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The Toxicology of Mercury and Its Chemical Compounds

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This review covers the toxicology of mercury and its compounds. Special attention is paid to those forms of mercury of current public health concern. Human exposure to the vapor of metallic mercury dates back to antiquity but continues today in occupational settings and from dental amalgam. Health risks from methylmercury in edible tissues of fish have been the subject of several large epidemiological investigations and continue to be the subject of intense debate. Ethylmercury in the form of a preservative, thimerosal, added to certain vaccines, is the most recent form of mercury that has become a public health concern. The review leads to general discussion of evolutionary aspects of mercury, protective and toxic mechanisms, and ends on a note that mercury is still an "element of mystery."

Keywords: Dental Amalgam, Ethylmercury, Inorganic Mercury, Mercury, Mercury Vapor, Methylmercury, Phenylmercury, Thimerosal

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I. INTRODUCTION

Mercury and its compounds have long presented a dilemma to those interested in making use of its many and varied properties. On the one hand, mercury has many attractive and useful properties, but on the other hand it presents a risk of toxic effects. The challenge we face today is to take advantage of these useful applications while at the same time assuring no adverse health effects occur. A key role for the toxicologist is to develop an understanding of its toxic properties so as to give advice to users and regulatory agencies to ensure that safe levels of exposure are not exceeded. This review focuses on the toxic properties of this metal and discusses current knowledge of the mechanisms of toxic action.

A. The Major Physical and Chemical Forms of Mercury

Table 1 summarizes the major chemical and physical forms of mercury. In its zero oxidation state, Hg°, it exists in its silvery liquid form as the only metal that is a liquid at ambient temperatures. The liquid is volatile and releases a monatomic gas usually referred to as mercury vapor. It is stable in ambient air and can remain in the atmosphere for months perhaps years. It plays a key role in the global cycling of mercury. It can undergo oxidation to form the two major oxidation states of mercury.

The first oxidation state, where the mercury atom has lost one electron, is called mercurous mercury and is most commonly found in the form of calomel or mercurous chloride, where two

TABLE 1
Physical and chemical forms of mercury: Some recent and present pathways of human exposure

	Inorganic mercury	
Hg vapor	Mercurous	Mercuric
Hg°	$Hg\text{-}Hg^{2,+}$	$Hg^{2,+}$
Occupational	Laxatives	Skin creams
Dental amalgam	Teething powders	
	Organic mercury[a]	
Short chain alkyl		Other organics
$CH_3(CH_2)_n\text{-}Hg^+$		$(R\text{-}C\text{-}Hg^+)$
Methylmercury in fish		Phenyl Hg antiseptic
Ethylmercury in preservatives		Mercurial diuretics

[a]Some organic mercury compounds involve two carbon atoms attached to mercury, such as dimethylmercury ($CH_3\text{-}Hg\text{-}CH_3$).

mercury atoms are linked together to give the chemical formula Hg_2Cl_2.

The mercuric ion, sometimes referred to as mercuric mercury or divalent mercury, is the second oxidation state, where two electrons have been removed from the mercury atom. It is responsible for nearly all the inorganic and organic chemical compounds of mercury. It is a product of the metabolism of inhaled mercury vapor and of the organic compounds of mercury as well as its release from the mercurous ion. It therefore plays a key role in the toxicology of most forms of mercury.

The chemistry of mercuric mercury is dominated by its high affinity for thiol groups, specifically the thiol anion $R\text{-}S^-$. The forward and back reactions are very rapid so that mercury can move quickly from one thiol group to another (Rabenstein et al., 1982; Govindaswamy et al., 1992). It is assumed that mercury binds to thiols *in vivo* based on the chemistry of this metal, but few mercury thiol compounds have been identified in tissues. Inorganic mercury and methylmercury complexes with reduced glutathione (Ballatori and Clarkson, 1985) have been identified in bile in animal experiments and a methylmercury–cysteine complex in fish protein (Harris et al., 2003). The only effective complexing and chelating agents that remove mercury from the body contain thiol groups (Kostyniak and Clarkson, 1981). The formation of complexes with thiol-containing small molecules such as cysteine and glutathione plays a major role in the processes of transport and disposition in the body.

Mercuric mercury also possesses a high affinity for the selenium in its reduced form of the selenide anion, Se^{2-}. Mercuric selenide, HgSe, is highly insoluble and may be the form of inorganic mercury that has a long residence time in human tissues (discussed later).

Those compounds in which the mercuric ion is covalently linked to at least one carbon atom are classified as organic mercury compounds. Perhaps the most notorious example is the methylmercury cation, as discussed later. Compounds of methylmercury are usually given the generic name of methylmercury. Other organic compounds are also given generic names based on the organic moiety species, such as ethyl- and phenylmercury. The generic distinction between inorganic and organic mercury is useful, as these forms of mercury usually have different toxicological properties.

B. Ancient and Modern Uses of Mercury

The late Leonard Goldwater published a detailed and fascinating history of many uses of mercury and its compounds over the millennia dating back to some of the earliest recorded civilizations (Goldwater, 1972). Mercury in the form of its brilliant red ore, cinnabar, may have played a role in the founding of bureaucracy, as the Chinese used it to prepare red ink over 3000 years ago. It has been found in Egyptian tombs, perhaps as a preservative but maybe as a protector against evil spirits (Figure 1). People also used compounds of mercury in skin

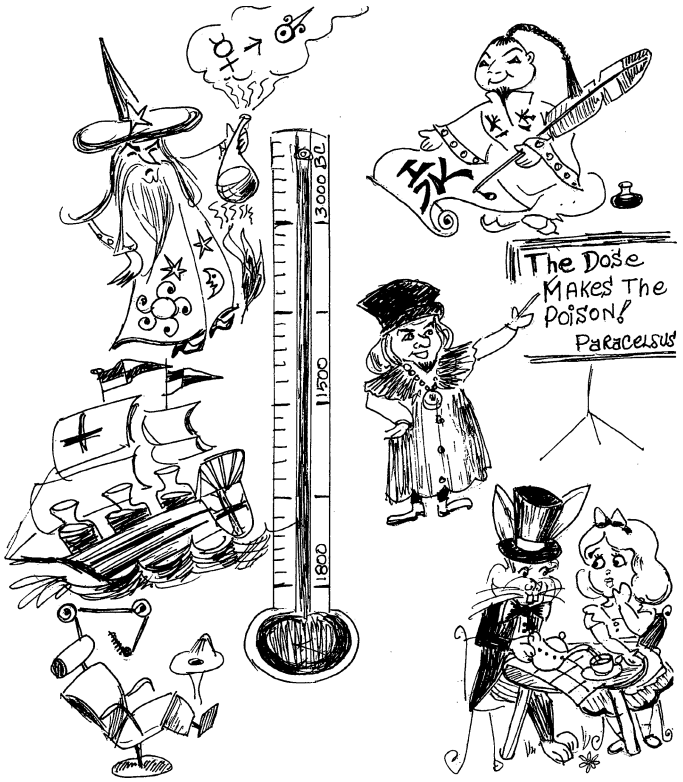

FIG. 1. A pictorial depiction of some of the major past uses of mercury and its compounds. Some of its earliest use was as a pigment in red ink and by alchemists. The Middle Ages saw its use in the treatment of syphilis, where a little might be useful but too much could be fatal. Spanish galleons shipped 76-lb flasks of liquid mercury to the Americas to extract gold and silver. Mercuric nitrate was used in the carroting of felt hats. Mercury amalgam was introduced as a tooth filling in the early 19th century. For further details, see text.

creams to treat infections. This early medical usage may have given birth to the idea that mercury might be useful in the treatment of syphilis after the entry of this disease into Europe following Columbus's epic voyage to the Americas. Paracelsus was an ardent advocate. He noted that a little mercury may be useful but too much was fatal, hence his immortal dictum, "the dose makes the poison." The medical applications of mercury compounds were common well into the 20th century, as, for example, the addition of calomel (Hg_2Cl_2) to teething powders.

Mercury compounds have found many other uses beyond the medical field. Following the revocation of the Treaty of Nantes (1685), many Huguenots emigrated to England, where they made use of their secret to produce high-quality felt hats. The secret was the use of mercuric nitrate to treat the fur used in the preparation of the felt. Alice in Wonderland's "mad hatter" may have been a victim of the secret, as his eccentric behavior is reminiscent of the symptoms caused by inhalation of mercury vapor. Apparently the fumes of mercury were good for felt hats but not for their makers. There is no doubt that the makers of top hats frequently succumbed to mercury poisoning, but whether or not the "mad hatter" was also a victim has been questioned (Waldron, 1983). In any event it makes for a good story! Certainly the numerous cases of severe poisoning in this industry gave rise to such phrases as "hatters shakes" and "mad as a hatter." The discovery that mercury fulminate was an excellent detonator for a variety of explosives was the occasion for a substantial increase in mercury production as recorded in the Almaden mines in Spain. Incidentally, Almaden, Arabic for "the metal," has been in production since at least Roman times and is still the major mercury mine to this day.

Human exposure to methylmercury is worldwide, as it is present in varying concentrations in virtually all edible marine and freshwater organisms. Until the 1970s organomercurials, especially methyl- and ethylmercury, were widely used in agriculture as antifungal agents in seed grain. This practice was discontinued as a result of a number of mass poisonings both in humans and in certain wildlife species, as discussed later. As antiseptic preservative, mercurochrome (dibromohydroxymercurifluorescein), has seen wide usage and has been the cause of several recorded cases of poisoning (Luk et al., 1997), Thimerosal (ethylmercury thiosalicylate) is still used in vaccines throughout the world, despite current concerns in the United States about its potential toxic effects in infants. Phenyl- and ethylmercury compounds still have a limited use as preservative antibacterial agents.

However, it was the liquid form of metallic mercury that attracted most attention. The chemical symbol for mercury, Hg, is an abbreviation of its ancient name *hydrargyrum* (Latin), *hydrargyros* (Greek), meaning literally water/silver. Later the silvery liquid form of metallic mercury gained it the name of quicksilver. As the only metal that is liquid at room temperature, its physical properties of low viscosity, high density, excellent electrical conductance, and reflective surface were the reason for its numerous applications. The mirror makers of Venice attained notoriety in Ramazzini's classic monograph *Diseases of Occupations* as scowling at their own reflections in the film of mercury that was the reflective surface (Ramazzini, 1713/1964). The long history of use of liquid metallic mercury has made it evident that the vapor can be highly toxic when inhaled but the ingestion of the liquid form offers a minuscule hazard. Indeed, in the 18th century a tablespoonful or so found use as a laxative, perhaps as the first application in medicine of Newton's new-found law of gravity.

Its uses in the industrial era include mercury barometers and thermometers, as an electrode in the electrolytic production of chlorine and caustic soda from saline, and in electrical switches still used widely in today's automobiles. The vapor from metallic mercury has also found wide application in mercury arc lamps and incandescent lights.

The propensity of liquid mercury to form stable amalgams with other metals, especially silver and gold, was well known to the alchemists of the ancient world. The silvery semisolid amalgam with gold could be dramatically transformed into pure gold when the mercury was evaporated by heating with an open flame. This alchemical trick was put to effective use in the extraction of gold and silver from their respective ores. Spanish galleons shipped enormous quantities of mercury to the new world for precisely this purpose. The extraction of gold from river sediments in the Amazon basin and elsewhere continues to this day. An amalgam of mercury principally with silver is the dental amalgam introduced in France some 150 years ago and is widely used to this day, despite concerns about its possible toxic side effects.

Generally speaking, most health authorities carefully regulate human exposure to all forms of mercury. However with the outsourcing of manufacturing and other activities previously located in developed countries there is now the ever-present danger of imported material contaminated with mercury. Unfortunately, the outsourcing of an industry is not always accompanied by the appropriate industrial hygiene measures.

II. INORGANIC MERCURY

A. An Overview

The inorganic forms of mercury include liquid metallic mercury and its vapor, compounds of mercurous and mercuric mercury and dental amalgam. The ingestion of liquid metallic mercury or "quicksilver" does not appear to be toxic in itself. Health hazards from quicksilver are due to its potential to release mercury vapor. Dental amalgam releases mercury vapor that can be inhaled and presumably behaves toxicologically like mercury vapor inhaled from external sources such as in occupational exposures. However, other than rare cases of contact allergy, no convincing evidence is yet forthcoming that dental amalgam can cause adverse health effects.

The other forms of mercury can give rise to kidney damage, including in some cases the nephrotic syndrome. Inhaled

mercury vapor can also cause damage to the central nervous system due to its ability to cross the blood–brain barrier. Mercurous mercury, in the form of mercurous chloride or calomel, has a long history of medicinal uses, especially as a laxative and in infant teething powders. Both mercurous and mercuric compounds are believed to be the causal agents in the childhood disease of acrodynia or "pink disease." The mercuric cation, Hg^{2+}, is believed to be the proximate toxic agent for all these inorganic forms of mercury.

All species of inorganic mercury have the capacity to elicit idiosyncratic reactions. Such reactions require exposure to mercury, but the prevalence and severity do not appear to be dose related. The nephrotic syndrome and acrodynia are examples (for details, see Magos and Clarkson, 2006).

The high mobility of inhaled mercury vapor in the body is assumed to be due to its physical properties as an uncharged, monatomic gas that can readily diffuse through the lipid monolayers of the cell membrane. The mechanisms of transport of mercurous and mercuric cations are not well understood. Mercuric mercury is known to exit liver cells into bile as a complex with reduced glutathione. Mercuric mercury has a limited capacity to cross the blood–brain and placental barriers but is avidly accumulated by the kidneys.

B. Pathways of Human Exposures

A WHO expert committee (WHO, 1990) estimated the dietary intake of inorganic mercury in the European and North American general population to be approximately 4 μg Hg as compared to an estimated daily intake of all forms of mercury as 6.6 μg Hg. They estimated that 0.6 μg Hg came from methylmercury in fish tissue and that the remainder was from nonfish sources. However, methylmercury is partially converted to inorganic mercury in mammals, so that meat and poultry products may contain some inorganic mercury ultimately derived from the metabolism of methylmercury from fish products used as animal food. An unusual dietary source is whale meat, where levels of inorganic mercury can be high (Grandjean et al., 1992). Medicinal uses of both mercurous and mercuric compounds have virtually disappeared, but inorganic mercury is the active principle in skin-whitening creams still used widely in many Third World countries (Weldon et al., 2000).

Mercury vapor is present in the ambient atmosphere at levels so low that human exposure is negligible (Fitzgerald and Clarkson, 1991).The two major pathways of human exposure to inhaled mercury vapor are occupational and from dental amalgam. Elemental mercury still has many industrial applications resulting in the escape of mercury vapor into the working atmosphere. Such industries include the production of caustic soda and chlorine, and in the manufacture of thermometers, thermostats, fluorescent light bulbs, batteries, and manometers. Occupational exposures have historically provided a considerable amount of information regarding the health effects of chronic human exposure. Another less common pathway takes place in the homes of workers in mercury industries if care is not taken to decontaminate the workers' clothing. Certain religious and ethnic practices lead to the use of liquid mercury in the homes resulting in the release of mercury vapor. Until the late 20th century, home exposure to mercury vapor could occur from the use of latex paints, where mercury compounds were used as a preservative. It is possible that this use of mercury may still occur in certain parts of the world.

C. Quicksilver

1. Disposition in the Body

Some rough estimates of the gastrointestinal absorption of mercury from ingested metallic mercury were summarized by a WHO expert committee (WHO, 1991). Animal data indicated less than 0.01%. This figure is bound to be variable, depending on the surface area of the ingested liquid. This low absorption is valid for intact gastrointestinal epithelium, but may not be true for mercury leaked from Miller-Abbott tubes used in patients suffering from ileus. In these patients, blood levels were observed 4 (Kummer and Michof, 1984) or even 10 times (Suzuki and Tanaka, 1971) higher than normal levels.

2. Toxic Effects

Cases of systemic toxicity from accidental swallowing of metallic mercury (such as from breakage of a thermometer or rupture of Cantor tubes) are rare, as it is poorly absorbed from the gastrointestinal tract (Cantor, 1951). (The Cantor tube is a double-lumen tube, which has a rubber bag at one end containing liquid metallic mercury, and is used to relieve obstruction in the small bowel.) However, aspiration of metallic mercury can be fatal in some cases. This is mostly due to the rupture of a mercury-filled bag of an intestinal tube in the naso-pharynx (Ozau et al., 1977). Cases of attempted suicide by intravenous injection of metallic mercury do not appear to result in systemic toxicity (Gutierrez and Leon, 2000).

D. Mercurous Mercury

1. Disposition in the Body

Calomel (mercurous chloride), in view of its low solubility, is probably slowly absorbed. However, high urinary levels in infants taking calomel in teething powders indicate that substantial amounts of inorganic mercury can be absorbed from calomel (Warkany, 1965). Once inside the body, the mercurous ion, $Hg–Hg^{2+}$, dissociates to release an atom of uncharged mercury Hg° and the mercuric ion, Hg^{2+}. Hand et al. (1943) demonstrated the presence of both mercurous and mercuric mercury in tissues of animals dosed with mercurous chloride. It is assumed that mercuric mercury accounts for the laxative, and antiseptic actions of calomel.

Little information is available on the disposition of mercury after exposure to mercurous mercury compounds. It is likely that the pattern of tissue disposition will resemble that of mercuric mercury to be described in the following section. Not only does the mercurous ion disproportionate into mercuric mercury but the atoms of uncharged mercury, released from the mercurous

ion, will be metabolized to mercuric mercury as described in the section on inhaled mercury vapor. However, there is the possibility that at least a fraction of the uncharged mercury atoms may persist long enough to cross the blood–brain barrier and possibly affect brain function as in the case of inhaled mercury vapor. Two cases exhibiting typical signs and symptoms of mercury vapor poisoning, following chronic exposure to mercurous mercury compounds, raise this possibility as discussed later.

2. Toxic Effects

Mercurous chloride was widely used in many medical preparations up to the middle of the 20th century. Its laxative powers were well known. The use of calomel in teething powders was shown by Warkany and Hubbard (1953; for details, see Warkany, 1965) to cause acrodynia or "pink disease." Bilderback (1920) gave the first detailed description of the disease in the United States. The report described signs and symptoms in 10 children characterized by profuse sweating, and swollen red feet and hands, which were cold, clammy, desquamating, and painfully sensitive to touch. Sometimes there was a rash on body, legs, and arms. There was progressive weight loss, marked weakness, and apathy. Insomnia and photophobia were also distressing attributes of the disease. The distraught and sleepless parents invariably sought medical help. A puzzling aspect of this disease was that of 500 or so children taking calomel in teething powder, only 1 would develop acrodynia. This may explain why it took so long to identify mercury as the cause.

The mechanism of action is not known, but some type of hypersensitivity reaction is suspected. Acrodynia is produced by other forms of mercury, including mercuric mercury and certain organomercurials. As discussed earlier, the mercurous ion dissociates to release mercuric mercury, and those organomercurials that cause acrodynia are rapidly metabolized to mercuric mercury. Also children, who play on a carpet on which liquid mercury was previously spilled can have both acrodynia and the typical signs of mercury vapor intoxication (Risher et al., 2003). All these forms of mercury give rise to mercuric mercury in the body, so one might conclude that mercuric mercury seems to be the proximate agent causing acrodynia. However, no reports of acrodynia have appeared with respect to ingestion of methylmercury, despite the fact that this form of mercury can give rise to mercuric mercury in the body.

An interesting case report of two individuals who had taken calomel-containing laxative tablets for 25 years revealed that mercurous mercury could elicit signs and symptoms of damage to the central nervous system. One of the patients had intentional tremor and both had dementia, renal failure, and colitis, signs characteristic of poisoning from inhaled mercury vapor. Ultimately they succumbed to renal failure (Davis et al., 1974). Perhaps the uncharged atom released from the mercurous ion behaves in the body as inhaled mercury vapor and thereby gains access to the brain.

E. Mercuric Mercury

1. Disposition in the Body

A study conducted in Finland (Rahola et al., 1973; Hattula and Rahola, 1975) remains the definitive study on the uptake and excretion of mercuric mercury ingested orally. Ten adult volunteers, equally represented by each sex, were given radioactive mercuric mercury attached to liver tissue, except for two who were given a solution of mercuric nitrate. The total dose was about 6 μg Hg, close to the average daily intake of the general population. Whole body counting revealed two half-times. The fast half-time of approximately 2 days probably represented unabsorbed mercury in the gastrointestinal tract, some of which may have been bound to intestinal epithelial cells that exfoliate with a similar half-time. The longer half-time of 41 days must represent the mercury absorbed into the body. Back-extrapolation of these whole-body counts to the time of dosing suggests that about 7% of the dose was absorbed. However, the range was considerable, going from 1 to 16%. Absorption may be higher in infants. Animal experiments indicate that gastrointestinal absorption was as high as 38% in newborn rats given an oral dose of mercuric chloride but fell to 1% in adult animals (Kostial et al., 1978, 1983).

Regional body counting revealed a half-time in the region of the liver in one subject to be about 53 days. The half-time in both red cells and plasma averaged about 25 days. The range of half-times in blood was also considerable, going from 2 to 40 days. Approximately equal amounts of mercury were found in red cells and plasma.

Only 0.2 to 0.4% of the dose was found in the blood compartment after 24 h. The total dose going to the blood compartment was probably higher, as absorption from the gastrointestinal (GI) tract may not have been complete in 24 h. The small amount deposited in blood contrasts sharply with the 10-fold larger amount after exposure to mercury vapor or to methylmercury.

Of special interest is that no significant radioactivity was found in the head region in the first 58 days. This is consistent with animal experiments where inorganic mercury penetration into brain is much less than other forms of mercury (reviewed by Magos, 1997) but inconsistent with the study described next.

Newton and Fry (1978) reported regional whole-body counts of two young adult male workers who had accidentally inhaled radioactive aerosols of mercuric oxide. A short half-time from the lung area of 2 days was assumed to be due to mucocilliary clearance followed by fecal excretion. The longer half-time of about 20 to 26 days may also involve clearance from the lung to the GI tract and fecal excretion, consistent with earlier observations by Morrow et al. (1964) on dogs. With increasing time after exposure, the kidney became the dominant site of deposition such that the half-time in the kidney region of about 60 days fell in the same range as that in the whole body.

The findings of both the Finnish study and Newton and Fry indicate that fecal excretion is dominant in the first few

days after exposure. Unabsorbed mercury probably makes a major contribution during this early period. Thereafter, secretion from liver to bile is an important source until most of the remaining body burden moves to the kidneys. At this point, urinary excretion accounts for the decline in kidney values and is the major route of excretion.

Of special interest was the deposition in the head region. Some 3 days after exposure, the head content of radioactivity may have accounted for as much as 18% of the body burden. At day 25, the head content fell to about 8%. A surprising finding was that the half-time in the head region, 23 days in one subject, 26 days in the other, was much shorter than the whole body half-time. As discussed later, these head half-times are similar to those reported after inhalation exposure to mercury vapor.

The finding that as much as 8 to 18% of the dose of inorganic mercury was found in the head region contrasts sharply with the findings in the Finnish study, where no radioactivity was found in the head region. It also contrasts with observations on animals where much more mercury was found in the brain of primates exposed to mercury vapor than after an equivalent dose of mercuric chloride (Berlin and Johansson, 1964). The explanation is not obvious. The dose was about an order of magnitude higher in the Finnish study. The pathways of exposure were different, oral versus inhalation. This difference may be one explanation. The inhalation of mercuric oxide aerosols should result in deposition on the surface of the head and in the mouth and nasal cavity, whereas no such deposition would have occurred in the Finnish study.

No information is available on the pattern of deposition in the human brain. In experiments on rats, Moller-Madsen and Danscher (1986), using an autometallographic silver enhancement technique, were able to detect deposits of inorganic mercury in the brain and cervical spinal cord when mercuric chloride was given in the drinking water for 8 months. In general, this method indicated an uneven distribution. More mercury was found in neurons than glial cells. The mercury accumulated in the lysosomes. More mercury was found in motor than sensory neurons with the heaviest deposits in the motor nuclei of the rhombencephalon. Mercury accumulated in the cells of the choriod plexus, an area without a blood–brain barrier. Deposits were also seen in the cerebral cortex and in part of the cerebellum but not in the Purkinje cells. It was also found in the anterior horn motor neurons of the spinal cord. The presence of mercury in lysosomes and in ependymal cells suggests that phagocytosis may be involved in the cellular disposition process.

The mechanisms of disposition in the body are poorly understood. Ballatori has shown in animal experiments that mercuric mercury is secreted in bile as a conjugate with reduced glutathione (Ballatori and Clarkson, 1985). Presumably, thiol ligands of two glutathione molecules attaches to the mercuric cation to form a structure that resembles that of oxidized glutathione (Figure 2) and is exported from the liver cells on glutathione carriers. This process is not operative in suckling rats, but data are lacking on human infants. In this connection,

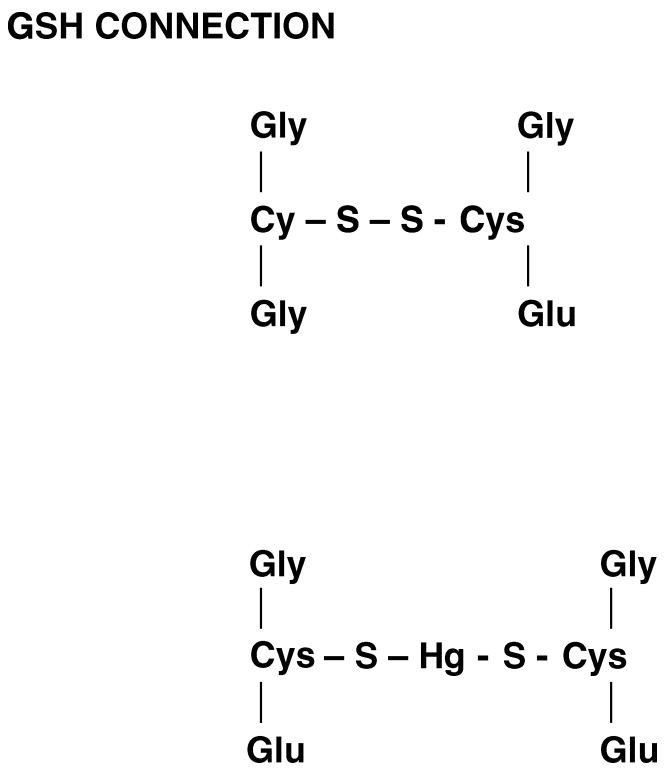

FIG. 2. Inorganic mercuric mercury, Hg^{2+}, attaches to the thiol ligands of two reduced glutathione molecules to form a complex similar to that of oxidized glutathione.

