7

MERCURY

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INTRODUCTION

Mercury occurs in a number of physical and chemical forms in three oxidation states:

Hg^{o} (elemental or metallic mercury)
Hg_2^{++} mercurous mercury and
Hg^{++} mercuric or divalent mercury.

Mercuric mercury also forms a number of organo-metallic compounds. The short-chain alkyl mercurials, e.g., methylmercury (MeHg) compounds, possess high stability and unique toxicologic properties. Other classes of organomercurials such as phenylmercury compounds are highly unstable in mammalian tissues, rapidly breaking down to inorganic mercury.

OCCURRENCE AND HUMAN EXPOSURE

Environmental Cycling of Mercury

Mercury is emitted to the atmosphere by "degassing" of the earth's surface and by reevaporation of mercury vapor previously deposited on the earth's surface (Linquist et al., 1984). Of the estimated 30,000 tons/year emitted, about 20 percent occurs from the burning of fossil fuels.

Methylation is the first, crucial step in aquatic bioaccumulation of mercury. Synthesis of methylmercury compounds (Wood and Wang, 1983) occurs in sediments in fresh and ocean water. MeHg is accumulated rapidly by most aquatic biota. It thus attains highest concentrations in large predatory fish such as trout, pike and bass in freshwaters and tuna, swordfish and shark in ocean waters (Buffoni and Bernhard, 1982). Long distance transport of mercury and acidification of freshwater by acid rain may contribute to

increased methylmercury levels in fish where direct sources of mercury contamination are not identified (Hultberg and Hasselrot, 1981; Swedish Expert Group, 1971).

Levels in the Environment

The concentrations and physio-chemical forms of mercury leading to general human exposure have been recently reviewed (USEPA, 1984); occupational exposures are discussed later.

The atmosphere. Mercury vapor, Hg^0, is believed to be the predominant form in the atmosphere. Background (unpolluted) levels of total mercury are 2 ng Hg/m^3 in the northern troposphere and 1 ng Hg/m^3 in the southern (Lindquist et al., 1984). In regionally polluted areas, concentrations are 3-4 ng Hg/m^3 and may reach 10 ng Hg/m^3 in urban atmospheres (reviewed by WHO, 1976). Human intake and absorption of mercury may be estimated assuming all atmospheric mercury to be Hg^0 and adult ventilation to be 20 m^3/day. Thus in the northern hemisphere uptake from air would be 2 x 20 = 40 ng Hg and 32 ng Hg absorbed (80 percent retention of vapor) (Table 1). In urban areas, intake five times higher would not be unlikely.

Drinking water. The chemical species of mercury in drinking water is assumed to be mainly Hg^{++} compounds (Toribara et al., 1970). Kudo et al. (1982) reported that in certain polluted rivers MeHg compounds accounted for 30 percent of total mercury. However, most analytical methods lack the sensitivity to detect MeHg in drinking water (McLean, 1980).

Background concentration of total mercury in freshwater ranges from 10 to 50 ng Hg/l and averages 25 ng Hg/l (Fitzgerald, 1979); concentrations are rarely above 200 ng Hg/l (WHO, 1976). Levels in drinking water should be similar to those in freshwater since mercury is not used in water distribution systems. Daily intake of mercury would be 50 ng Hg (Table 1) based on a daily intake of 2 l water containing 25 mg Hg/l. Assuming the mercury is in the form of Hg^{++} and 10 percent absorption, daily retention would average 5 ng Hg, ranging up to 10 ng Hg.

Food. Data from a number of surveys and reviews (National Academy of Sciences, 1978; Swedish Expert Group, 1971; U.K. Department of the Environment, 1976; USEPA, 1984) indicate that fish and fish products are the

Table 1. Estimated Average Daily Intake and Retention of Various Forms of Mercury in Populations Not Occupationally Exposed[a]

Source	Estimated Daily Intake and Retention ng Hg/day					
	Mercury Vapor		Inorganic		Methylmercury	
	Intake	Absorbed	Intake	Absorbed	Intake	Absorbed
Atmosphere	40	32				
Water			50	5		
Food			600	60	2400	2160
Total intake	40		650		2400	
absorbed		32		65		2160

[a]For assumptions underlying the calculations of average daily intake, see text.

main dietary sources of MeHg. Most non-fish foodstuffs contain less than 20 μg Hg/kg although poultry and other meats occasionally have levels up to 200 μg Hg/kg fresh weight (USEPA, 1984). Indeed, a low intake of mercury from non-fish sources is consistent with reports that non-fish eaters have the lowest blood concentrations of total mercury (Swedish Expert Group, 1971).

Intake of mercury due to fish consumption has been estimated to be: 2000 ng Hg/day worldwide (Bernhard and Andreae, 1984); 2900 ng Hg/day in Belgium (Fouassin and Fondu, 1978); and 4700 ng Hg/day for the average USA population (USEPA, 1984).

Average daily intake of mercury may be estimated assuming: intake from non-fish food sources is negligible; 3,000 ng Hg/day intake from fish of which 80 percent is MeHg and 20 percent inorganic mercury. Thus the average intake of MeHg is 2400 ng Hg with 2160 retained (90 percent absorption). Average of daily intake of inorganic mercury would be 600 ng with 60 ng Hg retained (10 percent absorption) (Table 1). The average figures for mercury consumption should be viewed in the context of fish intake that has wide geographic variations. The Food and Agricultural Organization of the United Nations estimated an average worldwide fish intake of 16 g/day fresh weight but populations largely dependent on fish in the diet have average daily intakes of 300 g (FAO, 1980).

In the USA, in 1973 to 1983, daily intake for adult diets averaged 2000 to 7000 ng Hg, for toddlers, 1000 ng Hg, and for infants less than 1000 ng Hg (Gartrell, 1984). Comparing the data for total Hg for adults with that estimated for fish consumption alone, it would appear that non-fish sources may make a significant contribution.

Table 1 summarizes the calculations of average daily intakes and absorbed amounts of mercury. Food dominates both the intake and absorbed amounts of methyl and inorganic mercury. Intake from food may be underestimated as non-fish sources were not included. Also not included is exposure to inorganic mercury through silver amalgam tooth fillings (for further discussion, Clarkson et al., this volume).

Occupational Exposures

During mining and treatment of cinnabar ore (Hgs) exposure may occur to aerosols of the insoluble ore and to the vapor of metallic mercury. Occupational exposures also occur in a number of industries using metallic mercury or inorganic mercury salts (Table 2). The National Institute of Occupational Health and Safety estimated that approximately 44,000 people were occupationally exposed to mercury in 1975. Mercury consumption may not necessarily be related to the number of people occupationally exposed. The dental profession uses relatively little mercury but a substantial number of dentists and technicians may be exposed.

Current occupational standards in most countries limit average air concentrations not to exceed 50 to 100 ug Hg/m^3 (for details, see ACGIH, 1980). A World Health Organization expert group has recommended an occupational exposure limit of 25 μg Hg/m^3 (WHO, 1980a). Workers may still be exposed to levels close to these limits.

ABSORPTION, DISTRIBUTION, RETENTION AND EXCRETION OF MERCURY AFTER EXPOSURE TO MERCURY VAPOR

Routes of Absorption

Lung. About 80 percent of inhaled mercury vapor is retained in the body (Hursh et al., 1976; Nielsen-Kudsk, 1965a). In human subjects (Hursh

Table 2. Mercury Utilization in the United States[a]

Use	Metric Tons 1973	Projected 1985
Electrical apparatus	619	662
Caustic chlorine	450	482
Paints	260	107
Industrial Instruments	247	447
Dental Preparations	92	129
Pharmaceuticals	21	17
Total (rounded)	2,100	2,100

[a]Taken from USEPA (1980).

et al., 1976) and non-human primates (Berlin et al., 1969b) about 80 percent of the retained vapor passed directly from alveolar air into the bloodstream and 20 percent was deposited in lung presumably in the oxidized form of Hg^{++}

Skin. Early studies indicate that mercury was absorbed across the skin of humans (Juliusberg, 1901) and animals (Schamberg et al., 1918) exposed to metallic mercury. No further studies have been reported even though droplets of metallic mercury in footware or on clothing probably come into contact with the skin of workers using metallic mercury.

Deposition and Retention

Following a single short exposure to mercury vapor, ^{197}Hg distributed within about 24 hrs. to most regions of the body except the head where radioactivity peaked 2-3 days later. The kidneys accumulated the highest radioactivity - a finding consistent with animal data. Autoradiographic studies on animals (Berlin and Johansson, 1964; Berlin et al., 1966) indicate that after inhalation, mercury distributed preferentially to certain ectodermal and endodermal epithelial cells: intestinal mucosa; epithelial layers of the skin; salivary, pancreas and sweat glands; and testes and prostate.

Blood. The distribution of mercury in blood after experimental or accidental exposures to mercury vapor has been reported. In volunteers, mercury concentrations after initial distribution were approximately 2 percent of the total dose per liter of whole blood (Cherian et al., 1978). Uptake in red cells was complete in 6-13 minutes but in plasma reached a maximum at 5-10 hrs. The concentration of mercury in red cells was twice that in plasma for at least 6 days.

Half-times for mercury in blood after mercury vapor exposure are shown in Table 3. The initial decline in blood followed a half-time of 2 to 4 days (Cherian et al., 1978). In another study, a similar decline accounted for 88 percent of the loss of mercury from the blood (Hursh et al., 1980). After a brief, accidental exposure of 2 adult females to mercury vapor, the initial rate of decline (90 percent loss of total mercury) was followed by a slower phase (Table 3). In another occupationally exposed woman, blood collection began after the initial rapid phase of decline had been completed (Clarkson and Kilpper, 1978).

These limited data suggest that the fall in mercury concentration in blood can be described by two half-times - one of 2-4 days accounting for

Table 3. Summary of Half-times in Human Tissues after Exposure to Mercury Vapor

Body Compartment	Exposure Duration[a]	Observational Period (days)	Half-time(s) days
Blood[b]	20 min	0 - 10	3.3
Blood[c]	few hrs	0 - 30	2.4 and 15
Blood[c]	months	30 -	30
Lung[d]	20 min	0 - 40	1.7
Kidney[d]	20 min	0 - 40	64
Head[d]	20 min	0 - 40	19
Whole Body[d]	20 min	0 - 40	58

[a]Exposure to a concentration of 0.05 mg/m^3 (ref. c) or 0.1 mg/m^3 (refs. b and d); [b]Cherian et al., 1978; [c]Clarkson and Kilpper, 1978; [d]Hursh et al., 1976.

about 90 percent of the initially deposited mercury and another of 15-30 days accounting for most of the remainder. Follow-up studies have not been continued for periods long enough to detect the possibility of a still longer half-time component.

Lung. In experimental animals (Berlin et al., 1969a) 25 to 33 percent of the retained inhaled mercury was deposited in lung tissue. In volunteers, Hursh et al. (1976) estimated half-time in lung tissue to be 1.7 days. Thus it appears that removal from lung occurred more rapidly than for other tissues. This was confirmed by observations on non-human primates (Berlin et al., 1969b).

Brain. Volunteers inhaling radioactive mercury accumulated about 7 percent in the head region after a single brief exposure. In animals, the amount deposited was usually between 0.1 to 1 percent of the inhaled dose per gram tissue (Berlin and Johansson, 1964; Berlin et al., 1969a,b). In rats (Cassano et al., 1966, 1969), the highest concentrations of radioactivity were found in the cerebellum and brain stem nucleii.

Measurements on 9 subjects (Hursh et al., 1976, 1980) yielded an average value of 19 ± 1.7 days. Re-examination of the original data confirms that this is the best interpretation of the brain loss rate for the first 35 to 45 days (that is a loss of 75 percent). At longer times, precise measurements are more difficult and there may be an additional longer half-life applying to a minor fraction of the brain mercury burden. Newton and Fry (1978) reported head half-times of 23 and 26 days for 2 subjects who accidentally inhaled aerosols of irradiated mercuric oxide, 3 and 8 days before the assessments of body radioactivity began.

