancy to methylmercury from eating ocean fish an average of 12 times per week. Maternal hair mercury concentrations representing the period of pregnancy averaged 6.9 ppm, SD = 4.5), with 88 of the children tested having been born to mothers with pregnancy hair mercury concentrations greater than 12 ppm. In this round of testing, recent postnatal methylmercury exposure was included, since it was associated with outcomes in the 66-month assessments on this population. The mean postnatal hair mercury concentration was 6.1 ppm (SD = 3.5), and the mean age at testing was 107 months.

In this report, a total of 21 endpoints were examined. The test battery included both global and domain-specific items, including the Boston Naming Test and other tests previously reported to have shown an association with methylmercury in other studies (Grandjean *et al.*, 1997; Crump *et al.*, 1998). Neurocognitive, language, memory, motor, perceptual-motor, and behavioral functions were all examined.

Significant associations between prenatal methylmercury exposure and test performance were found for only two of the 21 endpoints examined. There was a significant decrease in performance on the grooved pegboard time for the nondominant hand in males, but not for the dominant hand for males or either hand for females. In addition, there was also a significant improvement of the hyperactivity index of the Connor's teacher-rating scale as prenatal methylmercury exposure increased. It was found that a 10 νg/g (10 ppm) increase in exposure would result in a drop of one to six points (95% CI) in the hyperactivity index. The biological significance of this effect is uncertain, but would not be considered adverse. Myers *et al.* (2003) provided probability plots of *P* values which show a distribution that suggests that both of the positive outcomes were probably due to chance. No significant effects were reported for any of the other 19 tests conducted, including the Boston Naming Test that was used as the basis of the EPA RfD.

Among other findings of this study were consistent associations between socioeconomic score, early home environment scores, and maternal IQ with outcomes for neurocognitive endpoints, but only occasionally with outcomes on motor tasks.

As part of the SCDS, Myers *et al.* (1997) evaluated the Seychellois cohort for the attainment of developmental milestones reported in the Iraqi study. 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. Myers *et al.* (2000) also reported that a secondary analysis of the Seychellois cohort revealed no association between either prenatal or postnatal methylmercury exposure and behavior, as measured by the Child Behavior Checklist (CBCL).

Grandjean *et al.* (1997; 1998) reported another epidemiologic study of methylmercury exposure for a population in the Faroe Islands. Although the

Faroese are a fishing culture, the major source of methylmercury exposure for this population is pilot whale meat, which is intermittently consumed (1–2 meals/week) as part of the cultural tradition. The initial study cohort consisted of 1022 singleton births occurring in a 21-month window during 1986–87. At approximately seven years of age, neurobehavioral testing was conducted on 917 of the remaining cohort members. Cord blood and hair samples were collected to evaluate mercury exposure. Geometric mean values for cord blood and hair mercury were 22.9 νg/L and 4.27 νg/g (ppm), respectively (Grandjean *et al.*, 1997). No abnormalities attributable to mercury were found during clinical examinations or neurophysiologic testing. A neuropsychologic test battery was also conducted to evaluate possible effects on specific domains of brain function. The neuropsychologic testing indicated mercury-related dysfunction in the domains of language, attention, memory, and visuospatial and motor function (to a lesser extent), which the authors considered to remain after the children of women with maternal hair mercury concentrations above 10 νg/g (10 ppm) were excluded. This Faroese test population was concurrently exposed to polychlorinated biphenyls (PCBs) and other persistent organic pollutants (POPs), including DDT; however, Grandjean *et al.* (1997; 1998) did not consider these pollutants to be the cause of the observed deficits. A follow-up paper on this population suggested that the time-dependent susceptibility may vary for different brain functions (Grandjean *et al.*, 1999a). These researchers reported that the greatest susceptibility to methylmercury neurotoxicity occurs during late gestation, while early postnatal vulnerability is less.

Crump *et al.* (1998) conducted benchmark dose (BMD) analysis and further regression analyses of data collected in a study in which a series of scholastic and psychological tests were administered to children whose mothers had been exposed to methylmercury during pregnancy. Hair samples were collected from 10970 new mothers in New Zealand in 1977 and 1978. High hair mercury levels were considered to be those over 6 ppm, which was the hair level predicted to result at steady state from consumption of mercury at the WHO/FAO Provisional Tolerable Weekly Intake of 0.3 mg total mercury/week and 0.2 mg methylmercury/week. By this criterion, 73 of approximately 1000 mothers who had consumed fish more than three times per week during pregnancy were determined to have high hair mercury levels. In 1985, when the children were six to seven years of age, 61 children (including one set of twins) of the 73 mothers in the high hair mercury group were located; these children constituted the high exposure group, which was matched with three control groups [one with 3–6 ppm (νg/g) maternal hair mercury levels, one with 0–3 ppm whose mothers had been identified as 'high fish consumers', and one with 0–3 ppm whose mothers had not been high fish consumers]. The entire study cohort consisted of 237 children. A battery of 26 psychological and scholastic tests was administered to the children at school during 1985. Mothers were interviewed at the time of test administration to obtain additional data on social and environmental factors. In the high exposure group, one boy's mother had a hair mercury level of 86 ppm, which was more than four times higher than the next highest hair mercury level of 20 ppm. BMDs (10% response rate) calculated from five tests ranged from 32 to 73 ppm, when the child of the mother with the hair mercury level of 86 ppm was included. This corresponded to a benchmark dose level (BMDL) range of 17–24 ppm. The BMDL is a modeled number considered to correspond to an experimental no observed adverse effect level (NOAEL). Although none of the test scores of the child whose mother had the hair mercury level of 86 ppm were outliers according to the definition used in the analyses, his scores were significantly influential in the analyses. When this child was omitted from the analyses, BMDs ranged from 13 to 21, with corresponding BMDLs of 7.4–10 ppm. According to this most conservative interpretation of the New Zealand data, no neuropsychological effects would be seen (and were not seen) in the offspring of women with hair mercury levels at or below the bottom of this BMDL range (i.e., 7.4 ppm maternal hair mercury level).

Following the publication of the results of the Faroes (Grandjean *et al.*, 1997) and New Zealand (Crump *et al.*, 1998) studies, Palumbo *et al.* (2000) conducted a reanalysis of the possible association of methylmercury with performance on the McCarthy Scales of Children's Abilities (MSCA) in the 66-month Seychellois cohort. Since no

association between methylmercury exposure and performance on the MCSA General Cognitive Index had been found in this cohort (Davidson *et al.*, 1998), Palumbo and coworkers conducted further analyses to determine whether associations on specific subscales of the MSCA could be identified. After analysis of standard MSCA subscales, more specific subscales of the MSCA were defined and analyzed using a neuropsychological approach. In this process, subscales were recombined to approximate the domains of cognitive functioning evaluated in the Faroes and New Zealand studies. Palumbo *et al.* (2000) found that analyses of both the standard and recombined MSCA subscales showed no adverse associations with methylmercury exposure and the neuropsychological endpoints examined.

A number of other epidemiology studies investigated the potential adversity of methylmercury, but found no effect in the populations studied. No increase in the frequency of neurodevelopmental abnormalities in early childhood was observed in a cohort of 131 infant–mother pairs in Mancora, Peru (Marsh *et al.*, 1995). The mean concentration of mercury in maternal hair was determined to be 8.3 ppm (range 1.2–30 ppm), and the source of the mercury was believed to be from consumption of marine fish. Similarly, a study of 583 Faroe Island infants for the first 12 months after birth found no decrease in the age of attainment of developmental milestones.

Another study of the Faroese population was reported by Steuerwald *et al.* (2000). In this study, 182 singleton births were evaluated for effects of mercury, the primary source of which was maternal consumption of pilot whale during pregnancy. Maternal hair, serum, milk, and cord blood were analyzed for contaminants. Optimality scores for infants, adjusted for gestational age, were determined at two weeks of age. Exposure to both methylmercury and polychlorinated biphenyls (PCBs) was found to be increased in relation to maternal seafood intake. After statistical adjustment for selected confounding influences, the authors determined that a 10-fold increase in cord blood mercury concentration was associated with a decreased neurologic optimality score of 2.0 ($P = 0.03$). The authors concluded that maternal prenatal exposure to methylmercury from contaminated seafood was associated with an increased risk of neurodevelopmental deficit.

The findings of Bemis and Seegal (1999), however, cast a shadow over the conclusions reached by the Faroe Islands researchers. Bemis and Seegal found that, while exposure to PCBs alone reduced tissue dopamine (DA) and elevated media DA in a dose-dependent fashion, exposure to methylmercury alone did not significantly affect either measure. However, when striatal tissue was exposed simultaneously to both PCBs and MeHg, there were significantly greater decreases in tissue DA concentrations and elevations in media DA than those caused by PCBs alone. This led the authors to suggest that the significant interaction between these two toxicants may be due to a common site of action that influences DA function (such as toxicant-induced increases in intracellular calcium and changes in second messenger systems).

However, the most significant evidence bringing the conclusions of the Faroese study into question comes from the recent report by the Faroes research team themselves. Grandjean *et al.* (2001) examined the effects of prenatal exposure to PCBs on neurobehavioral development. Neurobehavioral deficits in 435 children from a Faroese birth cohort were identified through administration of the Boston Naming Test with cues (not adjusted for mercury) ($P = 0.03$) and the Continuous Performance Test reaction time ($P = 0.03$). These authors found that while no PCB effects were apparent in children with low mercury exposure, PCB-associated deficits within the highest tertile of mercury exposure indicated a possible interaction between these two contaminants. The authors concluded that the limited PCB-related neurotoxicity in this study cohort appears to be affected by concomitant methylmercury exposure.

At a 1998 workshop sponsored by the White House Council on Environment and Natural Resources (NIEHS, 1999), a panel (on Confounders and Variables) found that 'Although in most tissues, PCBs are measured most accurately on a lipid-adjusted basis, the lipid adjustment for cord tissue measures (as done in the Faroes) is not useful.' In the Faroes study, prenatal PCB exposure was associated with four of the same outcomes as mercury exposure. Regarding these, the Confounders and Variables Panel reported that 'These

outcomes related primarily to verbal and memory performance, the domains found in prior studies to be associated with PCB exposure. When PCBs and Hg are included together in the model, one of the outcomes is specifically related to Hg exposure. For the other three (including the Boston Naming Test), however, both the PCB and Hg effects fall short of conventional levels of statistical significance.'

The following information was provided by Grandjean *et al.* (1997) regarding the results of the Boston Naming Test

Boston Naming Test P Values

Before wet weight adjustment		After PCB wet weight adjustment		
	MeHg	MeHg	PCB	MeHg+PCB
w/o cues:	$P = 0.04$	$P = 0.21$	0.16	0.05
w/ cues:	$P = 0.007$	$P = 0.10$	0.08	0.008

Source: Grandjean *et al.* (1997).

The above table clearly shows that when properly adjusted for PCB content, the Boston Naming Test loses statistical significance for both the 'with cues' and 'without cues' categories. It further illustrates that when properly measured, PCBs played an important role in the test results and that the contribution of MeHg was merely to enhance the ability of PCBs to evoke the response on the Boston Naming Test, and not itself the sole or primary cause of the reported neuropsychological deficit in the test population. This hypothesis is supported by the findings of Grandjean *et al.* (2001), who found that cord PCB concentration was significantly associated with deficits on the Boston Naming Test, without cues ($P = 0.03$), and that wet-weight PCB concentration appeared to be a better predictor of neurobehavioral deficits. This is consistent with the findings of Stewart *et al.* (2000), who also found a decrease in performance in some portions of the Neonatal Behavioral Assessment Scale (NBAS) tests among babies born to women who consumed Lake Ontario fish contaminated with PCBs. Stewart *et al.* (2000) also found that the decreased NBAS performance was unrelated to concomitant exposure to methylmercury, lead, or pesticides. In addition, no effect of methylmercury on performance in the Boston Naming Test administered to the nine-year Seychellois cohort (Myers *et al.*, 2003) could be found.