Thomas et al. (1988) reported that the elimination of mercury from neonatal rats dosed with methylmercury increases sharply with age above 17 days.

Reduced glutathione (GSH) also plays an important role in the renal handling of mercury. Inhibition of the enzyme gamma-glutamyl transpeptidase (gamma GT), which degrades GSH, causes a marked reduction in renal uptake and a large increase in urinary excretion of mercury according to several research groups (De Ceaurriz et al., 1994; Tanaka et al., 1990; Berndt et al., 1985). The fact that inhibition of gamma GT is less effective when levels of GSH are reduced in renal cells has led to the idea that inorganic mercury is secreted from proximal tubular cells into the tubular lumen as a GSH complex (Tanaka et al., 1993). This complex is then degraded by gamma GT. Mercury is subsequently reabsorbed into the renal cells as a complex with one of these products, probably the mercury–cysteine complex, possibly via the large neutral amino acid transporter (Wei et al., 1999).

Ligation of the ureter has little or no effect on renal uptake of inorganic mercury, suggesting that the major uptake of mercury takes place via the basolateral membrane (Zalups and Barfuss, 1998). The transport mechanisms have not yet been identified. Certain dicarboxylic acids inhibited mercury uptake, suggesting to these same authors that organic anion transporters may be involved. Ligation of the bile duct decreases renal uptake,

probably by a mechanism involving hepatic GSH turnover (Zalups et al., 1999).

The mechanisms of intestinal absorption of both mercurous and mercuric mercury have not yet been identified. With respect to the mercuric species, both secretion and absorption process may be occurring at the same time (Zalups et al., 1999). It is highly likely that the complexes of mercury with small-molecule thiols such as GSH and cysteine are involved. The reason why the GI absorption of inorganic mercury in suckling animals is much higher than in adults remains unknown despite the passage of some 25 years since the first report (Kostial et al., 1978). Alpha-lactalbumin, a major protein component in breast milk, is known to bind divalent metals and perhaps enhance their absorption (Lonnerdahl, 2003). Extrapolation to human infants will remain speculative until we know more about the mechanisms involved.

2. *Toxic Effects*

Mercuric mercury in the form of its water-soluble salts is a highly potent poison (Magos and Clarkson, 2006). The ingestion of as little as 1 g can be fatal. Such a dose of mercuric chloride, aptly named corrosive sublimate, puts the victim into shock with a complete collapse of kidney function. The extensive corrosive damage to the GI tract is probably the immediate cause. Lower doses cause selective damage to the kidneys with the proximal tubule as the main site. Some resistance may develop with repeated exposure, perhaps due to the induction of metallothionien. However, the replacement of sloughed proximal tubular cells with cells more tolerant to mercury may also be a factor. That regenerating tubular cells are less sensitive to mercurial damage than normal cells was demonstrated by the protection given by another renotoxic agent, sodium chromate, against mercuric chloride, and vice versa (Sparrow et al., 1988). The selective action on the kidney no doubt arises from the avid renal accumulation of this form of mercury. The biochemical mechanisms of renal toxicity are unknown.

Although the kidneys are the main site of toxicity, mercuric mercury can cause stomatitis and gastroenteritis. Cases of occupational exposure to mercuric oxide have been associated with lung damage. Mercuric chloride used as an antiseptic in diaper washes has been a cause of acrodynia (Warkany and Hubbard, 1953). Skin contact can also cause vesication.

Many reports have indicated that mercury, especially in the divalent inorganic form, is linked to autoimmune disease in animals and possibly humans (reviewed by Pollard and Hultman, 1997). A considerable amount of experimental work has been devoted to the action of mercuric mercury on the immune system. Genetically susceptible animal models have been used to characterize the effects of Hg^{2+} on the immune system, namely, lymphoproliferation, hypergammaglobulinemia, and development of systemic autoimmunity. The latter is manifested as autoantibody production and immune complex disease. Although the action of mercury at systemic and cellular levels is now well characterized, its biochemical mechanisms of action are largely unknown (Pollard, 2002). Specifically, how does mercury contribute to the loss of peripheral tolerance? Apoptosis of immunocompetant cells plays a key role in regulating immune activity. In this context the CD95–caspase-3 signaling pathway that mediates apoptosis may play a key biochemical role in the autoimmune response (Whitekus et al., 1999; McCabe et al., 2003). Most recently, McCabe et al. (2005) have demonstrated that the attenuation of this pathway is not due to a direct action of Hg^{2+} on caspase-3 but instead targets a plasma membrane signaling event. This finding is not only of interest in terms of the immune system put provides another example that the initial action of mercury may be on the cell surface. This point is taken up later in discussing mechanisms of cellular resistance to mercury.

The report by McCabe et al. (2005) was one of the few instances where care was taken to measure the actual mercury levels in the target cells *in vitro*. Numerous reports on *in vitro* actions of mercuric mercury may be found in the literature. *In vitro*, mercury can affect numerous cellular processes such as inhibition of enzyme function and blockade of cellular receptors and ion channels. These actions in turn can change both intra- and intercellular signaling processes of considerable significance to the nervous system. Such effects have been observed at an impressively low concentration of mercury in the incubating media (reviewed by Berlin, 2004). The problem with all these studies is that the cellular concentrations of mercury were not measured. Cells or subcellular components contain many binding sites for mercury, such as the ubiquitous -SH ligands. The medium, on the other hand, usually contains few mercury binding sites. Consequently, mercuric mercury rapidly leaves the incubation medium to attach to cellular components. How much binds to the cell depends on the ratio of the number of cells to the volume of the media. A relatively low cell number suspended in a large volume of media usually means the cellular concentrations will be much higher than the concentration that was added to the media. It is therefore virtually impossible to translate such findings to equivalent levels in human target organs.

F. Inhaled Mercury Vapor

Mercury vapor is the monatomic uncharged gas that vaporizes from the surface of liquid metallic mercury. It can be produced by a number of chemical processes by reduction of the mercuric ion. It is chemically stable and can stay in the atmosphere for long periods of time. It plays a major role in the global cycling of mercury, as discussed later with respect to human exposure to methylmercury.

1. *Disposition in the Body*

The physical properties of mercury vapor ensure that it is readily absorbed after inhalation and distributes to all tissues in the body. As an uncharged monatomic gas it is highly diffusible and lipid soluble. Thus, much like any anesthetic

gas, it is well absorbed in the lung and easily crosses cell membranes. However, this diffusive mechanism of transport is an assumption based on the physical properties of the vapor. Other pathways may be available. For example, a protein-lined transport channel has recently been identified for the uncharged ammonia molecule (Knepper and Agre, 2004). Hitherto it had been assumed that ammonia diffused across the lipid bilayer. Perhaps mercury vapor may make use of such channels intended for lipid-soluble endogenous molecules such as ammonia.

The general picture of disposition of mercury following inhalation of the vapor involves two sequential processes. First, the inhaled vapor dissolved in tissue fluids and the bloodstream moves rapidly throughout the body. It readily crosses the blood–brain and placental barriers. The second process is the oxidation of the dissolved vapor to mercuric mercury by processes described in the next section. Thus the initial disposition in the body represents movement of the vapor, whereas the long-term tissue disposition becomes similar to that of inorganic mercuric mercury. The dispositional studies of inhaled radioactive mercury vapor reported next therefore represent a mix of dissolved vapor and mercuric mercury, depending on the time after exposure.

Studies on adult volunteers inhaling a single (0.1 mg Hg/m^3 for about 20 min) dose of mercury vapor indicated that an average of 74% was retained in the body (Hursh et al., 1976). Magos (1997) has concluded, based on a 4.2 blood to air partition coefficient, that vapor is retained by lung tissues as well as diffusing into the bloodstream.

Regional body counting in these same studies revealed that about 10% of the inhaled dose was found in the head region. With time after exposure, the greater proportion of the body burden is found in the kidneys. Animal experiments indicate that the kidneys can account for over 50% of the body burden, as has been observed for ingested mercuric mercury (Magos, 1997).

Regional body counting indicated that half-times of decreasing radioactivity depended on the location in the body. The whole-body half-times were about 58 days, range 35 to 90 days. The half-time in the kidney region was similar to that in the whole body. The half-time in the head region was about 21 days. The shortest half-time was in the chest region, about 1.7 days (range 1.2 to 2.1). This was presumed to represent removal of mercury from the lung tissues.

Mercury was also found in the expired air. The loss of mercury by this route involved a fast component representing a flushing out of the mercury-containing air in the dead space of the respiratory tract. A second, slower, phase accounted for 7% of the inhaled dose with a half-time of 7 days. Presumably this represents exhalation of vapor already absorbed in the body and perhaps some adsorbed mercury on the lung surface.

Further data were published from this same experiment by Cherian et al. (1978) in the distribution of radioactive mercury to biological fluids and excretion. In the first few hours after exposure, virtually all the radioactive mercury in blood was found in the red cells. After about 20 hours, the red cell levels had fallen and plasma levels had risen such that the ratio of mercury in red cells to plasma was 2:1. The ratio thereafter remained about the same, with the half-times in red cells and plasma having similar values of approximately 80 h. The initial rapid uptake into red cells probably represents the rapid entry of mercury vapor and supports the idea that the red cell may be a medium of transport of vapor around the body (Berlin, 1966). Using fresh blood exposed to mercury vapor, Berlin demonstrated that mercury could be released to a higher degree than could be explained by physically dissolved Hg vapor in water.

Urine and feces were the main pathways of excretion. Fecal excretion accounting for 50% of total elimination was dominant after the first week. In long-term occupational exposures, urinary excretion becomes dominant (Tehning and Oman, 1966). Given the rapid oxidation of the vapor, the mercury excreted in feces is probably in the form of mercuric mercury.

Cherian et al. (1978) could find no correlation between urinary excretion and plasma radioactivity of mercury or between the specific activity of the radioactive mercury in plasma and urine. The authors concluded that these findings suggest that a mechanism other than a direct glomerular filtration is involved in the urinary excretion of mercury. In a subsequent reanalysis, Hursh et al. (1985) found that the specific activity of urinary mercury followed closely the specific activity in kidney tissue, suggesting that urinary mercury comes directly from mercury previously deposited in kidney tissue.

Sandborgh-Englund et al. (1998a) also exposed adult males to a short (15 min) exposure to mercury vapor. The median lung retention was 69%.They also observed exhalation of mercury for several days after exposure. These and the data from Hursh et al. (1976) were used to develop a compartmental model to describe the kinetics of inhaled vapor (Jonsson et al., 1999). The model indicated a half-time in the respiratory compartment of 1.7 days and the whole-body elimination time of about 63 days. In general the data of both Hursh et al. (1976) and Sandborgh-Englund et al. (1998a) yielded similar kinetic parameters.

No information is available on the disposition of inhaled mercury vapor in pregnant and lactating women or in infants and children. After exposure of the mother during pregnancy, levels in the fetal and neonatal brain were substantially lower than in the corresponding maternal brain in primates, guinea pigs, and mice (reviewed by Newland et al., 1996). The lower deposition in the fetal brain is probably due to the conversion of the vapor to mercuric mercury in the first pass through the fetal liver.

Little is known about the pattern of distribution in the human brain after exposure to mercury vapor. In animals, the pattern of distribution is uneven in both the mature and developing brain. Warfvinge et al. (1994) observed deposition of mercury in the cortical areas and fiber systems of the neonatal and maternal adult cerebrum after exposure of pregnant squirrel monkeys to

mercury vapor. Accumulation in the eye of neonates was also observed (Warfvinge and Braun, 2000).

2. *Metabolism*

Once inside the cell, mercury vapor can undergo oxidation to mercuric mercury. It takes place by the catalase–hydrogen peroxide pathway. The catalase protein first forms an adduct with hydrogen peroxide to form catalase compound 1. The latter then reacts with an atom of mercury to form mercuric mercury as shown in the reaction sequences:

$$\mathrm{CatOH} + \mathrm{H_2O_2} = \mathrm{CatOOH} + \mathrm{H_2O}$$
$$\mathrm{CatOOH} + \mathrm{Hg^{\circ}} = \mathrm{CatOH} + \mathrm{HgO}$$

Apparently the mercury atom is sufficiently small to be able to diffuse down the cleft in the catalase enzyme to reach the active site where the heme ring is located. Oxidation most likely occurs in all tissue, as the catalase hydrogen peroxide pathway is ubiquitous.

Ethanol inhibits the catalase-mediated oxidation of mercury vapor, a fact that was discovered when Danish workers were found to retain less inhaled vapor when tested in the afternoon as compared to tests conducted in the morning. The culprit was found to be Danish lager consumed at lunchtime (Kudsk, 1965). In this respect it is of interest to note that a study on dentists, who routinely applied mercury amalgam tooth fillings, found that the chance of having a mercury concentration in urine greater than 15 μg Hg/L was about threefold higher in abstinent dentists than in those who consumed alcoholic beverages (Martin and Naleway, 2004).

Animals exhale mercury vapor after dosing with mercuric chloride (Dunn et al., 1981a, 1981b). The mechanisms of reduction are not well described but several processes may be involved. *In vitro*, the superoxide anion can perform this task as well as NADPH and NADH (Ogata et al., 1987).

The exhalation rate is increased by treatment of the animals with ethanol (Dunn et al., 1981a, 1981b). Acatalasemic mice also exhaled more vapor than normal mice when both received equal doses of a mercuric salt. The blood-to-brain concentration ratio was also higher in the acatalasemic animals (Ogata et al., 1987). It would appear that some of the vapor generated by reduction of mercuric mercury is reoxidized by the catalase pathway; thus, a cycle of oxidation and reduction of inorganic mercury exists inside the cell.

The role of this cycle is not well understood. As noted by Magos (1997), the exhalation process does not amount to a significant contribution to total mercury elimination from the body. But it may play a role in the mobility of inorganic mercury, as the reduced form of mercury is highly mobile whereas the oxidized form is not. For example, inorganic mercury can be secreted from the cell as a complex with glutathione but no mechanisms have been identified for the entry of mercuric mercury into the cell. Clearly, the vapor generated inside the cell exists for a sufficient period of time for at least a fraction to be exhaled. So it seems plausible that mercuric mercury may move from cell to cell or across the blood–brain barrier by a process of reduction and oxidation, with the reduced form being the mobile species.

The mercuric ion, Hg^{2+}, is believed to be the toxic species, as mercury vapor itself is unable to react with tissue ligands. If so, one would expect that the rate and location of the metabolic conversion of mercury vapor to mercuric mercury should be important determinants to the degree and pattern of the toxic effects on inhaled vapor. However, little is known about the role of metabolism, but the potential to affect the toxic outcome is real. For example, four alleles of catalase exist, having different catalytic properties. The peroxisomes are the main intracellular location, but catalase activity has been detected in other regions, including the nucleus (Yamamoto et al., 1988). Substrates that compete for the peroxidative activity of catalase may affect the rate of metabolism of mercury vapor; for example, moderate doses of ethanol are inhibitory. This is believed to be the reason why workers drinking Danish lager at lunchtime absorb less inhaled mercury vapor in the afternoon shift, as discussed earlier.

Mitochondria are believed to be the main source of hydrogen peroxide originating from oxygen metabolism. However, other sources do exist, for example, in red blood cells, where the rate of oxidation is limited by the availability of hydrogen peroxide. In contrast, hydrogen peroxide is not rate-limiting in liver cells (Magos et al., 1978).

Although Hg^{2+} is believed to be the proximate toxic agent after exposure to inhaled mercury vapor, a toxic role for the unoxidized mercury atom cannot be totally excluded. Clearly the uncharged mercury atom does possess a limited chemical reactivity, as evidenced by the fact that it is a substrate for catalase compound one. Perhaps it may interact with other heme proteins as well.

3. *Biological Indicators*

Urinary rate of excretion, usually expressed as μg Hg/g creatinine, or urinary concentration, μg Hg/L, is the most frequently used biological indicator for exposure to mercury vapor. Indeed, numerous studies of long-term occupational exposures indicate a close correlation between time weighted air concentrations and urinary excretion rates (WHO, 1991). Very likely the urine is a useful indicator medium for the kidney burden of mercury, as urinary mercury derives directly from mercury deposited in kidney tissue. Urinary mercury may also be a rough indicator of the total body burden of mercury, as the kidney tends to be the main site of deposition especially with chronic exposure to mercury vapor. These considerations should also apply to exposures to the inorganic compound of mercury.

In contrast to methylmercury disposition, to be discussed later, inorganic mercury is accumulated in hair only to a small extent if at all (Berglund et al., 2005). This low level of accumulation, along with the possibility of external

contamination from mercury vapor, argues against hair as a suitable indicator medium for inorganic species of mercury.

No satisfactory indicator medium is available for the other major target organ, the brain. As we have seen, the half-time in brain after exposure to mercury vapor differs from those in blood and kidney. There appears to be a relatively short half-time of about 20 days followed by a much longer half-time measured in years.

As discussed in the next subsection, the prevalence of effects from inhalation of mercury vapor have been compared with average air concentrations and urinary levels of mercury. In fact, such comparisons have formed the basis of estimated no-observable-adverse-effect levels (NOAEL).

4. Toxic Effects

a. Acute Toxicity. Liquid metallic mercury possesses a high vapor pressure, such that a saturated atmosphere at room temperature would contain about 14 mg Hg per cubic meter of air. This compares to current occupational safe limits in the range of 25 μg Hg/m^3. Most acute exposures have been due to spillage of liquid mercury in confined spaces or in hot areas (for details, see Magos and Clarkson, 2006). The first sign is dyspnea. This may be followed by paroxysmal cough, chest pain, pulmonary infiltration, chills, nausea, and vomiting. A fatal outcome has occurred in a few cases

b. Chronic Toxicity: Effects on Humans. The long occupational history has identified the major signs and symptoms of severe poisoning from inhaled mercury. Tremor and psychological disturbances are the main features. The latter has the ancient connotation as erethymus mercurialis or erethism. According to Waldron (1983), the term *erethism* was used up to the early nineteenth century to encompass all the manifestations of poisoning from mercury vapor. However, during the latter part of the 19th century, erethism became restricted to psychotic symptoms. The principal features are excessive timidity, diffidence, increasing shyness, loss of self-confidence, anxiety, and a desire to remain unobserved and unobtrusive. The victim also had a pathological fear of ridicule and often reacted with an explosive loss of temper when criticized. Mercury-induced tremor in milder cases is intentional, which occurs during guided movements (finger-to-nose test), but in more severe cases tremor becomes postural (tremor in the extended arm). Gingivitis, stomatitis, and excessive salivation are also associated with high occupational exposures (Goldwater, 1972). Severe kidney damage sometimes associated with the nephrotic syndrome may also be present. The nephrotic syndrome is an idiosyncratic reaction characterized by albuminuria and edema. Generally speaking such severe cases are rare and only found with chronic exposures, usually in the range of 500 μg Hg/m^3 and higher (WHO, 1991). The term "erethism" is mainly of historical interest, as the behavioral attributes are poorly defined and the nonspecific nature of the behaviors limits their diagnostic utility. The finger-to-nose test was part of the periodic medical examination of mercury-exposed workers but with decreasing levels of exposure there was a need for instrumental tests, which give numerical values for tremor, skill, coordination, and nerve conduction velocity (Magos and Clarkson, 2006).

Some reversing of these signs and symptoms takes place slowly after cessation of exposure. Nevertheless, long-term sequelae of nervous system damage have been reported (Albers et al. 1988). Workers who were examined some 25 to 30 years after exposure and had experienced peak urine levels of about 500 μg Hg/L still demonstrated adverse effects of the nervous system. These included decreased coordination, increased tremor, and decreased sensation when compared with controls. Likewise, the use of nerve conduction velocity and other diagnostic techniques, like measuring postural and acceleration tremor, identified polyneuropathy in a group of chloralkali workers 13 years after exposure associated with 108 μg/L urinary mercury excretion; 13% showed reduced distal sensation and 19% postural tremor/impaired coordination with or without defect in visual evoked response (Ellingsen et al., 1991).

Several different units of mercury levels are used to describe occupational exposures. The American Conference of Governmental Industrial Hygienists (ACGIH) express concentration of mercury in the work place in milligrams Hg per cubic meter. Others use micrograms Hg per cubic meter. The levels of mercury in urine are the most widely used biological exposure index (BEI), usually expressed as μg Hg/g creatinine. The ACGIH uses the ratio of 1 μg Hg/m^3 in air to 1.22 μg Hg/g creatinine in urine to convert air concentrations to urinary excretion rates for long-term exposures. Adults excrete on the average about 1.6 g creatinine per day, so that 1 g creatinine is equivalent to 15 h of urine flow. In general, 1 μg Hg/g creatinine is approximately the same as 1 μg Hg/L urine, except in cases of very dilute or concentrated urine samples. In the ensuing text, units of micrograms Hg per liter are used.

Effects on both the kidney and nervous system have also been reported at occupational levels in the range of 50 to 100 μg/L. Many of these are not specific to mercury, so that confounding causes may not always have been taken into account. However, a slight tremor may still persist, as well as such symptoms as insomnia, memory disturbances, irritability, and fatigue (WHO, 1991).

The possibility of adverse effects below 50 μg Hg/L still remains a matter of debate. At these low levels, effects of mercury signs and symptoms are nonspecific and difficult to detect above significant background prevalence. It is not surprising, therefore, that one finds conflicting reports in the literature.

Table 2 summarizes the outcomes of findings in workers where average urine levels are generally below 50 μg Hg/L. A wide range of endpoints was observed, including effects on the nervous and immune systems and on the kidneys. The average urine levels covered the range of 6 to 115 μg Hg/L. One cannot see any consistent relation between urinary levels and effect findings. Perhaps the most consistent finding is an

TABLE 2
Summary of findings in workers occupationally exposed to mercury vapor with average urinary levels generally below 50 μg Hg/L

Number	Urine levels (μg Hg/L)[a]	Endpoint	Outcome	Reference
41	27 (8–94)	Autoimmune	—	(1)
41	27 ± 18	Urinary NAG	+[r]	(2)
19	24 (4–72)	Nerve conduction	—	(3)
		Tremor frequency	—	
		Urinary NAG	+	
33	20 ± 10	Immune	+	(4)
27	18 ± 9	RBC GSH	+	
		RBC catalase	+	
21[b]	115 ± 62	Color vision	+	(5)
24[b]	20 (0.1–62)	Color vision	+	(6)
38[c]	12 (2–35)	Urinary markers	—	(7)
		Neurobehavioral	—	
47	10 (2–30)	Wait digit symbol	+	(8)
		Benton visual retention	+	
		Urinary NAG	+	
		Thyroid marker	+	
25	10 (4–25)	Circulating monocytes	+	(9)
		NK cells	+	
		PMNL chemotaxis	+	
121	8.3 (0.2–35)	Motor coordination	?	(10)
		Prolactin levels	?	
35	6 (2–12)	Immune parameters	—	(11)

[a]In most cases urine levels in units of μg Hg/L were numerically similar to urinary excretion in units of μg Hg/g creatinine.

[b]Estimated NOAELs 10 to 20 μg Hg/L.

[c]Estimated NOAEL >35 μg Hg/L.

Note: [r]Effects reversible. References; (1) Barregard et al. (1997); (2) Mandic et al. (2002); (3) Boogaard et al. (1996); (4) Queiroz and Dantas (1997a, 1997b); (5) Cavalleri and Gobba (1998); (6) Urban et al. (2003); (7) Camerino et al. (2002); (8) Ellingsen et al. (2000a, 2000b, 2001); (9) Vimercati et al. (2001), (10) a multicenter study, see LSRO (2004); (11) Soleo et al. (1997).

increase in urinary excretion of *N*-acetyl-β-D-glucosaminidase (NAG). This enzyme has two isoforms, A and B. The A form is a soluble component of lysosomes and is secreted into urine by exocytosis. The B isoform is attached to the lysosomal membrane and is released into urine as a result of damage to the membrane. To date, few studies have distinguished between these isoforms. Mandic et al. (2002) found that the urinary excretion of the A form was increased in workers exposed to mercury vapor. This raises the issue that NAG may be a sensitive marker for an influence of mercury on exocytosis but is not necessarily an indicator of renal damage.

The increased excretion of NAG induced by exposure to mercury vapor appears to be reversible. In workers previously exposed to mercury (Ellingsen et al., 1993) or in retired mercury miners (Franko et al., 2005), there was no increase in urine NAG activity. NAG excretion was normal in previously heavily exposed chloralkali workers whose vibration sense, motor speed, and tremor were worse than in unexposed subjects (Frumkin et al., 2001).

Increased urinary excretion of brush border enzymes, such as γ-glutamyl transpeptidase, alkaline phosphatase, and brush border antigens has also been taken as evidence of early toxic or irritant action on the surface of proximal tubular cells. With the progression of cellular injury, intracellular enzymes including lactate dehydrogenase, aspartate aminotransferase, and acid phosphatase escape into the bloodstream and urine (Schnellmann, 2001).

The dental profession has been the subject of many studies for effects of mercury vapor. Table 3 summarizes the more recent findings, with average urinary levels ranging from 2 to 22 μg Hg/L. In general these levels are about an order of magnitude lower than what they were a generation ago (Goldwater, 1972). The two studies that used objective measures of renal effects (urinary excretion of albumin and NAG) revealed no association with mercury. The increased prevalence of musculoskeletal complaints observed in dentists versus controls is probably due more to the work practices of the dentist than to any effect of mercury. The other apparent adverse outcomes relate mainly to mood and manual steadiness. It is at least conceivable that a dentist with decreased hand steadiness and suffering from moods of tension, fatigue, confusion, and hostility might spill more mercury or at least take less care about cleanliness than a "normal" practicing dentist. This becomes a question of "the chicken and the egg." This is not to mention the number of covariates probably involved in any apparent association with urinary mercury. For example, the number of fillings installed per day may correlate with both urinary mercury and moods such as fatigue and tension. In short, the recent studies in Table 3 provide no convincing evidence of adverse effects of mercury at average urinary levels in the range 2–22 μg Hg/L.

Tables 2 and 3 do not provide convincing evidence for adverse effects of mercury vapor at urinary levels generally even as high as 50 μg Hg/L. But neither do they exclude them, in view of the small number of subjects in these studies, usually below 100 subjects. The lack of association with current levels of mercury may be due to previous exposures to much higher levels. The report of Albers et al. (1988) discussed earlier indicates that residual damage to the nervous system may persist for years after a peak exposure to mercury vapor. As stated earlier,

TABLE 3
Summary of findings in dental personnel occupationally exposed to mercury vapor with average urinary levels generally below 50 μg Hg/L

Number	Urine levels (μg Hg/l)[a]	Endpoint	Outcome	Reference
22	22 ± 6	NAG	—	(1)
		Nonspecific symptoms	—	
44	5.3 (1.6–27)	Health complaints	+	(2)
		Urinary albumin	—	
		Urinary NAG	—	
180	4.6 (0.4–27)	Kidney disorders	+[b]	(3)
		Memory disturbances	+[b]	
268	3(0.7–23)	Musculoskeletal complaints	+[b]	(4)
43	2.1 (0.02–6)	Logical memory	+	(5)
		Anxiety, hostility, psychotism		
		Depression	+	

Note: References: (1) Rojas et al. (2000); (2) Langworth et al. (1997); (3) Ritchie et al. (2002); (4) Akesson et al. (2000); (5) Aydin et al. (2003).