Long half-time components, as yet undetected, may exist. Berlin et al. (1975) noted that mercury concentrations in brains of squirrel monkeys continued to increase after two months of exposure to mercury vapor. Indeed, Takahata et al. (1970), Watanabe (1971) and Kosta et al. (1975) reported that mercury persisted in brains of workers for many years following occupational exposure to mercury vapor.

Kidney. The kidney region accumulated the highest levels of radioactive mercury in the experiments by Hursh et al (1980). In animals, concentrations in kidney were one or more orders of magnitude higher than other tissues in

the body (Hayes and Rothstein, 1962; Rothstein and Hayes, 1964). The kidney is the major tissue depot of mercury in the body.

The kinetics of retention of mercury in the kidneys of rats can be explained by two compartments (Gage, 1961). Shaikh (1983) also reported greater concentrations of mercury in male mice and rats than in females exposed similarly. Because the kidney is the major depot for mercury, the retention half-time in the kidney (64 days) was similar to that for whole body (58 days) (Table 3). It also explains the observations of Magos (1973) that chemicals that reduce kidney concentrations, elevate mercury throughout the rest of the body.

Fetus. No data are available on the rate of transfer of inhaled vapor to the fetus in humans. In pregnant rats exposed briefly to vapor on day 17 of gestation, the amount of mercury was 4 times greater in these fetuses compared to fetuses from mothers given an equivalent dose of $HgCl_2$; the fetuses were examined immediately after exposure (Clarkson et al., 1972). Dencker et al. (1983) reported that, after 60-minute exposures of mice to mercury vapor, more mercury was accumulated by the fetus at later stages of gestation. No information is available on long-term exposures.

In two reported cases of exposure of pregnant women to mercury vapor, the concentration of mercury in infant's blood was similar to that in the mother's blood at the time of delivery (Clarkson and Kilpper, 1978).

Excretion

Urine and feces are the main media of excretion. In the first week following exposure of volunteers to mercury vapor (Cherian et al., 1978), fecal excretion exceeded urinary excretion (Table 4). However after long term exposure, urinary excretion predominated, reflecting kidney accumulation of the major burden of mercury in the body.

A small fraction (7 percent) of the total body burden of mercury inhaled by volunteers was excreted in the expired breath (Hursh et al., 1976), with a half-time of about 18 hours; it accounted for 37 percent of the total excretion during the first week after exposure. Kobal and Stegner (1985) reported that mercury concentrations are higher in expired breath of mercury miners a few days following occupational exposures.

Table 4. Mercury Excreted in Urine, Feces, and Expired Air After Exposure to Mercury Vapor

Excretion medium	Type of exposure	Percent of total excretion
Expired air	short-term[a]	37
Urine	short-term[a]	13
Feces	short-term[a]	50
Urine	long-term[b]	58
Feces	long-term[b]	42

[a]Average excretion in the first week following exposure to 0.1 mg Hg/m^3 for a 20 minute period (Hursh et al., 1976; Cherian et al., 1978).
[b]The daily excretion in urine and feces was measured in individuals exposed for several years to 0.05 to 0.2 mg Hg/m^3 (Tejning and Ohman, 1966). Information on excretion by other routes was not available.

Silver amalgam tooth fillings may contribute substantial amounts of volatile mercury in expired breath (Gay et al., 1979; Svare et al., 1981; Vimy and Lorscheider, 1985). Concentrations were related to occlusal surface areas of the fillings and are specially elevated after chewing. Concentrations in expired breath can approach 50 to 100 μg Hg/m^3 (see Clarkson et al., this volume).

Quantitative information on excretion via sweat and saliva is not available. However, in workers experiencing profuse perspiration, amounts of mercury excreted in the sweat may exceed those in the urine (Lovejoy et al., 1974).

Significance to Biological Monitoring

Concentrations of mercury in blood, red cells and plasma are influenced by recent (within a few days) exposure to mercury vapor. At low levels of exposure, whole blood may not be useful due to the confounding effects of fish consumption; rather, plasma should be analyzed separately and speciation carried out. When exposure is high, as in certain industries, whole blood analysis may be useful.

Urinary excretion of mercury is used widely in monitoring workers exposed to mercury vapor (see USEPA, 1984). However, the relationship between urinary excretion and absorbed dose is not well understood; urinary excretion may be directly related to the kidney burden of mercury unless renal damage has occurred.

Following brief exposures (hrs. or a few days), the rise in urinary excretion was delayed a day or so compared to concentrations in blood or expired breath (Kobal and Stegner, 1985); this is consistent with mercury accumulation in kidney tissue prior to excretion. Fecal excretion has never been used for biological monitoring for mercury vapor.

Hair concentrations probably reflect absorption of Hg^0 from the atmosphere, not blood levels (Cernichiari et al., in preparation). Hair concentrations at the low levels found in the general population are dominated by intake of methylmercury from fish consumption.

ABSORPTION, DISTRIBUTION, RETENTION AND EXCRETION OF INORGANIC MERCURY

Compounds of Mercurous Mercury

Few data are available on the pharmacokinetics of compounds of mercurous mercury. Calomel is highly insoluble in water and poorly absorbed from the gastrointestinal tract. Some absorption must occur, however, as very high tissue levels have been reported (527 μg Hg/g in the kidney) on one individual who took calomel as a laxative over a long period (Weiss et al., 1973).

The intravenous administration of mercurous mercury (Hg_2^{++}) as calomel, to laboratory animals (rats, rabbits, and guinea pigs) resulted in the deposition of mercuric ions in kidney and red blood cells, as evidenced by histochemical methods (Hand et al., 1944). An autopsy report of an individual who had chronically ingested calomel indicated the presence of mercuric sulfide crystals in cells in kidney, liver, and intestinal tissues (Weiss et al., 1973). The mechanism of conversion of mercurous to mercuric mercury in mammalian tissues is unknown.

Compounds of Mercuric Mercury

Absorption. Data are lacking on pulmonary retention of compounds of mercuric mercury in humans. In the dog, approximately 45 percent was cleared in 24 hours, and the remainder had a half-time of 33 days (Morrow et al., 1964).

Approximately 15 percent of a tracer dose of mercuric nitrate given orally as an aqueous solution or bound to liver protein was absorbed from the gastrointestinal tracts of adult volunteers (Miettinen, 1973). Animal data confirm that gastrointestinal absorption is 15 percent or less (reviewed by Clarkson, 1972). Gastrointestinal absorption in suckling animals is higher, about 50 percent of an oral dose (Kostial et al., 1978).

Friberg et al. (1961) and Wahlberg (1965a) reported that $HgCl_2$ was absorbed across the skin of guinea pigs; Wahlberg (1965b), reported that lethal amounts could be absorbed.

Distribution and Retention. Mercuric mercury is transported in roughly equal concentrations in plasma and red blood cells (reviewed by Berlin, 1986). In plasma it is bound to different proteins depending upon dose, time, and method of administration.

Deposition in various tissues and organs varies with time, dose and route of administration. Data on steady-state tissue levels are lacking for both man and animals. After a single dose, the kidney is the main site of deposition; in several animal species, 30 percent of the dose was deposited in the kidney within 3 days. After two weeks as much as 90 percent of the remaining body burden was located in the kidneys (Rothstein and Hayes, 1960); concentrations in other tissues were much lower. Mercuric mercury penetrates, to a small degree, the blood-brain barrier and the placenta. Only 0.01 percent of the total dose of inorganic mercury was found in the brain of rats given daily doses of mercuric chloride for six weeks; 3 percent was found in the kidneys (Friberg, 1956).

The biological half-time was 42 days (SE ±3 days) in ten volunteers receiving a single oral tracer dose of inorganic mercuric compounds (Rahola et al., 1973) (Table 5). The five females had a half-time of 37 (±3) days and five males, 48 (±5) days. The half-time in red blood cells for 6 members of the same group was 28 (±6) days, significantly lower than the whole body. Newton and Fry (1978) reported half times for various organs of 2 subjects who inhaled radioactive Hg^0 aerosols (Table 5).

Table 5. Half-Times in Human Tissues after Exposure to Inorganic Mercuric Compounds

Number of Subjects	Body Compartment	Exposure Durationn	Observation Period days	Half-times days
6	Plasma[a]	single tracer dose	0-31	24
	Red blood cells[a]	single tracer dose	0-31	28
1	Lung[b]	hrs	3-85	2.1 and 20
1	Kidney[b]	hrs	16-136	53
1	Head[b]	hrs	3-85	23
1	Pelvis-Legs[b]	hrs	3-42	22
1	Whole body[b]	hrs	3-212	1, 20 and 78

[a]Rahola et al., 1973; [b]Newton and Fry, 1978.

It is of interest that the half-time in blood is similar to the longer half-time (15 to 30 days) after exposure to inhaled mercury vapor. This probably reflects the fact that inhaled vapor is oxidized rapidly to divalent mercury in body tissues.

Excretion. The urinary and gastrointestinal tracts are the principal pathways of excretion. Rahola et al. (1973), noted that 50 days after a single oral dose of mercuric mercury, urinary and fecal excretion were approximately equal in ten adult volunteers. Inorganic mercury is secreted in saliva and bile, and by cells of the large intestine. Excretion may also occur in sweat, and a small amount in expired air. Dunn et al. (1978) reported that the rate of exhalation of mercury in expired breath was proportional to the body burden of mercury in mice given parenteral doses of $HgCl_2$.

ABSORPTION, DISTRIBUTION, RETENTION AND EXCRETION OF METHYLMERCURY (MeHg) AND OTHER SHORT-CHAIN ALKYL MERCURIALS

Absorption

Information on routes of absorption remains essentially the same since the comprehensive review by the World Health Organization (WHO, 1976). Generally, absorption of inhaled MeHg compounds is high. In fact, poisonings have resulted from inhalation in occupational settings. Severe cases of poisoning have resulted also from topical applications to treat skin fungal infections. The contribution of skin absorption to occupation poisonings from exposure to aerosols is unknown.

When subjects ingested measured amounts of a MeHg compound, about 90 percent was absorbed into the bloodstream whether presented as a simple salt or attached to dietary protein (see WHO, 1980). Animal experiments (Walsh, 1982) indicate that the efficiency of gastrointestinal absorption remains constant with age.

Deposition and Retention

Distribution of MeHg to all organs and tissues was completed in about four days (Kershaw et al., 1980). In general, MeHg distributed much more uniformly to tissues than does inorganic Hg. Concentrations of MeHg were uniform whereas inorganic (Charbonneau et al., 1976) Hg (split off from MeHg) exhibits preferential accumulation in the kidney. As noted by Berglund and Berlin (1969), the fact that the elimination from the body is described by first order kinetics indicates that MeHg is so mobile that excretion is the rate-determining step.

Blood. Observations on volunteers taking a tracer dose (see WHO, 1976) or ingesting a controlled amount in fish (Kershaw et al., 1980) indicate that about 5 percent of the dose was in the blood compartment. When distribution was completed in animal experiments, concentration ratios between blood and tissues remained constant when the body burden was steady (after long term exposure) and also when it was falling. Thus the blood to brain ratio is usually between 5 or 10 to 1, blood to hair is about 1 to 250, and maternal blood to cord is 1 to 1.2. Appreciable individual differences are known to exist for blood to hair ratios (see Table 6), and large species differences exist in blood to brain ratios (Evans et al., 1977).