The conclusion reached by Grandjean *et al.* (2001) that the limited PCB-related neurotoxicity in this study cohort appears to be affected by concomitant methylmercury exposure could as justifiably be restated to indicate that the limited methylmercury-related neurotoxicity (both prenatally and postnatally) in this study cohort appears to be affected by concomitant PCB exposure. At this point in time, however, it is our opinion that both methylmercury and PCBs may be jointly responsible for the effects reported in the Faroes (Risher *et al.*, 2003). Dourson *et al.* (2001) also provide an excellent discussion of the relationship between the results reported in the Faeroes and the potential impact of mixed chemical exposures in that population.

Another issue regards the predictive value of childhood versus adult blood pressure. Elevated blood pressure in childhood has been reported to be an important determinant of hypertension risk in later life (Sorensen *et al.*, 1999). To examine the effects of *in utero* methylmercury exposure on blood pressure in childhood, seven-year-olds from the same Faroese cohort described in Grandjean *et al.* (1997; 1998) were examined for blood pressure, heart rate, and variability in heart rate. After adjustment for body weight, diastolic and systolic blood pressure were increased by 13.9 and 14.6 mmHg, respectively, when cord blood mercury concentrations increased from 1 to 10 νg/L of cord blood, with the effect being stronger in lower birth weight children. In the case of boys, variability in heart rate decreased with increasing Hg exposures. The authors attributed these effects to prenatal exposure to methylmercury. (This was the same population co-exposed to high levels of PCBs, as discussed earlier in this review.)

Experimental animals

In nonhuman mammals, there is evidence of developmental effects following oral exposure to organic mercury during gestation, lactation, and/or postweaning. Several effects indicative of developmental toxicity have been observed.

Administration of 8 mg MeHg/kg as methylmercuric chloride on gestation day 10 resulted in increased skeletal variations (incomplete fusion of

the sternebrae) in fetal rats (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/kg per day) for a longer duration of gestation (days 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 per day was not significantly different from that found in controls in the same study. Administration of 2 mg Hg/kg per day as methylmercuric chloride to pregnant rats throughout gestation (days 0–20) resulted in increased malformed fetuses (Inouye and Murakami, 1975). The most common manifestations were generalized edema and brain lesions. Administration of 4 mg MeHg/kg per day to pregnant rats during gestation days 7–14 resulted in decreased fetal weight and increased total malformations, hydrocephalus, and wavy ribs (Fuyuta *et al.*, 1978). At 6 mg MeHg/kg per 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 MeHg/kg per day included absence of one or more sternebrae, bipartite sternebrae, and bilobed vertebral centra. Administration of a single dose of 24 mg MeHg/kg to pregnant rats during gestation days 6–12 resulted in decreased fetal weights and increased malformations (Inouye and Murakami, 1975). The incidence of defects (hydrocephalus, cleft palate, micrognathia, microglossia, generalized edema, subcutaneous bleeding, and hydronephrosis and hypoplasia of the kidneys) increased with later treatments (after gestation day 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.

Methylmercuric chloride administered orally by gavage to mice at a dosage equivalent of 5 mg Hg/kg per day during gestational days 6–17 resulted in 100% stillbirths or neonatal deaths and the failure of six of nine dams to deliver, without apparent maternal toxicity (Khera and Tabacova, 1973). At lower doses (2 and 4 mg Hg/kg per 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 methylmercury (16 mg/kg) administered to mice by gavage on either gestation day 10 or 12 resulted in decreased fetal weight, cleft palate, and dilation of the renal pelvis (Yasuda *et al.*, 1985).

Fredriksson *et al.* (1996) exposed four groups of 12 pregnant Sprague–Dawley rats to methylmercury (oral) or elemental mercury (vapor) alone or in combination at various times during gestation. The results indicate that co-exposure to methylmercury at levels which, by itself, did not alter a variety of motor and neurobehavioral functions, served to significantly aggravate the effects of prenatal exposure to elemental Hg (i.e., alterations to both spontaneous and learned behaviors).

Other studies have also reported functional disturbances in offspring following exposure to methylmercuric chloride during gestation. A single dose of 16 mg MeHg/kg administered on gestation days 13, 14, 15, 16, or 17 resulted in decreased spontaneous locomotor activity at five weeks of age, decreased righting response, abnormal tail position during walking, flexion, and crossing of the hindlimbs (Inouye *et al.*, 1985). Histopathologic 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. A decreased number of avoidances, increased number of escapes, and increased trials to reach criterion on a two-way avoidance task were exhibited in the offspring of mice receiving 3, 5, or 10 mg Hg/kg per day as methylmercuric hydroxide on day 8 of gestation (Hughes and Annau, 1976). No effects were present in the 2 mg Hg/kg equivalent dose group in this study.

Offspring of rats exposed to 4 mg Hg/kg per day as methylmercuric chloride on gestation days 6–9 showed impaired swimming behavior, increased passiveness, and an increased startle response (Stoltenburg-Didinger and Markwort, 1990). At 0.4 mg/kg per day, the offspring showed an increased startle response, whereas no effects were seen at 0.04 mg/kg per day. Exposure to 6.4 mg MeHg/kg on gestation day 15 resulted in decreases

in spontaneous locomotor activity, increased sensitivity to pentylenetetrazol-induced convulsions, and a transient increase in η-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 neurologic effects observed. The most sensitive test for neurologic effects of gestational exposure to methylmercury has shown a significant reduction in the operant behavioral performance (i.e., rewarded responses to total lever presses) in four-month-old rat offspring exposed to 0.008 mg MeHg/kg per day on gestational days 6–9 (Bornhausen *et al.*, 1980). A dose of 0.004 mg/kg per day did not alter the behavioral performance of the offspring.

Developmental neurotoxicity and changes in tissues including the liver and immune system have also been observed in studies in which exposure preceded gestation or was continued after gestation for extended durations. Retarded behavioral maturation (swimming behavior, righting reflexes) and learning disability (maze learning) were demonstrated in offspring or rats receiving a diet of 0.1 mg Hg/kg per day (unspecified forms of mercury) in a contaminated fish diet from day 1 of gestation 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 MeHg/kg per day from two weeks prior to mating through weaning (Elsner, 1991). Norepinephrine levels in the cerebellum of offspring were significantly increased. Daily doses of 0.25 mg MeHg/kg per 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 histologic changes in the harderian, exorbital lacrimal, and parotid salivary glands (Khera and Tabacova, 1973). 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 of rats to low doses of 0.7–1.4 mg MeHg/kg per day in the drinking water one month before mating and up to the end of pregnancy (Fowler and Woods, 1977).

Sakamoto *et al.* (2002) studied changes in brain mercury concentration of rat pups exposed to methylmercury throughout embryonic development, during breast-feeding, and after weaning, and the effects of methylmercury exposure on neurobehavioral test performance. In this study, adult female rats were given a diet containing 5 ppm mercury (as methylmercury) for eight weeks prior to mating, with no apparent adverse effects. This diet was continued throughout gestation and after parturition. Newborn offspring were weaned at day 30 of life, and then placed on the same diet as the mothers. Rat pups killed at birth were found to have brain mercury concentrations 1.4 times that of the mothers. This concentration declined during the suckling period to just one-fifth of that measured in the rats killed at birth, limited transfer of mercury in the milk and/or rapid growth of the brain. Once the weaned pups were placed on a contaminated diet, the brain mercury concentration began to gradually rise again. When behavioral tests were conducted during the fifth and sixth weeks of life, exposed rats showed a significant decrement in performance on the rotorod motor co-ordination test, as well as decreased learning ability in the passive avoidance response test, compared with controls. Focal cerebellar dysplasia, including heterotopic location of Purkinje cells and granule cells was observed postmortem.

Kakita *et al.* (2002) studied the effects of fetal methylmercury exposure on neuronal migration in the developing cerebral cortex. To accomplish this, pregnant rats were administered both methylmercury and 5-bromo-2-deoxyuridine (BrdU) on gestational days 11, 13, 16, or 21. Histopathological examination on offspring sacrificed on postnatal day 28 revealed no apparent cytoarchitectural abnormalities in either the primary motor or primary somatosensory areas of the cerebrum. Further, morphometric analysis of these areas revealed no differences in total neuron population, nor were there any differences in subpopulations of any of the cortical layers, when compared with controls. However, BrdU immunohistochemistry revealed an abnormally widespread distribution of

the labeled cells throughout cortical layers II–VI of offspring exposed to methylmercury on gestational days 16 and 21, indicating disruption of the inside-out pattern of neuronal migration.

Newland and Reile (1999) exposed groups of 10 female rats each to either 0, 0.5, or 6 ppm Hg (as methylmercuric chloride) in drinking water. For half of the rats in each exposure group, treatment was begun four weeks prior to mating; the remainder of the test animals were exposed for seven weeks before mating. Maternal exposure continued to 16 days postpartum. All mating males were unexposed. Levels of mercury in blood taken from pups on the date of birth and again on the day of weaning (21 days postpartum), as well as brain mercury levels, were found to be unrelated to the duration of maternal exposure before mating. Reproductive success, however, was found to be related to the duration of maternal premating exposure. In addition, brain and blood mercury levels were found to be dependent on maternal consumption during gestation. Brain mercury levels in the pups decreased from 0.49 to 0.045 ppm in the low-dose rats and from 9.8 to 0.53 ppm in the high-dose group animals, leading to the conclusion that maternal exposure during lactation apparently did not result in exposure of nursing pups. Brain:blood ratios averaged about 0.14 at birth, and 0.24 at weaning, suggesting differential loss from neuronal and non-neuronal tissue.

In another study, Watanabe *et al.* (1999) examined the effects of prenatal methylmercury exposure on the fetal brain of mice born to exposed dams. Injections of 3 mg Hg/kg as methylmercury were administered to pregnant mice on gestational days 12–14, inclusive. Postmortem analysis of fetal brains collected on gestational days 14 or 17 revealed significantly elevated levels of glutathione (reduced GSH); thiobarbituric acid reactive substances (TBARS) in fetal brain tissue showed a similar but nonsignificant trend.

The potential for low-dose exposure of pregnant rats to methylmercury to result in ictal effects in offspring was investigated by Szasz *et al.* (1999). Four-week-old offspring of dams exposed to methylmaercury during gestation were examined for possible methylmercury-induced changes in epileptogenic activity produced by 3-aminopyridine in the neocortex. Epileptogenicity was found to be significantly increased in offspring of mercury-treated animals when compared with controls. This activity was characterized by an increase in the frequency of periodic ictal activity, facilitated propagation of epileptiform discharges, and a strong tendency toward generalization. In addition, the amplitude of seizure discharges was significantly lower in methylmercury-treated animals than in controls, possibly due to a loss of cortical neurons. The authors concluded that the synaptic and membrane mechanisms responsible for the initiation and propagation of paroxysmal activity were probably facilitated as a result of maternal methylmercury exposure, while the efficacy of cortical inhibition in preventing initiation and the spread of epileptiform discharges was reduced by exposure of the developing nervous system to mercury.

The developing immune system was affected in newborn Sprague–Dawley rats exposed to 0.5 mg MeHg/kg per day via 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.

Impaired visual recognition memory was reported for 50–60-day-old monkeys born to mothers that received 0.04 or 0.06 mg MeHg/kg per day in apple juice for an average of 168 or 747 days prior to mating (Gunderson, 1988).

Spontaneous abortions and decreased mean litter size have been shown to be the predominant reproductive effects in different species of animals following oral exposure to organic mercury. Among rats orally administered 10, 20, or 30 mg methylmercuric chloride/kg body weight on gestation day 7, body weights were decreased in a dose-dependent fashion. Survival rates of fetuses and the number of implantation sites were also decreased in a dose-dependent manner. Preimplantation losses in the exposure groups were 17.2%, 24.8%, and 30.1%, respectively, while postimplantation losses were 16.7%, 34.1%, and 88.9%, respectively. The LD_{50} of methylmercuric chloride for fetuses in this study was determined to be 16.5 mg/kg.