[a]In most cases urine levels in units of μg Hg/L were numerically similar to urinary excretion in units of μg Hg/g creatinine.

[b]Reports from dentists versus nondental controls, no significant association with urinary mercury levels.

exposure of dentists to mercury was much higher a generation ago. Irreversible effects inflicted years ago may be picked up by current examinations and show no correlation with today's mercury levels. This may be one explanation of the finding (Table 3) that more complaints of memory disturbances were found in dentists versus nondental controls but no association was found with current mercury levels.

The urinary excretion of NAG appears to be increased at urinary levels below 50 μg Hg/L but the data do not allow identification of threshold level. The toxicological significance of increased excretion of this enzyme may be debatable, but changes in urinary NAG may be taken as an early warning sign of potential mercury damage to the kidneys.

Occupational exposures to mercury vapor do not appear to be associated with the onset of Alzheimer's (Gun et al., 1997) or Parkinson's disease (Gorell et al., 1997, 1999). Nor do such exposures have much effect on the human reproductive system (Magos and Clarkson, 2006). Male fertility appears unaffected, but occupational exposure to mercury vapor may increase the prevalence of dysmenorrhea in females. Paternal exposure to mercury vapor was claimed to increase the rate of spontaneous abortions, but correction was not made for previous adverse pregnancy outcomes.

c. Studies on Animals. Pregnant monkeys were exposed 5 days per week, 4 to 7 hours per day to mercury vapor at air concentrations of 0.5 and 1.0 mg Hg/m^3 (Newland et al., 1996). Maternal blood levels ranged from 25 to 180 μg Hg/L. The offspring were examined by use of lever press testing with structured reinforcement. The exposed monkeys learned behavior at a slower rate than the unexposed and exhibited more variability in performance and longer lever press durations, suggestive of adverse effects on motor function.

A study in rats examined the behavioral outcomes of combined exposure to mercury vapor and oral exposure to methylmercury (Frederiksson et al., 1996). Groups of rats were exposed separately to 1.8 mg Hg/m^3 for 1 hour per day during gestational days 14–19 or to methylmercury at a dosage of 2 mg/kg by gavage during gestational days 6–9 or to a combination of the two exposures. Standard screening tests of behavioral function were performed on the offspring between 4 and 5 months of age. Generally, the results indicate that prenatal exposure to Hg causes alterations to both spontaneous and learned behaviors, suggesting some deficit in adaptive functions. Coexposure to MeHg, which by itself did not alter these functions at the dose given in this study, served to significantly aggravate the changes.

The idea of combined exposures to mercury vapor and methylmercury raises the question of human health risks for the large numbers of people who have exposure to mercury vapor from amalgam and to methylmercury in fish. The possibility of potentiation of toxic effects from combined exposures has not been tested in humans. However, the effects found in the rat study are from doses that are orders of magnitude higher than those encountered in the general population.

G. Dental Amalgam

Dental amalgam has been widely used following its introduction into dentistry over 150 years ago (LSRO, 2004). A World Health Organization expert group (WHO, 1991) concluded that dental amalgam was the principal source of mercury vapor. It consists of about 50% metallic mercury in an amalgam with silver or copper, with small amounts of other metals such as zinc. Today most dental amalgam is sold as an encapsulated preparation. The powdered metals and elemental mercury are divided into separate compartments, and the physical divider is broken just prior to use. It has near ideal properties as a tooth filling. It is easily prepared, fits the oral cavity tightly as it expands slightly after introduction into the tooth, and has a long life, measured in tens of years. It is also much cheaper than its replacement. However, it does not have an attractive appearance and may be replaced by new materials if only for cosmetic reasons.

The debate on potential health risk from mercury in amalgam is not new. The so-called "amalgam wars" between the proponents and opponents of the use of amalgam in dentistry started with the first in the late 19th century, followed by the second in the early 20th, and now the third war covering a period of about 30 years (Goldwater, 1972). It is well recognized that an allergic response can occur, but it is so rare that a practicing dentist may not see one case in his or her professional lifetime. However, from this point onward there is little agreement of health risk from mercury. The basic problem is that billions of people carry amalgam fillings whereas the best-conducted epidemiological study can rarely exclude a risk below 5% of any kind of adverse health effect.

1. Disposition in the Body

In principle, mercury can be released from amalgam as mercury vapor and inhaled or in a particulate form and swallowed (Figure 3). Most studies have focused on the release of mercury vapor.

The German chemist Stock, in the late 1920s, was the first to report that the placement of dental amalgam could elevate urinary mercury levels (Goldwater, 1972). More recently, mercury "sniffing" devices, commonly used to assay occupational exposures, detected high levels of mercury vapor in the oral cavity. Some measures revealed mercury levels well in excess of occupational safe limits. The furor resulting from this finding eventually subsided when it was realized that the volume of the oral cavity was small and that the amount of vapor inhaled from this source did not exceed occupational standards.

Several publications have described factors that influence the rate of release of mercury vapor from amalgam. The rate of release is stimulated by hot liquids, and decreased by certain

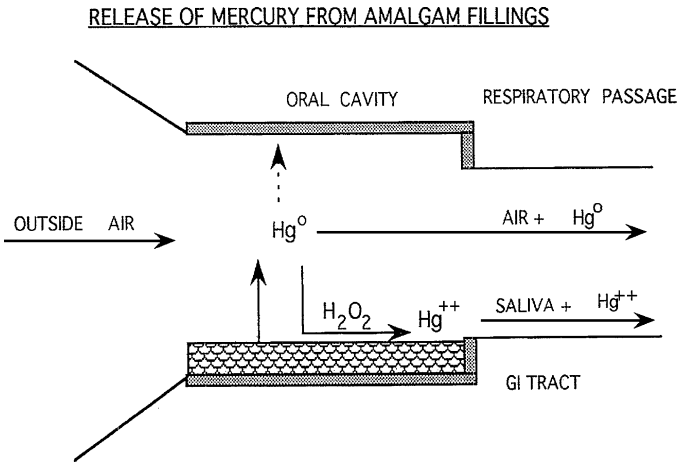

FIG. 3. A diagram of the release of mercury from dental amalgam in the oral cavity. Mercury vapor is emitted from the amalgam surfaces and inhaled or oxidized to Hg^{2+} and swallowed along with particulates of amalgam released by abrasion of the amalgam surfaces.

foodstuffs (LSRO, 2004). The number of amalgam surfaces, especially occlusal surfaces, directly determines the rate of release of vapor. The most important stimulant is excessive chewing. In fact, urinary levels of mercury can approach occupational safe limits when nicotine-containing chewing gum is used to break smoking habits (Sallsten et al., 1996).

Urine levels also correlate with amalgam surfaces. In the most extensive study to date, involving over 1000 individuals, it was estimated that 10 amalgam surfaces would, on the average, cause an increase in urine levels of 1 μg Hg/L urine (Kingman et al., 1998). This finding is in approximate agreement with a previous study (Skare and Engqvist, 1994) and is confirmed by the most recent one (Dye et al., 2005).

Oskarsson et al. (1996) have demonstrated that inorganic mercury in blood derived from dental amalgam can be efficiently transferred to breast milk. On average the concentration of inorganic mercury in breast milk was 55% of the corresponding concentration in blood. These authors also found that breast milk in the general populations contains both inorganic mercury and methylmercury. The relative proportion of these two species depends on the frequency of fish consumption, dental amalgam status, and occupational exposures to mercury.

Tissue levels of mercury have also been compared to amalgam status. Autopsy brain and kidney levels have been compared to the number of amalgam surfaces at the time of decease. In general there is a scattered but statistically significant correlation (WHO, 1990). More recently, Maas et al. (1996) observed a statistically significant correlation between the number of amalgam restorations and the concentration of mercury in the occipital lobe in autopsy brains from 55 cadavers. No associations were seen with other anatomical areas of the brain.

A limited number of animal experiments lend support to the findings from human studies. Hultman et al. (1998) introduced four amalgam filings into rats to observe that the occipital lobe of the cerebrum and the cerebellum had higher levels of mercury than untreated animals after 12 weeks of exposure. Galic et al. (1999) also introduced four amalgam fillings into rats. They found elevated levels in both brain and kidneys at months after 2 placement of the fillings.

Animal experiments also indicate that mercury from amalgam can cross the placenta to be deposited in fetal tissues. Vimy et al. (1990) placed 12 occlusal amalgam fillings in 5 pregnant sheep. Mercury accumulated to its highest levels in maternal kidney and liver and in the fetal liver and pituitary. This is the first study demonstrating transplacental movement of mercury from amalgam. These findings were confirmed by Takahashi et al. (2001), who introduced a single amalgam restoration in pregnant rats. Mercury levels were increased 6 times in maternal brain, liver, lung and placenta and 20 times in kidneys as compared to untreated animals. In fetal organs, the highest level was found in liver followed by kidneys and brain. Mercury concentrations in the fetal brain, liver, kidneys, and whole blood were lower than those in the mother. Takahashi et al. (2003), in

a follow-up study, placed zero, 1, 2, and 4 fillings into groups of five mice. The mercury levels in brain increased with the number of fillings. Mercury concentrations in fetal brain, liver, and kidneys were much lower than those of the dams, but liver and kidneys showed positive correlation between the mercury content and maternal amalgam surface areas. Taken together, these findings on the disposition of mercury in maternal–fetal tissues indicate that it is mercury vapor emitted from amalgam surfaces that is the mobile form of this metal that crosses the placenta and blood–brain barriers to ultimately deposit in the oxidized form of mercuric mercury.

It is generally assumed that mercury vapor emitted from amalgams finds its way into the brain via absorption in the lung, transport of the dissolved vapor in the bloodstream, and diffusion across the blood–brain barrier. However, the possibility of another pathway to the brain has been suggested. The sensory neurons of the olfactory system are unique in that their cell bodies are directly exposed to inhaled air and they are not synaptically isolated from neurons that connect to the brain. Sunderman (2001) has shown that inhaled manganese particles can cross synaptic junctions in the olfactory bulb and migrate to secondary neurons to the brain. Henriksson and Tjalve (1998) have speculated that mercury originating from amalgam might reach the brain via transport along the olfactory nerves. They conducted animal studies to show that mercuric chloride after intranasal instillation could enter the olfactory bulb. They suggested that continuous exposure of the nasal cavity to mercury vapor released from amalgam fillings and oxidized to Hg^{2+} in the olfactory mucosa, as well as a potential uptake of Hg^{2+} in the olfactory neurons from the blood, may lead to considerable concentrations of the metal in the olfactory bulbs.

However, transport into the brain is unlikely, as their experiments showed that mercury was unable to continue its journey from the olfactory bulb to secondary neurons. Moreover, in the study by Maas et al. (1996) discussed previously, no correlation was found between mercury levels on olfactory bulb and numbers of amalgam restoration. This perhaps is not surprising since intake of vapor from amalgam must be mainly via oral breathing.

The removal of amalgam fillings first causes a rise in blood plasma levels followed by an exponential decline lasting about 1 year. In the immediate postremoval phase, the peak plasma mercury rose to three to four times higher than the pretreatment level (Molin et al., 1990). Twelve months after the removal, the plasma levels were significantly reduced to 50% of the initial values. Subsequent studies confirm that amalgam removal causes increased exposure of the patient to mercury vapor (Berglund and Molin, 1997; Sandborgh-England et al., 1998b; Kremers et al., 1999).

2. *Toxic Effects*

Allergic response to the placement of amalgam takes the form of ulceration and inflammation of oral tissues in contact with amalgam. The amalgam has to be removed and replaced with alternative filling material.

Since the main exposure is from inhalation of mercury vapor, the issue arises that amalgam may produce toxic effects similar to those elicited by the vapor. Such effects have not yet been found despite intensive reviews of the literature by several national and international expert committees: CETS, (1997), WHO (1997), U.S. HHS (2003), European Commission (2004), Health Canada (2004), and (NHMRC (2001), This is not surprising, given the fact that urine levels on the average are about one order of magnitude lower than the lowest effect levels associated with occupational exposure. As mentioned earlier, urine levels can rise into the occupational risk zones in excessive chewing, such as the use of nicotine-containing chewing gum. However, sufficient numbers have not yet been studied for any adverse effects to be detected.

The literature is replete with case reports from patients claiming to experience a variety of illnesses from amalgam (for a recent review, see Berlin, 2004). Some reports record the patients feel better after removal of amalgam. It is difficult to draw firm conclusions from such reports as they are subjective in nature, lack controls, and generally describe a wide range of complaints with no consistent picture from one patient to another. On the other hand, it has been argued that almost any protein in the cell is a potential target for mercury so that, in principle, all kinds of adverse effects are possible. Thus the interpretation of these case reports remains controversial (LSRO, 2004).

Claims have been made that mercury released from amalgam may cause or exacerbate chronic degenerative diseases of the nervous system such as Alzheimer's disease (Mutter et al., 2004). Such claims arose from an early finding that brains of Alzheimer's patients had higher mercury than controls. Matching Alzheimer brains with appropriate controls is a difficult task. More recent papers have failed to find differences in brain levels (Fung et al., 1997). In any case, it is possible the damaged cells in Alzheimer's patients, as in the cases of aluminum encephalopathy, may take up more of the metal than normal cells (McLachlan et al., 1992). One is left with the "chicken and the egg" conundrum.

What studies have been carried to test a role of amalgam in degenerative disease has so far yielded little supporting evidence (Table 4). A study was conducted of 129 Roman Catholic sisters who were aged 75 to 109 years (Saxe et al., 1995). Eight different tests of cognitive function were carried out. Lower performance of any of these tests was not associated with the number or surface area of occlusal dental amalgams. Confounding covariates are probably absent in this study, given the homogeneous life styles and environment. The principal weakness is that the past history of amalgam status was not known.

Another smaller study gave support to the Catholic Sisters study. Fung et al. (1996) examined 18 nursing-home residents aged 60 to 96 years. Nine residents were diagnosed with

TABLE 4
Studies finding no association of amalgam status with neurodegenerative disease

Number of subject	Amalgam status (surfaces)	Age range, years	Reference
129	Number of amalgam surfaces	75–109	(1)
18	Number of amalgam surfaces and urine Hg	60–90	(2)
587	Number of restorations	46–89	(3)
300	With and without restorations	70–103	(4)
550	Number of amalgam surfaces and urine Hg	30–49	(5)

Note: References: (1) Saxe et al. (1995); (2) Fung et al. (1966); (3) Bjorkman et al. (1996); (4) Nitschke et al. (2000); (5) Factor-Litvak et al. (2003).

Alzheimer's disease (AD). The nine controls were also residents of the nursing home, sharing the same environment and diet. All subjects were placed on a seafood-free diet for 3 months before start of the study. Mercury exposure was measured as urinary concentrations; the mean for AD residents was 3.0 versus 1.9 μg Hg/L for controls. Amalgam status was determined as number of current dental amalgam surfaces: 6.2 for AD residents, 3 for controls. Neither the urine levels nor the number of surfaces differed significantly between the AD group and controls.

An ongoing Swedish adoption twin study on aging provided Bjorkman et al. (1996) with the opportunity to test for potential adverse effects of amalgam on mental health. Some 587 subjects were evaluated on different tests on mental health with a special focus on memory function. The average age was 66 with a range of 46 to 89 years. The authors found no association between the number of amalgam restorations and adverse mental health after controlling for covariates including age, gender, and education. They were also able to make use of a co-twin control design whereby one member of a pair of twins differed from the other by 10 or more amalgam surfaces. No adverse effects of amalgam could be detected.

Nitschke et al. (2000) made use of an ongoing "Berlin Ageing Study" (BASE). They selected 300 patients ranging in age from 70 to 103 years. The sample was well balanced with respect to lifestyle, education, and social status. Dental status was assessed as (1) edentate, (2) remaining teeth without, and (3) remaining teeth with amalgam restorations. The mental health tests were those used in the BASE. The outcome indicated no association of amalgam status with dementia or impaired cognitive performance. These endpoints included the physiological age-related decline in cognitive abilities.

Most recently, Factor-Litvak et al. (2003) examined some 550 adults, age range 30 to 49 years, with respect to amalgam status and cognitive functioning. The subjects were employees of an urban medical center with no history of occupational exposure to mercury. Exposure to mercury was measured as urinary concentration, and amalgam status was defined in terms of the total number of amalgam surfaces and the number of occlusal amalgam surfaces. The mean urinary mercury excretion was 1.7 μg/g creatinine (range 0.09–17.8); the mean total number of amalgam surfaces was 10.6 (range 0–46) and the mean number of occlusal amalgam surfaces was 6.1 (range 0–19). A battery of neuropsychological tests was applied to the subjects. Multivariate linear regression analyses including adjustments for sample design and other covariates revealed no association of urinary mercury or amalgam status with any deficits in cognitive or fine motor functioning.

In addition to epidemiological studies, a histological comparison was made of autopsy brains from individuals differing in amalgam status (Saxe et al., 1999) in 68 subjects with Alzheimer's disease (AD) and 33 controls. Detailed records were available of both current amalgam status and dental histories. The authors found no significant association of AD with the number, surface area, or history of having dental amalgam restorations. They also found no statistically significant differences in brain Hg level between subjects with AD and control subjects. However, as noted in a recent review (Bates, 2006), more studies are needed for investigation of effects in infants and children.

Other than rare cases of allergy, other adverse health effects have not yet been definitively established. Nevertheless, the life expectancy of amalgam may be limited. Today the dentist is faced with high cost in the use of amalgam. Environmental regulations require expensive methods to collect and dispose of the waste mercury. Ironically, it may not be the mercury placed in the patient's mouth that will sound the death knell of amalgam but the mercury that is not placed in the patient. The banning of amalgam in Denmark for environmental reasons is a case in point.

III. ORGANIC MERCURY

A. An Overview

The methyl- and ethylmercury compounds have similar chemical properties and are often referred to as the "short-chain alkyl mercurials." The primary target is the central nervous system. The ethyl compounds differ from their methyl relatives in that they are converted more rapidly to inorganic mercury in the body and produce kidney damage, whereas methylmercury appears to exclusively damage the central nervous system, at least in primates. The intact organomercurial cation is believed to be the proximate toxic agent responsible for damage to the central nervous system, and the mercuric cation released from ethylmercury plays this role in kidney damage.

The high mobility of methylmercury in the body is due to the formation of a complex with the amino acid cysteine. The structure of this complex resembles that of a large neutral amino acid, methionine, and thereby gains entry into cells on the large neutral amino acid carrier. Methylmercury exits from cells as a complex with reduced glutathione on the membrane carrier for this peptide. Much more toxicological information is available for methylmercury than for ethylmercury.

Other organomercurials include phenylmercury and methoxyethylmercury compounds. This group of mercurials is rapidly converted to inorganic mercury so that their toxic effects are similar to those of mercuric mercury compounds. In general, however, they are more efficiently absorbed into the body than inorganic mercury.

B. Methylmercury

1. Pathways of Human Exposures

Chemists first synthesized methylmercury in the mid-19th century in the form of dimethylmercury (Hunter, 1969). However, human exposure today occurs almost exclusively to monomethylmercury from consumption of fish and marine mammals. In the following text, the term methylmercury refers exclusively to monomethylmercury compounds.

Methylmercury is present in most if not all aquatic species as a result of the methylation of inorganic mercury by microorganisms present in sediments in bodies of fresh and ocean water. Inorganic mercury arrives in aquatic sediments as a result of the global cycling of mercury (Mason et al., 2005) depicted in Figure 4.

Mercury vapor is emitted to the atmosphere from both natural and anthropogenic sources. Volcanoes, depending on the local geology, are believed to be an important natural source, as also are soil and water surfaces. Current emissions due to human activity are from coal-burning power stations, municipal incinerators, and automobiles when they are recycled. Altogether, natural and anthropogenic sources are roughly equal in magnitude (Fitzgerald and Clarkson, 1991). Once the vapor enters the atmosphere it remains there for about 1 year. This long residence time ensures that mercury released from any point source such as the chimney of a power station will distribute throughout the hemisphere. The high rates of release in the Northern Hemisphere can lead to transport even to the most remote regions of the Arctic.

The vapor is slowly converted to mercuric mercury by oxidative processes not completely understood. The actual chemical compounds of mercuric mercury have not yet been identified, but mercuric oxide would seem to be a likely candidate. The mercuric compounds are returned to the earth's surface in rainwater. Some fraction of this mercury load is believed to be reduced back to the vapor, and returned to the atmosphere to complete the cycle. However, some of the mercury compounds find their way to aquatic sediments. Uptake of mercury vapor by vegetation is also considered to be an important deposition pathway.

The next important step on the pathway of methylmercury to humans takes place in the upper few centimeters of aquatic sediments. Inorganic mercury is converted to methylmercury by microorganisms. This is believed to be a protective mechanism on the part of microorganisms, as inorganic mercuric mercury is more toxic. In both marine and freshwater systems, the microbial communities responsible for methylation of mercury are mainly the sulfate-reducing bacteria.

The discovery of this process is a remarkable story in itself (Johnels and Westermark, 1969). Swedish investigators in the 1960s had observed that certain predatory birds were exhibiting abnormal neurological signs. They determined that these birds were consuming small mammals that in turn consumed seed grain. The latter had been treated with a methylmercury fungicide. They confirmed the mercury exposure by finding high levels in the birds' feathers. These were migratory birds. Feathers grown when the birds were in North Africa had background levels of mercury, whereas feathers grown in Sweden had high levels. Museum specimens revealed a sharp increase in mercury in feathers at the time mercury fungicides were introduced into agriculture.

However, fish-eating birds such as the osprey were also found to have high mercury levels. This discovery of elevated levels of methylmercury in the feathers of fish-eating birds was amplified by examination of museum specimens. These specimens exhibited a gradual rise in mercury levels corresponding to the period of industrialization in Sweden. Further investigation revealed that freshwater fish such as the northern pike caught downstream from a paper pulp factory were found to have high levels of methylmercury. However, the factory released only phenylmercury and inorganic mercury. The source of the methylmercury in the fish was a mystery. The source of methylmercury was eventually identified as a biomethylation process in sediments. The Swedish investigators were able to show that virtually all forms of mercury that find their way to aquatic sediment are substrates for the biomethylation process (Jernelov, 1969).

Methylmercury produced by this biomethylation process in aquatic sediments is able to enter the aquatic food chain. It undergoes a remarkable biomagnification process to achieve its highest concentrations in the muscle tissues of the long-lived predatory fish such as pike in fresh water and shark in ocean waters. Carnivorous sea mammals also have some of the highest concentrations. The degree of biomagnification depends on the location of the fish species in the food chain. For example, in unpolluted ocean water, the herbivorous reef fish may have concentrations of methylmercury as low as 0.01 ppm, whereas shark may achieve levels as high as 4 ppm. The concentration of methylmercury in ocean waters is about 1 pg/ml, so that biomagnification of the order of a millionfold occurs as mercury ascends the aquatic food chain.

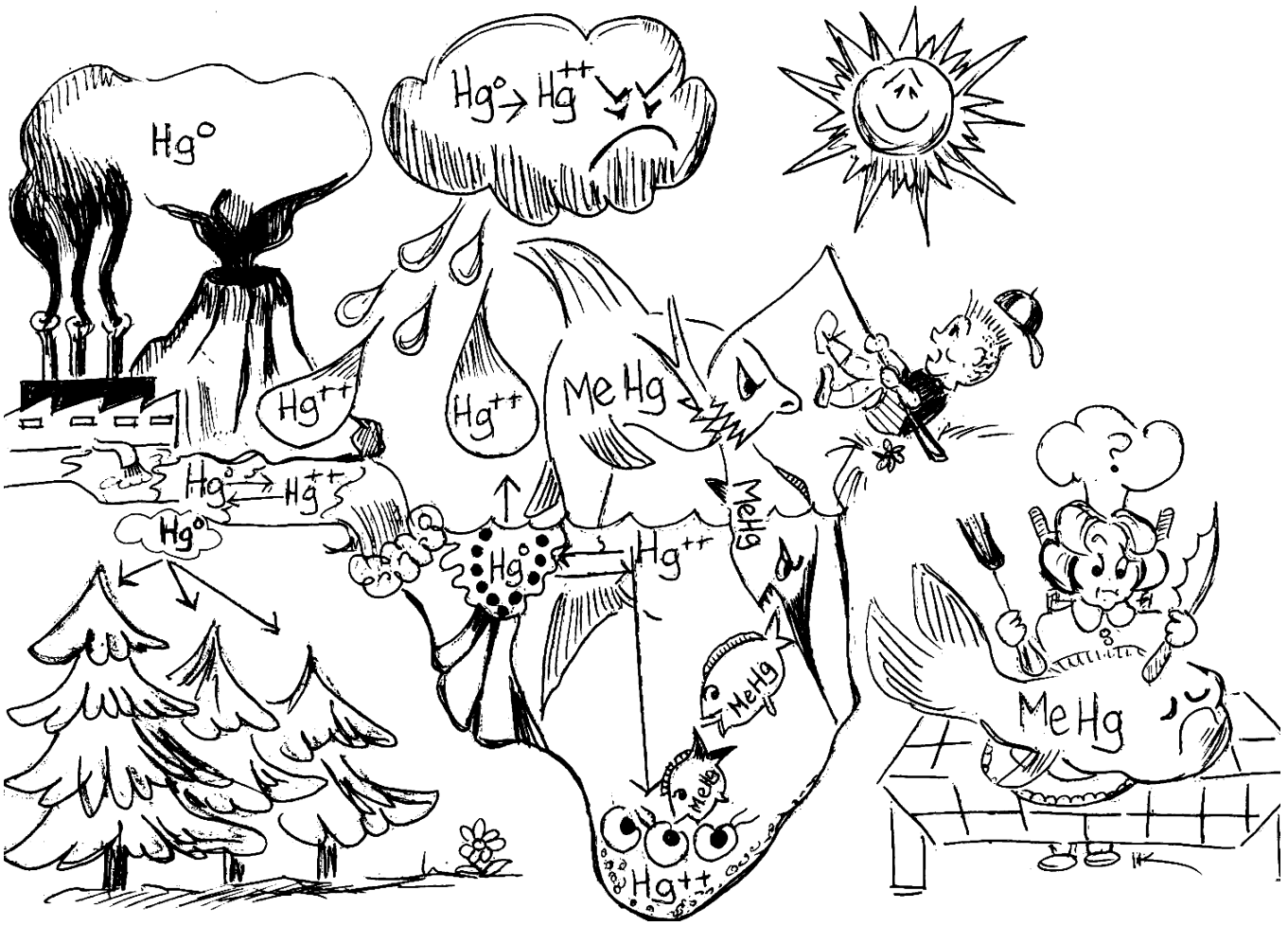

FIG. 4. The global cycling of mercury, its methylation in aquatic sediments and the bioaccumulation of methylmercury in aquatic food chains to eventually enter the human diet (from Fitzgerald and Clarkson, 1991 and Mason et al., 2005).