Biological half-times in blood have been measured both in volunteers given carefully measured doses and in individuals after cessation of accidental or high dietary exposure (Table 6). Half-times for blood were close to 50 days and within a range of 39 to 70 days. Blood half-times

Table 6. Average (range), Biological Half-times of Methylmercury, in Whole Body, Blood and Hair in Human Subjects

No. and Type of subject	Hg Intake µ/kg/day	Biological half-times (days) whole-body	blood	hair	(Ref)
Tracer					
3 adult	Tracer 1 dose	72 (70-74)	-	-	1
9 adult M	" "	79 (70-93)	50	-	1
6 adult F	" "	71 (52-88)	50	-	1
In Fish					
5 adult	<5 chronic	-	-	(33-120)	1
5 adult	<5 chronic	-	(58-164)[a]	-	1
5 adult	20 1 meal	-	52 (39-67)	-	2
20 adult	<3 100-day	-	50 (42-70)	-	3
Contaminated Bread					
16 adult	<50 to 2 mo	-	65 (40-105)	-	1
48 adult	" "	-	-	72 (35-189)[b]	1
14 children	" "	-	56 (39-72)	-	1
9 males	" "	-	68 (55-92)	-	1
10 females non-lactating	" "	-	79 (49-121)[c]	-	1
18 females lactating	" "	-	42 (30-58)	-	1

[a]One person had a biological half-time of 164 days, the other 4 were in the range of 58-87 days.
[b]The data were dimodally distributed. One group, (89 percent of the samples) had a mean of 65 days, and the other group had a mean of 119 days.
[c]One person had a half-time of 121 days and another 49 days, the other 8 were in the range 60-95 days.
[1]Quoted in EHC-1 (WHO, 1976); [2]Kershaw et al. (1980); [3]Sherlock et al. (1984).

overlap those for the whole body but average lower. This accounts for the observation that the amount of MeHg in blood constituted a decreasing fraction of the body burden with time after a single tracer dose (Miettinen, 1973). Lactating females had significantly lower half-times (average value 42 days) than non-lactating females (average value 79 days) - an observation confirmed in animal experiments.

Hair. MeHg is accumulated in hair at the time of formation of the hair strand; once incorporated its concentration remains constant and reflects the concentration in blood (Kershaw et al., 1980). Hislop et al. (1983) have noted a 20 day delay between concentration in blood and the concentration in an 8 mm sample of hair next to the scalp. With an average growth rate of approximately 1.0 cm/month, each 1 cm segment represents the average blood concentration during the month that this segment was formed. Depending on the length of the hair strand, it is possible to reconstruct blood levels over periods extending to several years (Amin-Zaki et al., 1976; Kershaw et al., 1980; Suzuki, this volume).

Observed relations between concentrations of Hg in samples of blood and hair of people having long-term exposure to MeHg in fish are given in Table

Table 7. The Relationship Between Mercury Concentrations in Blood and Hair Samples from People Having Long-term Exposure to Methylmercury from Fish

Country	Number of Subjects	Whole blood (x) (μg/liter)	Hair (y) (mg/kg)	Linear regression	Ref.
Canada	339	1-60	1-150	$y=0.30x+0.5$	1
Holland	47	1-40	0- 13	$y=0.26x+0.0$	2
Japan	45	2-800	20-325	$y=0.25x+0.0$	3
Sweden	12	4-650	1-180	$y=0.28x-1.3$	3
	51	4-110	1- 30	$y=0.23x+0.6$	3
	50	5-270	1- 56	$y=0.14x+1.5$	3
	60	44-550	1-142	$y=0.23x-3.6$	3
UK	173	0.4-26	0.1-11	$y=0.25x+0.6$	4
	98	1.1-42	0.2-21	$y=0.37x+0.7$	5

1. Phelps et al. (1980); 2. Den Tonkelaar (1974); 3. Quoted in EHC-1 (WHO 1976); 4. Haxton et al. (1979); 5. Sherlock et al. (1982).

7. The coefficient relating the blood concentration (x) to the concentration in hair (y) is about 0.25 but considerable differences are seen among studies. These differences may be due in part to measurement error and the possibility that individuals were not in true steady-state.

Half-times in hair may be determined by repeat sampling of short segments of hair from the same area of the head or from longitudinal analysis of the same strands of hair. They are only meaningful if high exposure has ceased. It is possible that some of the longer half-times seen in fish eating populations were an artifact from continuing exposures. As might be expected, half-times in hair closely follow those in blood. Hair half-times tend to have a wide range; for example, Shahristani et al. (1976) reported a bimodal distribution in 40 Iraqi subjects with average half-times of 65 days (89 percent) and 121 days (11 percent). It is possible that analytical artifacts may contribute to the wider range seen with hair (WHO, 1980). Nevertheless, animal data point to the importance of genetically determined individual differences (Doherty and Gates, 1984).

Brain. Quantitative data on deposition and retention in human brain are limited to observations on three male adults receiving a single oral tracer dose of a MeHg salt. A maximum of 10 percent of the dose was deposited in the head region - presumably in the brain. Maximum brain levels were attained in about three days. The decline in head radioactivity followed a first order process. The retention half-times were 70 to 90 days - not appreciably different from the whole body half-times. These human data are consistent with animal studies insomuch that brain and whole body half-times are approximately the same.

Autopsy data on brain levels of MeHg in Minamata cases who had died many years after the outbreak suggested that the half-time in brain was much greater than whole body half-times - of the order of 220 days (Takeuchi and Eto, 1975). However, the assumption that MeHg intake had ceased after the outbreak may not have been correct. In the single autopsy case of a Cree Indian who died two years after having blood levels as high as 600 μg/ml, the brain level was at background (Wheatley et al., 1979). In this individual, the brain half-time must have been less than 220 days.

Fetal Tissues. In fish eating populations the concentration of MeHg in cord blood was directly proportional to the concentration in maternal blood and average about 20 percent higher. Experiments on rats (Ohsawa et al., 1981) and on pigs (Kelman et al., 1982) indicate that placental transport of MeHg to the fetus increased toward the end of pregnancy. Autoradiographic data indicate that MeHg distributed readily to all tissues in the fetus in a variety of animal species (Berlin and Ullberg, 1963a; Dencker et al., 1983).

Excretion

The fecal excretion of Hg accounts for about 90 percent of the total in humans and other mammals after exposure to MeHg (for review, see WHO, 1976); urinary excretion accounts for most of the remaining 10 percent. Excretion in hair and sweat may make a minor contribution to total excretion.

Significance to Biological Monitoring

It is of special significance to biological monitoring that the accumulation and excretion of MeHg in man can be represented by a single compartment model. Accumulation in whole body or a tissue compartment is described by the equation:

$$A = (a \cdot b) \cdot (1 - \exp(-b \cdot t)) \quad (1)$$

where
A = the accumulated amount
a = the amount taken up by the body (or organ) daily
b = the elimination constant and
t = time

The elimination constant, b, is related to the biological half time, $t_{1/2}$ by the expression

$$t_{1/2} = \ln 2 / b \quad (2)$$

and a is related to the daily dietary intake (d) by the expression

$$a = f \cdot d \quad (3)$$

where f is the fraction of the daily intake taken up by the body (or organ).

The validity of equation (1) was tested by Sherlock et al. (1984) for blood mercury concentration for 100 days of MeHg intake and 100 days after intake was stopped in 20 volunteers consuming measured daily amounts of MeHg in fish. Predicted and observed values agreed closely.

Equation (1) predicts that a steady state (intake equals excretion) will be attained in about five half-times; in human adults, the whole body would attain steady state in about one year ($5 \times t_{1/2} = 350$ days). Thus an important prediction of the single compartment model is that continuous dietary exposure to MeHg for periods of years should not result in any greater accumulation than after one year's exposure. At steady-state, the accumulated amount Aoo, is given by

$$Aoo = a/b \quad (4)$$

In human adults, equation (4) predicts that the maximum amount accumulated in the whole body will be 100 times the average daily intake. In fact, steady blood levels may also be calculated from equations (2), (3) and (4) using the kinetic parameters of the single compartment model listed in Table 8. Thus the steady-state concentration in blood, Coo in ng Hg/ml is related to the average daily intake, d, in µg Hg as follows:

$$Coo = f \cdot d/b = 0{\cdot}95 \times d \quad (5)$$

Table 8. Principal Kinetic Paraments in the Single Compartment Model for Methylmercury in Humans

No. and type subjects	Dose (μgHg/kg)	No. of doses	Compartment: Whole body f	Whole body $t_{1/2}$ (days)	Blood f	Blood $t_{1/2}$ (days)	Ref.
3 adults	Tracer	1	0.95	72	--	--	1
15 adults	"		0.94	76	0.07	50	1
5 adults	20	"	--	--	0.05[a]	52	2
5 adults	3.3	100	--	--	0.05[a]	53	3
5 adults	1.5	"	--	--	0.055[a]	51	3
4 adults	1.1	"	--	--	0.057a	48	3
5 adults	0.6	"	--	--	0.064[a]	46	3

[a]Measurements were made of concentration in blood. The volume of f was calculated assuming blood values of 5 liters in a 70 kg. adult.
1. Quoted in EHC-1 (WHO, 1976); 2. Kershaw et al. (1980); 3. Sherlock et al. (1984).

assuming that 0.95 of the intake is absorbed, that 0.05 of the absorbed amount goes to the blood compartment, that the blood volume is 5 liters, and that the elimination constant is 0.01/day.

This predicted relationship has been compared with those observed in field studies of populations believed to have attained steady-state from long-term dietary exposures to MeHg in fish (Table 9).

The coefficients relating long term dietary intake to steady-state blood concentration are all lower than the predicted value of 0.95 calculated in equation (5) above. The reasons for this are not fully understood. The measurement of dietary intake in populations with uncontrolled intakes is liable to considerable error (Turner et al., 1980). However, this would not explain the consistently lower values from field studies. More likely, these populations were not in true steady-state as intake is frequently seasonal in fish eating populations. The close agreement among single dose-tracer studies, single dose fish intake of MeHg, extended controlled intake from fish and longitudinal hair analysis of individuals with very high intakes supports the validity of the single compartment model and of the kinetic parameters listed in Table 8.

Animal data indicate major ontogenic effects on biological half-times (Doherty et al., 1977). Suckling mice did not excrete MeHg; at weaning, excretion abruptly assumed the adult rate. Observations on infant monkeys confirm this finding (Lok, 1983). Biliary secretion was also virtually absent in suckling animals, and assumed the adult rate after weaning; biliary secretion of glutathione (GHS) shows parallel ontogenic changes (Ballatori and Clarkson, 1982). Microflora also had diminished capacity to demethylate MeHg in the suckling period (Rowland et al., 1983). Changes in diet have been observed to affect the biological half-time in animals (Landry et al., 1979, Rowland et al., 1983). In part, the diet might produce its effects by altering the demethylating capacity of the microflora. In view of these data it seems likely that human infants may also have a diminished excretion. Unfortunately, no direct observations have yet been reported.

It is clear from the one compartment model that the blood is an excellent biological indicator medium for the current body burden and probably

Table 9. Relation between Steady-State Blood Concentrations and Average Daily Intake of Methylmercury in Fish Consumers and Predicted Relations from Experimental Data.

No. of subjects	Duration of exposure	Average Hg intake (x) μ Hg/day/70 kg	Steady blood concentrations (y) μg Hg/l	Ref.
		Observed Relations		
32	years	1-800	y = 0.7x + 1	1
165	"	1-400	y = 0.3x + 5	1
20	"	1-800	y = 0.8x + 1	1
727	"	"	y = 0.5x + 4	1
22	"	"	y = 0.5x + 10	1
119	"	1-80	y = 0.15x+2.3[a]	2
	"	1-104	y = 0.24x + ?	3
98	"	1-160	y = 0.18x + 5.7[a]	4
		Predicted Relations		
15	1 dose	tracer	y = 1.0x	1
30	1-2 months	0-2340	y = 0.8x	1
5	1 dose	1400	y = 1.0x	5
20	100 days	1-230	y = 0.8x	6

[a]The authors noted the scatter in the data was so high that a linear relation between x and y could not be established.
1. Quoted in EHC-1 WHO (1976); 2. Haxton et al. (1979); 3. Working group on mercury in fish (1980) quoted by Sherlock et al. (1982); 4. Sherlock et al. (1984); 5. Kershaw et al (1980); 6. Sherlock et al. (1984).

for concentrations in the critical organ - the brain. Hair is useful to recapitulate past blood concentrations especially in epidemiological studies assessing exposure during pregnancy. In cases where a steady daily exposure is followed by an exposure-free period, the longitudinal concentration changes along the length of the hair strand can be described by the single compartment model. This allows calculation of the biological half time - the average daily intake, the period of exposure and the maximum blood level (Sharistani et al., 1976).

Urine is not a satisfactory indicator for MeHg as it is a minor route of excretion and mercury concentrations are therefore low.