In a study by Khera (1973), male rats were exposed for five to seven days to daily oral gavage dosages of 1, 2.5, or 5 mg MeHg/kg per day before

being mated to unexposed females. A dose-related reduction of mean litter size was attributed to preimplantation losses. At 2 mg MeHg/kg per day administered by gavage during days 6–9 of gestation, Fredriksson *et al.* (1996) found no differences in maternal body weight gain before parturition or differences in body weights of the offspring.

In male mice, no reduction in the incidence of fertile matings was observed after administration of five to seven daily oral doses of up to 5 mg MeHg/kg per day (Khera, 1973). A significant dose-related decrease in the number of pups born per litter was observed in mice receiving oral doses of 3, 5, or 10 mg Hg/kg administered as methylmercuric hydroxide on gestation day 8 (Hughes and Annau, 1976). Female mice administered 20 mg Hg/kg per day as methylmercuric chloride by gavage on gestation day 10 had increased resorptions, decreased live fetuses per litter, and decreased number of fetuses per litter (Fuyuta *et al.*, 1978). After guinea pigs were exposed to 11.5 mg MeHg/kg as methylmercuric chloride by gavage on gestational days 21, 28, 35, 42, or 49, half of the litters were aborted four to six days after treatment (Inouye and Kajiwara, 1988). In a study using primates, 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 MeHg/kg per day for four months (Burbacher *et al.*, 1988). The menstrual cycle length was not affected at these dose levels.

Pregnant BALB/c, C57BL/6J, and C57BL/6Cr mice were administered oral dosages of 0 or 3 mg MeHg/kg per day (as methylmercuric chloride) during gestational days 12–14 (Kim *et al.*, 2000). The behavior of male offspring in open field, in their home cage, and in a Morris water maze was subsequently evaluated. While treated BALB/c and C57BL/6Cr offspring exhibited less total locomotor activity than did their respective controls, no significant difference was observed between the C57BL/6J treated and control groups. In BALB/c male offspring, the methylmercury-treated mice exhibited significantly more central locomotion, but less peripheral locomotion, than did their control group. With respect to spontaneous home cage activity, all but the BALB/c methylmercury-treated offspring moved more actively in the dark phase than in the light phase. BALB/c activity was the same in both light and dark phases, suggesting a possible disturbance of nocturnal rhythm of spontaneous activity. In the Morris water maze, prenatal exposure to methylmercury resulted in significantly prolonged latency in the C57BL/6J and C57BL/6Cr mice, but not in the BALB/c strain. This study demonstrated that intraspecies differences in neurobehavioral performance can occur in mice treated with methylmercury.

In another recent study (Dore *et al.*, 2001), groups of pregnant C57BL/6 mice were administered daily oral doses of 4 or 6 mg MeHg/kg during gestational days 7–9 or 12–14. Female offspring six to 16 weeks of age were subsequently tested on a variety of behavioral tasks, including motor coordination, visual discrimination, open-field behavior, and spatial alternation training. This study found that, overall, more detrimental effects were observed in the female offspring of animals administered methylmercury on gestational days 12–14 than among those being dosed on days 7–9 of gestation. Among the most remarkable effects was a reduction in locomotor activity and impaired reference memory for egocentric and allocentric spatial information, and working memory for places.

Mean percentages of motile spermatozoa and mean sperm swimming speed were examined in *Macaca fascicularis* monkeys exposed by gavage to 0.025 or 0.035 mg Hg/kg per day as methylmercury for 20 weeks. Both parameters were significantly decreased in both treatment groups compared with controls (Mohamed *et al.*, 1987). Morphologic examination of semen smears indicated an increased incidence of tail defects (primarily bent and kinked tails) in both exposed groups. Tubular atrophy of the testes was observed in mice ingesting 0.69 mg Hg/kg per day in their feed for up to two years (Mitsumori *et al.*, 1990), and decreased spermatogenesis was observed in mice receiving daily doses equivalent to 0.73 mg Hg/kg in the diet (Hirano *et al.*, 1986).

In another study of the effects of organic mercury on reproductive parameters, semidomesticated female mink (*Mustela vison*) were fed daily fish-based diets containing 0.1, 0.5, or 1.0 ppm total mercury (Dansereau *et al.*, 1999). In this study, 20-month-old females exposed to the experimental diets for 400 days, as well as their 10-month-old offspring

that were exposed to mercury for approximately 300 days, were all mated to 10-month-old males who had been fed the 0.1 ppm diet for 60 days prior to the mating season. In this study, the proportion of females giving birth was low for all treatment groups, except the parental general females fed the 0.1 ppm diet. Mercury exposure did not influence the survival or growth of neonatal kits. Sundberg *et al.* (1999) studied the concentration of mercury in milk and the distribution pattern in the suckling pup following administration of a single *i.v.* injection to lactating mice with 0.5 mg/kg body weight of either ^{203}Hg-labeled methylmercuric chloride or mercuric chloride on day 10 of lactation. Mercury concentrations in milk and in whole body, blood, plasma, gastrointestinal (GI) tract, liver, kidneys, and brain of the offspring were followed up to 11 days after beginning of dosing. Lactational exposure following a maternal methylmercury or inorganic mercury dose resulted in almost similar mercury concentrations in liver, kidneys, and plasma of the suckling pup; however, the methylmercury group had twice the body mercury burden of the inorganic mercury group and higher concentrations in the brain (up to 14 times). The authors concluded that differences in kinetics indicate that lactational exposure to methylmercury is a greater hazard for the breast-fed infant than is inorganic mercury.

Ethylmercury-containing thimerosal has been shown to be a potent activator of intracellular calcium release in pig oocytes. Such activation mimics the effects of sperm-induced release of intracellular calcium, as well as other activation events that occur in pig oocytes (Machaty *et al.*, 1999). Wang *et al.* (1999) examined the temporal relationship between intracellular calcium transients, cortical granule exocytosis, and the zone reaction induced by thimerosal. These researchers found that thimerosal induced the same degree of exocytosis in oocytes that was caused by sperm penetration. Further, the zona block to sperm penetration in thimerosal-treated oocytes occurred within 35 minutes of cortical granule exocytosis and within 40 minutes of the first calcium transient. Machaty *et al.* (1999) found that the thimerosal-induced Ca^{2+} release did not require the formation of inositol 1,4,5-triphosphate (IP3). In addition, thimerosal destroyed the meiotic spindle, preventing further development.

In a study using mouse neural crest (NC) cells, Carey and Matsumoto (1999) reported that up to 50% of the cultured NC cells exhibited calcium transients during the period of neuronal differentiation. As neurogenic activity declined, the percentage of active NC-derived cells and their calcium spiking frequency also declined. Thimerosal was found to increase the frequency of oscillations in active NC-derived cells and induce them in a subpopulation of quiescent cells. As neurogenesis ended, NC-derived cells became nonresponsive to thimerosal.

Hepatic effects

Human

Autopsy reports for four adults and four infants who died as the result of methylmercury poisoning in Iraq in 1972 revealed fatty changes in the livers (Al-Saleem and the Clinical Committee on Mercury Poisoning, 1976). In contrast, the prevalence of liver disease in a population from the Minamata, Japan, area was not significantly increased relative to unexposed controls (Futatsuka *et al.*, 1992).

Experimental mammals

Verschuuren *et al.* (1976) found no treatment-related hepatic changes in rats exposed to dietary concentrations of 0.1 mg Hg/kg per day as methylmercuric chloride.

Renal/genitourinary/electrolyte homeostasis effects

Human

An outbreak of ethylmercury fungicide-induced poisoning was reported by Jalili and Abbasi (1961). Affected individuals exhibited polyuria, polydipsia, and albuminuria. In another report (Cinca *et al.*, 1979), two boys who ingested meat from a hog fed seed treated with ethylmercuric chloride had increased levels of blood urea, urinary protein, and urinary sediment (Cinca *et al.*, 1979). Autopsy additionally revealed nephritis.

A 13-month-old boy who ate porridge made from flour treated with an alkyl mercury compound (specific mercury compound not specified) experienced albuminuria, along with red and white cells and casts in the urine (Engleson and Herner, 1952). Autopsy of eight persons who died as the result of consuming methylmercury-treated seed grain dur-

ing 1972 in Iraq revealed tubular degeneration in one of the eight (Al-Saleem and the Clinical Committee on Mercury Poisoning, 1976).

Proteinuria and increased urinary mercury levels were measured in workers exposed to organomercurial seed dressings (Taylor *et al.*, 1969); however, the urinary mercury levels and proteinuria were not well correlated. Albuminuria has also been associated with the ingestion of ethylmercury, dermal exposure to phenylmercuric acetate, and inhalation of methoxyethyl mercury silicate (Skerfving and Vostel, 1972). The inhalation of methoxyethyl mercury has also resulted in nephrosis manifested as nephrotic syndrome. Following ingestion of 5 g of thimerosal in a suicide attempt, a 44-year-old male developed polyuric acute renal failure, with proteinuria, glycosuria, beta-2-microglobinuria, and a peak serum creatinine level of 2.4 mg/dL.

Hypernatremia (159 mEq/L) and renal insufficiency have been reported in infants treated with mercurochrome for omphaloceles (Debray *et al.*, 1979). Hypokalemia and dehydration (with a three-week course of nausea and diarrhea) were seen in an adult following long-term merthiolate ingestion (Nascimento *et al.*, 1990). More severe renal effects, including renal tubular necrosis, have been more commonly observed with aryl (e.g., phenylmercury) and alkoxyalkyl (e.g., methoxyethylmercury) compounds.

Experimental mammals

Organic mercury-induced nephrotoxicity has been demonstrated in rodents following acute, intermediate, and chronic exposure (Magos *et al.*, 1985; Yasutake *et al.*, 1991). Histopathologic changes were observed in the kidneys of female rats exposed to 0.86, 1.68, or 3.36 mg MeHg/kg per day as methylmercury dicyanidiamide by gavage, five days/week, for three to 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 (Magos and Butler, 1972). A 12-week diet containing 0.08 mg Hg/kg per day as methylmercury caused ultrastructural changes in kidney proximal tubule cells of female rats (Fowler, 1972). Methylmercuric chloride in the diet of mice for 26 weeks at a dosage of 0.6 mg Hg/kg per day resulted in proximal tubular degeneration, characterized by nuclear swelling and cytoplasmic vacuolization (Hirano *et al.*, 1986).

Rats fed daily doses of phenylmercuric acetate for up to two years exhibited slight-to-moderate renal damage, consisting of tubular dilatation, atrophy, granularity, and fibrosis. Effects were seen at doses as low as 0.02 mg Hg/kg per day, two orders of magnitude lower than those required to induce detectable effects in the mercuric acetate-treated rats (Fitzhugh *et al.*, 1950). Increased severity of renal nephrosis was also observed in another study in which rats were given 0.4 mg Hg/kg per day as phenylmercuric acetate in the drinking water for two years (Solecki *et al.*, 1991). Mice given methylmercuric chloride in the diet at a dose of 0.14 mg Hg/kg per day showed epithelial cell degeneration and interstitial fibrosis with ongoing regeneration of the tubules present (Mitsumori *et al.*, 1990). No effect was observed in this study at 0.03 mg Hg/kg per day. Similar effects were seen in mice given 0.11 mg Hg/kg per day as methylmercuric chloride in the diet for two years (Hirano *et al.*, 1986). Rats given methylmercuric chloride in the diet for two years at a dose of 0.1 mg Hg/kg per day had increased kidney weights and decreases in alkaline phosphatase, ATPase, NADH- and NADPH-oxidoreductase, and AMPase in the proximal convoluted tubules (Verschuuren *et al.*, 1976), but histopathologic examination revealed no treatment-related lesions.

Musculoskeletal effects

Human

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). Deep skeletal pain and muscle twitching/fasciculations were observed in Iraqis poisoned by consuming flour made from grain treated with ethylmercury *p*-toluene sulfonanilide (Jalili and Abbasi, 1961).

Experimental mammals

Usaki *et al.* (1998) administered 5 mg methylmercuric chloride/kg body weight (in distilled water) by gavage to 12-week-old male Wistar rats daily for 12 days. Treated rats gradually lost weight during the exposure period, and exhibited signs of muscle weakness and wasting. Histopathologic examina-

tion of animals killed seven days following the last treatment revealed a prominent decrease in mitochondria enzyme activity in skeletal muscle. Tissue concentrations of methylmercury were found to be as high in skeletal muscle as in the liver, kidney, and cerebrum.