The chemical form of methylmercury in fish tissue has recently been identified as attached to the thiol group of the cysteine residues in fish protein (Harris et al., 2003). Methylmercury accounts for nearly all of the total mercury in fish muscle except in pilot whales and probably other sea mammals, where inorganic mercury may account for 50% of the total mercury (Julshamn et al., 1987). The pacific marlin is an interesting exception where most of the mercury in its tissues is in the inorganic form (Schultz et al., 1976). The processes producing the high inorganic mercury levels in these species are unknown.

High levels of methylmercury in fish can also be produced by local pollution of fresh water lakes and rivers or in sheltered ocean bays. The most dramatic example is the Minamata Bay outbreak of human poisoning, where methylmercury itself along with other forms of mercury may have been released into an ocean bay (Hunter, 1969). In fact, it was this circumstance that demonstrated the biomagnification process where fish levels in excess of 20 ppm were found. Levels in excess of 5 ppm have been found in pike living downstream of paper pulp and chloralkali plants discharging mercury-containing effluents into rivers.

Other sources of methylmercury have virtually disappeared as a result of health warnings by international agencies (e.g., WHO, 1976). Intake of methylmercury therefore depends on the quantity and species of fish consumed. Populations that depend on fish as a major food source have the highest intakes. Such populations are globally distributed from tropical islanders to the Inuits of the far northern Arctic.

Steps have been taken since the 1970s to control local pollution, but the global cycling of mercury continues. The contribution of anthropogenic release to the global cycle is also a subject of regulatory debate. The exact contributions of these global emissions to fish levels of methylmercury still remain to be established. Health authorities in most countries regulate allowable methylmercury levels in fish. Usually fish with levels

in edible tissues in excess of 1 ppm are not allowed on the commercial markets.

2. Disposition in the Body

The parameters of uptake, distribution, and excretion of methylmercury in adult humans are sufficiently well described that it is possible to quantitatively relate levels in indicator media such as blood and hair to daily intake and even to estimate levels in the target tissue, the brain. In fact, such "pharmacokinetic models" have been used by regulatory agencies to estimate long-term allowable daily intakes from no-observed-adverse-effect levels in blood and hair (Rice et al., 2003).

Experimental studies on adults in the 1970s and 1980s determined that about 95% of methylmercury ingested in fish was absorbed into the bloodstream (WHO, 1990). After the distribution from the blood compartment to all the body tissues is completed, a process that takes some 30 to 40 h, on average about 5% of the absorbed dose remains in the blood compartment. The concentration in brain is about 5 times and in scalp hair about 250 times the corresponding concentration in blood. Hair levels closely follow blood levels. Allowance has to be made for the growth rate of hair. At an average growth rate of roughly 1 cm per month, consecutive 1-cm segments recapitulate average monthly blood levels. There is a delay period of approximately 20 days between the concentration of mercury in the first centimeter next to the scalp and the corresponding average monthly blood level. Elimination from the body tissues and from whole blood is adequately described by a single half-time. The best estimate for whole blood is an average of 44 days in six subjects that received a low nontoxic dose of methylmercury labeled with a radioactive isotope of mercury (Smith et al., 1994).

Less information is available for pregnancy, but it is known that cord blood levels of methylmercury closely follow those in the mother at the time of delivery. Animal data including primates indicate that brain levels in the newborn may be as high as five times the corresponding levels in the mother.

Little information is available on the disposition of methylmercury in lactating women and in infants and children. Methylmercury is secreted in breast milk, but the largest component is inorganic mercury, presumably originating from the breakdown of methylmercury in the body. Intake from breast milk, if this is the only source of nutrition, is sufficient to maintain infant hair and blood levels in the same range as those of the mother (Grandjean et al., 1995).

Early textbooks ascribed the high mobility of methylmercury in the body, such as its passage across the blood–brain and placental barriers, as due to its lipid solubility. This misconception probably arose through the common use of the lipid-soluble methylmercury chloride in experimental studies. The fact is that methylmercury forms water-soluble complexes in body tissues attached to thiol groups in proteins, certain peptides (reduced glutathione), and amino acids.

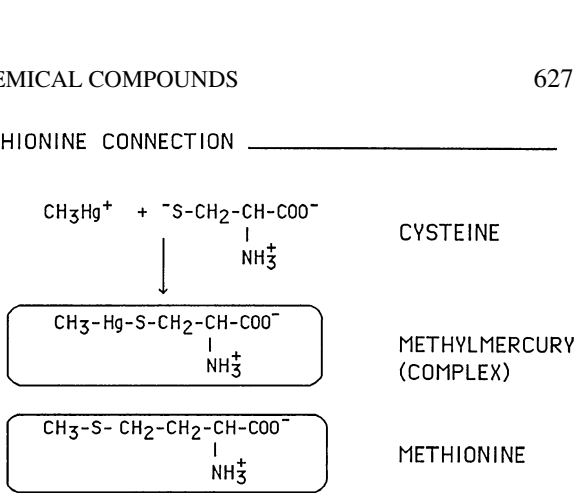

FIG. 5. The methylmercury cation, CH_3Hg^+, attaches to the thiol ligand of the amino acid cysteine, to form a complex that mimics the structure of the large neutral amino acid, methionine.

Experimental work on rats has shown that methylmercury gains entry into the endothelial cells of the blood–brain barrier as a complex with cysteine (Kerper et al., 1992; Simmons-Willis et al., 2002). Since this process is inhibited by large neutral amino acids, it was proposed that methylmercury–cysteine is transported across the cell membrane on the large neutral amino acid carrier. The structure of methylmercury–cysteine closely resembles that of methionine, a large neutral amino acid (Figure 5). The process is so selective that only the L-optical enantiomorph is transported. Intestinal absorption probably takes place by the same mechanism (Leaner and Mason, 2002).

Methylmercury is transported out of liver cells into bile as a complex with reduced glutathione using glutathione carriers (Ballatori and Clarkson, 1985; Ballatori et al., 1995). It may be that these two processes, entry into the cell as the cysteine complex and exit via the glutathione pathway, are sufficient to explain the mobility in the body. From an evolutionary standpoint it is puzzling why such a toxic metal is allowed to use universal amino acid and peptide carriers. This point is the subject of a later discussion on protective mechanisms.

These transport pathways also play a key role in the elimination of methylmercury from the body. The main route is via the feces, accounting for as much as 90% of total excretion according to animal observation. The methylmercury–glutathione complex, after secretion into bile, is broken down by extracellular enzymes into its constituent amino acids, thus releasing methylmercury as a complex with cysteine (Figure 6). This in turn is reabsorbed back into the bloodstream in the gallbladder and more distal areas of the gastrointestinal (GI) tract (Dutczak et al., 1991; Dutczak and Ballatori, 1992, 1994). Interestingly, experimental studies indicate that suckling rats are unable to secrete methylmercury into bile. The adult rate of secretion is switched on at the end of the suckling period (Ballatori and Clarkson, 1982). Nevertheless, some fecal excretion takes place as evident in rats where the bile duct was ligated (Norseth and Clarkson, 1971).

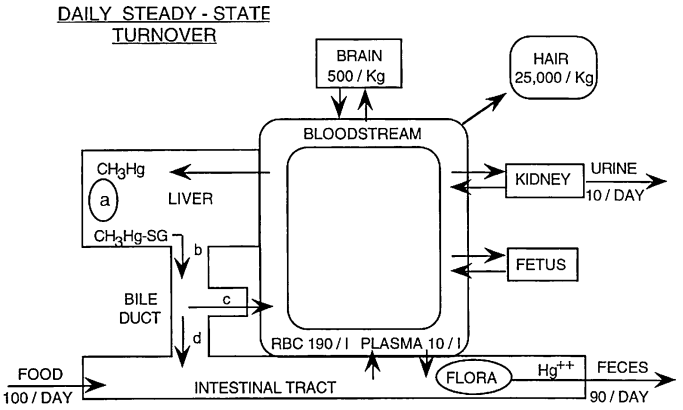

FIG. 6. A diagram of the entero-hepatic recirculation of methylmercury and its movement to maternal brain, kidney, and hair and to fetal tissues. (a) Methylmercury combines with reduced glutathione to form a complex, (b) that is secreted into bile. This glutathione is hydrolyzed to its constituent amino acids, releasing the methylmercury–cysteine complex. The latter, in part, is reabsorbed in the gallbladder into the bloodstream (c), and in part secreted into the intestinal tract along with any unhydrolysed glutathione complex (d). Once in the intestinal tract it is in part reabsorbed into the portal circulation as the cysteine complex and in part demethylated by intestinal microflora. Most of the inorganic mercury produced in this way is excreted in the feces. The numbers quoted in each body compartment are the relative concentrations of methylmercury and the intakes and excretion rates.

This completes an enterohepatic cycle. The existence of such a cycle was used to devise a means of accelerating the excretion of methylmercury. A nonabsorbable resin containing thiol ligands to bind methylmercury was designed to break the cycle by trapping the mercury entering the intestine and carrying it into the feces (Clarkson et al., 1981). Its success in reducing mercury levels in humans exposed to methylmercury testifies to the importance of this cycle.

Some methylmercury persists in the intestinal tract to come into contact with the intestinal microflora. These organisms are capable of breaking the carbon–mercury bond to release inorganic mercury. The latter is poorly absorbed, at least in adults, and is carried into the feces. In fact, inorganic mercury is by far the major form of mercury excreted in people exposed to methylmercury. As a consequence of this process, treatment of animals with antibiotics reduces fecal excretion and a high-fiber diet enhances excretion (Rowland et al., 1984, 1986).

The glutathione secretion pathway may explain the absence of methylmercury secretion in bile during the suckling period. The secretion of glutathione from liver to bile is not operative during this period and switches to the adult rate at the end of suckling (Ballatori and Clarkson, 1985).

A recent fascinating discovery revealed that a diet of tropical fruit greatly reduced blood levels of methylmercury ingested from fish (Passos et al., 2003). It is unknown what substances in tropical fruit are able to perform this protective action and which of the many processes in the enterohepatic cycle are affected.

Vahter et al. (1995) observed that obese monkeys given the same dose regimen of methylmercury as normal-weight monkeys had higher blood and brain levels. They attributed this finding to the possibility that methylmercury does not distribute to fat depots. Indeed, from the earlier discussion of the mechanisms of transport involving water-soluble complexes of methylmercury with cysteine, we would expect that methylmercury would be restricted to the lean body mass. Thus, for any given dosing regimen based on body weight, the lean body mass will receive a higher dose in obese animals than in those of normal weight. The authors raised the possibility that obesity may be a risk factor for exposures to methylmercury.

3. Metabolism

As already mentioned, methylmercury can be converted to inorganic mercury. The major site of metabolism is the flora in the intestinal tract. It is perhaps fortunate that these organisms undo the work of their ancient relatives, the methanogenic bacteria that synthesize methylmercury in the environment. Otherwise, fish eaters would soon accumulate toxic levels and perhaps humans and other fish-eating mammals might never have appeared on this planet. The mechanism of breakdown by intestinal microflora is unknown.

Some of the inorganic mercury found in mammalian tissues after exposure to methylmercury may be due to the absorption of inorganic mercury produced by intestinal microflora. However, Japanese investigators have presented evidence that methylmercury is converted to inorganic in phagocytic cells (Suda et al., 1992, 1993). Suda and Hirayama (1992) have reported that liver microsomes are also capable of demethylating methylmercury, perhaps via the action of NADPH-cytochrome P-450 reductase.

The inorganic mercury in brain appears to be virtually immobile, as substantially elevated levels have been found in autopsy brains of individuals who died at least 10 years after their last exposure to methylmercury (Davis et al., 1994). Observation in monkeys receiving long-term subclinical exposure to methylmercury also indicated that inorganic mercury derived from the breakdown of methylmercury had a half-time in brain of the order of hundreds of days (Vahter et al. (1995). An expert committee (WHO 1990) suggested that the long residence time is due to inorganic mercury forming a stable insoluble compound with selenium. Inorganic mercury in brain tissue probably arises from in situ metabolism of methylmercury, as the inorganic species does not pass readily across the blood–brain barrier. This conclusion is consistent with the findings of Vahter et al. (1995), who observed much higher levels of inorganic mercury in brains of monkeys dosed with methylmercury than in those dosed with inorganic mercury ($HgCl_2$).

The methylation of inorganic mercury does not appear to take place to any significant extent in either human or animal

tissues. Even in individuals exposed to toxic levels of mercury vapor, signs and symptoms characteristic of methylmercury intoxication have never been reported. Nor have elevated levels of methylmercury been reported after exposure to inorganic mercury (Barregard et al., 1994).

4. Biological Indicators

Levels of total mercury in whole blood or scalp hair are the indicator media of choice in adults for the absorbed dose or as indicators of levels in the target tissue, the brain (Cernichiari et al., 1995a). With respect to prenatal exposure to methylmercury, both cord blood and maternal hair have been used. In fact, a debate has continued for many years as to which is the better indicator of the dose to the fetal brain. One side of the argument notes that methylmercury in cord blood is in closer contact with the fetal brain than mercury in maternal hair. Backers of this approach also note that hair is potentially subject to external contamination and that various types of cosmetic treatments may reduce mercury levels in hair (Dakeishi et al., 2005).

The proponents for hair point out that cord blood levels will be influenced by the hematocrit since at least 80% of the methylmercury in blood is associated with the red blood cells. Depending on the method of collection of cord blood, the hematocrit may vary over a wide range (Nelle et al., 1993; Linderkamp et al., 1992). Hair has the advantage of being able to recapitulate mercury levels throughout pregnancy, whereas cord blood gives the level only at delivery. Indeed, hair has been used from preserved museum specimens to recapitulate historic exposures to methylmercury (Lindberg et al. 2004). Claims have been made that cosmetic hair treatment removes mercury from the hair. A recent comprehensive study of mercury levels in human hair found no effects of cosmetic treatment on hair levels. It would appear that under conditions of actual use of these cosmetic preparations, no mercury is lost from hair (McDowell et al., 2004).

What is known about the mechanism of disposition of methylmercury would support the use of hair as the biological indicator of choice. We have seen that the species of methylmercury transported into cells is methylmercury–cysteine via the large neutral amino acid carrier. This mechanism of transport has not yet been demonstrated for methylmercury entry into the hair follicle, but it seems highly likely that it will be operative. The demand for amino acids as substrates for proteins, especially keratin, in growing hair is probably higher than that of any other tissue. The large neutral amino acid carrier is a necessary component of all cells that require amino acids for protein synthesis. Thus methylmercury level in hair will be a measure of the methylmercury–cysteine complex present in plasma, the same complex that is the first step in methylmercury transport into the brain.

Mercury in hair after exposure to methylmercury consists of somewhat in excess of 80% methylmercury and the remainder inorganic mercury in both humans (Cernichiari et al., 1995b) and animals (Lindberg et al., (2004). The inorganic fraction appears to be constant over wide ranges of concentrations of total mercury. As noted earlier in this review, inorganic mercury is poorly accumulated in hair, indicating that the inorganic fraction is due to conversion of methyl to inorganic mercury in the hair follicle. This conclusion is consistent with the report by Lindberg et al. (2004) that total mercury concentration in hair reflects methylmercury exposure and not inorganic exposure.

Methylmercury–cysteine accounts for less than 1% of methylmercury in whole blood. Most mercury is bound to the hemoglobin in red cells, with a small fraction attached to glutathione. Of the small fraction found in plasma, most is protein bound (Ancora et al., 2002). Thus assays of mercury in whole blood actually measure the nontransportable species. Using whole blood as the biological indicator assumes a rapid equilibration between the mercury–cysteine complex in plasma and the nontransportable species inside the red cell.

In addition there are significant strain and species differences in blood to brain ratios (Doi and Tagawa, 1983). These differences in blood to brain ratio seem to be largely determined by differences in the degree of binding of methylmercury to hemoglobin, with the greatest binding seen in rat erythrocytes and the lowest in humans.

The debate on hair versus blood has become most intense with regard to interpretation of the results from the Faroes and Seychelles studies. The difference in outcomes of these two studies is discussed later. Several attempts have been made by the proponents of cord blood to assess the relative variance or "error" in maternal hair versus cord blood as biological indicators (Grandjean et al., 2004a; Budtz-Jorgensen et al., 2004). Comparisons have been made between hair, cord blood, and dietary estimates of methylmercury intake. Comparisons have been made on how well maternal hair and cord blood "predict" adverse outcomes in the offspring. The latter argument begs the question since a priori the outcomes are unknown and may be either adverse or beneficial, as the source of methylmercury is nutritious seafood. In the case of the former, comparison between media and diet information misses the key comparison with levels in the target organ, the brain.

Mercury in maternal hair and blood has been compared to levels of mercury in the brain (Cernichiari et al., 1995b). In this study, autopsy brains were available from infants dying of natural causes within a few weeks of birth. The mercury concentration in samples of maternal blood and hair mercury was measured. The hair sample was the 1-cm segment that overlapped in time with the delivery of the infants. Regression analyses indicated that the correlation coefficients of the mercury levels in these samples with levels in six anatomical regions of the infant brain were similar. In other words, maternal blood was no better predictor of brain levels of mercury than maternal hair levels. Blood samples were also collected from the infants after death and may have been subjected to postmortem change. Unfortunately, no data are available relating levels in cord blood to infant brain levels.

5. Toxic Effects

The toxic effects of methylmercury differ between adult and prenatal exposures. They differ both in terms of the type of damage to the brain and in terms of the lowest toxic doses. They are therefore treated separately here. Distinction between acute and chronic toxicity of methylmercury is not really meaningful. A single dose can elicit the same syndrome of clinical methylmercury poisoning as chronic exposure, is discussed later.

a. Adult Exposures. The potential for methylmercury compounds to damage the central nervous system was demonstrated immediately following the first synthesis of dimethylmercury in a chemical laboratory in London in the 1860s (Hunter, 1969). Dimethylmercury is a liquid with a high vapor pressure. It is an uncharged lipid-soluble compound, so absorption could have taken place via inhalation or skin contact. In any event, whatever the route of absorption, the two chemists working in the laboratory developed severe neurological signs that eventually proved fatal. In both cases, numbness of the hands and feet was an early symptom. Their medical condition rapidly deteriorated, with the appearance of incoordination, dysarthria, and loss of vision and hearing, among other signs of severe damage to the nervous system.

This dramatic incident may have served as a warning to chemists to avoid alkylmercurial compounds. Indeed, it was not until well into the next century that two additional cases appeared. The first case was a chemist who synthesized dimethylmercury over a 3-month period (Pazderova et al., 1974). He developed numbness and tingling in the fingertips and lips, and suffered from ataxia and insomnia. After admission to hospital, his condition deteriorated, and included slurred speech and hearing problems. Later he did not recognize relatives, and eventually he developed pneumonia, which was probably the cause of death. When he died, about 50 days after the end of exposure, the concentration of mercury in the brain was 13.2–14.2 μg/g.

The second severe case took place in a chemical laboratory at Dartmouth College, in New Hampshire (Nierenberg et al., 1998). In this case, dimethylmercury was being used to calibrate a scientific instrument by a professor of chemistry conducting studies on the toxicology of metals. According to her notebook, she had accidentally spilled a few drops of the liquid mercury compound onto her latex gloves in a fume cupboard. She experienced no ill effects and continued with her work.

This single exposure took place on August, 1997. On January 20, 1998, she was admitted to a hospital with a 5-day history of progressive deterioration in balance, gait, and speech. She had lost 15 lb over a period of 2 months and had experienced several brief episodes of nausea, diarrhea, and abdominal discomfort. Otherwise she had experienced no ill effects since her exposure to dimethylmercury some 5 months earlier. Clinical examination revealed moderate upper extemity dysmetria, dystaxic handwriting, a widely based gait, and mild "scanning speech." In the ensuing days, the patient experienced progressive difficulty with speech, walking, hearing, and vision (constricted visual fields). On February 6, 1998, 22 days after the first neurologic symptoms developed and 176 days after exposure, the patient became unresponsive to all visual, verbal, and light touch stimuli. She died several months later, despite intensive medical support including the application of chelating agents.

This case dramatically illustrates an insidious, hazardous property of methylmercury, namely, the long latent period between exposure and the onset of symptoms, in this case as long as 5 months. Analysis of single strands of head hair confirmed that she had received only a single exposure to methylmercury at the date stated in her notebook. Thereafter mercury levels in hair declined exponentially, with a half-time of about 75 days (Figure 7). The maximum hair level of 1100 ppm is consistent with severe methylmercury poisoning as indicated by data from previous outbreaks of poisoning (see later discussion). A blood level somewhat in excess of 1000 ppb at the time of admission is consistent with a corresponding hair level of approximately 300 ppm (Figure 7). Subsequent blood levels were higher, probably due to mobilization from tissue deposits as a result of chelation therapy. Based on the hair and blood levels, it was calculated than she had absorbed no more than 0.44 ml of the liquid dimethylmercury.

According to animal studies, dimethylmercury must first be converted to monomethylmercury in order to accumulate in hair (Ostlund, 1969). The lag time (half-time 6.5 days in Figure 7) is consistent with a rapid conversion. The single half-time in hair of 75 days following the peak level falls within the range of half-times previously reported for methylmercury (WHO, 1990).

The latent period of 5 months is the longest ever reported for methylmercury and might raise some doubts that this case

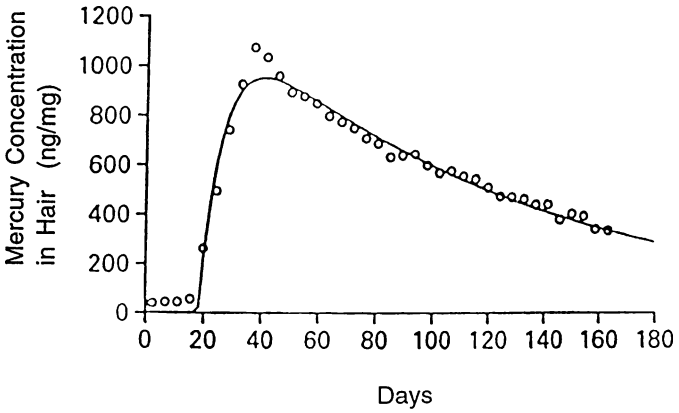

FIG. 7. The concentration of mercury in a single strand of hair before and after a single exposure to dimethylmercury. The beginning of the sharp rise in mercury levels indicates the day that the exposure took place. The data were obtained by x-ray fluorescent analysis of consecutive 2-mm segments. (From Nierenberg et al., 1998). Copyright 1998 Massachusetts Medical Society. All rights reserved.

was an example of methylmercury poisoning. However, the symptomatology, levels of mercury in hair and blood, and autopsy findings on the brain are all consistent with a diagnosis of methylmercury poisoning. The visual cortex around the calcarine fissure was grossly gliotic and the cerebellum showed diffuse atrophy of both vermal and hemispheric folia.

However, many other cases of methylmercury poisoning had preceded the Dartmouth case. The potent antifungal properties of organo mercury compounds had been discovered early in the 20th century. The most potent were the short-chain alkylmercurial, especially methyl- and ethylmercury compounds. A factory manufacturing the methylmercury fungicides was the site of the first report of occupational poisoning (Hunter, Bomford, and Russell, 1940). The signs and symptoms of poisoning were the same as already described and confirmed that only the nervous system was involved.

The death of one of the victims 15 years later was the occasion for the first report on the morphology and histology of brain damage in humans (Hunter and Russell, 1954). Damage was restricted to specific focal areas of the brain. For example, cerebellar cortical atrophy selectively involved the granule-cell layer of the neo-cerebellum, accounting for the signs of ataxia. The adjacent Purkinje cells were hardly affected. The bilateral cortical atrophy around the calcarine fissures accounted for the concentric constriction of the visual fields.

Other cases of occupational or accidental poisoning were also reported in the first half of the 20th century (for a review, see Hunter, 1969) The first large mass outbreak took place in Minamata, Japan, in the 1950s, when fishermen and their families consumed fish containing high levels of methylmercury. The source of the mercury was eventually traced to a chemical plant manufacturing acetaldehyde, where inorganic mercury was used as a catalyst (Irukayma, 1977). The catalytic reaction resulted in the unwanted synthesis of methylmercury compounds: Acetate in the presence of an oxidizing agent is converted to peracetate and peracetate in the presence of inorganic mercury produces methylmercury. The fluid filtrate of the discharge from the manufacture of acetaldehyde had 500–1000 μg/g total mercury, of which 100–170 μg Hg/g was methylmercury, so that methylmercury accounted for approximately 18% of total mercury. The estimated loss of total mercury from the acetaldehyde plant over several years was 81.3 tons, so that, based on this percentage, the amount of methylmercury discharged was 14.6 tons. The same plant, probably resulting in more methylmercury being discharged to Minamata Bay, also manufactured phenylmercury compounds. In fact, methylmercury was identified in the filter system of the acetaldehyde plant and in every step of the drainage system, including sediments in the bay. Based on these data, the Japanese Ministry of Public Health and Welfare announced in 1968 that the causative agent of Minamata disease was methylmercury compound contained in the waste from the acetaldehyde plant.

One cannot completely exclude the possibility that some inorganic mercury discharged to the bay may have undergone biomethylation by natural processes in the sediment and thereby contributed to methylmercury levels in the fish. However, given the unusually high levels of methylmercury found in the fish, at least an order of magnitude higher that those found in fish where biomethylation was the sole source, it seems highly likely that the high levels in fish in this outbreak and the subsequent and similar one in Niigata were due to the discharge of methylmercury itself.

This conclusion is of special interest since no clinical cases of methylmercury poisoning have ever been reported from consumption of fish where the source of methylmercury was the natural biomethylation process. It was the uniquely high levels of methylmercury produced in the fish from methylmercury itself discharged in the Minamata and Niigata outbreaks that was responsible for the cases of poisoning.

Although Minamata Bay has restricted access to the open ocean, nevertheless the high levels in fish from this industrial discharge provide conclusive evidence of the environmental hazard from methylmercury, namely, its potential to accumulate in aquatic food chains to the point of causing human poisoning.