PHENYLMERCURY AND RELATED COMPOUNDS

In the last decade, a number of general reviews on mercury have included the pharmacokinetics and metabolism of phenylmercury compounds (U.S. Environmental Protection Agency, 1980; Nordberg, 1976; World Health Organization, 1976). Phenylmercury compounds are usually assumed to be toxicologically representative of other aryl- and alkoxyarylmercurials (Goldwater, 1973). This assumption is somewhat tenuous as most of the available data are restricted to phenylmercury compounds. Furthermore, most of what is known about phenylmercury compounds comes from experimental animal studies.

Phenylmercury is rapidly metabolized to inorganic mercury. Following a single dose of phenylmercury, the absorption, transport, and initial tissue distribution of mercury are similar to those of other organic mercurials such as methylmercury. Within one week, tissue distribution and excretion approximate those seen after doses of inorganic mercury compounds.

BIOTRANSFORMATION

Vapor of Metallic Mercury and Inorganic Mercury Compounds

Inhaled mercury vapor is oxidized to divalent mercury by hydrogen peroxide - catalase compound I. (Clarkson et al., 1980; Halbach and Clarkson, 1978; Magos et al., 1978; Nielsen-Kudsk, 1965c). The catalase pathway appears to be the only pathway in red blood cells (Halbach and Clarkson, 1978) liver (Magos et al., 1978) and brain cells (Sichak et al., 1986). The reaction sequences are believed to occur as follows:

$$Cat\text{-}OH + H_2O_2 \rightarrow CatOOH + H_2O$$
$$Cat.OOH + Hg^o \rightarrow Cat\text{-}OH + HgO$$

The importance of the oxidation step in the retention of inhaled vapor is demonstrated by the inhibition by low concentrations of ethanol. For example, Nielsen-Kudsk (1965b) showed that workers who had ingested a moderate dose of alcohol retained about 50 percent less vapor than workers not ingesting alcohol. When volunteers took alcohol before being to exposed to mercury vapor, the deposition of mercury in the red cells was reduced almost by a factor of ten in individuals having alcohol blood levels less than 80 mg percent (Hursh et al., 1980). However, observations on both animals and man have followed single or brief exposures to vapor and alcohol. No information is available on the role of repetitive dosing with alcohol in animals or man continually exposed to mercury vapor.

That oxidation of mercury occurs in the lung tissue is suggested by the fact that treatment of animals with ethanol dramatically reduced the amounts of mercury deposited in lung tissue after brief exposures to vapor (Magos et al., 1973). Lung deposition was also reduced by ethanol in humans exposed to tracer doses of mercury vapor (Hursh et al., 1980).

The extent to which oxidation and reduction processes occur either in the placenta or in the fetus has not yet been thoroughly studies. The autoradiographic findings of Dencker et al. (1983) suggest that oxidation took place in fetal liver tissues.

The divalent (oxidized) form of mercury is also subject to reduction in mammalian tissues. This is evidenced by exhalation of vapor in mice and rats treated parenterally with mercuric chloride (Clarkson and Rothstein, 1964; Dunn et al., 1978, 1981b; Sugata and Clarkson, 1979). It is not known to what extent this reduction process applies to all tissues in the body; however, mercury will volatilize from liver and kidney homogenates of animals given divalent mercury and the volatilization rate is increased by adding ethanol to the homogenate (Dunn et al., 1981a). The mechanism responsible for the reduction of divalent mercury has not been described. Ethanol elicited a ten-fold increase in the exhalation of vapor in volunteers exposed briefly to mercury vapor. Exhalation of vapor was also increased by ethanol in volunteers given tracer doses of mercuric chloride (Smith and Kilpper, 1979) and in mice dosed parenterally with mercuric chloride (Dunn et al., 1981b).

The effect of alcohol in increasing volatilization of mercury *in vitro* and *in vivo* is probably due to its inhibitory action on the oxidation step,

preventing the reoxidation of the vapor. This proposed mechanism is supported by the finding that other inhibitors of catalase also increased volatilization of mercury (Dunn et al., 1981a; Sugata and Clarkson, 1979).

In summary, an oxidation-reduction cycle for mercury exists in mammaliar tissues. Mercury, as Hg^0, is probably the more mobile species whereas divalent mercury Hg^{++} is the more reactive species that binds more avidly to tissue ligands. This cycle, mediated by at least two enzyme systems, may be of profound importance in the disposition of inorganic mercury in the body.

Methylmercury and Short-chain Alkyl Mercurials

MeHg is converted to inorganic mercury, assumed to be Hg^{++}, in mammals (WHO, 1976). The fraction of Hg^{++} in tissues depends upon the duration of exposure to MeHg and time after cessation of exposure. The kidneys usually contain the highest fraction of Hg^{++}; the percentage of Hg^{++} in human tissues and body fluids in people exposed to high oral daily intakes for about two months (Amin-Zaki et al., 1976; Magos et al., 1976; WHO, 1976) were as follows: whole blood, 7 percent; plasma, 22 percent; breast milk, 39 percent; liver, 16 to 40 percent; and urine, 73 percent. Inorganic mercury accounted for about 5 percent of total mercury in whole blood and about 20 percent in samples of head hair taken from a population with high intakes of MeHg in fish (Phelps et al., 1980).

Conversion of MeHg to Hg^{++} is a key step in the processes of excretion. The fecal pathway accounts for about 90 percent of the total excretion of mercury after exposure to MeHg; virtually all the mercury in human feces is in the inorganic form. Fecal excretion begins with biliary secretion of both MeHg and Hg^{++} in bile mainly as complexes with glutathione (GHS). Inorganic mercury is poorly absorbed across the intestinal wall so that approximately 90 percent of the inorganic mercury secreted in bile passes directly into the feces. MeHg secreted into the intestinal contents is largely reabsorbed and may subsequently contribute to biliary secretion thereby forming a secretion-reabsorption cycle. This cycle has the effect of increasing the amount of MeHg passing through the intestinal contents and provides a continuous supply of MeHg to serve as a substrate for intestinal microflora. The latter were capable of converting MeHg to inorganic Hg (Rowland et al., 1978) which then became the major contributor to total fecal excretion. Presumably about 10 percent of the inorganic Hg produced by action of microflora was absorbed into the bloodstream and contributes to the concentrations of this form of mercury seen in tissues, plasma, bile, breast milk, and urine. As noted above, intestinal flora appeared to have a greatly diminished capacity to demethylate in suckling animals.

A number of studies investigating whether mammalian tissues break down MeHg have been reviewed by Takehashi and Suda (1980). Their recent studies indicate that macrophages present in spleen are active in methylmercury breakdown. It has been shown that selenide compounds were capable of cleaving the carbon-mercury bond (Iwata et al., 1982). To what extent this reaction occurs in vivo is not known. Given that the biological half-time in man of approximately 70 days and that virtually all the mercury is excreted in the inorganic form, it follows that the overall rate of breakdown of methylmercury in the adult human is approximately 1 percent per day.

Animals given radiolabelled methylmercury have a small percentage of inorganic mercury (usually less than five percent) present in the brain within a few days of a single dose. Thus, it is possible that a slow demethylation rate may produce a relatively high concentration of inorganic mercury in the brain in people with low exposures.

The methylation of inorganic mercury apparently does not occur in mammalian tissues, although Rowland et al. (1978) reported that rat intestinal flora were capable, in vitro, of methylating a minute fraction of inorganic mercury. Individuals occupationally exposed for many years to inorganic mercury did not exhibit elevated levels of methylmercury.

Phenylmercury

Phenylmercury is rapidly metabolized to inorganic mercury in experimental animals. In humans exposed to phenylmercury, the urinary mercury was mainly in the inorganic form (Gotelli, et al., 1985).

SPECIATION

Data on chemical and physical species of mercury in biological fluids and tissues are limited. The lack of sensitivity and selectivity of current analytical techniques is a major barrier to work in this field.

Vapor of Metallic Mercury and Inorganic Mercury Compounds

Blood. Hursh (1985) has shown that mercury vapor is more soluble in plasma, whole blood and hemoglobin solutions than in distilled water or isotonic saline. Magos (1967) and Satoh et al. (1981) identified dissolved elemental mercury vapor both in blood from mice exposed to vapor and in samples of human blood exposed in vitro to vapor. The fraction of mercury in blood present as dissolved vapor (defined as volatile mercury) is small, considerably less than 1 percent. A non-diffusible protein-bound or high-molecular-weight species has also been identified as the predominant form in plasma (Berlin and Gibson, 1963). The specific protein complex may depend upon the dose of mercury and, in one case, has been reported to be an albumin complex (Clarkson et al., 1961). However, other complexes with globulins have also been reported (Cember et al., 1968).

Brain. Little is known about the chemical forms of mercury in the brain. Ashe et al. (1953), in their studies of rabbits exposed to mercury vapor distinguished between "water-soluble and -insoluble" forms of mercury in brain homogenates. These probably correspond to the nonprotein and protein-bound forms of mercury reported by Cassano et al. (1966) in brain tissue from mice and rats repeatedly exposed to mercury vapor. These investigators were unable to detect any mercury associated with lipid material in the brain.

In autopsy, tissues from miners exposed to mercury vapor in Yugoslavia, there was a remarkable correlation between mercury and selenium being present in a one-to-one atomic ratio over a wide range of concentrations (Kosta et al., 1975). Observations on Japanese workers who died ten years after their last exposure indicated high residual concentrations of mercury in the brain (Takahata et al., 1970). Thus, it appears that some "trapping" of mercury may occur.

Kidney. The kidney has an enormous capacity to accumulate divalent forms of mercury perhaps related to metallotheine production in this tissue. Exposure of rats to high concentrations of mercury vapor induced metallothionein in kidney tissue that resulted in the binding of divalent mercury (Cherian and Clarkson, 1976; Sapota et al., 1974). Clarkson and Magos (1966) identified three classes of sulfhydryl groups in kidney differing in their affinity for ionic mercury. The highest-affinity sites corresponded to metallothionein in kidney tissue.

Urine. The different forms of mercury in urine are consistent with the observations of Henderson et al. (1974) on occupationally exposed workers;

these authors identified three distinct forms of mercury. One was elemental mercury, which could be removed by "degassing" the urine sample. A second form was released from urine by reduction with stannous chloride. A third could be liberated only after complete organic destruction and was probably the mercuric cysteine complex identified by Mudge and Weiner (1958), as this complex is known to be readily reducible by stannous chloride (Clarkson and Greenwood, 1970).

Although small-molecular-weight forms of mercury have not been identified in renal tissue, evidence from urine samples suggests that they exist. Mudge and Weiner (1958) identified mercuric cysteine complex in urine of dogs given mercurial diuretics. Other workers have identified a variety of molecular weight species of mercury in the urine (Piotrowski et al., 1973). Cysteine complexes in urine could originate from tissues other than kidney and might ultimately derive from the breakdown of protein complexes.

Stopford et al. (1978), studying workers exposed to 0.016-0.68 mg Hg/m^3 of mercury vapor, found Hg^0 in urine at levels less than 2 μg Hg/l. Yoshida and Yamamura (1982) confirmed these findings on thermometer workers. Elemental mercury accounted for less than one percent of total urinary mercury in these workers. The authors suggested that Hg^0 in blood was filtered at the glomerulus, whereas inorganic divalent mercury, Hg^{++} was first taken up by kidney tissue before being excreted in urine. The authors concluded that Hg^{++} in urine reflects kidney levels of mercury, whereas Hg^0 in urine reflects blood levels of Hg^0 and, therefore, very recent exposure to the metal.

Recently, Mulder and Kostyniak (1985) and Berndt et al. (1985) have shown that an inhibitor of the renal brush border enzyme, γ-glutamyl transpeptidase, dramatically increased urinary excretion of inorganic mercury. These data raise the possibility that inorganic mercury enters the tubular fluid as a glutathione complex and is reabsorbed in the renal tubule either in the form of the complex or after excess glutathione has been removed and the complex dissociated.