Using a smaller dosage, Verschuuren *et al.* (1976) found no histopathologic changes in the skeletal muscle of rats exposed to dietary methylmercuric chloride at a concentration of 0.1 mg Hg/kg per day for two years.

Hematologic/lymphatic effects

Experimental mammals

Rats that received phenylmercuric acetate in their drinking water for two years showed decreases in hemoglobin, hematocrit, and red blood cell count at a dose of 4.2 mg Hg/kg per day (Solecki *et al.*, 1991). No treatment-related changes were observed in hematologic parameters measured in rats exposed via the diet for two years to 0.1 mg MeHg/kg per day as methylmercuric chloride (Verschuuren *et al.*, 1976). *In vitro* studies suggest that organic mercury compounds may interfere with the bacteriotoxic capacity of polymorphonuclear leucocytes (Obel *et al.*, 1993).

Immune effects

Human

Dermal sensitization from exposure to organomercurials has been shown to occur in some individuals (Skerfving and Vostel, 1972; Wilson *et al.*, 1981; Torres and De Corres, 1985; Aberer *et al.*, 1990). Intense dyspnea, upper body flushing, and exanthema were observed within three minutes of applying a mercurochrome-containing antiseptic to the abraded skin of a male adult (Torres and De Corres, 1985). Patch tests conducted for organic (as well as inorganic) mercury were positive for this individual. Maibach (1975) reported a case of acute laryngeal obstruction, requiring emergency tracheostomy, in an adult 30 hours after the treatment of a minor sore throat with a thimerosal-containing spray. Subsequent patch testing to thimerosal was positive.

In another study, a 54-year-old woman with a family history of atopy was found to display erythema 30 minutes after topical treatment with a 0.01% solution of phenylmercuric acetate (Torresani *et al.*, 1993). Urticaria was observed at 60 minutes post-treatment. This reaction was accompanied by the 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.

Suneja and Belsito (2001) described a five-year study at the University of Kansas Medical Center in which 574 patients were patch-tested using the standard allergen tray of the North American Contact Dermatitis Group, including thimerosal. A comparison of demographic data from thimerosal-allergic and nonallergic subjects revealed statistically significant increases in thimerosal allergy among women, healthcare workers, secretaries, and cooks. In addition, thimerosal-allergic persons were more likely to be allergic to neomycin, bacitracin, and tixocortol pivalate.

Experimental mammals

Although immunologic effects of mercury have, in general, been well studied (ATSDR, 1999), relatively less research has been conducted on the various organomercurials. In BALB/c mice administered a diet containing 0.5 mg MeHg/kg per day daily for 12 weeks, the thymus weight and cell number decreased by 22% and 50%, respectively, compared with 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 was increased at this dose of mercury.

Dermal effects

Human

Organomercurials have produced a variety of dermatologic effects. Skin contact with ethylmercury, methylmercury, phenylmercury, and tolylmercury compounds have produced dermatitis and eczema (Skerfving and Vostel, 1972; Lebrec *et al.*, 1999).

Earlier case reports suggest that dermal exposure to methylmercury or phenylmercury can cause dermatologic problems. Hunter *et al.* (1940) reported the presence of burns and blisters on the forearm of a 33-year-old male occupationally exposed to methylmercuric nitrate dust for two years. These effects subsided/healed within nine

days of removal from exposure. Morris (1960) reported itchy, pruritic, papular eruptions or rashes on the skin of individuals acutely exposed dermally to phenylmercuric salts.

Concentrations of 0.1% phenylmercuric acetate and phenylmercuric nitrate produce primary irritant effects, beginning with burning and erythema and progressing to vesicle formation within 48 hours (Morris, 1960; Koby, 1972). Methoxyethylmercury silicate is also a local skin irritant. A 0.01% solution of phenylmercuric acetate has been demonstrated to produce a positive allergic response, with symptoms including urticaria, facial edema, and bronchospasm (Torresani *et al.*, 1993). A positive response to sodium ethylmercury salicylate (thimerosal) has also been documented (Skerfving and Vostel, 1972; Wilson *et al.*, 1981; Aberer *et al.*, 1990).

Acrodynia has been reported in a 10-year-old boy, 10 days after the inside of his home was painted with interior latex paint containing phenylmercuric acetate at a level three times that recommended by EPA (Agocs *et al.*, 1990; CDC, 1990). Acrodynia is a hypersensitivity reaction to mercury seen primarily in children and expressed by irritability; mood changes/erythism; erythema of the hands, feet, and sometimes nose and other parts of the body; leg pains/cramps; peeling of the palms of the hands and soles of the feet; and insomnia. This syndrome was believed in this case to be the result of elemental mercury vapor released from the phenylmercury as the paint dried.

Patrizi *et al.* (1999) reported a study of five infants (two females, three males), aged seven to 28 months, who were affected by atopic dermatitis and who had experienced an exacerbation of their clinical condition within two to 10 days after receiving vaccines containing thimerosal. In this study, these same infants were tested by patch test for sensitivity to thimerosal in petrolatum. Positive reactions were seen at 0.1% thimerosal in all five children, and three of them also tested positive at 0.05% and 0.01% solutions. Despite their thimerosal hypersensitivity, all children were able to complete the entire series of mandatory vaccinations. A two-year follow-up examination did not reveal other episodes of exacerbation of the dermatitis after vaccination. The authors concluded that their study confirms the high frequency of sensitization to thimerosal in atopic children and suggests that vaccination with thimerosal-containing vaccines can cause clinical symptoms in sensitized children. They additionally noted, however, that sensitization to thimerosal does not prevent children from continuing with mandatory vaccinations (Patrizi *et al.*, 1999).

Allergic contact dermatitis has been associated with both the mercuric and thiosalicylic acid groups of thimerosal (Lebrec *et al.*, 1999).

Experimental mammals

Concentrated methoxyethylmercury acetate solutions have produced blisters on the skin of animals (Skerfving and Vostel, 1972). Santucci *et al.* (1999) reported that, due to the close chemical similarities to EtHgCl and to its water solubility, MeHgCl seems to be a suitable model for evaluating the reactivity of alkyl mercury compounds in the skin. Santucci *et al.* (1999) further speculated that both ethylmercury and methylmercury derivatives represent xenobiotics with similar reactivity.

Sensory effects (ocular, otic)

Human

Constriction of the visual field has been reported following occupational inhalation exposure to organic mercury fungicides and acute dermal exposure to dimethylmercury in a laboratory setting (Grant, 1986; Nierenberg *et al.*, 1998). This effect has also been reported following ingestion of methylmercury-contaminated food (Bakir *et al.*, 1980; Davis *et al.*, 1994). Grant (1986) reported that the constriction may continue to progress after cessation of exposure, and spontaneous recovery of full peripheral vision rarely occurs. In a study of seven-year-old children from a fishing village on the Portuguese Island of Madeira, Murata *et al.* (1999a) suggested that latencies of evoked potentials may be delayed due to increased exposures to methylmercury during development. Concerning that same study population, Murata *et al.* (1999b) also reported that methylmercury poisoning may cause constriction of visual fields and deafness, especially if exposure occurs prenatally.

In a case of fatal dimethylmercury poisoning, audiological examination conducted to determine the degree and type of hearing loss incurred as the result of the poisoning revealed an inability to

understand speech, both in formal and informal assessments, and bilateral abnormality of auditory brain stem response. In contrast, this examination revealed relatively good bilateral hearing sensitivity for pure tones and only minimal deficits in both ears. Collectively, these findings were believed to indicate central neuronal involvement, with little effect on the sensory (cochlear) mechanism (Musiak and Hanlon, 1999).

In addition to constriction of visual field, ingestion of ethylmercury and methylmercury in contaminated food has resulted in complete loss of vision (Amin-Zaki *et al.*, 1978; Bakir *et al.*, 1980; Davis *et al.*, 1994), and maternal ingestion of alkyl mercurials during pregnancy has also resulted in blindness in the neonate (Bakir *et al.*, 1980). Complete loss of sight has also been reported in occupational settings following inhalation (Grant, 1986) and dermal exposure (Nierenberg *et al.*, 1998) to alkyl and/or dialkyl mercurials. Spontaneous recovery of central vision may occur following termination of exposure, but the extent of any recovery is contingent upon the magnitude of the existing damage.

Contact with very concentrated solutions of some phenylmercuric salts can produce eye irritation, as well as irritation of the surrounding skin (Grant, 1986). Whereas short-term exposure to low concentrations of phenylmercuric salts used as an antimicrobial in eye drops might not be expected to cause similar symptoms, long-term exposure to such preparations may produce adverse effects on the eye. For example, long-term use of eye drops containing 0.001% phenylmercuric acetate as a preservative has been reported to cause discoloration of the lens (Grant, 1986), an effect known as mercurialentis. In addition, long-term use of eye drops containing 0.002% phenylmercuric acetate has resulted in altered lens transparency (Grant, 1986).

Use of pilocarpine eye drops containing phenylmercuric nitrate as a preservative has been reported to cause atypical band keratopathy or corneal calcification in humans who used this product for about 10 years (average exposure duration). Ptosis, nystagmus, jerky visual tracking, and other disturbances of eye movement have also been occasionally observed in individuals using ocular solutions containing phenylmercury. Most eye preparations now use thimerosal, instead of phenylmercury, as a preservative/antimicrobial. While thimerosal is not nearly as toxic to ocular tissue as phenylmercury, thimerosal in eye drops and contact lens solutions may cause eye irritation in hypersensitive individuals (Grant, 1986).

Another organomercurial, p-chloromercuribenzoate, has been shown to produce retinal effects (including retinal edema, chorioretinal atrophy, impairment of aqueous outflow), and systemic absorption of that mercurial has caused slight retinal toxicity in rabbits (Grant, 1986).

Partial to complete loss of hearing in adults, children, and neonates has been seen in poisoning outbreaks involving the consumption of ethyl- and/or methylmercury (Nagi and Yassin, 1974; Bakir *et al.*, 1980). In a tragic case of accidental dermal exposure to dimethylmercury in a laboratory, mild to moderate sensorineural hearing loss was observed (Nierenberg *et al.*, 1998).

Experimental mammals

Phenylmercuric salts have been shown to cause damage to the cornea in laboratory mammals. Corneal opacification and vascularization have been observed in rats and guinea pigs administered eye drops containing 0.1% phenylmercuric dinaphthyl-methanedisulfonate (Hydrargaphen). This is only three times the concentration (0.033%) of Hydrargaphen used in human ophthalmic preparations 'without apparent serious adverse effect' (Grant, 1986).

Other effects

Experimental mammals

A number of animal studies have reported decreases in body weight or body weight gain after ingestion of methylmercury or phenylmercury. A 20–25% decrease in body weight gain of male and female rats was observed after five daily gavage dosages with 8 mg Hg/kg as either methylmercuric chloride or ethylmercuric chloride (Magos *et al.*, 1985), and significant decreases in body weight gain have been observed in rats after exposure to dosages as low as 0.8 mg MeHg/kg per day for six weeks (Chang and Hartmann, 1972a) and in mice after exposure to 1 mg/kg per day for 60 days (Berthoud *et al.*, 1976). A two-year exposure to 0.4 mg Hg/kg per 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 12 mg MeHg/kg per day for two days resulted in a decrease in body temperature in rats (Arito and Takahashi, 1991).

Genotoxic effects

The body of evidence showing the induction of primary DNA damage in mammalian and bacterial cells and weak mutagenesis in mammalian cells suggests some genotoxic potential. Although the data on clastogenesis are less consistent, recent well-conducted studies suggest that mercury compounds can be clastogenic.

Chromosomal aberrations and spindle disturbances have been induced by methylmercury, ethylmercury, dimethylmercury, methoxyethylmercury, and phenylmercury in cultured human lymphocytes and other mammalian cells (IARC, 1993). Methylmercury-induced dominant lethal mutation has been reported in rats in several studies (IARC, 1993).