Pathological studies of the victims of the Minamata outbreak confirmed that the central nervous system was the primary site of damage. However, Eto et al. (2002) have recently presented evidence of peripheral neuropathy that may account for the signs of sensory loss in the extremities.

The outbreaks in Minamata and Niigata were the subject of an intensive review by a Swedish Expert Committee (Report of an expert group, 1971). This report has been a model for many other criteria documents attempting to assess human health risks from mercury and other environmental pollutants. The committee was able to use data on hair and blood levels of the victims and related the maximum mercury levels to the prevalence of signs and symptoms. Specifically, an attempt was made to calculate the lowest hair and blood levels associated with the onset of paresthesia, the first and mildest symptom of methylmercury poisoning. The investigators concluded that the onset of symptoms should occur at blood levels above 200 μg Hg/L of whole blood and above 50 μg Hg/g of hair.

An opportunity to check these quantitative predictions from the Japanese outbreaks presented itself in a large outbreak of methylmercury poisoning that took place in rural Iraq in the winter of 1971–1972. Iraq grows its own wheat, as it has done throughout recorded history. This area of the Middle East was known as the Fertile Crescent and was the breadbasket of the ancient world before North America inherited that title. In 1970 the crop failed, so a large commercial order was placed for seed grain for the next year. It was the largest commercial order ever placed (Bakir et al., 1973).

Wheat was delivered in 50-kg sacks to be used as seed grain. The seeds were colored red with a dye. This is the typical warning sign used in Western countries to tell the purchaser that the seeds had been treated with a fungicide. In Iraq, this warning was counterproductive. The Iraqi farmers washed the dye off the wheat, believing they had removed

the methylmercury fungicide. The warning against eating was written in Spanish since the grain had originated in Mexico. A skull-and-crossbones sign was attached to the sacks, another warning of dubious value since the Arabic farmers were not closely familiar with "pirates on the Spanish Main." Following the failure of the warning signs, the treated grain was used to prepare homemade bread throughout the rural areas of Iraq. The latent period on methylmercury also contributed to the disaster, as the farmers fed the contaminated grain to their livestock without observing any immediate effects. Another factor was that the warning labels did not distinguish between the highly toxic methylmercury fungicide and the virtually innocuous phenylmercury fungicide that was also used to treat the wheat.

The consumption of the contaminated bread resulted in an insidious and irreversible development of neurological signs and symptoms. The victims experienced no ill effects during the intake period. After consumption had stopped, the first neurological signs did not appear for over a month. There were examples in Iraq of individuals who had unknowingly consumed what would prove to be a fatal dose. Usually the first symptom to appear was paresthesia. Subsequently and in rapid succession, more serious signs appeared such as ataxia, dysarthria, and loss of vision, exactly as was observed in the cases in London a century earlier!

Some functional recovery did occur as the victim learned to live with the disability, but the underlying damage is irreversible. For this reason, the peak mercury level was used as the appropriate measure of dose. For an irreversible poison, the maximum level in the brain should cause the greatest damage. The peak value is plotted on the *x* axis, here expressed in units of concentration in hair (Figure 8). Each sign or symptom has its own relationship with the mercury levels. Paresthesia has background prevalence in the population of about 5%. At a certain threshold level, the prevalence increases above background to reach levels affecting the entire population at the highest mercury levels. The more serious signs have higher threshold levels, with fatality being at the highest level of all. The threshold for paresthesia, 100 ppm, is the lowest-effect level for adult poisoning. We contrast this 100-ppm level with the lowest-effect level for prenatal exposures. This lowest-effect hair level is consistent with the estimate from the Swedish Expert Committee (1971) based on data from Japan, where they concluded that the onset of clinical symptoms should occur at hair levels above 50 ppm.

The Iraqi outbreak was the result of an exposure lasting for a month or so. Under these circumstances, the peak mercury levels predict the toxic outcome. But the question arises as to what might happen in chronic, even lifetime exposure. This question is especially important when we consider fish-eating populations.

Exposure from fish consumption produces two basic patterns. One pattern is seasonal, illustrated from Canadian data, where peaks are repeated over several years (Figure 9). These data were taken from a study of aboriginal peoples consuming freshwater

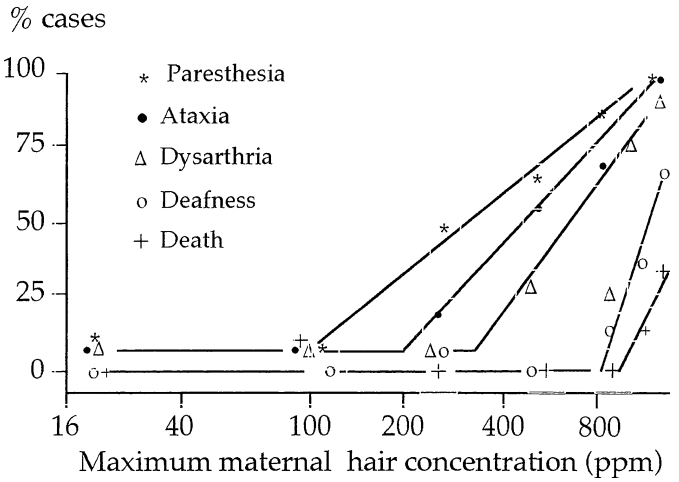

FIG. 8. The relationship between the prevalence of signs and symptoms of methylmercury poisoning and the corresponding hair concentration. The latter is the estimated maximum hair concentration attained during a brief (few months) exposure to methylmercury from consuming contaminated homemade bread (Bakir et al., 1973).

fish in the summer months (Phelps et al., 1980). This repeated peak exposure pattern may have continued for a lifetime.

A second exposure pattern is shown by people dependent on ocean fish as their main source of protein. Figure 10 depicts hair levels in three separate individuals from four different populations of ocean fish consumers. One is a coastal population in Peru, while the other three are island populations. All have high consumption of ocean fish. In general, the hair levels are more uniform throughout the year than in the aboriginal Canadian populations. The levels for each individual are not completely flat but may range by a factor of two or so over a 1-year period. The levels are below the Iraqi adult threshold of 100 ppm, but a few individuals in the populations can exceed 50 ppm. The exposures are expected to occur year after year throughout the lifetime of the individual.

In two studies of fish consumers, associations of adverse symptoms with hair levels of mercury have been reported. Hair but not blood levels were associated with adverse outcomes of psychomotor tests in an Amazonian population (n-84) consuming freshwater fish. The average hair level was 9 ppm. However as the authors point out, higher hair levels may have existed prior to the time of this study (Dolbec et al., 2000). Another cross-sectional study in the same area involving 132 families also claimed to find evidence of "mild Minamata disease" such as a prevalence of "glove and stocking" paresthesia (Harada et al., 2001). It should be noted that these studies were conducted in an area of intensive gold mining, where large quantities of liquid metallic mercury are used to extract the gold.

Otherwise, poisoning cases have not resulted from these extended exposures to methylmercury in a diet of fish. Epidemiological studies in Canada, Peru, Samoa, Canada, and the

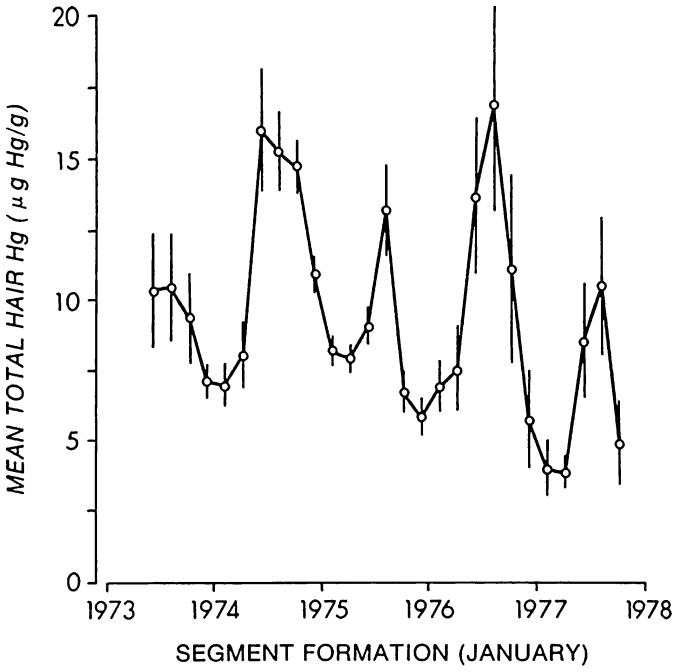

FIG. 9. Seasonal changes in mercury concentrations in hair samples from indigenous populations consuming freshwater fish in northern Canada (Phelps et al., 1980). Fish were caught and consumed mainly in the summer season, as fish in lakes and rivers became inaccessible in the winter season. Reprinted with permission of the Helen Dwight Reid Educational Foundation. Published by Heldref Publications. Copyright 1980.

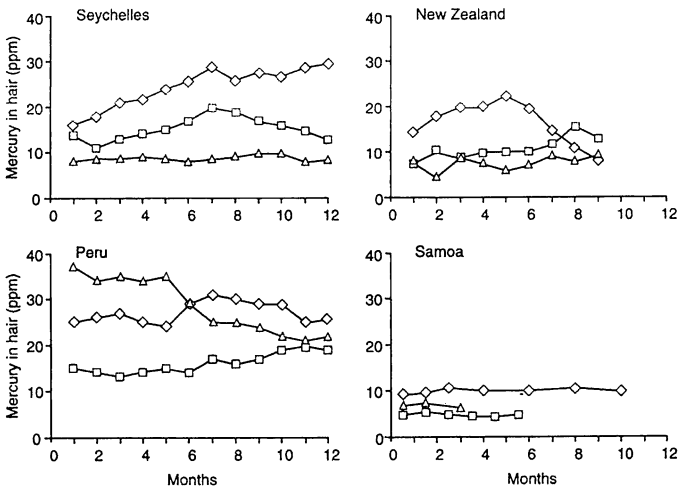

FIG. 10. The mercury concentrations in hair samples taken from individuals consuming ocean fish. The data represent coastal and island populations dependent on fish protein as a major dietary item. Consumption takes place throughout the year with no consistent pattern of seasonal changes.

Mediterranean have failed to find adverse effects associated with methylmercury from fish (WHO, 1976, 1990). Furthermore, some of these populations have been under close medical scrutiny. Individuals in Canada with hair levels in excess of 30 ppm were given intensive neurological examinations without detecting effects due to mercury (Wheatley et al., 1979).

Thus our experience to date in adult fish consumers is that extended exposure does not increase the risks of poisoning of the kind seen in Iraq. The specter of a long delay in onset of symptoms from chronic, continuous exposures does not seem to be real. The peak value of mercury appears to be the determinant of toxic damage.

As methylmercury is partially broken down to inorganic mercury in the brain, the question arises as to which mercury species is the proximate toxic agent. The brain pathology and the signs and symptoms of methylmercury poisoning differ from those of inorganic mercury, suggesting that the intact methylmercury species is the cause of the brain damage. Studies by Magos et al. (1985) support this idea. In comparing the brain levels and neurological and morphological effects of methyl- and ethylmercury compounds, such effects were elicited by similar brain levels of the intact mercurial and were unrelated to levels of inorganic mercury released from these organomercurials. These findings do not exclude the possibility that inorganic mercury might play a less potent role in brain damage. However, as discussed in a previous section, it would appear that the long-term deposits of inorganic mercury are in an inert form.

An extensive series of studies has been carried out on adult *Macaca fascicularis* monkeys (Vahter et al., 1994, 1995; Charleston et al., 1994, 1995, 1996). Three groups of five monkeys received oral daily doses of 50 μg Hg/kg body weight as methylmercury hydroxide, added to the diet for 6, 12, and 18 months, respectively, prior to sacrifice and autopsy. A fourth group received the same dosage for 12 months followed by a 6-month mercury-free diet prior to sacrifice. A fifth group of the animals received a daily intravenous infusion by an indwelling catheter for 2 to 3 months of a solution of $HgCl_2$ at a dosage rate of 200 μg Hg/kg. The latter dosage gave rise to a steady-state blood level of about 600 μg Hg/L, as compared to blood levels around 1000 μg Hg/L after methylmercury. A sixth group of four monkeys served as controls.

No adverse clinical, neurological, or behavioural effects were observed in any of the five exposed groups versus the control animals. This finding is of interest in itself as these animals had attained steady-state blood levels in excess to 1000 μg Hg/L and average brain levels in two of the groups in excess of 4 μg Hg/g wet weight brain tissue. This contrasts sharply with the findings in adult human subjects discussed earlier.

However, a detailed neuromorphological examination of the brains revealed an increase in the number of microglia cells in all exposed groups as compared to the controls. In Table 5, we compare the percentage increase of microglia cells over controls in relation to the levels of inorganic and organic (presumed to be methyl) mercury in the occipital lobe as reported by Charleston et al. (1994).

TABLE 5
Mercury concentrations (conc) in the occipital pole and increase in microglia cells in adult monkeys given 50 μg Hg/kg/day as methylmercury or 200 μg Hg/kg/day as $HgCl_2$: Increase in the number of reactive glia in the visual cortex of *Macaca fascicularis* following subclinical long-term methylmercury exposure

Exposure	Hg conc (μg/g tissue)		Microglia cells, % increase
	Inorg Hg conc	MeHg conc	
2–3 months $HgCl_2$	0.11	0.01	165
6 months MeHg	0.29	3.0	72
12 months MeHg	0.21	4.5	152
18 months MeHg	0.52	4.35	120
6 months after 12 month MeHg	0.23	1.5	89

The interpretation of these subclinical findings is difficult. The animals had been exposed for different periods of time, but blood levels in all groups had attained steady state (Vahter et al., 1994). No correlation can be seen between brain levels of either inorganic or organic mercury versus the percent increase in microglia. Apparently the inorganic species is especially effective, as judged from the animals treated with $HgCl_2$. This group of treated animals had the highest percent increase in microglia with the lowest tissue levels of mercury. The role of inorganic mercury in the methylmercury-treated animals is not clear, as there is no obvious correlation with brain levels. In any case, one cannot be sure whether inorganic mercury is the cause or consequence of the increase activity of the microglia, as the phagocytotic activity of the latter may be responsible for the conversion of methylmercury to inorganic mercury. In fact, the glial cells that surround neuronal cells and outnumber them by a ratio of about 50:1 probably play a protective role. For example, Berlin et al. (1975a) demonstrated in squirrel monkeys given tritiated methylmercury that at nontoxic doses, autoradiography detected methylmercury only in glial cells, whereas when the toxic doses were given, methylmercury had spilled over into the neuronal cells. Perhaps the conversion of methylmercury to inorganic mercury is part of the defensive role of glial cells.

Kawasaki et al. (1986) exposed four groups of macaque monkeys to doses of 10, 30, 100, and 300 μg Hg/kg/day in the form of methylmercury chloride added to the diet. The two lower dosage groups were exposed for 52 months before sacrifice and autopsy. However, the exposure of the 100-μg Hg/kg/day group was terminated between 6 and 8 months and that of the 300 μg Hg/kg/day around 2 months because 4 of the 5 animals in each group died or had to be sacrificed in a moribund condition. One monkey in each of the two high exposure groups became ill and weak for exposure to be continued and were sacrificed with the other monkeys 52 months after the start of exposure, so they had over 40 months of clearance time (Table 6).

Histological damage was observed in the neurons of the occipital lobe but not in the cerebellum of the two higher dosage groups. One may see that although methylmercury concentrations were greatly elevated in the occipital lobe, the concentration of inorganic mercury fell in the same range of the two lower dosage groups where no histological damage was seen. The highest levels of inorganic mercury were found in the cerebellum, where, as noted earlier, no damage to neurons could be found on histological examination. In agreement with the studies on rats discussed earlier (Magos et al., 1985), damage to neurons is associated with levels of methylmercury but not inorganic mercury.

The fact that no damage was seen in the cerebellum despite elevated levels of methylmercury appears to be a characteristic of nonhuman primates, as noted for rhesus monkeys (Hunter et al., 1940; Shaw et al., 1975) and squirrel monkeys (Grant, 1973). In human adults, the destruction of granule cells of the cerebellum is a characteristic pathological lesion. The reason for the species difference is unknown.

TABLE 6
The concentrations of methyl and inorganic mercury in the occipital lobe and cerebellum of macaque monkeys receiving methylmercury chloride added to their diet (data taken from Kawalski et al., 1986)

Dose in μg/kg/day (total exposure period)	n	Methyl Hg (μg Hg/g)		Inorganic Hg (μg Hg/g)	
		Occipital lobe	Cerebellum	Occipital lobe	Cerebellum
Control	5	0	0	0.014	0.014
10 (52 months)	5	0.40	0.02	0.30	0.62
30 (52 months)	5	1.01	0.07	1.33	1.95
100 (6–8 months)	4	13.2	5.08	0.13	4.87
300 (2 months)	4	24.00	9.80	0.5	8.5
100 (7 months)	1	0	0	1.5	0.8
300 (2 months)	1	0	0	0.14	0.17

The Kawasaki et al. study also indicates another characteristic feature of methylmercury poisoning in adult animals. As the dosage level of methylmercury rises to a certain critical level, the amount deposited in the brain sharply increases out of proportion to the increase in dose. The data in Table 6 illustrate this feature. Over the threefold range of the two lower dosages, the levels of methylmercury in both the occipital lobe and the cerebellum increase roughly in proportion to the dose by about a factor of three. However, brain levels of methylmercury jump sharply with the next increase in dosage rate from 30 to 100 μg Hg/kg/day, despite the fact that the exposure period is much shorter (52 versus 6–8 months). This is the dosage level associated with damage. The Kawasaki et al. data confirm the conclusions reached by Berlin et al. (1975b) on squirrel monkeys, that brain levels jump disproportionately to the increase in dose in their experiment at blood levels above 1000 μg Hg/L. Blood levels are given by Kawasaki et al. only for the two low exposure groups. The first estimation at 12 months gave 218 and 756 μg Hg/L. and the last at 52 months 298 and 607 μg Hg/L. Although blood levels were not reported for the next higher exposure group where the jump in brain levels occurred, it is highly likely the blood level would have passed through the 1000-μg Hg/L critical level as noted by Berlin et al. (1975b).

The mechanism responsible for this abrupt jump in brain levels is not known. Do damaged cells take up mercury, or is the abrupt increase in brain levels responsible for the damage? It appears that this "chicken and egg" conundrum cannot be resolved at this time. However, a plausible explanation may be found in what is known about the mechanism of transport of methylmercury across the blood–brain barrier. Methylmercury in plasma must bind to a number of thiol-containing molecules.

Yasutake et al. (1990) demonstrated that methylmercury preferentially binds to serum mercaptalbumin as compared to small thiol molecules such as cysteine and homocysteine. Nevertheless, some equilibrium distribution will occur as the forward and back reactions of mercury cations with thiol ligands are rapid. Although the preponderance of binding is to mercaptalbumin, some complex formation with cysteine should occur. As discussed in a previous section, it is the methylmercury–cysteine complex that is transported across the blood–brain barrier on the large neutral amino acid carrier. It seems reasonable to suppose that as the levels of methylmercury rise in plasma, at some point the high-affinity binding sites on mercaptalbumin will approach saturation so that methylmercury distribution will shift in favor of the transportable species, resulting in an abrupt rise in brain levels with the ensuing damage.

The localized damage seen in the adult brain has been attributed to differences in repair capacity of different populations of neuronal cells. Syversen (1982) observed that protein synthesis was reduced in all areas of the brain in rats dosed with methylmercury. However, in certain cell populations, protein synthesis recovered and even exceeded pretreatment levels, whereas protein synthesis in other cells remained depressed. Of special interest was the finding that cerebellar granule cells, known to be affected by methylmercury, did not show recovery in protein synthesis, while the cerebellar Purkinje cells, known to be resistant to methylmercury, exhibited a bounce back in protein synthesis.

However, other ideas have been put forward to explain the differential sensitivity of granule versus Purkinje cells. For example Yuan and Atchison (2003) have found that $GABA_A$ (gamma-aminobutyric acid) receptors in granule cells appear to be more sensitive to blockade by methylmercury than are those of the Purkinje cells. Previously this same laboratory has demonstrated that $GABA_A$ receptors are highly sensitive to the action of methylmercury (Xu and Atchison, 1998). It is of additional interest that these studies point to a primary action of methylmercury on the outer surface of the neuron, a point that is taken up in a later section on protective mechanisms.

In summary, effects on adults are due to damage to discrete anatomical regions of the central nervous system concerned with sensory and motor coordination functions. The outcome is usually irreversible as neuronal cells are destroyed. The appearance of signs and symptoms of poisoning can be preceded by a latent period after exposure has ceased. This latent period can amount to weeks or even months. Studies on nonhuman primates have revealed that above a critical blood level of about 1000 μg Hg/L, an abrupt jump in brain levels occurs, accompanied by histological evidence of damage to neuronal cells. The weight of evidence from animal studies indicates that the intact mercurial and not inorganic released from methylmercury in brain tissue is responsible for the damage to neuronal cells. The cellular location of the initial site of damage is not well established, but *in vitro* studies suggest receptors or ion channels associated with the outer cell surface.

b. Prenatal Exposures.

i. Consumption of contaminated loaves. The outbreaks in Japan had suggested that the prenatal period was the most sensitive period in the life cycle to methylmercury (Swedish Expert Group, 1971). Infants with severe brain damage were born of mothers minimally affected by methylmercury. Subsequent animal experiments also pointed to the sensitivity of the developing brain (Spyker et al., 1972; Spyker, 1975).

Cases of severe prenatal methylmercury poisoning similar to those seen in Japan were also observed in the Iraqi outbreak. Autopsy brain tissues became available from some severely affected infants that had died shortly after birth. The cytoarchitecture of the brain was extensively disrupted (Choi et al., 1978). Focal lesions are seen in adults, whereas in the case of severe prenatal poisoning, the entire brain is affected. The uniform cortical layers of neuronal cells seen in normal brain are grossly distorted. Ectopic neurons were observed that had not attained their final anatomic destination, indicating an inhibition of neuronal migration.

Less severe cases were also found. Specifically infants that outwardly appeared normal to the examining pediatrician had a history of delayed development (Marsh et al., 1987). These

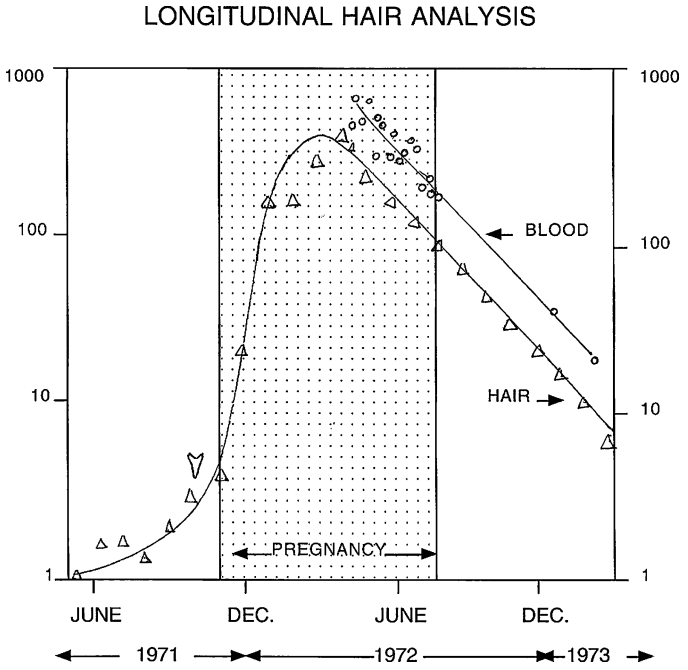

FIG. 11. The concentration of mercury in consecutive 1-cm segments of a maternal sample and corresponding concentration in blood samples. The dotted band is the period of pregnancy. The vertical arrow indicates the estimated date for the start of consumption of the contaminated bread.

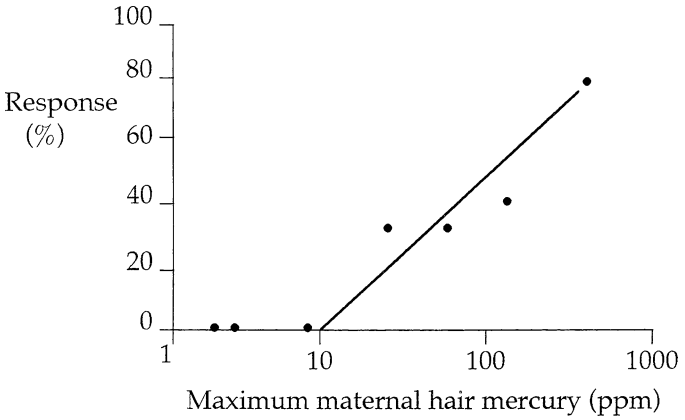

FIG. 12. The prevalence of delayed achievement of developmental milestones versus the maximum concentration of mercury in maternal hair during pregnancy. The developmental milestone depicted in this figure is delayed walking determined in Iraqi infants prenatally exposed to methylmercury.

milder cases provided the opportunity to see if maternal hair levels during pregnancy predicted delayed development in the offspring.

Hair analysis (Figure 11) in Iraq allowed us to recapitulate exposures during pregnancy. This hair sample was collected in 1973, many months after pregnancy. Hair grows about 1 cm per month. Therefore one can go back in time, in this case for over 2 years, by measuring mercury in the hair centimeter by centimeter from the scalp end. The period of intake is indicated by the rising levels in early 1972. Later the woman was admitted to a hospital, where it was possible to collect blood samples. One can see the remarkable parallel between the hair and blood levels. Thus the collection of one hair sample, even months after the birth of the child, provided a complete recapitulation of blood levels throughout pregnancy.

The offspring were examined clinically at about 30 months of age, but the examinations were conducted under trying circumstances in the deserts of rural Iraq. Interviews were conducted with family members to determine the birth date of the infant, to confirm that prenatal exposure had occurred. The interviews also obtained information on the age at which the infant achieved developmental milestones, such as age at first walking.

The outcome of this prenatal study was a dose-response relationship based on peak level in the mother during pregnancy versus prevalence of delayed achievement of developmental milestones and the presence of neurological abnormalities such as exaggerated tendon reflexes (Cox et al., 1989). An example of a prenatal dose-response relationship is given in Figure 12. In this example, delayed walking was defined as not walking until after 18 months of age. One sees a low background prevalence unrelated to mercury levels. At higher mercury levels, the prevalence increases as the mercury levels in the mother rise. Given the scatter of the points, one cannot identify a precise threshold level, but, as the graph indicates, it could be as low as 10 ppm in maternal hair. This outcome, 10 ppm for delayed walking, contrasts with the adult threshold of 100 ppm for paresthesia, thus confirming the greater sensitivity of the fetal brain.

ii. Populations consuming fish or sea mammals. The Iraq studies raised the possibility that prenatal exposures maybe of concern in fish-eating populations. If the estimated prenatal threshold from Iraq were indeed as low as 10 ppm in hair, then people frequently eating fish could exceed this value. Several studies were carried out to test the possibility that prenatal exposure to methylmercury from fish consumption is associated with adverse effects.