Fetal tissues. Little is known about the species of mercury in fetal tissues. Sapota et al. (1974) reported higher concentrations of metallothionein in fetal liver of rats than in maternal liver; exposure of the mother to mercury vapor lead to binding of mercury to metallothionein in the fetal liver. However, no increase in metallothionein concentration was observed in maternal or fetal liver. Webb (1983) recently reviewed the role of metallothionein in mercury metabolism in pre-and postnatal development. High concentrations of hepatic metallothionein in the newborn provided immediately available sites, binding mercury in replacement for zinc.

Methylmercury and Other Short-chain Alkyl Mercurials

The solubility of methylmercury chloride and other halide salts in nonpolar solvents has sometimes been interpreted to indicate that "methylmercury" is lipid soluble. The "lipid solubility" of methylmercury has been invoked to explain its rapid transport across cell membranes and to predict that MeHg will be sequestered in lipid and fat depots in the body. In fact, the methylmercury cation (CH_3Hg^+) is highly water soluble, and binds preferentially to sulfhydryl groups in proteins, peptides and amino-acids to form water soluble complexes. Analysis of tissues of animals exposed to MeHg indicate binding to the protein but not the lipid fraction (Yoshino et al., 1966).

MeHg binds to hemoglobin. Studies in inbred strains of mice have revealed that the type of hemoglobin may account for strain differences in red cell/plasma and in blood/tissue ratios of MeHg (Doi and Kobayashi, 1982;

Doi and Tagawa, 1983). MeHg is believed to bind to cystinyl residues in the hemoglobin molecule; the number and position of these residues vary in genetically different hemoglobins.

Information is sparse on the binding of MeHg to tissue ligands other than hemoglobin. MeHg was complexed to glutathione in red blood cells in both humans and animals (Naganuma et al., 1980). Animal data indicate that MeHg-glutathione complexes also existed in brain tissue (Thomas and Smith, 1979), in bile (Berlin et al., 1975b; Refsvik and Norseth, 1975) and probably in liver and kidney (Richardson and Murphy, 1975). Methylmercury formed a non-polar complex with selenium ($CH_3HgSeHgCH_3$) according to in vitro experiments (Magos et al., 1979). The formation of this complex may explain the redistribution of MeHg in experimental animals after administration of selenide salts. Human data are not available. Unlike inorganic mercury, MeHg does not induce or bind to metallothionein.

TRANSPORT PROCESSES

The Vapor of Metallic Mercury and Inorganic Compounds

Dissolved mercury vapor is the main transport species after inhalation of the vapor. To what extent dissolved vapor produced from reduction of divalent mercury plays a role in transport is not known. Mercury vapor is highly diffusible and soluble in lipids (Hursh, 1985). These properties account for its rapid and virtually complete diffusion across the alveolar membranes in the lung (Friberg and Vostal, 1972).

The transfer of mercury from the blood to brain is believed to occur primarily by diffusion of the dissolved vapor from the plasma since brain concentrations of mercury were approximately ten times greater in animals exposed to mercury vapor versus those exposed to equivalent doses of ionic mercury (Berlin and Johansson, 1964).

After doses of mercuric chloride to rats, Ballatori and Clarkson (1984) have shown that inorganic mercury was secreted in bile mainly as a complex with glutathione. Biliary secretion was determined by the rate of secretion of glutathione. This process commenced abruptly at weaning so that there was virtually no secretion of inorganic mercury in suckling animals.

The transport of mercury from mother to fetus is probably similar to uptake into brain. The amount of mercury found in the fetus was greater in animals exposed to mercury vapor than in those given an equivalent dose of injected mercuric salts (Clarkson et al., 1972). Also, the amount retained by the placental tissues was much less in the case of exposure to mercury vapor than in animals dosed with mercuric salts.

Methylmercury and Other Short-chain Alkyl Mercurials

Little information is available on the processes of transport of methylmercury compounds in the body. MeHg appears to cross cell membranes by diffusion of an uncharged complex of MeHg in vitro (Lakowicz and Anderson, 1980; Bienvenue et al., 1984). However, it is not clear that these experiments allow conclusions to be extrapolated in vivo. The lipid soluble compound, MeHgCl, may have been present in their system.

Complexes with low molecular thiol compounds appear to play a role in the transport of MeHg from blood to tissues including transport across the blood-brain barrier (Hirayama, 1980). The strongest evidence for the importance of thiol complexes comes from studies of biliary secretion (for review, see Ballatori and Clarkson, 1985). Over 90 percent of MeHg in bile

was present as a complex with reduced glutathione. A close correlation was observed between the rates of biliary secretion of MeHg and glutathione, both when glutathione secretion was inhibited and when it was stimulated.

TOXIC EFFECTS OF THE VAPOR OF METALLIC MERCURY

Local Effects

Acute exposure to high concentrations of mercury vapor may lead to metal fume fever and pneumonitis. Ten percent of the fatalities are due to lung damage (Garnier et al., 1981, Milne et al., 1970). In most cases, an acute gingio-stomatis was generally observed. In a recent review, Jaffe et al. (1983) noted that infants 4 to 30 months of age appeared to be more susceptible than older children and adults to the direct effects of mercury vapor.

Contact dermatitis may result from exposure to liquid metallic mercury when prolonged skin contact occured (Hunter, 1969). This manifests itself as a papular erythema with slight hyperkeratosis. However, this particular effect of metallic mercury has not been widely reported in the literature.

Systemic Effects

The major systemic effects of inhaled mercury have been described since antiquity (for reviews, see Ramazzini, 1713; Hunter, 1969; Goldwater, 1972). The major effects classically present as erethism, tremor and gingivitis. More recently, Trachtenberg (1969) and Smith et al. (1970) have described a milder form of poisoning involving a number of non-specific neurological and psychological symptoms. Most of the effects result from chronic exposure but one case report (Adams et al., 1983) indicates neurological effects after a brief but intensive exposure to mercury vapor.

Effects on the nervous system. Hunter (1969) noted that the mercurial tremor, though seldom the first sign to appear, is the most characteristic sign of mercurialism. Chaffin et al. (1973) noted a statistically significant correlation between mean tremor frequency and current urinary mercury concentration. Studies using specific psychomotor tests (Miller et al., 1975; Langolf et al., 1977; Schuckmann, 1979; Roels et al., 1982) indicate that tremor and other psychomotor abnormalities were likely to occur at urine concentrations above 500 μg Hg/1 and possibly at urine levels as low as 100 μg Hg/1 (Miller et al., 1975; Roels et al., 1982) (Table 10). These psychomotor effects are usually reversible (Wood et al., 1973).

Erethism - excessive introversion, delusions and mania - is now rare (Hunter, 1969), but less obvious behavior effects occur. Smith et al., (1970) found an increase in complaints of insomnia, loss of appetite, weight loss and shyness at the lowest levels of mercury vapor. Others (Angotzi et al., 1980; Chaffin et al., 1973; Langolf et al., 1978; Shapiro et al., 1983; Vroom and Greer, 1972; Williamson et al., 1982) have noted short-term memory loss and introversion as early effects of mercury vapor. The fact that motor changes were more easily detected and more likely to be reversed upon exposure cessation suggests, as stated by Hanninen (1982), that "the more insidiously developing cognitive decrements and emotional alterations may be the most harmful health hazard for mercury workers today."

Case reports and studies of occupationally exposed groups indicate that the peripheral nervous system was also affected by mercury vapor (Goldstein et al., 1975; Hryhorczak and Meyers, 1982; Iyer et al., 1976; Shapiro et al., 1982; Vroom and Greer, 1972). These reports recorded sensory neuropathies, limb weakness, and distal paresthesias. Levine et al. (1982) found that pro-

Table 10. A Summary of the Relationship Between Observed Effects and the Concentration of Mercury in Air and Urine

Observed effects	Frequency of effect	Minimum Mercury Levels Air[a] µg/m^3	Urine µg/g creatinine or µg/l[b]
Objective tremor	high	250	500
Objective tremor and psychomotor tests	low	50	100
Non-specific symptoms	low	50	100
Urinary proteins	low	25-50	50-100

[a]The ratio of air to urine concentrations in individuals having long-term occupational exposures is assumed to be 1:2 following the recommendations of a World Health Organization expert committee (WHO, 1980). However, as discussed in the text, the ratio may vary from 1:1 to 1:3 depending upon how the measurements were made (static versus personal samplers, hygienic conditions, etc.).

[b]Urinary concentrations of mercury in µg Hg/g urine is numerically similar to mercury in urine expressed as µg Hg/g creatinine. The latter may average about 20 percent higher on a group basis and will depend upon the degree of hydration of the subjects, lean body mass and other factors (for discussion see text).

longed motor and sensory distal latencies (nerve conduction tests) correlated with increasing urinary mercury concentrations from 20-450 µg Hg/l (group mean of 290 µ Hg/l).

Smith et al. (1970) have published the most comprehensive dose(exposure)-response relationship for neurological and behavioral effects. A dramatic increase in frequency of some symptoms and signs, such as loss of appetite, weight loss, insomnia, and objective tremors, took place at the highest time-weighted air concentrations (0.24 to 0.27 mg Hg/m^3), but such non-specific symptoms as loss of appetite, insomnia, and shyness may have increased at the lowest levels (0.01 to 0.05 mg Hg/m^3). The dose-response relationships did not exhibit any clear threshold.

Measurements of total mercury in spot urine samples may not be adequate to identify a threshold for effects on psychomotor function. More studies are needed where time-weighted air and urinary concentrations are used as these indicators may provide a better estimate mercury exposure and therefore, a better estimate of levels of mercury in the nervous system. Unfortunately, animal experiments have so far failed as appropriate models for man. The signs observed in animals are not obviously related to effects in humans, and very high concentrations are needed to produce behavioral and neurological effects.

Renal Effects. The nephrotic syndrome - albuminuria, edema and fatigue - has long been associated with occupational exposure to mercury vapor (Friberg et al., 1953; Kazantzis et al., 1962; Smith and Wells, 1960). Stewart et al. (1977) found a mild proteinuria in workers exposed to air concentrations up to 100 ug Hg/m^3 (median urinary excretion 53 µg Hg/24 hr).

Buchet et al. (1980) reported dose-response relations for mild effects (proteinuria, enzymuria) on kidney function after occupational exposure to

mercury vapor. The prevalence of abnormal urinary excretion rates did not relate well to the blood concentrations of mercury, but the excretion of high molecular proteins did correlate with urinary mercury levels. In contrast, no effects were seen in the excretion of the low-molecular-weight protein, β2-microglobulin. The authors concluded that mercury effects may occur at urinary excretion rates exceeding 50 μg Hg/g creatinine. The same group (Roels et al., 1982) performed a similar study on 51 control and 51 mercury vapor-exposed workers. Again there was no statistically significant effect on the excretion of low-molecular-weight proteins (β2-microglobulin) or on urinary excretion of albumin, but excretion of total protein was significantly affected. The median urinary concentration for the mercury-exposed group was 71 μg Hg/g creatinine (upper 95 percentile level, 245 μg Hg/g creatinine). Stonard et al. (1983) were unable to confirm effects on albumin but did find that two enzymes - N-acetyl-glucosaminidase and gamma glutamyl transpeptidase - were elevated in urine samples when mercury excretion exceeded 100 μg Hg/g creatinine in an occupationally exposed group.

Other effects. Limited information is available on the effects of mercury vapor on the early stages of the human life. There is one report (Mishonova et al., 1980) of effects on pregnancy and parturition in women occupationally exposed to mercury vapor; insufficient detail was available to evaluate dose-response relationships.

TOXIC EFFECTS OF INORGANIC MERCURY COMPOUNDS

Salts of both mercurous and mercuric mercury produce acrodynia (pink's disease) in infants and children (for review, see Cheek, 1980). The signs and symptoms include bluish-pink hands and feet, crimson cheeks, profuse sweating, painful joints, photophobia, glove and stocking paresthesias, irritability and other nervous disturbances. Only a small fraction of children exposed to inorganic mercury develop the full syndrome of acrodynia which has been reported to occur at urinary levels as low as 50 μg Hg/l.