Methylmercuric chloride was found to induce single-strand breaks in the DNA of intact rat glioblastoma cells, Chinese hamster V79 (fetal lung) cells, human lung cells, and human nerve cells (Costa *et al.*, 1991). Both structural and numerical chromosomal aberrations were observed following the exposure of human lymphocytes to methylmercury chloride or dimethylmercury *in vitro* (Betti *et al.*, 1992). Methylmercuric chloride treatment of human lymphocytes resulted in chromosome and chromatid aberrations (Betti *et al.*, 1993).

Methylmercury chloride (0.08–0.4 νg Hg/mL) and methoxyethylmercury chloride (0.04–0.23 νg Hg/mL) each induced weak but dose-related mutagenic responses in Chinese hamster V-79 cells near the cytotoxic threshold (Fiskesjo, 1979). In another study, both methylmercury and phenylmercuric acetate induced primary DNA damage in the *B. subtilis* rec assay (Kanematsu *et al.*, 1980).

Methylmercuric chloride was also found to be capable of producing aneuploidy (particularly hyperdiploidy). At low doses, more chromosomal aberrations were observed in the second metaphase than in the first, suggesting that several premutational lesions induced by methylmercury survived through one cell cycle. Betti *et al.* (1993) concluded that methylmercuric chloride was capable of producing long lasting damage, which in turn gives rise to both structural and numerical chromosome abnormalities. Bala *et al.* (1993) reported that methylmercuric chloride in concentrations of 10^{-5}, 10^{-6}, and 10^{-7} M induced aberrant metaphases (including gaps) in cultured human peripheral lymphocytes in a dose-dependent manner. Furthermore, methylmercuric chloride induced a significant number of sister chromatid exchanges (SCEs) per cell in a dose-dependent manner.

Endocrine effects

Mihai *et al.* (1999) found that thimerosal increases the responsiveness of the calcium receptor in human parathyroid cells and rat medullary thyroid carcinoma (rMTC6-23) cells. The observed increase in responsiveness of calcium receptor agonists was believed to be due to modulation of the sensitivity of the IP3 receptor in both parathyroid and rMTC6-23 cells.

Cancer

Significant increases in renal tumors have been observed in rodents exposed either to methylmercuric chloride or phenylmercuric acetate. Dietary exposure of male mice to methylmercuric chloride for two years at 0.69 mg Hg/kg per day resulted in significant increases in the incidence of renal epithelial cell adenomas and carcinomas (Mitsumori *et al.*, 1990). Methylmercuric chloride at doses equivalent to 0.73 mg Hg/kg per day and 11.6 mg Hg/kg per day produced significant increases in the incidence of epithelial cell adenocarcinomas in male mice (Mitsumori *et al.*, 1981; 1990; Hirano *et al.*, 1986). In yet another study, no increase in tumor incidence was observed in rats exposed for two years to methylmercuric chloride at dietary doses as high as 0.1 mg Hg/kg per day (Verschuuren *et al.*, 1976); whereas exposure of male Wistar rats to phenylmercuric acetate in drinking water at 4.2 mg Hg/kg per day for two years resulted in an increase in renal cell adenomas (Solecki *et al.*, 1991).

Populations that are particularly susceptible

The elderly with declining organ function and the youngest of the population with immature and

developing organs will generally be more vulnerable to toxic substances than will healthy adults. This is believed to be due to both the increased susceptibility of the developing nervous system (Cox *et al.*, 1989; Grandjean *et al.*, 1997) and, maybe, in part due to the slower elimination of organomercurials in the young than in adults, as seen in rats (Thomas *et al.*, 1982).

Very young children are more susceptible to mercury toxicity than are adults, because of increased absorption and retention of mercury by children and because of the vulnerability of their developing nervous systems. Exposure to children may occur through inhalation of organomercurial vapors, ingestion of organic mercury or mercury salts, dermal application of mercury-containing ointments, or injection of thimerosal-containing vaccines. Neonates may also be especially susceptible to mercury toxicity. Both inorganic and organic forms of mercury are excreted in the milk (Sundberg and Oskarsson, 1992; Yoshida *et al.*, 1992).

Probably the most susceptible individual is the unborn child. Data from large-scale poisonings in Japan (Harada, 1978) and Iraq (Marsh *et al.*, 1987) indicate that infants exposed *in utero* to high levels of alkyl mercury compounds developed severe neurologic toxicity, whereas their mothers may have experienced no or only mild toxicity.

Observations of laboratory animals indicate that development of the brain is a carefully regulated and complex genetically based process. In this process (which begins early in the third week of human development), profound changes in structural arrangement of the brain cells/components occur according to a rigid temporal and spatial schedule, deviation from which will usually have adverse consequences on nervous system integrity and (eventually) function (NIEHS, 1999).

During this development process, neurons in different regions of the brain pass through the maturational sequences at different times. Furthermore, each region of the brain has its individual timetable of development that is relatively rigid and intolerant of delay. Should migration of a selected population of cells be delayed for any reason, subsequent generative neurons may either be physically blocked upon entering the migratory stage or pass the arrested neurons (NIEHS, 1999). Such changes in the normal genetically based processing can cause changes ranging from very subtle to profound in subsequent neurophysiologic, neurobehavioral, or neuropsychologic function. Critical milestones at which chemical insult may occur to the developing embryo, fetus, or infant are shown in Table 1.

Individuals with diseases of the liver, kidney, lung, and nervous system are also considered to be at greater risk of suffering from the toxic effects of both organic and inorganic mercury. Individuals with a dietary insufficiency of zinc, glutathione, antioxidants, or selenium, or those who are malnourished may be more susceptible to the toxic effects of mercury poisoning because of the diminished ability of these substances to protect against mercury toxicity.

Patients receiving regular injections of gammaglobulin are potentially at risk of mercury toxicity because of the merthiolate content (49% organically bound mercury) of the injected solution (Matheson *et al.*, 1980).

Kinetics and metabolism

Absorption

Oral exposure is the primary route for methylmercury. Although all organomercurials can be absorbed to some extent through the intact skin, dermal penetration is usually highly significant only for dialkyl organomercurials. Organic mercury compounds are readily absorbed through the lungs as a vapor, but the typical route of absorption for methylmercury is through the GI tract as the result of ingestion of contaminated aquatic organisms. Organomercurials are much more readily absorbed by the oral route than are 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). Dialkyl mercury compounds are rapidly and completely absorbed via all routes of exposure (inhalation, oral, and dermal).

Alkyl and aryl mercurials are extensively absorbed through the intestine and, with the exception of phenylmercury, which is rapidly oxidized to the divalent cationic form, enter the hepatic portal

Table 1. Milestones in neurodevelopment.

Week 3	Week 4	Week 5	By end of week 8	Week 20	At birth	Second year
Neuronal migration begins	Development of neural tube[1]	Forebrain, hindbrain, and midbrain all evident	Gross structure of nervous system already established[4]	Sensory and motor axons begin myelin development[5]	Number of CNS neurons established[6]	Myelination of corticospinal tract completed
Formation of neural plate	Development of three primary vesicles[2] from anterior portion of neural tube	Dorsal and ventral horns of spinal cord forming			Blood–brain barrier formation nearly complete in most brain areas[7]	
	Neuroectodermal cells differientiate into neurons and all four glial cell types	Formation of peripheral and sensory nerves and secondary vesicles[3]				

[1] Complete by gestation day 23; infolding of neural tube becomes CNS; neural crest becomes PNS.
[2] Prosencephalon (forebrain); mesencephalon (midbrain); rhombencephalon (hindbrain).
[3] Anterior telencephalon; diencephalon, mesencephalon; metencephalon; myelencephalon.
[4] Growth of axons and dendrites, along with elaboration of synaptic connections, continue at a rapid pace, making the CNS vulnerable to teratogenic or hypoxic influences throughout pregnancy.
[5] Process continues throughout first two years of life.
[6] Subsequent increase in brain size due to growth in axonal length and increase in number of glial cells.
[7] But remains functionally immature and permeable; will not be fully developed until age 6.
Sources: Brasel and Gruen (1986), Guyton and Hall (1996), Tortora and Grabowski (1996), Behrman *et al.* (2000), Zheng (2001).

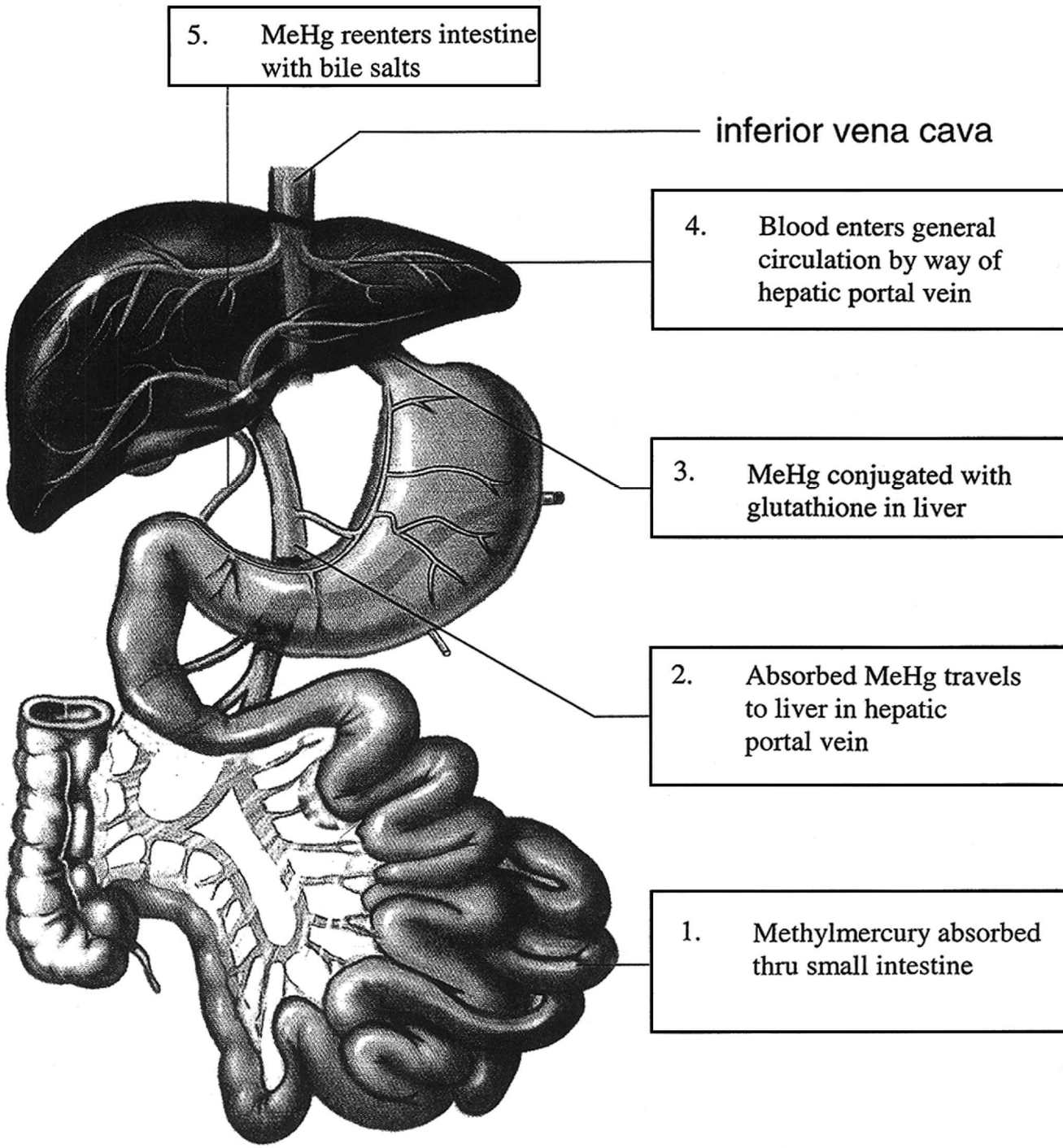

Figure 1. Enterohepatic circulation of methylmercury.

circulation. In the liver, the organomercurial is conjugated with bile salts by glutathione, and then excreted bound to low molecular weight nonprotein sulfhydryl compounds (Ballatori and Clarkson, 1982; Urano *et al.*, 1990) in the bile into the gall bladder or the bile duct for ultimate delivery to the duodenum. In the intestine, some of the organomercurial is oxidized to Hg^{2+} in the lumen of the gut (Nakamura *et al.*, 1977; Rowland *et al.*, 1980) and excreted in the feces. Most, however, is reabsorbed through the intestinal wall and repeatedly 'recycled' through this enterohepatic process (Figure 1).