In the 1970s, an epidemiological study was conducted by the McGill group on prenatal exposures in a Cree Indian population in the province of Quebec, Canada (McKeown-Eyssen et al., 1983). Neurological and developmental status was examined in 234 Cree Indian children aged 12 to 30 months from four communities in northern Quebec. No consistent relation could be found between hair levels in the mother during pregnancy and developmental outcomes in the offspring.

More recently, a study has been reported on a small group of infants exposed prenatally to methylmercury in fish in the Philippines. The study cohort consisted of 78 infant–mother pairs (Ramirez et al., 2000). Attempts were made to measure mercury in a number of biological media, including hair, blood, milk, and meconium. In most media, mercury was

detectable in only a minority of samples. Meconium gave the highest percentage of detectable mercury levels (46%). A barely significant ($p = .0469$) statistical correlation was found between mercury levels in meconium and the prevalence of small head circumference of the newborns. However, meconium has never been established as a reliable biological indicator medium for methylmercury.

In a follow-up study of 46 of these infants at 2 years of age and 45 control infants, the authors reported that the evidence suggested that "prenatal Hg exposure is correlated with lower scores in neurodevelopmental screening, but more so in the linguistic pathway." Other confounding factors could not be eliminated (Ramirez et al., 2003).

Most attention has been paid to three major epidemiological studies because of the large number of infant–mother pairs studied and careful control over confounding factors. One study was conducted in populations in New Zealand in the South Pacific (Kjellstrom et al., 1986, 1989), another in the Faroe Islands in the North Sea (Grandjean et al., 1997) and the third in the Seychelles Islands in the Indian Ocean (Myers et al., 2003).

(a). New Zealand Study. The New Zealand study group is comprised of three ethnic groups: Maori, Polynesian, and descendents of Caucasian immigrants. Fish consumption is somewhat episodic, such as weekly "fish and chip" meals, where the fish species is mainly shark with high levels of methylmercury, some as high as 4 ppm. The first report (Kjellstrom et al., 1986) described the collection of exposure information on 11,000 women, but the authors focused on 935 women who reported eating fish more than three times a week. Seventy-three women had average hair levels in pregnancy in excess of 6 ppm. The maternal hair strands were measured from the scalp end longitudinally centimeter by centimeter. As hair grows about 1 cm per month, these analyses yielded mercury levels on a monthly basis during pregnancy as well as average levels.

The 74 children of these women were designated as the high-mercury group. An attempt was made to match each child with a low-mercury reference child on the basis of maternal ethnicity, hospital at delivery, maternal age, and child age. The Denver Developmental Screening Test (DDST) was conducted on 38 high-mercury and 36 reference children at age 4 years, including 30 well-matched pairs; 52% of the high- versus 17% of the low-mercury children had either abnormal or questionable DDST results.

The New Zealand cohort was examined in more detail at 6 years of age (Kjellstrom et al., 1989). Each child with high maternal mercury (>6 ppm) was matched against three control children on the basis of ethnicity, sex, maternal age, maternal smoking, current maternal residence, and duration of residence in New Zealand. One control had a maternal hair level between 3 to 6 ppm, whereas the other two controls had maternal hair levels during pregnancy of less than 3 ppm. Fifty-seven fully matched sets of four and four incomplete sets gave a total number of 237 children that were examined. The mean maternal hair in the high group was 8.3 ppm with a range of 6 to 21 ppm, except for one mother having a hair level of 86 ppm. Information was collected on social class, medical history, and nutrition. In total, 26 psychological and scholastic tests were administered, covering domains of general intelligence, language development, fine and gross motor coordination, academic attainment, and social adjustment. Multiple regression analyses on average maternal hair levels were carried out on the results of these tests. Adjustments were many for covariates including maternal ethnic group, maternal age, maternal smoking and alcohol consumption during pregnancy, length of maternal residence in New Zealand, social class, primary language, siblings, sex, birth weight, fetal maturity, Apgar score, and duration of breastfeeding. Robust regression was used by assigning a weighting factor between zero and unity to test outcome, depending on the degree to which it was an outlier. Maternal hair levels were associated (p values ranging from .0034 to .074) with poorer scores on full IQ, language development, and gross motor skills. The poorer scores in the high-mercury group appeared to be largely attributed to children with average maternal hair levels in the range of 13 to 15 ppm during pregnancy, with the corresponding peak monthly average of 25 ppm. The maternal hair levels accounted for a relatively small amount of the variance in the outcome measures as compared to social class and ethnic group (NRC, 2000).

Crump et al. (1998) conducted a benchmark analysis of the New Zealand data. They noted that the statistical association of test outcomes with maternal hair was greatly influenced by one high maternal mercury level of 86 ppm, over four times greater than the next highest hair level. This high point was not an outlier as defined by the usual technical criteria (NRC, 2000). When the regressions on all 26 psychological and scholastic tests were repeated omitting the high point, 6 were adversely associated with mean maternal hair levels at $p < .1$. The benchmark results are reported later in Figure 13, and are discussed later with respect to the findings from Iraq, the Faroes, and the Seychelles.

(b). The Faroes Study. Episodic consumption of whale meat with average levels of about 1.6 ppm is the main source of methylmercury in the Faroese population (Grandjean et al., 1992). There is a concomitant intake of PCBs and other persistent organic pollutants from ingestion of whale blubber. A cohort of 1022 singleton births was assembled over a 21-month period from 1386 hospital births in the Faroe Islands (Grandjean et al., 1992).

Milestones of development during the child's first year of life were obtained by interview of the mother or visiting nurse (Grandjean et al., 1995). Complete records were obtained for 583 children. Three milestones usually achieved in the 5- to 12-month period were selected: the ages at which the child sits without support, creeps, and gets up into a standing position. The age of achievement of these three milestones was not associated with either cord blood or maternal hair mercury levels. However, as discussed later, breastfeeding of these same

infants was associated with beneficial outcomes despite intake of methylmercury from this source.

A preliminary functional neurological examination was conducted on 917 of these children at age 7 years (Dahl et al., 1996). The mean cord blood level was 23 ppb and the mean maternal hair level in pregnancy was 4.3 ppm. The exam focused on motor coordination and perceptual motor performance. The examination included tests for diadochokinesia, alternately opening and closing fists, finger opposition, catching a ball, and finger agnosia and double finger agnosia. Finger opposition was the only test where children with questionable or poor performance ($n = 425$) had higher cord blood mercury (23.9 ppb) versus children with a normal response (21.4 ppb, $n = 465$).

A comprehensive battery of neuropsychological tests followed this preliminary examination also at 7 years of age (Grandjean et al., 1997). The primary measure of prenatal exposure was umbilical cord blood mercury levels (mean 22.9 ppb, interquartile range 13 to 41 ppb, $n = 894$). The geometric mean of the maternal hair was 4.3 ppm, (interquartile range 2.6 to 7.7 ppm; $n = 527$). These tests included three computer-operated tests on finger tapping, hand–eye coordination, and a continuous performance test. Additional tests were tactile performance, three subsets of the WISC–R (digit span, similarities, and block design), the Bender Gestalt Test, the California Verbal Learning Test, the Boston Naming Test, and the Non-Verbal Analogue Profile of Mood States. Selected items from the Child Behavior Check List were administered to the parents. Multiple linear regression analysis with adjustment for covariates revealed that increasing cord blood mercury levels were associated with worse scores on finger tapping (preferred hand $p = .05$), continuous performance test in the first year of data collection (false negatives $p = .02$, mean reaction time $p = .001$), WISC–R digit span ($p = .05$), Boston Naming Test (no cues $p = .0003$, with cues $p = .0001$), and California Verbal Learning Test (short-term reproduction $p = .02$, long-term reproduction $p = .05$). For two endpoints, WISC–R Block Design and Bender Gestalt Errors, associations indicating adverse effects were found when an alternative approach to adjustment for confounders was applied.

The authors stated that regression coefficients were generally lower and less significant when maternal hair mercury was used as the independent variable, except for finger tapping. Otherwise the number of outcomes attaining statistical significance was not stated. Details were not given for the maternal hair analyses but presumably were reported in an earlier publication (Grandjean et al., 1992).

According to the authors, covariates included maternal intelligence, with additional ones chosen from "empirical and theoretical considerations." They reported that cord blood mercury levels were affected by alcohol intake in pregnancy, current place of residence, whether the mother was Faroese, whether the mother was "unskilled," whether children had day care, and whether there were older siblings. The finding that maternal alcohol intake reduces cord blood concentrations of mercury is especially interesting as it illustrates the potential for complex interactions between alcohol, mercury, and brain development. Not only can alcohol directly affect brain development but, by reducing cord blood concentrations, it may skew linear regression on cord blood levels to lower effect levels or perhaps even protect the fetus if lower cord blood means less mercury is crossing the placenta.

An additional set of analyses was conducted on these same data (Grandjean et al., 1998). A case group of 112 children with average maternal hair concentration of 10 to 20 ppm (median level 12.5 ppm) during pregnancy was compared with a "control" group with maternal hair less than 3 ppm (median 1.8 ppm). The two groups were matched for age, sex, year of examination, and maternal intelligence. Median cord blood levels of mercury also differed between the two groups (cases 50 ppb versus 12 ppb for controls). Six out of 18 endpoints in the case group scored significantly lower than the control group (one-tailed p value of .05). These endpoints were finger tapping (both hands), hand–eye coordination, WISC–R Block Design, Boston Naming Test (cues, no cues), and the California Verbal Learning Test (long-term reproduction). The outcomes were not identical to those in the main study (Grandjean et al., 1997). Moreover, in contrast to the main study, significant sex differences in response were found. In fact, in all the scores, adverse effects were seen for boys but not girls.

The results of the neuropsychological testing are summarized in Table 7. Numerous tests were reported to show adverse associations with cord blood mercury levels and to a lesser extent with maternal hair levels. These different outcomes are discussed in more detail later.

Electrophysiological tests were also carried out. From a total of 11 outcomes, only one was associated with cord blood mercury levels. This outcome, brainstem auditory evoked potential latencies at 40 Hz, peak V, had girls showing a significantly lower latency than boys. However, instrumental changes took place during the course of these measurements, and detailed examination of the evoked potentials revealed significant differences between the results obtained with the original versus those with a replacement instrument. A reevaluation of brainstem auditory potential was made on the readings from the original instrument (Murata et al., 1999). Delays in the brainstem auditory peak III and in the peak I–III interval were associated with either cord blood or maternal hair mercury levels during pregnancy. However, the authors noted that visual evoked potential latencies are known to be affected by nutritional factors, which were not measured in this study.

Some 878 children of this same cohort were examined for auditory evoked potential at age 14 years (Murata et al., 2004). Peak latencies and interpeak intervals were regressed against cord blood and maternal hair mercury levels. Latencies of peaks III and V increased with increasing mercury levels.

Cardiovascular risk factors were also examined when the Faroese children were 7 years of age (Sorensen et al., 1999). A generalized additive nonparametric statistical model was used to regress systolic and diastolic blood pressure against

TABLE 7
Outcomes in terms of adverse associations of neurodevelopmental tests with cord blood and or maternal hair levels of mercury in Faroese children (for further details, see text)

			Test outcomes		
Age (years)	Number	Type of test	Adverse	Total	Reference
7	890	Neurological	1	9	(1)
1	583	Milestones		3	(2)
7	894	Comprehensive[a]	5	20	(3)
7	894	Comprehensive[a,b]	7	20	(3)
7	914	Comprehensive[c]	1<5	20	(3)
7	112/272	Case control	6	18	(4)
1–109	Both	All	19	33	

Note: References: (1) Dahl et al. (1996); (2) Grandjean et al. (1995); (3) Grandjean et al. (1997); (4) Grandjean et al. (1998).

[a]Regression on cord blood mercury levels.

[b]With alternative adjustment for confounders.

[c]regression on maternal hair mercury levels during pregnancy.

cord blood mercury levels. Both measures of blood pressure increased by about 14 mm Hg for a 1 to 10 ppb rise in cord blood mercury levels. Above this mercury level, no further increase was seen. Birth weight acted as a modifier, with the mercury effect being stronger with lower birth weights. Heart rate variability decreased with increasing mercury levels but in boys only.

Cardiac autonomic activity was evaluated in this same cohort when the children were 14 years of age (Grandjean et al., 2004b). Increasing cord blood mercury levels were associated with decreasing in low-frequency (LF) and high-frequency (HF) powers and in the coefficient of variation of the R–R interval. However unlike the findings at 7 years of age, no discernible effect on blood pressure was apparent. The authors speculated that the decreased sympathetic (LF) and parasympathetic (HF) modulation of heart-rate variability might be caused by methylmercury toxicity to brainstem nuclei.

(c). The Seychelles Study. The Seychellois consume a wide variety of species of ocean fish on a daily basis, with average levels about 10 times lower than the other two populations (Shamlaye et al., 1995). Studies of prenatal exposure to methylmercury date back to the late 1980s, when a pilot cohort of 804 infant–mother pairs was established over a 2-year period (Myers et al., 1995a). Fifteen infants were excluded for maternal illnesses or infant characteristics that might affect the developmental outcomes. In total, 789 infants were evaluated between ages of 5 and 109 weeks by a pediatric neurologist masked to the mercury levels. Prenatal exposure was measured as the average maternal hair level during pregnancy. The mean maternal hair level was 6.1 ppm (range 0.6 to 36 ppm). A number of neurological tests were made, but the subsequent statistical analysis focussed on three endpoints: overall neurological examination, increased muscle tone, and deep tendon reflexes. The findings were judged abnormal, questionable, or normal. No association was found between maternal hair levels and abnormal or questionable findings. The same pediatric neurologist also administered the revised Denver Developmental Screening Test (DDST–R). A statistically significant association was found between maternal hair levels and the combined score of abnormal and questionable in the DDST–R test. The statistical analyses made use of multiple logistic regression. Adjustment was made for a number of covariates-including gender, birth weight, 1- and 5-min Apgar scores, age at testing, and child's medical history. Maternal covariates included tobacco and alcohol consumption during pregnancy and maternal medical history.

The pilot cohort was established to provide guidance for the main cohort. The latter was established over a 1-year period (1989–1990) and consisted of 779 infant–mother pairs, corresponding to about 50% of the live births during the same period (Marsh et al., 1995). After exclusion of 39 infants, 740 were examined at age 6.5 months by the same pediatric neurologist using the same neurological tests as for the pilot cohort and the DDST–R. The median maternal hair was 5.9 ppm (range 0.5 to 27 ppm). As in the case of the pilot study, the frequency of abnormal or questionable neurological findings was not associated with average maternal hair levels during pregnancy (Myers et al., 1995b). The Fagan Test of Infant Intelligence (FTII) was also administered to 723 children by the same examiner. This test is a measure of visual recognition memory or novelty preference. The mean percentage novelty preference and visual attention time (the time to reach visual fixation criteria) were not associated with maternal hair mercury levels.

When the children were 19 months of age in the main cohort, the primary caregiver was questioned about the age of

achievement of a number of developmental milestones (Myers et al., 1997). The latter included age the child first walked without support, or spoke words in addition to "mama" and "papa." Some 738 children were included. The mean maternal hair level was 5.8 ppm (range 0.5 to 27 ppm). Three statistical models were used: standard multiple regression and segmented linear models to estimate threshold as used in the Iraq study (Cox et al., 1989) and logistic regression using binary variables in which an abnormal response was defined as age 14 months or more. The only statistically significant association was found in boys but not girls, between maternal hair levels and age at walking, in regression models stratified for age and adjusted for covariates. The significance of the association disappeared when four statistical outlying points were excluded from the regression analysis.

Axtell et al. (1998) reexamined the same data on milestones of development employing semiparametric generalized additive models. This approach is less restrictive than those used by Myers et al. (1997), as the latter require assumptions about the functional form of the dose-effect relationship. Axtell et al. (1998) found that the relation between age at walking and maternal hair mercury was nonlinear, with walking appearing at later ages as maternal hair rose from 0.5 to 7 ppm, but above 7 ppm the direction of the relationship reversed. The increase in developmental delay in walking over the maternal hair range of 0.5 to 7 ppm was slight, amounting to less than 1 day. Given the finding that there was no consistent association over the entire range of maternal hair levels, that the apparent adverse association was found only at the lower hair levels, and that the magnitude of the effect was clinically and developmentally insignificant, this analysis does not confirm an adverse effect on milestones of development.

Davidson et al. (1995a, 1995b) administered the Bayley Scales of Infant Development (BSID) to children in the main cohort at ages 19 months ($n = 738$) and at 29 months ($n = 736$). The scales have two primary scores: the mental development index (MDI) and the psychomotor development index (PDI). Multiple linear regression analysis with adjustment for covariates found no association of the MDI scores measured at 19 and 29 months of age with maternal hair levels. A ceiling effect was found with the PDI scores, with some 200 children achieving the maximum score of 150. Consequently the PDI data were converted to a binary variable, splitting the distribution at the median score. The risk of the PDI score being below the median was not significantly associated with maternal hair levels. The BSID is believed to be the best test for detection of adverse neurodevelopment effects in children in this age group from exposure to a variety of environmental toxicants such as lead (Bellinger et al., 1997) and polychorinated biphenyls (PCBs) (Rogan and Gladen, 1991).

Six items of the Infant Behavior Record (IBR) were also completed for the main cohort at 29 months of age (Davidson et al., 1999). These six items assessed activity level, attention span, responsiveness to examiner, response to caregiver, cooperation, and general emotional tone. The maternal hair concentration was significantly associated only with activity level and only in males. Multiple linear regression revealed a decrease of 1 unit (in a scale of 1 to 9) in activity for a 10-ppm increase in maternal hair level.

Child development in the main cohort was intensively evaluated when the children were 66 ± 6 months of age (Davidson et al., 1998). The study on the main cohort followed a limited study on the 66-month-olds in the pilot cohort (Myers et al., 1995c). (In each case, the pilot cohort was used to prepare for testing the main cohort.) The pilot study revealed some adverse correlations with maternal hair, which largely disappeared when outlying points were excluded. Also, some important confounders were not included in the multilinear regression analyses.

Nevertheless, experience with the pilot group was a useful preparation for the detailed evaluation in the main cohort, where 711 of the original 779 children were included. The mean maternal hair level during pregnancy was 6.8 ppm (range 0.5 to 27 ppm). The six major neurodevelopmental domains assessed were general cognitive ability, expressive and receptive language, reading achievement, arithmetic, visual–spatial ability, and social and adaptive behavior. Multilinear regression with adjustment for covariates did not find any adverse correlations with maternal hair levels. In fact, one outcome in the expressive and receptive language domain (the preschool language scale) indicated improved scores with increasing maternal hair levels. The finding of "beneficial" association with mercury levels is taken up later, with the discussion on postnatal exposures.

The data of Davidson et al. (1998) were subjected to analysis for the impact of error variance in the maternal hair levels. Huang et al. (2003) were able to estimate the true error variance by comparing maternal hair levels to autopsy neonatal brain levels. The variance was estimated to be 6.6 ppm with a 95% confidence limit of 4.1 to 12.4 ppm. They made use of established statistical measurement error models for the independent variable, in this case maternal hair levels of mercury, to test the impact on regression analyses previously reported by Davidson et al. (1998) on the Seychelles cohort. The authors concluded that "adjustment for measurement errors in explanatory variables had no appreciable effect on the original results."

There was one interesting exception. In the original multilinear regression analysis, the preschool language scale total score improved with increasing maternal hair levels. When the error measurement correction was applied, this association became statistically insignificant. This had been the only finding of a beneficial association with prenatal exposures.

Of the original 779 children in the main cohort, 643 (83%) were reexamined at age 9 years. The mean maternal hair level in pregnancy was 6.9 ppm (SD 4.5 ppm). Individual neurodevelopmental tests measured intelligence, learning and achievement, memory, motor function, language, visual-motor integration, sustained attention, and behavior. The covariates included sex, examiner, family resource scale, family status code,

the Henderson Early Learning Process Scale (HELPS), Home Observation for Measurement of the Environment (HOME), child's age at testing, and medical history, maternal age, caregiver intelligence, socioeconomic status, and hearing ability. The covariates were selected a priori for the known influence on child development and were expected to provide an index of the effectiveness of the neurodevelopmental assessments. Multilinear regression analyses detected two endpoints that were associated with maternal hair mercury levels. Decrease motor performance, as measured by the grooved pegboard test, and improved scores in the hyperactivity index of the Conner's Teacher Rating Scale were associated with increasing maternal mercury levels. The authors were concerned that the large number of tests conducted in this study would give rise to multiplicity whereby a statistically significant outcome ($p < .5$) might actually be due to chance. If indeed this were the case—in other words, if no effects were present—the p values for all the tests should be evenly distributed between zero and one. They were able to demonstrate graphically that this indeed was the case.

The battery of tests used in this study, nevertheless, were able to detect effects of covariates known to affect child development, such as socioeconomic score, early home environment scores, and maternal IQ.

The Seychelles Child Development Study has found no convincing evidence of adverse effects on child development due to prenatal exposure to methyl mercury in ocean fish. Table 8 summarizes the outcomes of all the tests carried out on the Seychellois children from ages 6 to 109 months in both the pilot and main cohorts. From a total of over 60 tests of child development, only 4 indicated adverse associations, 2 indicated beneficial associations with maternal hair levels during pregnancy, and 1 was difficult to characterize. This is one of the largest cohorts ever examined and is the only cohort subjected to several evaluations over a 9-year period. Nevertheless, epidemiological studies, no matter how well conducted, can never prove the absence of risks but only set limits to the degree of risk. This topic is taken up later when the outcome from the Seychelles is compared to those of the New Zealand and the Faroes studies.

c. Comparison of Outcomes of the Three Major Studies with Iraq. It is possible to compare the outcomes of these three studies with the risk predictions from Iraq. All four studies have been analyzed to determine a no-observed-adverse-effect-level (NOAEL). Benchmark analysis attempts to estimate a NOAEL from dose-response data from an epidemiological study (Crump, 1984). The benchmark dose or BMD is defined as the dose corresponding to an arbitrary increase in prevalence

TABLE 8
Outcomes in terms of adverse, beneficial or no (neutral) of associations of neurodevelopmental tests with average maternal hair levels during pregnancy in Seychellois children (for further details, see text)

			Test outcomes		
Age (months)	Cohort	Type of test	Adverse	Beneficial	Neutral
1–22	Pilot	Neurological			3
		DDST	1		
6.5	Main	Neurological			3
		DDST			1
		Visual Recog.[a]			1
		Visual Memory[a]			1
19	Main	Milestones[b]			3
19	Main	Milestones			3
19	Main	MDI[c]			1
19	Main	PDI[c]			1
29	Main	MDI[c]			1
29	Main	PDI[c]			1
29	Main	Inf. Behavior	1(M)	5	
66	Pilot	Comprehensive[b]	1		8
66	Main	Comprehensive		1	5
109	Main	Comprehensive	1	1	19
1–109	Both	All	4	2	56

Note: (M) In males only.
[a]The Fagan Test of Infant Intelligence.
[b]After removal of outliers.
[c]The Bayley Scales of Infant Development.

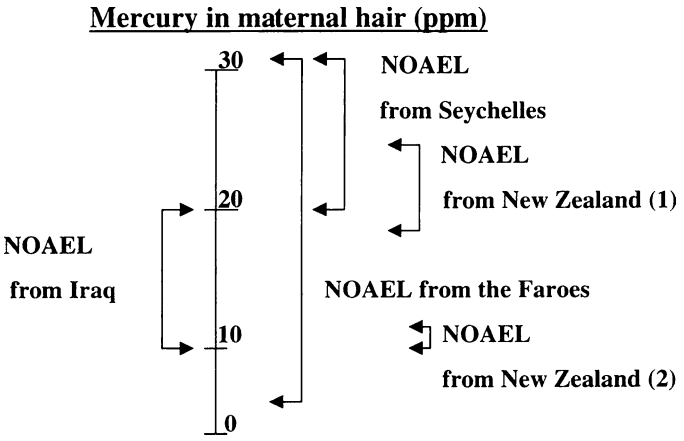

FIG. 13. A comparison of no-observed-adverse-effect levels (NOAEL) estimated from different epidemiological studies in populations prenatally exposed to methylmercury. The NOAEL is expressed as mercury levels in maternal hair during pregnancy. The range of 10 to 20 ppm was estimated from the Iraqi data by a World Health Organization expert committee (WHO, 1990). The NOAELs for the other studies are estimated as the lower 95% confidence limit (BMDL) of the benchmark dose (BMD), where the benchmark response rate was set at 10%. The BMDLs for the Seychelles are from Crump et al. (2000), for New Zealand from Crump et al. (1998), and for the Faroes from Budtz-Jorgensen et al. (2000). The New Zealand (1) estimate included all the data points, whereas New Zealand (2) omitted the highest mercury data point.

of an adverse response. It is usually assumed to be a 5 or 10% prevalence over and above any background prevalence in the study population. The statistical lower limit, termed the *benchmark lower limit* or BMDL, is calculated as two standard deviations below the BMD, the lower 95% confidence limit. This is assumed to represent a NOAEL. This type of analysis was conducted for the three major epidemiological studies. A NOAEL was estimated from the Iraqi data by a WHO expert group (WHO, 1990), based on the segmented linear analysis described in Figure 12. The group concluded that the Iraqi data predicted a risk of approximately 5% in adverse developmental effects in the range of maternal hair levels of 10 to 20 ppm.

Figure 13 is a graphical depiction of the NOAELs estimated from all four studies. The figure gives the range of uncertainty in these estimates. The estimates from all three studies on fish consumers include the range predicted from Iraq. Given the innate variance in studies of this kind, the agreement is remarkable. Keiding et al. (2003) in their comments on the outcomes of the 9-year Seychelles cohort (Myers et al., 2003) also pointed to the overlap of some test scores with those in the 7-year Faroes cohort. However, this agreement may be more apparent than real. The outcomes of benchmark analysis are highly dependent on a number of ad hoc assumptions that must be made to estimate the benchmark dose (Budtz-Jorgensen et al., 2001). Other assumptions might not give the overlap seen in Figure 13. Moreover, subsequent reanalysis of the Iraq data suggested another plausible threshold level considerably higher than the 10 to 20 ppm range estimated by the World Health Organization expert committee (Cox et al., 1995; Crump et al., 2000).