Salts of mercuric mercury can produce acute renal failure when ingested as a single dose; the lethal dose of $HgCl_2$ in human adults has been estimated to be approximately 1 to 4 g (Gleason et al., 1957). Studies in animals indicate that long-term oral intake of divalent mercury can lead to kidney damage (Fitzhugh et al., 1950) probably mediated by immunological mechanisms (Druet and co-workers, 1978, 1982). Marked strain differences in susceptibility were reported by Druet's group.

Mercuric salts were teratogenic in animal tests (Gale, 1981). The relevance of these findings to human health risk is unknown.

Information is lacking on chronic human exposures to inorganic divalent mercury except for the report by Barber (1978) on two workers, exposed predominantly but not entirely to mercuric oxide, who developed neurological changes suggestive of the syndrome of amyotrophic lateral sclerosis (ALS). An additional 19 workers developed signs and symptoms that were considered an early phase of ALS. The neurological effects were reversible. Air levels checked at a later date were in the range of 0.5 to 5 mg Hg/m^3 with peaks to 10 mg Hg/m^3 of total mercury.

TOXIC EFFECTS OF METHYLMERCURY AND OTHER SHORT-CHAIN ALKYL MERCURIALS

The effects of methylmercury on the adults differ both quantitatively and qualitatively from effects seen after prenatal exposure. Thus effects on the mature human and animal are discussed separately from effects on developing organisms.

Effects on Adults

The effects of methylmercury on adults have been well described (WHO, 1976).

Nervous system. The nervous system is the principal if not the only target tissue for effects of methylmercury in adult humans. The sensory, visual, and auditory functions, and coordination, are most commonly affected. The earliest effects are non-specific symptoms, such as paresthesia, malaise, and blurred vision. Subsequently, signs appear such as concentric constriction of the visual field, deafness, dysarthria and ataxia. In the worst cases, the patient may go on to coma and ultimate death. In less severe cases, some degree of recovery occurs in each symptom after cessation of exposure; this is believed to be a functional recovery that depends on the compensatory function. The subjective complaint of paresthesia was found to be permanent in patients in the Japanese outbreak, whereas in the Iraq outbreak, paresthesia in many cases was transient. The reason for the difference is not known.

At high doses, methylmercury affects the peripheral nervous system in human subjects (Rustam et al., 1975). In Iraq, symptoms of neuromuscular weakness could be ameliorated by treatment with acetylcholinesterase inhibitors.

Methylmercury poisoning has several important features: 1) a long, latent period usually lasts several months; 2) damage is almost exclusively limited to the nervous system, especially the central nervous system; 3) areas of damage are highly localized (focal), e.g., in the visual cortex, and the granular layer of the cerebellum especially in the infolded regions (sulci); 4) effects in the severe cases are irreversible due to destruction of neuronal cells; 5) the earliest effects are non-specific subjective complaints such as paresthesia, blurred vision and malaise.

The reason for the long latent period is not understood. The mean latent period was 16 to 38 days in Iraq; in many cases initial symptoms appeared after the cessation of the intake of contaminated bread. In some cases in Japan a latent period of up to several years was reported (for discussion, see WHO, 1980). Such a long latent period may be partially explained by psychogenic overly which modified the symptoms, or subclinical lesions which may have been revealed by the aging factor. The long latent period itself, however, cannot be explained by slow accumulation of methylmercury in the brain or by a slow accumulation of inorganic mercury which has split off from the methylmercury.

The mechanism of the selective damage is not well understood. Syversen, in a series of publications, has claimed that the selective effects are due to the capacity of certain cells in the central nervous system to repair the damage initially inflicted by methylmercury (for discussion see Clarkson, 1983).

Numerous reports have appeared since the publication of EHC-1 (WHO, 1976) on the mechanism of action at the cellular and molecular levels (for more detailed discussion and references, see Clarkson, 1983; Berlin, 1986).

Non-nervous tissues. The only effect in humans not involving the nervous system is the claim of chromosome damage after long term exposure to methylmercury. No further reports have appeared on this subject since the review in EHC-1 (WHO, 1976) but animal and cell-culture experiments confirmed that MeHg damaged chromosomes (Curle et al., 1983; Gilbert et al., 1983; Mailhes, 1983; Morimoto et al., 1982; Watanabe et al., 1982). The mutagenic response of V79 Chinese Hamster cells to methylnitrosourea was enhanced by

methylmercury. MeHg has been reported to interfere with gene expression in cultures of glioma cells at low concentrations - 0.005 to 0.1 μM (Ramanujam and Prasad, 1979).

Effects in animal studies include interference with spermatogenesis in mice given a dose of methylmercury, (1 mg Hg/kg) much lower than doses giving rise to neurological effects (Lee and Dixon, 1975). Studies on effects of methylmercury on spermatogenesis in humans have not been reported (for a more detailed discussion of reproductive effects in animals, see Clarkson et al., 1983).

Methylmercury has been reported to produce renal carcinomas in rats (Mitsumori et al., 1981) and increase the tumor response to sodium nitrite and methylnitrosourea (Nixon et al., 1979). These are the only reports of potential carcinogenicity. Changes in the ultrastructure of kidney cells has also been reported in primates after chronic exposure (Chen et al., 1983). Kidney damage in humans has not been reported although it is not clear to what extent this potential effect has been pursued in clinical or epidemiological studies.

The significance of effects in animals is confounded by well established species differences (for further discussion, see Berlin, 1986).

Effects in Developing Tissue

The nervous system. Observations on both human subjects and animals indicate that the developing central nervous system is more sensitive to damage from methylmercury than the adult nervous system (for review, see WHO, 1976). The clinical picture is dose-dependent. In those exposed to high maternal blood levels the resulting cerebral palsy was indistinguishable from that caused by other factors. Microcephaly, hyperreflexia, gross motor and mental impairment sometimes associated with blindness or deafness, were the main features. At lower maternal blood levels, milder degrees of the infliction were seen. Such cases showed mainly psychomotor impairment and persistence of pathological reflexes - findings quite similar to the minimal brain damage syndrome.

Postmortem observations in Japan indicated that damage was generalized throughout the brain in the case of prenatal exposure in contrast to adult exposure where focal lesions were predominant. Prenatal poisoning resulted in disturbed development of cytoarchitecture of the brain; in severe cases the brain size was diminished (Takeuchi, 1968). Similar pathological findings were reported from autopsies of two prenatally exposed Iraq infants (Choi et al., 1978). These authors attributed these pathological findings to incomplete and abnormal migration of neuronal cells to the cerebellar and cerebral cortices. Subsequent in vitro studies lend some support to these conclusions (Choi and Lapham, 1980).

A second general mechanism whereby brain development could be impaired has been indicated in recent studies by Sager et al. (1984a). Methylmercury is known to inhibit cell division by mitotic arrest, similar to that observed with colchicine, presumably by disruption of the mitotic spindle (Ramel, 1969). Miura et al. (1978) noted that methylmercury inhibited the division of cultured mouse glioma cells. Sager et al. (1982) hypothesized that methylmercury might arrest division of immature neurons at critical stages of brain development. They administered methylmercury to newborn mice at a time when external granule layer (EGL) of the cerebellar cortex was rapidly dividing, and found a decreased number of granule cells in the treated animals as well as a decrease in the percentage of late mitotic figures. This incomplete mitosis may be responsible for decreased cell numbers which persisted to the time of weaning. Similar results have been obtained in other proliferative zones in the developing CNS after earlier exposure (Rodier et al., 1984).

The deranged cell migration reported by Choi et al. (1978) and the arrested cell division found by Sager et al. (1984) might both be an expression of the action of methylmercury on microtubules and thus be consistent with microtubule protein being an important molecular target for methylmercury in the developing brain (for discussion, see Sager and Syversen, 1986).

Effects on other developing tissues. Reported effects in humans are associated with damage to the developing nervous system. Experiments on hamster and rats indicate that methylmercury is a non-specific teratogen producing edematous, undersized fetuses.

TOXIC EFFECTS OF PHENYLMERCURY AND RELATED COMPOUNDS

Cotter (1947) found liver damage in ten subjects exposed to phenylmercury salts, and perhaps other substances. Renal damage and intestinal complaints have been reported (see review by Skerfving and Vostal, 1972). Goldwater (1973) found no evidence of toxicity in 13 workers employed for 11-23 years in the manufacture of phenylmercury. Exposure levels were not reported but urinary concentrations of mercury varied from 85-100 μg/l. In general, there seems to be no difference in the toxicity of various phenylmercurials which have been studied. The symptoms, where noted, resemble those of inorganic mercury.

In a recent exposure of an estimated 6000 infants to a phenylmercury compound, Astolfi and Gotelli (1981) reported that several infants developed acrodynia. In a follow-up study, Gotelli et al. (1985) reported that increased excretion of the renal brush border enzyme - γ-glutamyl transpeptidase - correlated with increased urinary excretion of mercury. Diuresis occurred at higher mercury excretion rates. Estimated threshold values from enzymuria were 4 μg Hg/kg-24 hr and for diuresis were 14 μg Hg/kg-24 hrs.

Long-term oral intake of phenylmercury acetate lead to kidney damage in rats (Fitzhugh et al., 1950) and pigs (Tryphonas and Nielsen, 1970). No effects on the nervous system have been reported. Phenylmercury compounds are teratogenic in animals and are potent spermicides in humans when given in contraceptive preparations.

SUMMARY OF DOSE-RESPONSE RELATIONS

The Vapor of Metallic Mercury

Dose-effect, dose-response data are available only for long-term exposure to mercury vapor. These data are summarized in Table 10. The critical effect may be increased excretion of urinary enzymes or other large molecular weight proteins or non-specific emotional and cognitive changes. For objectivity as well as ease of measurement, enzymuria or specific proteinuria would be the preferred critical effect. Thus the kidney, not the central nervous system, would be the critical organ. However, from the point of view of severity of health effects, the nervous system should still be the organ of major concern - that is to say that enzymuria may be used as an early warning sign to take preventive measures against the more serious effects on the nervous system.

The data in Table 10 suggest that a low risk of mild effects is to be expected at air concentrations (measured by static samples) in the range of 50 $\mu g/m^3$ which is the current industrial exposure limit in many countries.

Inorganic Mercury Compounds

No useful quantitative information is available for long term exposure to inorganic mercury compounds. Differences in individual susceptibility may be great as evidenced by clinical experience with acrodynia. Acrodynia has been reported in children at urinary excretion rates as low as 50 μg Hg/g creatinine.

Methylmercury and Other Short-chain Alkyl Mercurials

Adult exposures. Since the comprehensive analysis of dose-response and risk estimates in 1976 by the World Health Organization (WHO, 1976), new data have been reported. These new data included re-analysis of samples of hair, brain and other tissues obtained from patients in Minamata and Niigata, clinical follow-up of some individuals exposed to methylmercury in Niigata, a new statistical analysis of the Iraqi data and reports on high fish consumers in Canada and elsewhere. In general, these data support the original conclusions (for discussions, see WHO, 1980).

A re-analysis of the Iraqi data has been published by Nordberg and Strangert (1976, 1978, 1982). This analysis took into account not only the individual range of thresholds to such symptoms as paresthesia but also the interindividual variation in whole body biological half-times. These two distributions were combined to give an overall estimate of the risk of paresthesia for a given steady state daily intake of methylmercury. An intake of 50 μg/day in an adult person gave a calculated risk of about 0.3 percent for the symptoms of paresthesia, whereas an intake of 200 μg/day gave a risk of about 8 percent for symptoms of paresthesia.

The re-analysis by Nordberg and Strangert is in agreement with the conclusions of the WHO (1976) document - that "the prevalence of the earliest effects could be expected to be approximately 5 percent" following long term daily intake of methylmercury at 3 to 7 μg Hg/kg body weight. Such a long term daily intake should give rise to blood concentrations of approximately 200 ng Hg/ml and the maximum hair concentration of about 5 μg Hg/g.