Distribution

The lipophilic nature of methylmercuric compounds enables ready distribution to all tissues, including the brain and fetus, after absorption from the gastrointestinal tract. About 90% of the methylmercury in blood is found in the red blood cells (Kershaw *et al.*, 1980). The ability of methylmercury, ethylmercury, and phenylmercury compounds to cross the blood–brain and placental barriers allows distribution, and subsequent accumulation, in the brain and fetus. Tissue concentrations tend to remain constant relative to blood levels. Because of the uniform distribution in tissues, blood levels are a good indicator of tissue concentrations independent of dose (Nordberg, 1976; Smith and Farris, 1996).

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 methylmercury 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 180° angle, thus creating a chemical structure similar to that of the essential amino acid methionine (Clarkson, 1995). In such a manner, methylmercury can then cross the blood–brain barrier 'disguised' as an amino acid. 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).

Mercury accumulates in hair following exposure to methylmercury in humans (Cernichiari *et al.*, 1995). Although it is likely that other alkyl organomercurials will follow a similar pattern of distribution, empirical evidence is lacking. 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. Mercury concentrations in maternal hair were found to correlate significantly with cord blood levels of mercury in pregnant women who had frequently ingested whale meat throughout pregnancy (Grandjean *et al.*, 1992; 1997; 1998). It takes approximately 20 days following ingestion of methylmercury for the first incorporation into a hair follicle. The incorporation of mercury into hair is irreversible (Nielsen and Andersen, 1991b).

Methylmercury can accumulate in the placenta and readily traverses the placental barrier. Ask *et al.* (2002) reported that although methylmercury is easily transferred to the fetus, it is also accumulated in the placenta. Among 119 Swedish women, not selected with respect to high mercury exposure, an average of 60% of placental mercury was in the form of methylmercury. Ask *et al.* (2002) further found that the placental concentration of methylmercury was more than twice the maternal blood concentration.

Concentrations of methylmercury in the fetal blood are also somewhat higher than in the maternal blood (Kuhnert *et al.*, 1981; Inouye and Kajiwara, 1988). Following an oral dose of methylmercuric chloride during gestation, accumulation of mercury was much greater in the fetal kidney than in the maternal kidney of guinea pigs (Inouye and Kajiwara, 1988). Mercury levels in the liver were also slightly higher in the fetus compared with the dam when exposed to organic mercury at late

Table 2. Total mercury concentrations in human breast milk.

Population	Total mercury content in human breast milk (ppb)[1]	Reference
Minamata, Japan (contaminated seafood)	63	Fujita and Takabatake, 1977
Iraq (contaminated grain)	<200	Bakir *et al.*, 1973
Tokyo, Japan (urban population)	3.6 ± 2.2 (0.4–9.8)	Fujita and Takabatake, 1977
Iowa, USA	0.9 ± 0.23	Pitkin *et al.*, 1966
Alaska, USA (coastal population) (urban population)	 7.6 ± 2.7 3.3 ± 0.5	Galster, 1976
Madrid, Spain	9.5 ± 5.5 (0.9–19)	Baluja *et al.*, 1982
Sweden (15 female fish consumers)	0.2–6.3	Skerfving, 1988
Sweden (fish consumers with an average of 12 dental amalgams)	0.6 ± 0.4 (0.1–0.2)	Oskarsson *et al.*, 1996
Faroe Islands (consumption of pilot whale)	Median: 2.45 Maximum: 8.8	Grandjean *et al.*, 1995
Rochester, NY, USA	0.3 (0.24–0.42)	Pichichero *et al.*, 2002

[1] Values are given as arithmetic mean values, with ranges (where available) in parentheses, unless otherwise noted.

gestation, but were similar at early gestation. Distribution of mercury in the maternal and fetal brain was uneven, with the highest concentrations found in the neopallium, diencephalon, and mesencephalon, and lowest in the rhombencephalon. Exposure at later gestational weeks resulted in higher concentrations for both maternal and fetal brains (Inouye and Kajiwara, 1988). Treatment of mice with ethanol results in increased accumulation of mercury in the fetus (Khayat and Dencker, 1982).

Methylmercury may also be secreted in mother's milk (Bakir *et al.*, 1973). Demethylation of methylmercury in the dam and transport of inorganic mercury to the sucklings via milk also occur. The placental transfer of methylmercury was more efficient compared with 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). Again, it is probable that ethylmercury follows a similar process, but empirical evidence is lacking. Table 2 shows a number of studies which report human breast milk mercury concentrations in a variety of geographic locations and exposure scenarios.

Ukita *et al.* (1969) administered radiolabeled ethylmercury by intraperitoneal injection to one cynomegalus monkey (800 vg Hg as ethylmercuric chloride) and one cat. Twenty hours following administration, the animals were killed, and tissue mercury was autoradiographically determined. In the monkey, mercury distribution was pronounced in the heart and skeletal muscles, lung, tongue, mucosa of the oral cavity and throat, salivary gland, spleen, and digestive mucosa. Blood mercury was restricted to the corpuscles, and almost negative in the plasma. Mercury was also found in high levels in the eyes, particularly in the conjunctiva, iris, eye muscles, and lachrymal gland. The ethylmercury found in the central nervous system of the monkey was distributed throughout the gray matter. A similar distribution was seen in the cat. Autoradiograph of the monkey further revealed conspicuous concentrations of the radiolabel in the cerebral and cerebellar cortices, subcortical gray matter, various nuclei of the brain stem, and gray matter of the spinal cord, but far lesser amounts in the white matter of the cerebrum and spinal cord. No radiolabel was found in the corpus callosum, anterior commissure, or the optic nerve.

Vahter *et al.* (1994) found the accumulation of methylmercury in the brain appeared to be biphasic, with an elimination half-life of 35 days for brain methylmercury in female monkeys (*Macaca fascicularis*) exposed for 12 months. In the brain, methylmercury was demethylated to inorganic mercury. Once formed, the inorganic species was much more slowly eliminated, however, due to the

relative impermeability of the blood–brain barrier to inorganic mercury. The elimination half-life of brain inorganic mercury was reported by Vahter *et al.* (1994) to be on the order of years. It was also found that inorganic mercury accounted for approximately 9% of the total brain mercury at six to 12 months, 18% at 18 months, and 74% six months after termination of exposure.

Less is known of the distribution of ethylmercury than of methylmercury. However, there is no evidence to suggest that these two alkyl mercurials would behave in a significantly different fashion, if different at all. It is known that the ethyl group is more electrophilic than the methyl group, making it more susceptible to cleavage of the carbon–mercury bond by hydroxyl radicals produced by the P450 enzyme system. This would suggest a possibly shorter half-life of ethylmercury in blood, with a somewhat lesser probability of crossing the blood–brain barrier. Unlike methylmercury, ethylmercury is not believed to be helped to cross the blood–brain barrier via an active transport mechanism (Karper *et al.*, 1992; Magos, 2001). Further, Magos (2001) indicated that passage of ethylmercury across the blood–brain barrier is hindered by its larger size and faster decomposition (than methylmercury).

Once in the central nervous system, however, oxidation of the ethylmercury to the divalent cationic inorganic form of mercury, as occurs with methylmercury, would undoubtedly occur, trapping the mercury in the brain for an extended period of time. These presumptions are supported by studies of mice (Suzuki *et al.*, 1963) and rats (Magos *et al.*, 1983). After administration of equivalent doses, more mercury was found in blood and less in brain of test animals following ethylmercury than after methylmercury treatment. Tissue distribution of phenylmercury is initially similar to that of methylmercury. One week after administration, however, 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 into the plasma. Phenylmercury also predominantly distributes to the liver (Berlin, 1963). It is less permeable to the placental and blood–brain barriers than is 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).

Pedersen *et al.* (1999) examined autopsy samples from 29 adult brains for total and organic mercury. While there was a general distribution of mercury in the brain, the highest average concentration of total mercury was found in the cerebellum. The total concentration of mercury was positively correlated with age, whereas the fraction of methylmercury was negatively correlated with age, suggesting an age-dependent accumulation of total mercury and a slow transformation of methylmercury to inorganic mercury in the brain. Autometallographic analysis determined that the primary location of mercury was in the glial cells (Pedersen *et al.*, 1999).

Stajich *et al.* (2000) measured neonatal mercury levels both before and after administration of thimerosal-containing hepatitis B vaccine in 15 term and five preterm infants. Following vaccination, total mercury levels were significantly increased in both term and preterm infants; however, postvaccination mercury levels were significantly higher in preterm infants. This would be expected on a body weight difference, alone; but no other determinations were made.

Metabolism

The available evidence indicates that the metabolism of all forms of mercury is similar for humans and laboratory mammals. 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. 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. The conversion of alkyl or aryl mercurials to divalent inorganic mercury can probably occur soon after absorption, also feeding into the oxidation-reduction pathway.

It is known that absorbed methylmercury can be converted to inorganic mercury (specifically the divalent cation [Hg^{2+}]) in tissues (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 (Turner *et al.*, 1975) and phenylmercury (Nordberg, 1976) exposure.

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

Phenylmercury is rapidly metabolized to inorganic mercury (Nordberg, 1976). The metabolism of phenylmercury involves hydroxylation of the benzene ring to an unstable metabolite that spontaneously releases inorganic mercury. Consequently, the tissue disposition following initial metabolism of an ingested dose of phenylmercury resembles that seen after the administration of inorganic salts (Gage, 1973). While it may be more rapidly absorbed than inorganic mercury compounds, the enterohepatic circulation typical of alkyl mercurial compounds is not a significant factor, due to its rapid conversion in vivo to the divalent mercury cation.

Elimination and excretion

The elimination and excretion of organic forms of mercury vary with the particular organomercurial species. As shown in experimental animals, phenylmercury is excreted initially via the bile, then shifts to urine, whereas methylmercury is primarily excreted in the bile. 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 (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).

The fecal (biliary) pathway (see Figure 1) is the predominant excretory route for methylmercury and ethylmercury, with less than one-third of the total mercury excretion occurring via the urine, following oral, inhalation, or other parenteral exposure (Norseth and Clarkson, 1970; Pichichero *et al.*, 2002). Methylmercury is also excreted in the breast milk of rats, humans, and guinea pigs (Sundberg and Oskarsson, 1992; Yoshida *et al.*, 1992).

Ishihara (2000) confirmed the fecal elimination of methylmercury in four Japanese men; but in contrast to other studies, Ishihara found that the calculated amounts of methylmercury excreted daily in feces were similar to those found in urine in the case of those four individuals.

In rats and nonhuman primates, methylmercury has been shown to be secreted in the bile and can be reabsorbed in the intestine (Norseth and Clarkson, 1971; Berlin *et al.*, 1975; 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, methylmercury is extensively reabsorbed, providing evidence for the biliary-hepatic recycling of this metal (Dutczak *et al.*, 1991). This biliary-hepatic cycle probably contributes to the long biological half-life. Methylmercury can also be converted to 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). 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.

Elimination of methylmercury compounds generally follows first-order kinetics, with excretion directly proportional to body burden, and is independent of the route of administration (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, due to the appearance of an initial rapid elimination phase followed by a slower elimination phase.

Elimination rates for methylmercury vary with species, dose, gender, and strain (Nielsen, 1992). There is also evidence of gender differences in the elimination of methylmercury in humans (Miettinen, 1973), with males excreting faster during the fast component, and females excreting faster during the slow component. Similar sex-related differences in elimination were also observed in laboratory rodents (Thomas *et al.*, 1982; Nielsen, 1992).