If we take the outcomes of the three major epidemiological studies at "face value," it would appear that fewer, if any, adverse associations with prenatal exposure were found in the Seychelles as opposed to the adverse outcomes reported from the Faroes and New Zealand. The key question is whether such differences are due to differences in methodology, population characteristics, or something else like concomitant exposures to other pollutants. This question has been at the center of debate since the first findings were published some 10 years or more ago.

One methodological issue is, which is the better marker for exposure: the average maternal hair level in pregnancy, or the cord blood level? The Faroes used both but claimed to get more adverse associations and statistical significance when cord blood was used as the independent variable. On the other hand, the New Zealand study, along with smaller studies in other populations, claimed to find adverse association with maternal hair. In the only comparison to date with actual autopsy brain levels, blood and hair samples appeared to predict brain levels equally well. Indeed, a meeting of experts specially convened by the National Institute of Environmental Health Sciences (NIEHS, 1998) to examine the differences in outcome of these studies concluded that these differences probably could not be explained by the use of different biomarkers.

Another methodological issue that had been raised was differences between the ages at testing. The NIEHS group at the time of its meeting only had available the $5\frac{1}{2}$ year age outcome from the Seychelles. They suggested that this younger age might not allow detection of adverse effects as compared to the tests conducted in the Faroes at 7 years of age. However the Seychelles has now published findings at 9 years of age (Myers et al., 2003), which, like the earlier study, found no adverse associations with prenatal exposure.

The outcome measures themselves appear to be sufficiently sensitive to detect subtle changes in neurodevelopment. Such tests detected the impact on outcome scores of covariates known to affect child development in all three studies. Indeed, both the NIEHS meeting and the NRC committee described the studies as well designed and well executed.

One therefore must seriously consider the alternative explanation, namely, that the different outcomes are due to differences in population characteristics or other factors. The Faroes population is exposed to PCBs and other fat-soluble organic pollutants through consumption of blubber from pilot whales. Attempts have been made to make statistical corrections for PCBs, which were measured in stored samples of umbilical cord tissue from about half the mothers in the cohort. Little influence of PCBs was found on the outcome scores, but the

success of the PCB correction depends on the assumption that the PCB concentration in cord tissue is a reliable indicator of PCBs in the target organ, the brain. The New Zealand mercury distribution is unusual in having one outlier in maternal hair many times higher than the next nearest level. There were no formal reasons for its removal. This, in itself, is a problem, as only when it is removed from the data analysis does the association with mercury attain statistical significance. The New Zealand Cohort is also unusual in that it consists of three ethnic groups, Caucasian, Polynesian, and Maori. These differences in ethnicity accounted for more variance in the outcomes than did mercury levels. How exactly correction was made for ethnicity is a key question in interpreting the outcome of this study. Marsh (1994) reviewed the design of the New Zealand study in some detail and raised concern about the findings.

Maternal smoking habits and alcohol consumption were only briefly reported in these studies, and paternal smoking (secondary smoke) not at all. As argued elsewhere (Magos and Clarkson, 2006), prenatal exposure to tobacco smoke and its metabolic products and exposure to alcohol are well-established risk factors for child development. It is highly unlikely that all three populations had similar smoking and alcohol consumption characteristics.

Perhaps the most important covariable in these studies and the one that has received least attention is diet. However, a person with a high fish intake must have a different diet than a low-fish-intake person, not only with regard to fish intake itself but with regard to the rest of the diet. The relevant dietary factors are more complex in the Faroese population, as the main source of methylmercury in the Faroese people is whale meat but they also consume whale blubber and codfish, both low in mercury but rich in micronutrients. A person with high whale meat consumption might eat less codfish. Likewise, a low whale meat consumer might eat more codfish. The overall result might be that child development appears to be inversely related to mercury levels whereas in fact it is determined by the intake of micronutrients.

However, by far the greatest differences within the three studies are the differing mercury levels in the seafood. Whale meat consumed in the Faroes has an average level of methylmercury of 1.6 (SD = 0.4) ppm (Julshamn et al., 1987). Thus, occasional meals may have methylmercury levels in excess of 2 ppm. The New Zealand "fish and chip" meals generally used shark with average methylmercury levels above 2 ppm and some reaching 4 ppm (Kjellstrom et al., 1986). In contrast, the Seychelles diet of ocean fish has average levels about 10 times lower (0.3 ppm; Myers et al., 2003). Thus the bolus dose of methylmercury reaching the brain after each seafood meal was about 10 times higher in the Faroes and New Zealand populations than in the Seychelles. A large bolus dose of methylmercury arriving at the surface of neuronal, astrocytic, and glial cells at critical stages of brain development may have lasting consequences to the cytoarchitecture and function of the central nervous system. This point is taken up in detail in a discussion of protective mechanisms presented in later pages.

In other words, the risk of damage to the developing brain may depend more on the manner in which mercury is presented to developing nervous tissue. Infrequent episodic high-mercury meals may be more damaging than frequent low-level meals even though the average body burdens may be similar. As discussed later, it may make more sense to reduce risks from dietary methylmercury by avoiding high-methylmercury fish as opposed to trying to control average daily intakes. The Harvard Center for Risk Assessment also concluded that avoiding intake of high-mercury fish is the best way to minimize risks from methylmercury but still enjoy the health benefits from consumption of low-mercury fish (Cohen et al., 2005).

To date, studies on the effects of prenatal exposure have not yet been extended to observations on adolescents. Adolescence is known to be a time when major changes occur in psychological development, physical growth, and diet. There is some animal evidence that effects of low exposures to MeHg early in postnatal development may not appear until later in life. This phenomenon has been demonstrated and replicated experimentally in mice (Spyker, 1975) and monkeys (Rice, 1996), but it has never been looked for in children. Latent deficits in animals ranged from sensory and perceptual motor deficits, to altered activity level and aggression, to immune system deficiencies. Animals exposed both prenatally and postnatally showed greater effects than those exposed only prenatally (Spyker, 1975).

In addition to the pioneering studies of Spyker (1975, discussed earlier), a number of reports describe the outcome of prenatal exposure in nonhuman primates (Table 9). Depending on the date of publication, these studies either anticipated or confirmed the findings on humans. In general, subtle changes in neurological and behavioral performance were seen in offspring of mothers who appeared themselves to be unaffected by methylmercury. One of the studies on primates (reference 2 in Table 9) used a visual recognition memory test (the so-called Fagan test) that was used in the Seychelles cohort. Of special interest to the ongoing human studies is the finding that adverse effects can appear in nonhuman primates late in life, in one case at 19 years of age (reference 5 in Table 9).

The lowest-observed-adverse-effect levels (LOAELs) reported in Table 9 in nonhuman primates may be compared with the estimated human NOAELs in hair reported in Figure 13. If we take the NOAEL as in the 10 to 20 ppm range from Figure 13, the equivalent long-term daily intake to achieve these hair levels would be roughly 1.2 to 2.4 μg Hg/kg/day for a 50-kg female. (The calculation assumes a blood half-time of 44 days, that 5% of the daily intake distributes to the blood compartment, and that the blood volume is 7% of the body weight.) The NOAELs in humans are an order of magnitude lower than the LOAELs in nonhuman primates. One reason is that they are calculated differently. The human NOAELs are the results of benchmark analysis, whereas the animal numbers come from a

TABLE 9
Effects of prenatal exposure to methylmercury in non human primates (M *fasicularis*)

LOAEL[a] (μg/kg/day)	Effect	Reference[b]
50–70	Retarded development	(1)
50–70	Impaired visual recognition memory	(2)
50	Adverse social behavior	(3)
10–50	Decreased visual contrast sensitivity (at 5 years of age)	(4)
10–50	Increased pure tone thresholds (at 19 years of age)	(5)
50–90	Deceased visual contrast sensitivity	(6)

Note: Adapted from table 5–11 of NRC (2000). Reference: (1) Burbacher et al. (1986); (2) Gunderson et al. (1986, 1988); (3) Burbacher et al. (1990); (4) Rice and Gilbert (1990); (5) Rice (1998); (6) Burbacher et al. (1999).

[a]Lowest-observed-adverse-effect level, expressed as dose to the pregnant animal.

[b]Although six references are cited, all these data came from only two research groups.

visual inspection of the lowest dose that produces a measurable effect. Another reason is that as much as half the body burden of methylmercury may be found in the fur of nonhuman primates so that the animal LOAELs should be halved to compare with human values. In any event, one expects a NOAEL to be lower than the LOAEL as a matter of definition.

Studies of prenatal exposures in rodents have been summarized and discussed by an expert group (NRC, 2000). The message is essentially the same as from the nonhuman primate studies except quantitative comparison with human findings is not possible due to large species difference in tissue deposition of methylmercury. Studies on animals related to mechanism of action are discussed later.

Observations on the severely affected brains from the infants in Iraq had indicated widespread damage to brain development. Animal studies confirmed that both cell division and neuronal migration were inhibited by prenatal exposure to methylmercury. At the molecular level, methylmercury caused the disappearance of microtubules. The latter are formed by a treadmilling process, with assembly from the protein monomers at one end and depolymerization at the other end. Methylmercury blocks the assembly process, preventing the addition of the tubulin proteins. The depolymerization process continues so that microtubules disappear (for a recent review, see Philbert et al., 2000).

The mechanism of action at lower levels of methylmercury associated with developmental delays is unknown and may differ from that seen in severe poisoning. This question is revisited when protective mechanisms are discussed.

d. Exposure in Childhood and Adolescence. Limited data are available on postnatal exposure except for mature adults. Clinical reports from the Iraq outbreak did not find many adverse effects in infants exposed during the first year of life. In a few cases infant blood levels were in excess of 1000 μg Hg/L, substantially above the adult threshold of 200 μg Hg/L (Amin-Zaki et al., 1974).

The study in the Faroe Islands found that enhanced achievement of developmental milestones was actually associated with increasing levels of mercury in the hair of 1-year-old infants (Grandjean et al., 1995). These authors noted that mercury levels correlated with the length of breastfeeding and suggested that the nutritional benefits of breastfeeding were responsible for the apparent beneficial association with mercury. When examined at 7 years of age, fewer adverse associations were found with current hair levels of mercury as compared to associations with prenatal levels (Grandjean et al., 1997).

The study of infant development in the Seychelles Islands also found beneficial associations with postnatal mercury levels (Davidson et al., 1998). When the 66-month-old children were examined by a battery of neuropsychological tests, several of these test outcomes indicated enhanced development associated with increasing mercury levels in hair. The authors suggested that this unexpected outcome was probably due to the nutrition benefits of fish consumption. Mercury levels in hair are known to correlate with fish consumption when fish are the only source of methylmercury, as is the case in the Seychelles. However, when examined at 9 years of age several test outcomes indicated an adverse effect on child development (Myers et al., 2003).

A complete picture of the age dependency of susceptibility to methylmercury still remains illusive. The available data suggest that in infancy and early childhood, the nutritional benefits of breastfeeding and fish consumption may outweigh any potential adverse effects of methylmercury. In later years when the rate of brain development is much slower, nutritional needs may be less important, thus allowing the toxic effects of methylmercury to become manifest. An important unanswered question is the potential adverse effects during adolescence as discussed for prenatal exposure.

e. Effects in Pregnant Subjects. Little information is available on the effects of methylmercury during pregnancy. The Japanese data, as discussed previously, gave a qualitative indication the offspring was more severely affected than the mother. All the epidemiological studies of prenatal exposure have focused exclusively on the offspring, on the assumption that the developing brain is more susceptible to damage from methylmercury than the adult brain. Likewise, animal studies of prenatal exposures have also focussed on the offspring. Studies of methylmercury disposition in pregnant animals have indicated that fetal brain levels are substantially higher than

maternal brain levels. However, the current working hypothesis that the maternal brain in pregnancy is less susceptible than the fetal brain still remains to be tested.

6. The Role of Nutrition

Given the frequent mention of nutritional factors in the epidemiological studies discussed earlier; it is remarkable that so little attention has been paid to diet as an important covariate in studies of methylmercury exposure. It is unlikely that estimates of the actual health risk from methylmercury due to either pre- or postnatal exposure can be made without taking into account the important health and protective impact of diet.

Fish consumers enjoy the benefits of fish as a source of protein and a number of highly beneficial micronutrients. A recent study of over 7000 children revealed that fish intake by the mother during pregnancy and by the infant postnatally was associated with higher mean developmental scores (Daniels et al., 2004). The Harvard Center for Risk Assessment convened a panel of experts to assess the beneficial impact of fish consumption on stroke, heart disease and prenatal cognitive development. At the same time, they assessed the negative impact of intake of methylmercury from fish (summarized by Teutsch and Cohen, 2005). As a result of a quantitative risk analysis, the panel concluded that regulatory agencies should take great care to determine the impact of regulatory action on fish consumption in pregnant women and in the population in general (Cohen et al., 2005). Specifically if such regulatory action results in an overall decrease in fish consumption in pregnancy or in the general population, "the net public health impact is negative." Some of these micronutrients may enhance brain development, whereas others may neutralize the toxic effects of methylmercury (Strain et al., 2005).

Diet can affect methylmercury metabolism in the body. For example, Rowland et al. (1986) have demonstrated in experimental animals that a high-fiber diet can accelerate the elimination of mercury from the body. A recent report (Passos et al., 2003) from a study of fish eaters in the Amazon basin indicated that individuals consuming fresh fruit have much lower hair levels at the same daily intake of methylmercury as those not consuming fresh fruit.

C. Ethylmercury

1. Pathways of Human Exposures

Diethylmercury was first synthesized in Germany in the late 19th century for the treatment of syphilis (Hunter, 1969). The potent antifungal properties of both the methyl and ethyl compound were discovered soon after, leading to widespread applications in agriculture. The most used forms were the monoalkyl compounds such a methylmercury dicyandiamide and ethylmercury chloride.

An additional source of human exposure started in the early 1930s when ethylmercury thiosalicylate, under the trade names of thimerosal and merthiolate, was introduced as a preservative in many medicinal preparations and vaccines (Press and Risher, 2000). Multiple-use vials containing vaccines require a preservative, as the syringe needle comes into contact with the vaccine fluid many times. It was not until 2001 that this application of thimerosal came into question as a possible toxic hazard to infants (Ball et al., 2001). Currently it has been removed from most vaccines in the United States, but it is still used elsewhere, especially in developing countries, where the advantages of multiple use vials outweighs the putative toxic hazards from the preservative (WHO, 2002).

2. Disposition in the Body

Much less information is available on the disposition of ethyl- as compared to methylmercury. Maybe this is the reason why several papers (discussed next) compare the disposition of ethyl- with the larger body of knowledge on methylmercury.

As noted earlier, most human exposure to ethylmercury is in the form of thimerosal. Suzuki (1973) observed in animal experiments that the tissue disposition patterns of mercury after equivalent doses of ethylmercury chloride or thimerosal were the same. Apparently the thiosalicylic acid anion attached to ethylmercury in the thimerosal molecule has no special influence on the fate of the ethylmercury in the body. Presumably the thimerosal molecule rapidly dissociates to release ethylmercury after injection (Tan and Parkin, 2000; Reader and Lines, 1983).

Magos et al. (1985) compared the disposition of ethyl- to methylmercury when given to rats as the chloride salts. Methylmercury produced higher brain levels of the organic species than did ethylmercury, whereas the brain levels of inorganic mercury were higher after ethylmercury. The kidneys were the site of the highest levels of inorganic mercury.

Rats appear to be a unique species as far as deposition of methyl- and ethylmercury in blood is concerned. The organ to blood concentration ratios, including the brain, are substantially lower than in any other species. For example, in several studies, the brain to blood mercury concentration ratio in methylmercury-exposed rats was less than 0.1, while in monkeys it ranged from 2.7 to 5.3 (reviewed by FAO/WHO, 2000). In this respect, alkylmercury behaves like akyltin compounds, which also have high affinity with rat hemoglobin (Rose and Aldridge, 1969). Interestingly in contrast to the alkyl mercurials, the thiethyltin cation has little affinity with thiol radicals.

Burbacher et al. (2005) recently reported on the disposition of mercury in infant monkeys given ethylmercury in the form of thimerosal. A comparison group was dosed with a methylmercury compound. In general, this experiment confirmed the findings of Magos et al. (1985) in rats. Levels of inorganic mercury were higher and of the organic moiety lower in the brain after thimerosal. The kinetics of buildup and clearance of total mercury in the blood compartment also differed. The one-compartment model best described blood concentrations after methylmercury, whereas a two-compartment model gave

the better description after ethylmercury. Moreover, the initial and terminal half-times of mercury in blood after thimerosal exposures, 2.1 and 8.6 days, respectively, were significantly shorter than the clearance half-time of mercury after methylmercury exposures of 21.5 days. The half-times in brain also differed. The clearance half-times for organic mercury in the brain were 58 days on average after methylmercury as compared to 14 days after ethylmercury.

If the observations on infant primates are any gauge in the fate of ethylmercury in human infants, the brain half-time of 14 days indicates that little ethylmercury would be accumulated in the approximately 60-day period between the two monthly vaccination schedule for the first 6 months of life. On the other hand, the clearance of inorganic mercury metabolized from both methyl and ethylmercury is so slow, at least 120 days, that accumulation in brain tissue would be expected. The neurotoxic potential of the organic versus the inorganic species of mercury is discussed elsewhere.

The concentration of total mercury in samples of whole blood has been used widely as a biomarker for absorbed dose of methylmercury and by implication the concentration in the target tissue, the brain. In fact a high correlation has been reported (Cernichiari et al., 1995a) between concentrations of total mercury in whole blood and corresponding concentrations in autopsy brain samples. However, such correlations can be expected only under steady-state conditions when blood and brain levels are no longer changing. However, whole blood cannot be used as a biomarker under non-steady-state conditions when blood and brain levels are changing since the half-times of clearance from brain and blood are significantly different. In the case of ethylmercury, as noted earlier, a half-time of 14 days for organic mercury in brain compares to two lower half-times of total mercury in blood of 2.1 and 8.6 days. As concluded elsewhere (Magos and Clarkson, 2006), whole blood levels of mercury cannot be used for predicting brain levels under the conditions of intermittent exposure from vaccines.

Information in humans is sparse. Suzuki et al. (1973) reported data consistent with a 7-day clearance half-time for total mercury in red blood cells in patients given thimerosal present in infused human plasma. Suzuki et al. (1973) also reported plasma concentrations, which allowed Magos (2003) to compute a clearance half-time from whole blood of approximately 18 days. Since methylmercury clears from blood with a half-time of 44 days, the ratio of methyl- to ethylmercury half-times in blood of adult humans is approximately 2.4 (44/18). The corresponding ratio in infant monkeys is 2.5 using the terminal half-time for ethylmercury of 8.6 days (21.5/8.6), suggesting that the disposition kinetics found in the infant monkeys may be applicable to humans.

Stajich et al. (2000) measured blood levels in pre- and full-term infants on average 60 h (48–72 h) after vaccination. The average concentration of total mercury in samples of whole blood was 7.36 and 2.24 μg Hg/L for pre- and full-term infants, respectively. The average body weights were 748 g and 3588 g, respectively. Assuming that the volume of the blood compartment in infants is 8% of the body weight (Diem and Lentner, 1971), the respective blood volumes of the pre- and full-term infants were 60 ml and 287 ml and would contain 0.44 μg Hg and 0.64 μg Hg, respectively. These amounts of mercury in the blood compartment correspond to 3.5% and 5.1% of the injected dose. These percentages overlap the figure of 5% for the initial deposition of methylmercury into the blood compartment of adults as noted in the previous section on methylmercury deposition. These estimates can only be approximate, given the high variance in the observed blood levels and that an unknown amount of mercury may have been excreted in the 48- to 72-h period before the blood samples were collected. However, in the absence of any other data, we may tentatively conclude that the initial distribution of ethylmercury to the blood compartment does not appear to differ greatly from that of methylmercury.

Pichichero et al. (2002) studied two groups of 20 infants, one age 2 months and the other 6 months, who received a normal pediatric schedule of vaccines containing thimerosal. Mercury was also measured in a control group of 11 infants 2 months old and 10 who were 6 months old who received thimerosal-free vaccines. Total mercury was measured in samples of whole blood, urine, and stools for several weeks after the last vaccination. The highest recorded blood level was 5.1 μg Hg/L in a 2-month old approximately 5 days after the last vaccination. Most blood levels were below 2 μg Hg/L.

The authors used a novel approach to calculate a half-time of clearance from whole blood for the combined groups. They estimated the expected blood levels based on the dose and assumed that the clearance from blood followed first-order kinetics (i.e., a single half-time), that 5% of the dose was initially distributed to the blood compartment, and that the volume of the blood compartment was 8% of the body weight. The calculated half-time was that which gives the best agreement between the calculated and observed blood levels. This half-time was determined to be approximately 7 days. Magos (2003) published the results of allometric calculations based on difference in body size, and concluded that 7 days in infants is what might be expected from the published adult half-times. This is in agreement with the terminal half-time estimated from infant monkeys of 8.6 days. These short half-times would indicate that virtually all of the mercury would be cleared from blood in the usual 2-month period between consecutive vaccinations during the first 6 months of life. If the data from infant monkeys predict half-times in brain as well as they do for whole blood, then, as concluded previously, one would expect most of the organic mercury but not the inorganic mercury to be cleared from brain tissue in the same 2-month interval.

Observations by Pichichero et al. (2002) on levels of mercury in samples of stool and urine indicate that substantial excretion is taking place via the fecal route. Urinary excretion appeared to be negligible. Thus, ethylmercury appears to behave like methylmercury where, as discussed in a previous section of this

review, fecal excretion accounts for most of the elimination from the body.

In summary, ethylmercury appears to be roughly similar to methylmercury in terms of its initial distribution to the blood compartment and in its fecal excretion. Methyl- and ethylmercury differ sharply in the patterns of tissue deposition and in the rate of metabolism to inorganic mercury. These large differences in disposition and metabolism indicate that the data on methylmercury are not a suitable reference for risk assessment for thimerosal.

3. Toxic Effects

The toxic effects of ethylmercury compounds were first revealed in animal experiments in the 1870s in Germany (Hunter, 1969). Prior to its therapeutic application in the treatment of syphilis, diethylmercury was given to animals. The toxic sequelae and pathology indicated that the central nervous system was the target. Incoordination of movement was a common finding. In fact, these early animal findings would be confirmed almost a century later in human outbreaks of poisoning.

An outbreak of ethylmercury poisoning that took place in rural Iraq in the 1950s gave more details on the toxic effects (Jalili and Abbasi, 1961). In this outbreak, homemade bread had been prepared from seed wheat treated with an ethylmercury fungicide, ethylmercury *p*-toluene sulfanilamide. The neurological signs and symptoms are similar if not identical with those already discussed for methylmercury. In addition, there was clinical evidence of kidney damage that included albuminuria and the nephrotic syndrome. It is possible that some of the fatalities in this outbreak were the sequelae of kidney failure.

Risk assessment for effects on the nervous system have been made by assuming that the dose-effect and dose-response relationships published for methylmercury apply to ethylmercury. It was on this basis that thimerosal, an ethylmercury compound, was removed from vaccines commonly given to infants and children (AAP and U.S. PHS, 1999). At the time of this decision it may have been the most prudent course of action. Since then, as discussed earlier, it has been shown that the kinetics of tissues disposition and metabolism differ from those for methylmercury. Also, case reports of ethylmercury exposure show striking differences from methylmercury in terms of blood levels associated with toxic effects in the nervous system (for details, see Magos and Clarkson, 2006).

Few animal studies have been reported. Magos et al. (1985) confirmed that both the brain and kidney were targets for ethylmercury compounds. They demonstrated that the higher brain mercury concentration in methylmercury-treated rats was associated with severe damage in the granular layer of the cerebellum. In ethylmercury-treated rats the brain mercury concentration was lower, and did not elicit the same degree of damage even when the dose of ethylmercury exceeded the dose of methylmercury and approached lethal toxicity.

Hornig et al. (2004) observed the effects of thimerosal on an autoimmune disease sensitive strain of mice (SJL/J). They reported growth delay, and behavioral changes such as reduced locomotion and exaggerated response to novelty. Examination of the brain tissues revealed densely packed, hyperchromic hippocampal neurons with altered glutamate receptors and transporters. Other strains of mice, C57Bl/6J and BALN/cJ, did not show such effects at the same dosing schedule.

Hornig et al. (2004) tried to emulate the 1-year dosing schedule of infants with a 10-day schedule in mouse pups. While the time intervals between vaccinations of infants assure nearly complete clearance from brain and blood of the organic species, even a 7–8 times higher clearance rate in mouse pups would not prevent significant accumulation in mice.

The mechanisms of toxic action of ethylmercury are unknown. Presumably the intact ethyl radical acts in the same way as the methylmercury radical on the nervous system. As discussed previously for methylmercury, intracellular reduced glutathione also appears to be protective for ethylmercury toxicity to neuronal cells (James et al., 2005).

Magos et al. (1985) concluded from their study that inorganic mercury split off from the ethylmercury radical appears to be the proximate toxic agent for kidney damage, whereas the intact mercurial was responsible for damage to the cerebellar neurons of the rat brain. These observations were conducted at high, near-lethal doses, resulting in severe damage. One might assume that the intact mercurial is also the neurotoxic species at lower subclinical levels. However, as noted in the previous section, the repeated intermittent exposure of infants to ethylmercury in vaccines would result in virtually complete clearance of the organic species from brain but the inorganic species would accumulate. The infant's brain at the end of the usual pediatric vaccination program would contain the inorganic but not the organic species of mercury, a situation that differs from the experiments of Magos et al. (1985).

As discussed in a previous section, the long-term exposure to methylmercury also results in accumulation of inorganic mercury in brains of adults. This inorganic species appears to be associated with selenium and is assumed to be present as an insoluble inert form (WHO, 1990). However, the role of inorganic mercury freshly deposited in infant brains on repeated exposure to vaccines is unknown at this time. The neurotoxic role of inorganic mercury metabolized from inhaled mercury vapor and from organomercurials is discussed further in the section on "Mercurial Mysteries" later in this review.

D. Other Organomercurials

1. Pathways of Human Exposures

A variety of other organomercurials have also found use as preservatives and antifungal agents. Phenylmercury compounds have been widely used as preservatives in pharmaceutical

preparations, as antiseptic agents in skin creams, in diaper washes, and as a spermicide. The class of alkoxyalkyl mercurials such as methoxyethylmercury compounds has rivaled their alkyl cousins as antifungal preparations for seed dressing. By and large the usage of these compounds has diminished as a result of stricter regulations against mercury compounds in general. However, these mercurials along with thimerosal still find their way into a number of commercial products (B. Lamphear, personal communication).