More recently, clinical and epidemiological assessments have become available from studies on various groups of Canadian Indians, population groups exposed over a long period of time to methylmercury through fish consumption (Harada et al., 1976; Reudy and McKeown-Eyssen, 1980; Wheatley, 1979). In addition to the extensive Canadian studies, some other reports have been published since 1976 on the blood levels of mercury in populations exposed to varying extent to methylmercury through fish (Bacci et al., 1976; Haxton et al., 1979; Riolfatti, 1977; Turner et al., 1980). From these reports there seems to be about 100 adults outside of Japan or Iraq who were exposed to methylmercury in fish and with levels in blood above 200 μg/l. A diagnosis of Minamata disease has not been made, but it is possible that some may have had mild methylmercury poisoning. Assuming that none of these persons suffered any adverse effects from the exposure, such a negative finding is still consistent with the risk estimates discussed above.

These risk estimates may not apply to pregnant women. Some severe cases of poisoning in pregnant women exposed to high doses were reported in Iraq (for discussion, see WHO, 1980). At lower doses, transient paresthesias and other mild symptoms have been reported (Marsh et al., 1979, 1980, 1981; Tsubaki and Irukayama, 1977). Maternal paresthesia was related to peak hair concentrations of methylmercury and effects may have occurred at hair concentrations in the range of 18-68 mg/kg (Table 11). These observations suggest a greater risk to pregnant than for non-pregnant women.

Prenatal exposure. Since the last comprehensive review of this subject (WHO, 1976), results have been obtained from a clinical follow-up study on 29 infant-mother pairs in Iraq (Marsh et al., 1977, 1980). These reports describe children with psychomotor retardation caused by prenatal exposure, social bias excluded. A relationship was noted between maximum mercury concentrations, measured in 1 cm segments of maternal hair during pregnancy, and the frequency of neurological effects in the infants. The latter included delayed achievement milestones with or without neurological signs. The children were four and one-half to five years of age on last examination.

Subsequently, a more complete report (Marsh et al., 1981) became available on 84 infant-mother pairs including the 29 pairs previously described. The peak maternal hair levels ranged from 0.4 to 640 mg/kg. At hair mercury levels of 68-640 mg/kg, there was clear evidence of effects on psychomotor function, such as delayed walking or talking (Tables 11 and 12, Marsh et al., 1979). Severe neurological deficits were observed in five children of mothers with peak hair concentrations between 165 and 320 ppm.

McKeown-Eyssen et al. (1983b) have reported an epidemiological study on infants in a population of Cree Indians prenatally exposed to methylmercury. This study included 234 Cree Indian children aged 12 to 30 months from four Northern Quebec communities. The neurological, physical, mental, and psychosocial development of the children was assessed by a pediatric neurologist "blinded" to the children's level of exposure. Methylmercury was measured in 1 cm segments of maternal hair corresponding to the period of pregnancy. Abnormal tendon reflexes were positively associated with methylmercury exposure in males but not in females. Other neurological disturbances were less prevalent and none was positively associated with exposure. The higher exposure group had maximum maternal hair concentrations in pregnancy between 14 and 24 ppm.

Phenylmercury Compounds

Dose-response data available for phenylmercury compounds indicate that enzymuria is the critical effect and the kidney is the critical organ in infants. The threshold dose measured as urinary excretion rate of mercury was estimated to be 4 μg Hg/kg/24 hr. Animal experiments also point to the kidney as the first site of damage.

Thus, with the exception of acrodynia, mild changes in kidney function are probably the critical effect for both inorganic and phenylmercury compounds. If it is assumed that the divalent inorganic species of mercury is responsible for the renal effects after vapor, inorganic and phenyl salts, it is appropriate to use the data for mercury vapor to indicate that the threshold for these effects occurs at urinary mercury concentrations of about 50 μg Hg/g creatinine in adults.

MEDIA FOR BIOLOGICAL MONITORING

The Vapor of Metallic Mercury

A complete metabolic model including a compartmental mathematic model does not yet exist for mercury vapor, inorganic or phenylmercury compounds. Thus levels in indicator media such as blood and hair cannot be used to calculate the body burden, as can be done for methylmercury, or to estimate the amount in the critical organs and tissues. Nevertheless limited metabolic data provide some basis for understanding relations between concentrations in various media, between media and exposure, and between some media (urine) and certain health effects (renal).

Table 11. Symptoms (not signs) in Mothers and Children Related to Maternal Hair Peak Hg in Pregnancy[a]

Peak Hair range	Mother Hg ppm mean	Neurol. symptoms	N	Children Retarded walk.	talk.	mental	Seizures	Total no symptom
0.4-6.0	3	1	14	2	1	1	0	4
5.0-10.0	3	2	14	4	0	0	0	5
10.0-18.0	13	1	14	0	0	0	0	0
18.0-67.6	37	5	14	5	7	2	2	16
68.0-180	125	7	14	6	5	3	3	17
204-640	293	6	14	9	6	3	3	21

[a]Data from Marsh et al. (1981).

Any of the reported retention half-times for inhaled vapor in man are on the order of two months or less. Following the principle that a steady state should be attained after approximately five half-times, individuals with either continuous or regular exposure to mercury vapor (for example occupationally) should have attained a state of balance (steady state) after one year's exposure. One would expect a linear relation between average exposure (time weighted average air concentrations of mercury vapor) and concentrations in blood, urine or other media. In fact, Smith et al., (1970) have shown this in groups of workers having occupational exposures of more than one year. Despite the lack of a complete model, these findings support the idea that a steady state can be attained after the expected period of exposures and that under these circumstances, concentrations of mercury in blood and urine should be related to the absorbed dose.

The relations reported by Smith et al. (1970) are useful in converting steady state levels in air, blood and urine (e.g. Table 10). The data on blood-versus-air concentrations indicate that steady-state blood levels will increase by about 50 ng Hg/ml for each 100 $\mu g/m^3$ increase in the air concentration. Urinary mercury concentrations are also linearly related to time-weighted average air concentrations; however the slope of the line relating urine to air concentrations depends upon how the urinary mercury concentrations are expressed. Thus, uncorrected urine concentrations have the flattest slopes indicating that for every 100 $\mu g/m^3$ increase in mercury air concentrations, the urine concentration would increase about 200 $\mu g/l$. If urinary concentrations are corrected to a specific gravity of 1.024, the proportional increase in urinary concentration would be about 300 $\mu g/l$ for every 100 mg/m^3 increase in mercury air concentration. The latter value is similar to other values reported in the literature.

Lundgren et al. (1967) and Hernberg and Hassanan (1971) also reported relationships between mercury in blood and mercury in urine. These may be combined with the blood level versus air concentrations reported by Smith et al. (1970) to predict that the urinary concentration of mercury will increase approximately 300 $\mu g/l$ for every 100 $\mu g/m^3$ increase in time-weighted average air concentration.

The above relationships were based upon the measurement of air concentrations using static samplers. Henderson (1973) first reported quantitative differences in mercury concentrations in the general working environment compared to the "microenvironment" in the breathing zone of the individual worker. This was confirmed by Stopford et al. (1978), who noted that breathing zone samples may average several-fold higher in concentration than

Table 12. Signs in Children Related to Maternal Hair Peak Hg in Pregnancy[a]

Maternal Hair Peak Hg			Signs in Children			Total
Range	Mean	N	Normal-mild score 0-3	Moderate 4-6	Severe 7-11	number of signs
0.4-6.0	3	14	14	0	0	25
6.0-10.0	3	14	14	1	0	19
10.0-18.0	13	14	14	0	0	19
18.0-67.6	37	14	11	3	0	31
68-180	125	14	10	2	2	44
204-640	293	14	8	3	3	58

[a]Data from Marsh et al. (1981).

concurrent area samples, reflecting a "microenvironmental" contribution of exposure to mercury vapor, presumably from contaminated clothing and hands. Thus, several reports have found that when personal samplers are used, a 100 $\mu g/m^3$ increase in air concentration is associated with an increase in urinary concentration of 100 µg/l (Bell et al., 1973), 130 µg/l (Berode et al., 1980), or 160 µg/l (Lindstedt et al., 1979), all considerably lower than the increase of 300 µg Hg/l urine based on area (static sampling) levels of mercury. A report by Schuckmann (1981) indicated that, by intensifying compliance with personal hygiene and safety regulations and by improving technology of production in the factory, static samplers can approach the ratio of air-to-urine concentrations observed by use of personal samplers.

These presumed steady-state relationships noted above have been based on observations on groups of workers. However, high individual variation and great fluctuations from day to day have been reported in individual workers under similar conditions of exposure (Goldwater et al., 1964; Jacobs et al., 1964).

Evidence reviewed above suggests that the rate of urinary excretion of inorganic mercury is proportional to the amount of mercury in the kidneys. Thus, one might expect to see a relation between urinary excretion of mercury and effects of mercury on kidney function. Indeed, Buchet et al. (1980) and Roels et al. (1982) demonstrated dose (urinary excretion rate of mercury)-response (frequency of enzymuria) relations in workers occupationally exposed to mercury vapor. Gotelli et al. (1985) (see also Clarkson et al., 1985) have shown dose-effect and dose-response relations for infants exposed to phenylmercury compounds, despite the fact that these infants were not in steady state. In these three examples, a threshold dose (excretion rate of mercury) was noted below which effects on the kidney were not detectable. These findings support the idea that urine is a useful indicator medium for kidney effects. However, current methods of measuring urinary mercury excretion could be improved (Clarkson et al., 1986).

Blood concentrations of mercury have been rarely measured in studies of occupational exposures. Buchet et al. (1980) did not find a correlation between blood levels and renal effects.

A suitable indicator media for effects on the nervous system has not yet been identified. Roels et al. (1982) did not find any correlation between urinary levels and effects on the nervous system in workers chronically exposed to mercury vapor. On the other hand, Miller et al.

(1975) and Langolf et al. (1977) found that the prevalence of tremor in workers exposed to mercury vapor was related to the frequency with which the urine sample exceeded 500 μg Hg/l. Smith et al.'s (1970) findings of correlations between nervous system effects and time weighted air concentrations on the one hand and correlations between time weighted air concentrations and urine or blood concentrations on the other hand would suggest that blood or urine values should be useful indicators. It may be relevant that Smith and coworkers study involved a larger number of subjects and covered a wider range of air concentrations of mercury than the other studies.

Data are lacking for other media: hair, expired breath, sweat or feces.

Compounds of Inorganic Mercury

Little information is available on indicator media for human exposure. Metabolic studies reviewed above indicate that the urine would serve the same role as indicator medium as in the case of exposure to mercury vapor.

Methylmercury and Other Short-chain Alkyl Mercurials

The metabolic data reviewed above indicate that whole blood or red blood cells are valid indicator media for the current body burden and brain levels of MeHg.

Longitudinal analysis of head hair recapitulates prior blood concentrations. The possibility of external contamination of hair samples including the use of shampoos should always be considered. Unfortunately, no satisfactory washing procedure is available although a number have been used (for review, see Jenkins, 1979 and Suzuki, this volume).

Longitudinal analysis of bunches of hair strands can lead to artifacts in case of intermittent exposures (Giovanoli and Berg, 1974). These artifacts arise because individual strands become misaligned due to differences in growth rate and mechanical displacement during collection and subsequent handling. Recently, new analytical methods allow the analysis of single strands of head hair that avoid such artifacts (Toribara et al., 1982).

Phenylmercury Compounds

Gotelli et al. (1985) have reported that the rate of urinary excretion of mercury appears to be a predictor of early mild effects on the kidney. Phenylmercury is rapidly transformed to inorganic mercury; the latter is accumulated in the kidney and excreted in urine. It is assumed that the urinary excretion is proportional to the kidney burden.

ANALYTICAL METHODS

A comprehensive review of all the sampling and analytical methods for mercury was published by Smith (1972) and a less detailed review by WHO (1976) and USEPA (1984). Prior to the 1960's, a colorimetric reagent (dithizone) was widely used.