Recent human data show that ethylmercury, however, is excreted from the body at a much faster rate than is methylmercury. Pichichero *et al.* (2002) administered vaccines containing thimerosal (with ethylmercury) to 40 full-term infants, 20 at age two months and 20 at age six months, and measured subsequent concentrations of mercury in blood, urine, and stools of those infants. A control group of 21 infants did not receive thimerosal-containing vaccines. Samples were collected from some individuals of each group within seven days of vaccination, between days 8 and 14 postvaccination, and between days 15 and 27. Blood mercury concentrations were below the range of reliable quantification in five of 17 blood samples from two-month-olds and seven of 16 blood samples from six-month-olds. The mean concentration of blood mercury in quantifiable samples was higher in two- than six-month-olds, but was low in both groups. The highest level of blood mercury measured in any infant in this study was 20.55 nmol/L in a two-month-old five days after vaccination. Although mercury blood concentrations were reported to be uniformly low, the highest levels were recorded soon after vaccination. Chichichero *et al.* (2002) estimated a half-life of mercury in blood to be seven days (same as seen in two-month-olds) for ethylmercury from thimerosal for the overall study, although the half-life was only five days in six-month-olds. When compared with a 40–50 day blood half-life of methylmercury, this study strongly suggests a significantly shorter half-life for ethylmercury.

Pichichero *et al.* (2002) reported that mercury was undetectable in most of the urine samples. Only one of 12 urine samples for exposed two-month-olds, three of 15 samples from exposed six-month-olds, and none of 14 samples from the controls contained detectable mercury. The highest urine mercury concentration was 6.45 nmol/L in a six-month-old infant in the exposure group. In contrast, all stool samples obtained from infants who received thimerosal-containing vaccines had detectable levels of mercury, with concentrations in the two-month-olds being slightly higher than those from six-month-olds. Most of the mercury in stools was inorganic. In addition, hair and breast milk samples were collected from some mothers. Mean hair mercury concentrations of mothers of exposure group children was 0.45 νg/g hair [0.45 ppm, versus 0.32 νg/g (0.32 ppm) in mothers of control infants]. Breast milk samples from eight mothers of infants in the six-month-old group averaged 0.3 νg Hg/g milk.

Pichichero *et al.* (2002) also found that the primary route of excretion of mercury from thimerosal-containing vaccines was the feces (suggesting biliary excretion of ethylmercury in human infants). The data of Pichichero *et al.* (2002) are particularly significant, in that it strongly suggests that the rapid elimination of ethylmercury in infants precludes the blood residence time necessary to result in significant accumulation of mercury from childhood vaccinations containing thimerosal as a preservative. The findings of Pichichero *et al.* (2002) are consistent with studies in laboratory rodents (Miller *et al.*, 1960; Norseth and Clarkson, 1970), which showed more rapid biotransformation to inorganic mercury and excretion (primarily in the feces) of ethylmercury than methylmercury following injection of the organomercurial.

Phenylmercury compounds are also eliminated primarily in the feces during the first few days after intravenous dosing as a result of biliary secretion and concentration in the gastrointestinal tract (mucosa and lumen) (Berlin and Ullberg, 1963). The initial 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).

Mechanisms of toxicity

High affinity binding of the divalent mercuric ion to thiol or sulfhydryl groups of proteins is believed to be the underlying mechanism for the biologic activity of mercury (Hughes, 1957; Passow *et al.*, 1961; Clarkson, 1972). Since proteins containing sulfhydryl groups are rather ubiquitous, occurring in both extracellular and intracellular membranes and organelles, and since most sulfhydryl groups play an integral part in the structure or function of most all proteins, the precise target for mercury is not easily determined. Possibilities include the inactivation of various enzymes, structural proteins, or transport processes (Bulger, 1986). Mercury may also bind to other sites (e.g., amine, carboxyl

groups), which are less favored than sulfhydryl groups. Through alterations in intracellular thiol status, mercury can also promote oxidative stress, lipid peroxidation, mitochondrial dysfunction, and changes in heme metabolism (Zalups and Lash, 1994). Hg^{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 an Hg^{2+}-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^{2+}-induced oxidative stress in kidney cells. Miller and Woods (1993) found that mercury–thiol complexes possess redox activity, which promotes the oxidation of porphyrinogen and possibly other biomolecules.

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 (Lebel *et al.*, 1990; 1992; Ali *et al.*, 1992). It has been suggested that organic mercury exerts its toxic effects on the nervous system and other organs by being metabolized to inorganic mercury, since both forms elicit very similar histopathologic changes.

In investigating the possibility that intracellular glutathione (GSH) synthesis may determine sensitivity to methylmercury exposure, Ou *et al.* (1999a) found that, while oxidative stress may mediate aspects of methylmercury toxicity, disruption of GSH homeostasis alone is not responsible for the sensitivity of embryonic central nervous system cells to methylmercury. In a separate study, Ou *et al.* (1999b) reported that the activation of cell cycle regulatory genes may be one mechanism by which methylmercury interferes with the cell cycle in both adult and developing organisms.

Thimerosal, whose active ingredient is ethylmercury, has been shown to be a versatile sulfhydryl reagent, a mobilizer of intracellular calcium, and a modulator of cell function (Elferink, 1999). Thimerosal has been shown to induce the release of intracellular calcium stores in many cell types. This mobilization of calcium can in turn modulate a number of cell functions. Tornquist *et al.* (1999) found that thimerosal mobilized sequestered calcium and evoked modest store-dependent calcium entry in thyroid FRTL-5 cells. The mechanism of action was suggested to be mediated via activation of protein kinase C, as thimerosal potently stimulated the bonding of [3H]phorbol-12,13-dibutyrate and was without effect on store-operated calcium entry in cells treated with staurosporine or in cells with downregulated protein kinase C. Whole cell patch clamping experiments revealed that thimerosal did not depolarize the membrane potential. It was concluded that thimerosal attenuates any increase in internal calcium ion concentration, probably by activating a plasma membrane Ca^{2+}-ATPase (Tornquist *et al.*, 1999).

Inositol 1,4,5-triphosphate (IP3) is involved in intracellular calcium homeostasis; and the binding of this compound to the IPS receptor is modulated by a number of compounds. The binding of IP3 to IP3 receptors can be differentially modulated by thimerosal (Vanlingen *et al.*, 1999). The sensitivity of intracellular calcium stores to IP3 has been shown to increase the affinity of the IP3 receptor in rat hepatocytes (Green *et al.*, 1999); and thimerosal was further shown to enhance agonist-specific differences in the oscillation of intracellular calcium in rat hepatocytes. Mason and Mahaut-Smith (2001) also reported the voltage-dependent Ca^{2+} release in rat megakaryocytes following sensitization of IP3 receptors with thimerosal.

Altering intracellular calcium levels in brain capillary endothelial cells has a direct effect on blood–brain barrier permeability and transport (Paemeleire *et al.*, 1999). Thus, substances such as organic mercury compounds, which cause the release of intracellularly bound Ca^{2+}, might not only have a direct affect on neuronal function, but may also increase the availability of mercury, and possibly other neurotoxicants, in the central nervous system.

Calcium channels in skeletal muscle, cardiac muscle, and certain nerve fibers have a high affinity for the plant alkaloid ryanodine (Sitsapesan and Williams, 2000). The receptors for which ryanodine has this particular affinity are known as ryanodine

receptors (RyR). Eager and Dulhunty (1999) found that thimerosal reacts with specific cysteine residues on RyR, contributing to either activation or inhibition of the channel, depending on the domain and particular class of cysteine associated with that receptor.

Targets of developmental methylmercury exposure include neural cell adhesion molecules (NCAMs), which are sialoglycoconjugates whose proper temporal and spatial expression is important at all stages of neurodevelopment, especially during the formation of synapses. Dey *et al.* (1999) dosed rat pups subcutaneously with 7.0 mg/kg on every other day from postnatal days 3 to 13, and investigated the effects of methylmercury on the temporal expression of NCAMs during development. Postmortem examination of whole-cerebellum homogenates, cerebellar synaptosomes, and isolated cerebellar growth cones collected at postpartum days 15, 30, and 60 was conducted. Golgi sialytransferase activity analysis revealed significant reductions in samples collected at postnatal day 15; however, no such changes were found at postnatal days 30 or 60. *In vitro* studies revealed decreasing methylmercury sensitivity of cerebellar sialytransferases with increasing developmental age. The authors concluded that methylmercury-induced perturbation of the developmentally regulated
expression of polysialylated NCAM during brain formation may disturb the stereotypic formation of neuronal contacts and contribute to the behavioral and morphologic disturbances seen following methylmercury poisoning (Dey *et al.*, 1999).

Castoldi *et al.* (2000) exposed *in vitro* cultures of rat cerebellar granule cells to methylmercury concentrations of 0.5–1 νM or 5–10 νM. One hour of exposure to the higher concentration resulted in impairment of mitochondrial function and plasma membrane lysis, resulting in cell death. While the lower (0.5–1 νM) concentrations did not compromise cell viability or mitochondrial function at early time points, neuronal network fragmentation and depolymerization of microtubules were observed within 1.5 hours. This damage continued to progress over time, and complete dissolution of microtubules and neuronal processes was seen after 18 hours. The authors postulated that cytoskeletal breakdown and deprivation of neurotrophic support may play a role in delayed toxicity following methylmercury exposure. Similarly, Miura *et al.* (1999) studied the relationship between changes in the cell cycle and the induction of apoptosis caused by methylmercury in cultured mammalian cells, and reported that GM2/M-phase arrest through the disruption of microtubules is an important event in the development of apoptosis by methylmercury.

Using whole-cell patch clamping to study the effects of thimerosal on tetrodotoxin (TTX)-sensitive and TTX-resistant sodium channels in dorsal root ganglion neurons, Song *et al.* (2000) found that thimerosal blocked the two channel types in a dose-dependent fashion. The inhibition was considerably more pronounced in the TTX-resistant channels, but the effect was not reversed in either case with washing with thimerosal-free solution. The thimerosal-induced inactivation of both types of sodium channels would serve to diminish neuronal activity.

In a study of the *in vivo* degenerative effects of methylmercuric chloride on rat brain and cranial nerves, Kinoshita *et al.* (1999) demonstrated a disturbance in the integrity of microtubules and neurofilaments in the rat nervous system, particularly in the optic nerves. Specifically, electron microscopic examination revealed a marked decrease in microtubules and a moderate decrease of neurofilaments in the myelinated fibers of optic nerves in treated animals.

Allergic contact dermatitis induced by the mercuric and thiosalicylic acid groups of thimerosal was studied by Lebrec *et al.* (1999). T-cell responses to such substances involve CD4+ and CD8+αβ+ T-lymphocytes, as well as CD4/CD8 gammadelta+ T-cells. While T helper-2 cytokine production by drug-specific human T cells from patients with allergic contact dermatitis has been previously shown, these authors also reported that T helper 1-like and T cytotoxic 1-like responses clearly also play key roles in this cutaneous reaction.

In investigating the possible factors associated with reproductive dysfunction in men following methylmercury exposure, Dufresne and Cyr (1999) found that exposure of adult rats to methylmercury can modulate metalothionine mRNA levels in both the testis and epididymis. Further, changes in metalothionine mRNA levels following methylmer-

cury exposure differ between epididymal segments, suggesting either differences in methylmercury accumulation or differences in metalothionine modulation.

Biomarkers of exposure

The biomarkers of exposure to organomercurials (with the notable exception of phenylmercury) are different than those considered most reliable for metallic and inorganic mercury exposure. Urine samples are considered the best determinant of body burden of mercury due to long-term exposure to elemental and inorganic mercury. Blood samples are useful primarily in cases of acute, higher level exposures to these forms, but are not as reliable as an indicator of total body burden in longer term exposures. In the case of methylmercury, the most appropriate determinant of previous exposure is scalp hair.