2. Disposition in the Body

In general, these compounds are well absorbed after oral and inhalation exposure. An outbreak of poisoning, discussed later, indicates that phenyl mercurials can also be absorbed across the skin. Virtually no data are available on humans. The overall disposition is dominated by rapid conversion to inorganic mercury. The conversion to inorganic mercury is so rapid that, within a few days of exposure, the disposition in the body is similar to that found after exposure to inorganic mercury (for a review, see Nordberg, 1986)

3. Toxic Effects

Exposure of infants to phenylmercury has been a cause of acrodynia. Indeed, the sudden appearance of cases of acrodynia in Buenos Aires led to the discovery that infants were being exposed to a phenylmercury compound used as a fungicide in a commercial diaper service (Gotelli et al., 1985). This fungicide was added to diapers to prevent spoilage as the used diapers awaited collection. The commercial diaper service decided to decrease the frequency of diaper collection so the service doubled the amount of phenylmercury added to the diapers. This increased quantity must have exceeded the threshold amount needed to cause acrodynia. This outcome is of toxicological interest in itself. Acrodynia was shown to occur in only 1 of about 500 infants equally exposed to mercury in teething powders (Warkany, 1965). The Argentine outbreak indicates that a threshold dose exists even for those sensitive infants prone to get acrodynia. Ethylmercury has been reported as a cause of acrodynia in one other case (Matheson et al., 1980). One assumes that the inorganic mercury released from the ethylmercury radical is the proximate cause as discussed previously in the section on inorganic mercury.

The discovery that exposure to phenylmercury was taking place in infants in Buenos Aires led to the examination of a cohort for evidence of effects on kidney function. Gotelli et al. (1985) measured the urinary excretion of the enzyme, gamma-glutamyl transpeptidase, as a marker of renal damage. This enzyme is located on the brush borders of proximal tubular cells so that even slight damage to the cell membrane results in a sharp increase in urinary excretion of this enzyme. Gotelli et al. observed that the rate of urinary excretion of this enzyme exhibited a "threshold" type of relationship with urinary mercury levels. The urinary excretion of the enzyme remained steady up to a "threshold" level of mercury in urine and thereafter increased with increasing mercury levels. The threshold mercury level was approximately 190 μg Hg/L of urine. It is not possible to compare this value with data in the literature, as no other studies on infants have been reported. As discussed previously in the section on inorganic mercury, the adult lowest effect level appears to be in the range of 50 μg Hg/L.

The mechanism of action almost certainly involves the mercuric ion as the proximate toxic agent. As stated earlier, conversion of phenylmercury to inorganic mercury is complete within a few days at least in animal experiments.

IV. PROTECTIVE MECHANISMS

As in the case of the body's protection against any toxicant, several general mechanisms may come into operation. At the cellular level, damaged cells can be replaced. This mechanism is especially effective with cells having high turnover rates, such as the epithelial cells of the intestine and proximal tubule in the kidney. In fact, repeated exposures to inorganic mercury appear to result in the replacement of proximal tubular cells with cells possessing a higher resistance to mercury (for a review see Nordberg, 1986). This mechanism of protection, however, is virtually absent in the adult brain, where neuronal cell turnover is absent, except possibly for certain neurons in the hippocampus.

At the molecular level, all cells possess mechanisms for replacement of damaged proteins. As most mercury in the cell is protein bound, this may be an important mechanism of defense against all forms of mercury. Although the mechanisms of protein removal and replacement are well understood in general, the effectiveness of this process may vary depending on the type and cellular location of each protein. Receptor proteins, located on the outer surface of the neuron, may be least protected by this mechanism, which is an intracellular process. As is discussed later, cell surface receptors may be a prime target for methylmercury.

Other protective mechanisms specially tailored to deal with mercury are also operative. Both inorganic mercury and methylmercury rapidly bind to reduced glutathione (GSH), present in most cells in millimolar concentrations. Not only does GSH divert mercury from binding to target proteins inside the cell, but it also serves as a means of removing mercury from the cells. Binding of mercury to other intracellular molecules also contributes to the overall cellular resistance to mercury. Inorganic mercury but not methylmercury can induce the metal-binding protein metallothionein. Binding to this protein is generally regarded as a detoxication process.

The conversion of methylmercury to inorganic mercury may be regarded as a detoxication process, at least in two cases: First, the demethylation by microflora in the intestines is a key step in fecal excretion; second, inorganic mercury split from methylmercury in the brain over long periods of time combines

with selenium to form an inert complex (WHO, 1990). How soon this protective mechanism comes into play is not known.

Inorganic mercury recently released from methylmercury should first appear in glial cells since, as discussed previously, phagocytotic activity is associated with the conversion of methylmercury to inorganic mercury. If the combination of inorganic mercury with Se takes place in these cells, the neuronal cell may never encounter inorganic mercury in its toxic form. Glial cells outnumber neurons by a ratio of approximately 50:1, so there is ample opportunity for glial cells to play a protective role not only for their contribution to the blood–brain barrier but as a buffer and metabolic defense for neuronal cells. Nevertheless, in other tissues such as the kidneys, inorganic mercury that is rapidly released from ethyl- and phenylmercury compounds is probably responsible for kidney damage seen after exposure to these organomercurials.

A. A Role for Evolution?

The earliest life forms on this planet, including the prokaryotic bacteria, may have been exposed to a storm of inorganic mercury at the time when oxygen levels started to rise in the earth's atmosphere. One can speculate that high levels of mercury vapor should have been present in the Archaean atmosphere from intense volcanic activity and with no mechanism of removal until the advent of oxygen. Upon the appearance of oxygen in the atmosphere, current global models for mercury would predict a deluge of oxidized mercury reaching the earth's surface in rainwater. The primitive cells at that time experienced a double toxic onslaught from mercury as well as oxygen. The survivors must have developed protective mechanisms against both toxicants.

The mechanisms of protection discussed earlier involve antioxidants such as reduced glutathione, metallothionein, and selenium. These ligands play a double role, both binding mercury cations and functioning as antioxidants. It is understandable that some of these protective mechanisms against both oxygen and mercury share a common evolutionary origin.

The fact that the evolutionary process has allowed the transport of methylmercury into cells on the ubiquitous large neutral amino acid carrier is at first sight quite a puzzle. Perhaps we have been looking in the wrong place for the site of action of this highly toxic form of mercury. It may be that pumping methylmercury into the cell is actually a protective mechanism. Perhaps the most vulnerable targets are on the surface of the cell. Rothstein has elegantly demonstrated such surface targets almost half a century ago for other metals (Rothstein and Maier, 1951). Examples have already been presented to indicate that the first toxic actions of methyl, ethyl, and inorganic mercury are on receptors on the cell surface. Thus Yuan and Atchison (2003) have shown that a primary action of methylmercury is its inhibition of the GABA receptors located on the membranes of Purkinje and granule neurons in the mature brain. According to Hornig et al. (2004), the primary action of ethylmercury compounds is on glutamate receptors and transporters in the membranes of neuronal cells in the hippocampus in the developing brain. McCabe et al. (2005) has concluded that the initial action of inorganic mercury on immunocompetent cells is on receptors in the outer membrane.

Consider, for example, a bolus of methylmercury appearing in the interstitial fluid bathing a neuronal cell following a meal of fish. The first point of attack would be receptors on the outer surface of the neuron. Pumping methylmercury into the cell would divert this metal to the cell interior, where an ample supply of thiol ligands will protect. In contrast, there are few if any protective ligands in interstitial fluid. Thus it is vital to remove the extracellular bolus as quickly as possible.

This mechanism whereby mercury is taken into cells as the first step in a protective mechanism was demonstrated many years ago in the case of bacterial resistance to mercury (for a recent review, see Barkay et al., 2003). A protein located on the outer membrane of the cells grabs hold of extracellular mercury using thiol ligands and transports the metal into the cell. The mercuric ion is transferred to a mercury reductase enzyme that reduces the divalent ion to the uncharged atom, which readily diffuses out of the cell to evaporate into the atmosphere. Organomercurials are first lysed to inorganic mercury before reduction to the elemental metallic species.

If these mechanisms involving extracellular toxic action and intracellular protection are operative in the brain, it would imply that the neuron is most vulnerable to high bolus doses of methylmercury and not to average brain levels where most of the methylmercury has moved inside the cells. In this connection, high concentrations of methylmercury were present in seafood consumed in the Faroes and New Zealand populations. Adverse effects were reported. On the other hand, no adverse effects were found in a study of seafood consumers in the Seychelles Islands, where mercury levels were much lower. If this indeed is the case, the time-honored way of regulating human risk based on excluding fish with high levels as practiced by the U.S. FDA is to be preferred over regulation based on controlling average daily intake as practiced by the U.S. EPA.

In fact an attempt was made to see if variability of mercury levels during pregnancy affected developmental outcomes in the main Faroese cohort (Grandjean et al., 2003). As a test of variability they compared differences in mercury levels between average levels in 8 to 9-cm lengths of hair roughly covering the duration of pregnancy, with a 2-cm segment corresponding to weeks 27–35 of pregnancy. Variability of mercury levels measured in this way had little impact on the regression outcomes. However, this type of measure of variability does not address the bolus issue discussed earlier. High bolus doses from whale meat can be repeated regularly throughout pregnancy and not give rise to the kinds of monthly variability as measured by Grandjean et al. (2003).

V. NO-OBSERVED-ADVERSE EFFECT LEVELS

The Swedish Expert Group (1971) was the first to attempt to determine levels in indicator media, blood and hair, above which

adverse effects of methylmercury might appear. They followed the time-honored tradition of examining dose-response data to determine an indicator level below which no adverse effects were observed (NOAEL). Based on the data from the outbreaks in Japan, they concluded that a no-adverse-effect level would correspond to 200 μg Hg/L whole blood and 50 μg Hg/g scalp hair.

Subsequently, WHO expert groups followed the same approach and established NOAELs for occupational exposure to mercury vapor, where urine was the indicator medium. Originally the NOAEL for occupational exposure was set at 150 μg Hg/L for nonspecific symptoms and 300–600 μg Hg/L for tremor (WHO, 1976). In a subsequent analysis, a WHO expert group was unable to determine a specific NOAEL due to lack of epidemiological data (WHO, 1991). Instead of using urinary concentrations they then adopted creatinine corrected urinary levels. At urinary excretion rates above 100 μg Hg/g creatinine, they concluded that the chance of developing classical neurological signs and proteinuria is high. (Since an average of 1.6 g of creatinine and about 1.6 L urine are excreted daily by an adult, the numerical values in urinary concentration in micrograms Hg per liter are practically identical to those in units of micrograms Hg per grams creatinine.) They also concluded that a variety of nonspecific symptoms might first appear at urinary excretion rates in the range of 30 to 80 μg Hg/g creatinine. Below these levels it was noted that there were insufficient data to conclude that adverse effects would not be found.

With respect to methylmercury, a WHO expert group concluded that peak maternal hair levels during pregnancy above 70 ppm is associated with a high risk (more than 30%) of neurological disorders in the offspring (WHO, 1990). They also concluded that a prudent interpretation of the Iraqi data implies that a 5% risk may be associated with a peak level of 10 to 20 ppm in maternal hair. As discussed in the previous text, more recent epidemiological studies do not necessarily invalidate this conclusion.

The NOAEL approach to determine minimum risk levels has the weakness that it actually depends on a few data points as a means of distinguishing a level at which adverse effects are seen. Its use still continues for occupational exposures to mercury vapor, but new approaches have been developed for methylmercury. These new approaches make use of all of the dose-response data to estimate threshold or NOAEL.

As discussed previously, Cox et al. (1989) made use of a threshold statistical model to estimate a NOAEL from data relating maternal hair levels in pregnancy to delayed development in the offspring (for example, Figure 12). Several measures were available for child development, including delayed achievement of developmental milestones and abnormal reflexes. The data were analyzed by segmented linear statistical model. The analyses revealed a low background prevalence, usually less than 5%, that was unrelated to mercury levels. However, as maternal hair levels exceeded a threshold level, the prevalence of delayed development started to increase as a linear function of the hair levels when plotted on a logarithmic scale. The intersection of the horizontal line for the background prevalence with the inclined line describing the increase with higher mercury levels was taken as the threshold or LOAEL. Its value fell between 10 and 20 μg Hg/g maternal hair for the various measures of delayed development and abnormal reflexes. These values were adopted by a WHO expert group as the NOAEL for methylmercury (WHO, 1990).

Subsequently, Crump et al. (1984) introduced the benchmark procedure. This too makes use of the entire range of data. A background prevalence rate of the adverse effect is assumed. The benchmark is the dose (or mercury concentration in the indicator media) corresponding to a 5 or 10% increase over the background prevalence. A statistical model is assumed to describe the dose-response relationship and thereby to calculate the benchmark dose. The 95% lower limit of the benchmark dose (LLBD) is then used as an estimate of the NOAEL. This procedure has been applied to the data on child development in the three major epidemiological studies on prenatal exposure to methylmercury, namely, the Faroes, New Zealand, and Seychelles studies. The results have been summarized in Figure 13. As stated previously, the ranges of NOAELs calculated from these three studies overlap the range of NOAELs calculated by the segmented linear model from the Iraqi data.

The benchmark method has been criticized (Budtz-Jorgensen et al., 2001). It involves a number of assumptions, and the choice of the statistical model to describe the dose-response relationship also affects the value of the estimated benchmark dose. The segmented linear model involves fewer assumptions as it calculates the NOAEL directly from the data.

The most recent approach has been the application of generalized additive models to examine the relation between measures of outcome and mercury levels. Such an approach was used to describe the relationship between measures of child development and mercury levels in maternal hair in pregnancy in the Seychelles Child Development Study (Axtell et al., 2000; Huang et al., 2005). These models do not in themselves identify a specific threshold concentration of mercury in hair. However, they do give an overall picture of the relation between effects and mercury levels and at least an approximation of where the threshold might lie. An example is given in Figure 14, taken from Huang et al. (2005), where one can see that developmental scores tend to decrease as mercury levels in hair exceed about 12 ppm.

VI. SAFETY AND UNCERTAINTY FACTORS

The application of a so-called "safety factor" is a time-honored tradition to reassure the public that food is "safe." The "folklore" story dating back over 50 years is that the then commissioner of the FDA was faced with a challenge of assuring the public that certain dyes were "safe" when added to certain food items. His only data came from animal studies. Objections were raised that these data might not be applicable to humans. In a moment of inspiration, he conceived the idea of a safety factor.

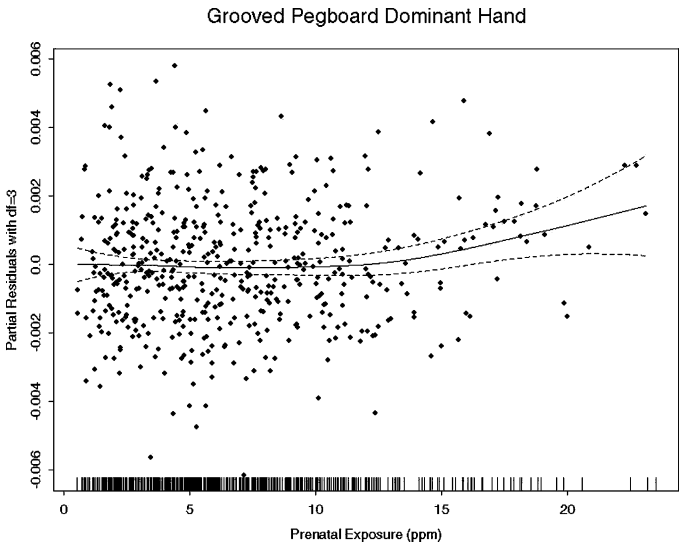

FIG. 14. The outcomes of the grooved pegboard test, corrected for covariates and expressed as partial residual scores, are plotted against the average maternal hair level during pregnancy. The curves were drawn using a semiparametric additive model with three degrees of freedom. The dashed lines are twice the pointwise standard error bounds. The verticle marks along the x axis illustrate the distribution of the concentration of total mercury in samples of maternal hair. This figure is taken from figure 1(a) of Huang et al. (2005). Copyright 2005 with permission from Elsevier.

Such a factor would be applied to the animal data to determine a safe daily intake for humans.

The idea soon gained wide acceptance. Guidelines were promulgated and frequently revised as to the magnitude of this factor that should be used for a given data set. The first estimates of a safe daily intake (with corresponding levels in indicator media) of methylmercury involved the use of safety factors. For example, the WHO took the NOAELs estimated by the Swedish Expert Group to estimate "tolerable weekly intakes" of methylmercury by applying a safety factor (for details see WHO, 1976). At that time NOAELs were available only for adult exposure to methylmercury. So a safety factor of 10 was used to take into account the suspected greater vulnerability of the developing brain. Thus the upper safe limits for blood and hair were 20 μg Hg/L whole blood and 5 μg Hg/g hair. They then used a pharmacokinetic model to convert these indicator media to a corresponding long-term "tolerable weekly intake" of 30 μg Hg/kg body weight per day.

This procedure has been followed ever since, although the numbers for the NOAELs and safety factors have changed. For example, a recent attempt to define a safe daily intake was undertaken by the U.S. EPA (for detailed discussion see Rice et al., 2003). It is customary for this agency to select one study as the basis of their so-called "reference" dose. This is really a safe daily intake as it is supposed to be protective for life time exposure to methylmercury. The agency chose the Faroes study and performed a benchmark analysis to obtain a NOAEL of 58 μg Hg/L whole blood and 14.5 μg Hg/g hair. Although the original WHO analysis applied a safety factor of 10 to the adult data from Japan, the US EPA applied the same factor of 10 to the prenatal data. This resulted in a roughly fourfold reduction in the safe daily intake down to 0.1 μg Hg/kg/day, as compared to the original WHO levels of 0.4 μg Hg/kg/day (equivalent to the tolerable weekly intake of 30 μg Hg/kg/day). The corresponding indicator media levels are 5.8 μg Hg/L whole blood and 1.4 μg Hg/g hair.

Some changes in terminology also took place. Tolerable or safe daily intake became called the "reference dose." The basic idea was the same, to make some attempt to account for the individuals that were most sensitive to methylmercury. An attempt was made to justify the assumed "uncertainty factor" of 10 when applied to the prenatal data. A half-time was used in the pharmacokinetic model to convert indicator media levels to long-term daily intakes. A factor of 3 was used to take into account the variance or "uncertainty" in the half-time. Another factor of three was used to cover any remaining range of prenatal sensitivity over and above the allowances made in the benchmark estimates. The two factors of three were "rounded off" to give an overall factor of 10.

It is clear that in the U.S. EPA derivation of the reference dose, two safety factors are involved, namely, the two standard deviations of the benchmark dose used to calculate the NOAEL and the 10-fold factor applied to the NOAEL. While it is prudent to err on the side of safety, the application of safety factors eventually should take into account the corresponding health risks if regulation of mercury intake at these levels takes away the benefits of fish consumption.

The U.S. EPA reference dose is based on studies of what is believed to be the most sensitive stage of the life span, namely, the prenatal period of brain development. It was derived using safety factors as described earlier. The purpose is to provide adequate protection from long-term exposure to methylmercury at any stage of life. The risks at the intake levels of the reference dose is assumed to be virtually nonexistent but in fact is unknown since an approximately 100-fold safety factor has been applied to the benchmark dose corresponding to a risk of 10%.

Even though the actual risks are unknown but assumed to be virtually zero, the reference dose has been used to estimate risks from methylmercury in the general population. Survey data are used such as hair levels of mercury in the general population to determine what percentage of the population is at risk. In this case the focus is on women of childbearing age. In this way a number is produced to indicate the number of children "at risk" of developmental retardation. These numbers can be large. An NRC expert committee calculated the number of 60,000 for the United States (NRC, 2000).

Such numbers can be misleading, as the reference dose was never designed to be used in this way. It is calculated as described earlier to give a prudent and approximate estimate of a safe daily

intake, namely, to err on the safe side. Exceeding this number by a small margin is meaningless in terms of actual risk, as the magnitude of the risk is unknown at these intake levels of mercury.

VII. THREE PUBLIC HEALTH DILEMMAS

Current health concerns have focused on human exposure to three different species of mercury: dental amalgam, methylmercury in fish, and ethylmercury in the preservative thimerosal in vaccines. These three modern faces of mercury have a number of things in common: Billions of people are exposed to them, health risks are uncertain, and each has an associated benefit. Further attempts to reduce human exposure present a dilemma that important benefits, including in some cases even health benefits, may be lost (Clarkson et al., 2003).

Dental amalgam, more than a century after its first introduction, remains the most efficacious tooth filling material. Other than a rare case of allergy, which is self-correcting, there is still no definitive evidence of adverse effects despite some 30 years of intensive evaluation of health risks. Its lifetime in the mouth is measured in tens of years. No alternative filling can match the degree of longevity. The costs are weighed not only in terms of the filling material but also in the number of visits to the dental office required for materials with shorter life.

The current contentious issue on health risks from methylmercury in fish also underlines a major public health dilemma. Despite almost 30 years of studies searching for adverse effects in human health from ingestion of methylmercury in fish, no clear answer has yet emerged. Figure 13 indicates a general concordance of the major epidemiological studies that the ranges of uncertainty in outcomes overlap. These large observational investigations in the Faroes, New Zealand, and the Seychelles are by their very natures fraught with uncertainties. The endpoint, child development, can be affected by numerous factors other than methylmercury. There is always a question as to whether all these have been taken into account and if so whether they were accurately measured. The statistical models for data analysis vary from one study to another with no clear reasons for the choice. These studies are conducted at the very limit of detection so that any signal for adverse outcomes on child development may be buried in the background noise. On the other hand, the cardiovascular benefits from fish consumption are well established. The most recent studies now identify a fish diet as beneficial for brain development and protection from stroke and heart disease (Daniels et al., 2004; Cohen et al., 2005). Thus the trade-off between risks to child development and benefits to brain development from micronutrients in fish becomes particularly acute for human intake of methylmercury, as fish is now the only source of human exposure.

In 1999, an apparent public health risk arose over the presence in vaccines of an ethylmercury preservative known as thimerosal. FDA scientists had calculated that the average daily intake of ethylmercury would exceed the newly established U.S. EPA guideline for methylmercury from the current schedule of vaccinations of infants over the first 6 months of life. This discovery amounted to a concern of almost crisis proportions leading to the removal of thimerosal from infant vaccines. This move was expensive since multiple-use vials had to be replaced with single-use vials, as the latter did not require a preservative. On the other hand, the World Health Organization realized that a switch from multiple- to single-use vials would be a logistic nightmare in developing countries as well as a severe if not crippling strain of the budget. Indeed, any reduction in vaccination coverage of infants would amount to a vastly greater health risk than any potential problems from ethylmercury. Perhaps it was the prudent thing to do in the United States but not in developing countries. As discussed earlier, subsequent studies indicate the assumption of toxicological equivalence between ethyl- and methylmercury appears not to be correct, with ethylmercury offering the lesser health risk.

VIII. MERCURIAL MYSTERIES

A great deal still needs to be understood about the toxicology of mercury, especially with regard to mechanisms of action. In fact, it is particularly difficult to identify specific mechanisms of damage with a highly reactive toxic agent such as mercury. Both the divalent inorganic and the organic cations can avidly and rapidly react with any thiol group. The binding is reversible so the metal can jump from one protein thiol group to another. Virtually any protein can be damaged if sufficient amounts of mercury are added, as already discussed with regard to *in vitro* studies. Eliciting the true mechanisms of action will require exquisite attention to the lowest effect mercury levels. Indeed, there may be no single specific mechanisms of action, and several parallel processes in the cell may be affected at the same time, even at the lowest effect levels of mercury.

Although many gaps in our knowledge have been indicated in the preceding text, some deserve special attention as they present interesting and challenging research questions.

Perhaps one of the most fascinating of the mercury mysteries is the long latent period between exposure to methylmercury and the appearance of signs and symptoms seen in adult poisoning cases. This latent period was most dramatically illustrated by the Dartmouth case. In this case, a single exposure occurred in August and signs of poisoning did not appear until the following January. This is the longest recorded latent period. Others such as in the Iraq outbreak were of the order of weeks to months but followed an exposure period also measured in weeks to months. When the first symptom, usually paresthesia, finally appears, it is followed rapidly, usually within 2 weeks, by more severe effects, even ending in coma.

This long latent period followed by a rapid onset of signs and symptoms that has been recognized for at least half a century still eludes a mechanistic explanation. Intuitively one might expect that the latent period would decrease with increasing dose. However, the length of the latent period does not appear to be related to the size of the dose, according to data from the

Iraq outbreak (Bakir et al., 1973). The Dartmouth case with the longest latent period had received such a high dose that it proved to be fatal.

Clearly some minimal threshold dose of methylmercury is needed to elicit a toxic effect. It appears that once this threshold dose is exceeded, a process is triggered that takes months to finally result in an abrupt appearance of signs and symptoms of severe poisoning. It seems unlikely that methylmercury plays any further role once it has triggered this "latent" process; otherwise, the length of the latent period would be dose dependent. Indeed, in the Dartmouth case, following the single exposure, hair levels of methylmercury fell exponentially such that the level at onset of symptoms was about an order of magnitude lower than the original peak level.

It remains a mystery why it takes so long for damage to occur and why the onset is so rapid after the appearance of the first sign of damage. If we knew more about the underlying processes, we would understand much more about the mechanisms of action of methylmercury.

Divalent inorganic mercury, Hg^{2+}, is believed to be the proximate toxic agent in the case of poisoning from inhaled mercury vapor, whereas it does not appear to be so in the case of methylmercury. It is a metabolic product from both the vapor and methylmercury and is produced in the same target organ, the brain. In fact, the distribution pattern within the brain following exposure to mercury vapor is the same as the pattern seen for inorganic mercury released from methylmercury (Warfvinge et al., 1992). It remains a puzzle why it is the proximate toxic agent in one case but not in the other.

This may be due to differences in metabolism. Hg^{2+} is produced from Hg° by the catalase hydrogen peroxide pathway. The process is rapid and takes place in all types of cells. On the other hand, methylmercury is converted to Hg^{2+} in phagocytic cells, and the process is much slower than the oxidation of mercury vapor. Perhaps its production in nonneuronal cells at a relatively slow rate may allow protective processes to spare the neuronal cells from damage.

Nevertheless, inorganic mercury is found in neuronal cells after methylmercury exposure. It is especially high after ethylmercury exposure, but the nature of the brain damage appears to be the same as with methylmercury. Thus the role of inorganic mercury split off from either methyl- or ethylmercury remains a mystery—why it is toxic when produced from mercury vapor but apparently not so or much less so from methyl- or ethylmercury

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