Unlike with inorganic forms of mercury, urine is not an accurate or reliable indicator of exposure to most organomercurials (with phenylmercury being the notable exception). In the case of alkyl mercurials, less than 10% of the absorbed dose is typically excreted in the urine. The majority of the absorbed dose of methylmercury is excreted as inorganic mercury in the feces, with some being excreted in the hair.

The mean total mercury levels in whole blood and urine of the general population worldwide are approximately 1–8 νg/L and 4–5 νg/L, respectively (Gerhardsson and Brune, 1989; WHO, 1990). The US Centers for Disease Control and Prevention (CDC, 2003) reported a geometric mean blood concentration of 1.02 ppb (νg/L) for all females aged 16–49 years, with a 95th percentile blood concentration of 7.10 νg/L. A geometric mean urine mercury level of 0.72 νg/L was reported for 16–49-year-old females, with a 95th percentile urine mercury value of 5.00 νg/L. The International Commission on Occupational Health (ICOH) and the International Union of Pure and Applied Chemistry (IUPAC) Commission on Toxicology determined that a mean value of 2 νg/L was the background blood level of mercury in persons who do not eat fish (Nordberg *et al.*, 1992). These levels are 'background' in the sense that they represent the average levels in blood in the general population, and are not associated with a particular source for mercury. However, the intra- and interindividual differences in these biomarkers are substantial, possibly due to dental amalgam (urine) and ingestion of contaminated fish (blood) (Verschoor *et al.*, 1988; WHO, 1991).

Grandjean *et al.* (1997; 1999a) used umbilical cord blood collected at parturition as a measure of methylmercury exposure during pregnancy. While cord blood is considered an excellent indicator of exposure during at least the latter part of the third trimester, it does not serve as an index of exposures that occurred during the first two trimesters of pregnancy (and possibly the early portion of the third trimester, as well), times during which critical neuronal migration and brain organization were taking place.

Among the general population, mean blood levels as high as 20 νg/L have been reported for some populations with no known occupational exposure and without excessive fish consumption (WHO, 1990). Long-term consumption of fish is believed to be the source of nearly all of the methylmercury measured in the general population, and individuals in communities with high fish consumption rates have been shown to have blood levels of 200 νg/L with daily intake of 200 νg mercury (WHO, 1990).

With the exception of phenylmercury, which volatilizes as metallic mercury vapor, urine mercury measurement is not considered a reliable indicator of organic mercury exposure, since most forms of organic mercury are excreted primarily in the feces.

Nonoccupational exposure to mercury includes the use of mercury-containing products and consumption of mercury-contaminated food. Increased urinary excretion and blood levels of mercury were observed in volunteers who used phenylmercuric borate solutions or lozenges intended for the treatment of mouth or throat infections (Lauwerys *et al.*, 1977).

Swedes consuming fish contaminated with 0.3–7 mg Hg/kg (total Hg) had blood cell levels of total mercury ranging from 8 to 390 ng/g (Skerfving, 1974). Long-term exposure to methylmercury at 4 νg Hg/kg per day was associated with a mercury level in blood cells of approximately 300 ng/g (Skerfving, 1974). The steady state concentration of methylmercury in blood may be related to daily

intake in the following equation (Task Group on Metal Accumulation, 1973; WHO, 1990):

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

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

Hair is a biomarker of long-term exposure to methylmercury. Once mercury is incorporated into hair, it remains unchanged (Clarkson *et al.*, 1973; Nielsen and Andersen, 1991a,b). Approximately 95% of the mercury found in hair is organic (typically methyl) mercury; thus, hair mercury is typically presumed to be due to methylmercury exposure. However, mercury may also be deposited on hair from the air when significant sources of mercury are present in the air, as in certain occupational settings, or when certain hair treatments have been used (WHO, 1991). In such (atypical) instances, the relationship between hair mercury levels and methylmercury ingestion may be less clear.

A number of studies have demonstrated a fairly strong correlation between the amount of fish consumed, the level of mercury in the fish, and the level of mercury in the hair (Haxton *et al.*, 1979; Sherlock *et al.*, 1982; Airey, 1983; Oskarsson *et al.*, 1990). The relationship between hair level and blood levels has also been well documented (Den Tonkelaar *et al.*, 1974; Skerfving, 1974; Amin-Zaki *et al.*, 1976; Haxton *et al.*, 1979; Kershaw *et al.*, 1980; Phelps *et al.*, 1980; Sherlock *et al.*, 1982; Soria *et al.*, 1992).

Thompson *et al.* (1999) identified another biomarker of exposure to methylmercury in mice, although this biomarker is not readily usable as a biological indicator of exposure for humans due to its invasive nature. In a study originally designed to evaluate the effects of subchronic (12-week) exposure to drinking water containing either 0, 3, or 10 ppm methylmercury on GSH levels, glutamate-cysteine ligase (GLCL) activity in brain, liver, and kidney tissue, as well as GLCL catalytic and regulatory subunit mRNA and protein levels in C57B1/6 mice, Thompson and colleagues found a 30% increase in GLCL activity. This led the authors to conclude that upregulation of GSH synthetic capacity in the brains of mice is a sensitive biomarker of subchronic methylmercury exposure.

Relevance to human health

The nature and severity of the toxicity that may result from organic mercury exposure are functions of the magnitude and duration of exposure, the route of exposure, and the form of the mercury compound (e.g., alkyl, dialkyl, phenyl) to which exposure occurs. Since the ultimate toxic species for all mercury compounds is thought to be the mercuric ion, the kinetics of the parent compound is the primary determinant of the severity of parent compound toxicity. It is differences in the delivery to target sites that result in the spectrum of effects. This is apparent in the differences in the neurotoxicity of organic and inorganic forms of mercury, as well as such differences between alkyl mercurial compounds. Magos (2001), after an extensive review of the literature on ethylmercury, reported that both toxicological and kinetic studies indicate that the relationships of dose and blood mercury concentration to the risk of intoxication established for methylmercury overestimates the risk of ethylmercury intoxication.

The nervous system is the primary target for alkyl mercurials and most other organomercurials. Neurologic and behavioral disorders in humans have been observed following inhalation of organic mercury compounds, ingestion or dermal exposure to organic mercury-containing compounds, or ingestion of seafood contaminated with alkyl mercurials. Specific neurologic symptoms include tremors (initially affecting the hands and sometimes spreading to other parts of the body), emotional lability (characterized by irritability, excessive shyness, confidence loss, and nervousness), insomnia, memory loss, neuromuscular changes (weakness, muscle atrophy, and muscle twitching), headaches, polyneuropathy (paresthesias, stocking-glove sensory loss, hyperactive tendon reflexes, slowed sensory and motor nerve conduction velocities), and performance deficits in tests of cognitive and motor function (see ATSDR, 1999 for extensive listing). Although improvement has been observed upon removal of persons from the source of exposure,

Table 3. Concentration of total mercury in hair.

Mean (M) or median (m) (ppm)	Maximum (ppm)	*n*	Description of population	Reference
1.4 (M)	27.4	942	United Kingdom. Area chosen for 'above average fish consumption'	Sherlock *et al.*, 1982
1.35 (M)	5.8	55	United Kingdom. Fishing community, area with lesser contamination	Haxton *et al.*, 1979
1.48 (M)	3.28	34	Islands of the central Adriatic (Yugoslavia). Women; degree of fish consumption not reported. Hair sampled at end of pregnancy	Horvat *et al.*, 1988
2.0 (M)	11.3	119	United Kingdom. Fishing community, area with greater contamination. Fish consumption range 10–225 g/day with 50% eating greater than 50 g/day	Haxton *et al.*, 1979
2.85 (M)	20	50	Spain. Women; degree of fish consumption not reported. Hair sampled at end of pregnancy	Soria *et al.*, 1992
3.2 (M)	10.8	50	Sweden. High consumption of freshwater fish. Mercury levels in fish generally below 1 ppm	Oskarsson *et al.*, 1990
3.9 (M)	21	98	United Kingdom. Consumed an average of 0.36 kg fish/week	Sherlock *et al.*, 1982
5.6 (M)	20	35	Japan. Fish consumption not known	Suzuki *et al.*, 1993
0.8 (m)	2[1]	18	Faroe Islands. No fish consumption	Grandjean *et al.*, 1992
1.61 (m)	3.7	49	One fish meal per week	
2.5 (m)	4.7	75	Two fish meals per week	
2.1 (m)	3.6	49	Three fish meals per week	
5.2 (m)	8	17	Four fish meals per week	
1.4 (M)			Eat fish once a month	Airey, 1983
1.9 (M)			Eat fish once every two weeks	
2.5 (M)			Eat fish once a week	
11.6 (M)			Eat fish once a day	
0.247 (m)	2.5	150	German sample from 150 cadavers (75 males, 75 females) from the general population (i.e., no occupational or unusual exposures to metals)	Drasch *et al.*, 1997
0.45 (M)	1.9	40	Mothers of children receiving thimerosal-containing vaccine	Pichichero *et al.*, 2002
0.32 (M)	1.4	21	Mothers of control group children	Pichichero *et al.*, 2002

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

some changes may be irreversible. Autopsy findings of degenerative changes in the brains of poisoned patients exposed to mercury support the functional changes observed (Davis *et al.*, 1974; Al-Saleem and the Clinical Committee on Mercury Poisoning, 1976; Miyakawa *et al.*, 1976; Cinca *et al.*, 1979).

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 neuropathologic feature is degenerative changes in the cerebellum that caused the motor dysfunctions. In humans, disruptions of higher functions have also been noted, as evidenced by depression and irritability.

The developing fetus and infants are particularly vulnerable to the harmful effects of mercury. The fetus can be exposed to mercury from the pregnant woman's body through the placenta, and nursing infants may be exposed through the mother's breast milk. Both inhaled mercury vapors and ingested methylmercury can cross the placenta. Methylmercury and ethylmercury, as well as inorganic mercury, will move into breast milk. Pregnant women and nursing mothers need to be cautious in their use of consumer products containing mercury and should pay attention to possible exposures to mercury at work and in the home.

The best biological indicator of exposure to alkyl mercurials is mercury level in hair. Approximately 95% of total hair mercury is present in the form of organic mercury (Clarkson *et al.*, 1973), and hair levels may be used to track exposure over a period of time based on hair content and the known growth rate of scalp hair.

Levels of mercury in hair have been monitored in a variety of populations and generally range from 1 to 4 ppm, depending on the level of fish consumption. The geometric mean hair mercury concentration was recently reported to be 0.2 ppm for American women aged 16–49 years, with the 90th percentile hair mercury concentration being 1.4 ppm in the population studied (CDC, 1999). Table 3 summarizes the mean (or median) values and the maximum value from a number of other studies. Multiple routes of exposure to organomercurials can contribute to the body burden of mercury.

Although medical treatments, such as the use of various chelating agents, may reduce the body burden of mercury from organo-mercurials, these treatments pose health risks of their own, including increasing temporarily the amount of mercury in the blood accessible to the central nervous system. The best way to prevent the effects of organic mercury exposure is to mitigate or eliminate exposure to the maximum extent possible, consistent with sound dietary practices and proper nutrition.

Summary

The toxicity of organic forms of mercury is well documented. In sufficient quantities, organic mercury compounds can cause a variety of adverse health effects, including neurologic, renal, and developmental outcomes. It should be kept in mind, however, that while exposure to these compounds should be minimized, the mere existence of exposure does not necessarily mean that adverse health outcomes will occur. Almost 500 years ago, the Swiss physician Paracelsus said: 'All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy.' While organic forms of mercury are not used in the modern world as a remedy, we still must keep in mind that it is the dose that makes the poison. We may certainly say without hesitation that exposure to all forms of mercury should be avoided to the maximum extent practicable. And while mercury is a naturally occurring element in the earth's crust and some exposure is unavoidable, man has to ensure that unnecessary exposure for the current residents of our planet and our future generations is minimized. In the assessment of the potential health impacts of organic mercury, however, we must also ensure that full consideration is given to the entire mercury database and that we do not overestimate the potential health impacts of organic mercury and unnecessarily alarm people by overstating the risk of these compounds. It is hoped that this review will more fully enable the reader to make an informed decision regarding the health risk associated with exposure to organic forms of mercury.

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