The "cold vapor" atomic absorption procedure introduced in the 1960's revolutionized mercury analysis. When applied to biological specimens, the basic approach is to convert the mercury to the mercuric ion, reduce it to elemental mercury (e.g. with stannous chloride), sweep the vapor into an atomic absorption spectrophotometer or u.v. meter set at the mercury absorption line of 254A. (for details see Bourcier et al., 1982; Gage and Warren, 1970; Hatch and Ott, 1968; Henderson et al., 1974; Rathje and

Marcero, 1976; Smith, 1972;). A suitable alternative is to vaporize the mercury using a graphite furnace accessory of an atomic absorption instrument (Diggs and Leadbetter, 1983; Kubasik et al., 1972; Lindstedt, 1970; Menke and Wallis, 1980; Trujillo and Campbell, 1975). Mercury vapor, once generated from the sample, can be measured by amalgamation or in thin layers of gold deposited on a quartz crystal making use of the piezoelectric effect (Scheide and Taylor, 1974).

Mercury in the workplace environment is usually at sufficiently high concentrations (above 1 ug/m^3) to be measured by commercially available direct-reading mercury vapor u.v. meters. However, caution should be exercised so that other u.v. absorbing contaminants as well as strong magnetic fields do not affect the readings. Furthermore, it should be appreciated that these meters will not measure particulate mercury. To include particulate mercury, collection procedures should involve the use of high efficiency filters (Menke and Wallis, 1980; Trujillo and Campbell, 1975). Personal monitors (McCammon and Woodfin, 1977) are also available. These monitors may give a better measure of the actual inhalation exposure than static room samplers.

Measurement of the low concentrations of mercury in ambient air (~1-10 ng/m^3) and in expired breath requires methods that remove mercury from large volumes of air and that are extremely sensitive (Bell et al., 1973a; Long et al., 1973; Scheide and Taylor, 1974; USEPA 40 CFR 61, 1981).

Most methods for analysis of blood, urine and other biological samples rely on the same analytical methods but require pretreatment of the sample to liberate the bound mercury. The methods of Gage and Warren (1970), Hendersen et al. (1974), Moffitt and Kapel (1971) apply to most types of samples. Special methods for urine samples have been described by Rathje (1969) and for both blood and urine samples by Bourcier et al. (1982), Krause et al. (1971), Kubasik et al. (1972) and Stopford et al. (1978).

A convenient, rapid and sensitive method for measurement of mercury has been described by Magos and Cernik (1969) for urine and by Magos and Clarkson (1972) for blood samples. The "Magos Method" has the advantage of distinguishing between inorganic and organic forms of mercury.

Blood samples are best collected in vacutainers containing heparin and refrigerated at 4°C prior to analysis. This method of collection is specially important if mercury is to be measured in plasma and red blood cells. The blood samples can usually be stored for one or two days before hemolysis becomes important.

Urine samples may be collected in any clean glass or plastic container. It may be preserved by freezing or by adding hydrochloric acid to a final concentration of 1 percent (Magos and Cernick, 1969). If the sample has been frozen, hydrochloric acid should be added to the thawed contents to elute any mercury from the container or from the surface of any precipitate.

The reference or normal value of mercury in blood and urine samples should usually be established for each laboratory and analytical method. In a study sponsored by the World Health Organization and summarized by Goldwater (1964), 1107 urine samples from 15 countries and 649 blood samples from 16 countries were analyzed for mercury by the method of Jacobs et al. (1961). These samples were collected from individuals with no known exposures (either occupational or other deliberate exposures to mercury) and results are given in Table 13. More recent data from "control groups" in clinical and epidemiological studies confirm these data (e.g. Buchet et al., 1980; Gotelli et al., 1985).

Table 13. Concentration of Total Mercury in Blood and Urine Samples Taken in a Worldwide Survey from People Having No Known Exposure to Mercury[a]

	Blood	Urine
No. of Samples	812	1107
No. of Countries	15	15
Percent less than 0.5 μg/l[b]		78
5.0	77	86
10	85	89
15		94
20	89	95
25		96[c]
30	95	
40	97	
50	97.2[c]	

[a]Adapted from Goldwater (1972).
[b]Detection limits: blood 5μg/l; urine 0.5 μg/l.
[c]Highest recorded value: blood 39.6 μg/l; urine 221 μg/l.

SUMMARY AND CONCLUSIONS FOR BIOLOGICAL MONITORING

The Vapor of Metallic Mercury and Compounds of Inorganic Mercury

Despite the lack of an adequate compartmental model for disposition of inorganic mercury in man, the limited data do suggest that biological monitoring is feasible in certain limited circumstances and should become more generally applicable in the future.

Current use. Urine is the most commonly (perhaps exclusively) used indicator media for occupational exposures (Henderson, 1985). For reasons presented above, the rate of urinary excretion probably reflects the amount of mercury in the kidneys. Current practices and opinions differ on how the urinary excretion of mercury should be measured.

It is well recognized that urinary concentrations of mercury are highly variable when samples are collected at different times from the same individual. Wallis and Barber (1982) in studies of workers exposed to mercury vapor, reported that correcting the concentration for specific gravity significantly reduced the variance in spot urine samples. The first sample collected after a night's rest was more representative of the composites of all spot samples collected on one day than spot samples collected at other times. This can probably be attributed to the fact that the first spot sample of the morning typically represents collection from six to eight hours as compared to only two to four hours from other spot samples. Similar observations have been made by Piotrowski et al. (1975). In a follow-up publication, Barber and Wallis (1984) noted that correction of concentrations of mercury in spot urine samples for specific gravity or osmolality reduced the variability to about the same extent and that correction for creatinine was the most effective in reducing this variability. In general, recent reports tend to favor the use of the creatinine correction (Buchet et al., 1980; Gotelli et al. 1985; Roels et al., 1982). The rationale for the application of the creatinine correction to spot urine samples to estimate the true urinary excretion rate for mercury has been presented in some detail by Clarkson et al. (1986).

Briefly, the reasons are as follows: The rate of urinary excretion of creatinine is proportional to the lean body mass, and therefore does not change greatly for a given individual or for groups of workers having roughly similar body weights. Conversely, urine volume flow rates vary greatly depending upon the degree of hydration of the individual. The concentration of mercury in spot urine samples may be greatly affected by the degree of hydration of the subject and, therefore, not accurately indicative of the true excretion rate. Human adults excrete approximately 1 to 2 grams of creatinine per day. Therefore, expressing the mercury excretion per gram creatinine gives a rough indication of daily excretion rate and avoids errors due to fluctuations in dilution of the spot urine sample. Since normally hydrated individuals excrete between 1 and 2 liters of urine per day, the mercury excretion in μg Hg/g creatinine is approximately equal to 1 μg Hg/l urine. Expressing mercury excretion by this "creatinine correction" also has another advantage. Since creatinine excretion is proportion to lean body mass, urinary creatinine excretion is a "sizing factor." Thus, urinary mercury expressed as μg Hg/g creatinine amounts to a (lean) body weight-corrected excretion rate.

Future improvements: urine. The creatinine correction might be improved by greater understanding of factors affecting creatinine excretion. Lean body mass largely determines the daily output of urinary creatinine. If the intent is to estimate the actual excretion rate of mercury from spot urine samples, it would be valuable to take into account lean body mass. This may be roughly estimated by the "skin fold" technique or by normalizing for the height of the individual. Either procedure will be an improvement on current practice where no adjustments are made. It will also be useful to take into account dietary habits, at least to make the gross distinction between heavy meat eaters and vegetarians. Sex differences are important as they probably show systematic differences in lean body mass.

An alternative to using spot urine samples, is to collect timed samples. The most convenient choice might be a 6-8 hour timed sample collected during the working day. Here some supervision of collecting technique and timing is possible. Great care must be taken to avoid contamination. Less desirable are the overnight or 24 hr. collection where supervision is minimal and the possibility of collection errors is high.

Urinalysis might be made more exact by the use of special chelating agents that mobilize inorganic mercury selectively from kidney tissue to urine. Cherian et al. (1986) have preliminary evidence that dimercapto-propane sulfonate can perform this role.

Hursh et al. (1985) have described a technique of using the urine sample to give an actual estimate of the amount of mercury in the kidneys. They have shown that a tracer dose of radioactive inorganic mercury will equilibrate with stable mercury in kidney tissue such that three days after dosing, the specific activity in the urine is virtually identical with that in the kidney tissues. Thus, knowing the fraction of radioactive dose in the kidneys and by directly measuring the specific activity in the urine, one can calculate the amount of mercury in the kidneys. In different animal species, it was shown that the same fraction of the tracer dose was always found in the kidney after three days so that it is reasonable to assume that this same fraction is found in humans.

Other biological media. Future developments will depend upon a better knowledge of the metabolic model and advances in the sensitivity and selectivity of analytical techniques. Mercury in the expired breath offers a simple, noninvasive possibility. Indeed, animal experiments have shown that the rate of exhalation is proportional to the body burden of inorganic mercury. Further technical developments are still needed for application to

humans (see Kobal and Stegner, 1985) and interference from mercury amalgam tooth fillings will have to be considered.

Methylmercury and Other Short-chain Alkylmercurials

Current use. Biological monitoring for methylmercury is mainly if not exclusively used for fish eating populations. Samples of blood and/or head hair are collected for analysis.

Blood samples are collected from the vein into heparin-containing test tubes or more frequently into commercially available vacutainers^{R}. The containers are refrigerated, not frozen, and are transported on ice to the analytical laboratory. Analysis is carried out usually within 24 hours to avoid hemolysis. The hematocrit is recorded because methylmercury accumulates in the red blood cells. Measurements may be restricted to samples of whole blood and plasma. The latter is preferred because, knowing the hematocrit, it is possible to calculate the concentration in red cells from the concentrations in whole blood and plasma. This reveals the red blood cell to plasma concentration ratio which is helpful in confirming that exposure has occurred to methylmercury compounds.

If commercial vacutainers are used, it is usual to test each batch for mercury contamination since organic mercury fungicides may have been used to ensure sterilization. This test is usually carried out by adding a few milliliters of isotonic saline solution containing 0.1M cysteine.

Hair samples are collected from the head in one of two ways - as short (a few millimeters) segments or as a bunch of the longest strands of the hair. The short segments reveal only recent exposure, i.e., blood levels existing within the last 29 days or so. The hair may be stored in an envelope or a plastic bag or a test tube. The amount of hair is usually between 1 to 10 mg.

The longest hairs on the head are collected for longitudinal analysis to recapitulate previous blood concentrations. In order to accomplish this, it is necessary to collect the hair in such a way as to avoid misaligning the hair strands. One procedure is to identify approximately 50 strands of hair as close to the scalp as possible. Before releasing the hemostat, the bunch of hair is tied with cotton thread. The bunch may then be placed in an envelope or a plastic bag for storage and shipment. Longitudinal analysis is usually conducted on consecutive 1 cm segments although some laboratories use three cm segments. Each laboratory devises its own cutting procedure. Each segment is usually weighed on an electrobalance and should be in the range of 1-10 milligrams depending upon the sensitivity of the analytical methods.

Hair analysis has now become the most widely used means of biological monitoring in populations suspected of exposure to methylmercury through fish consumption. It is specially useful to recapitulate maternal blood concentrations during pregnancy. One blood sample should be collected so as to establish the individual's hair to blood concentration ratio.

Future improvements. The current practice of using either blood or hair samples or both is generally satisfactory. Improvement for hair analysis might involve the analysis of single strands of hair plucked out by the root. New techniques are now becoming available which allow longitudinal analysis millimeters by millimeter. Not only does this give a more precise temporal recapitulation but it avoids artifacts arising from the misalignment of hair strands when they are measured as a bunch. It also has the advantage that at least one measurement can be made at the root end where the hair segment has not yet emerged above the surface of the scalp thus giving one datum point free of external contamination. The instruments now used for single

strand hair analysis have certain disadvantages. They are expensive, somewhat difficult to calibrate precisely and lack the sensitivity required to measure mercury in the hair of non-exposed individuals. More development, particularly with regard to the sensitivity of the instruments, is still needed.

Methods are also being developed that allow measurement of a cross-section of a hair strand. The cross-sectional profile may also give evidence as to the degree of external contamination.

Phenylmercury

Very few data have been published on biological monitoring of individuals or populations exposed to phenylmercury compounds. In occupational exposures (Goldwater, 1973) or in accidental exposures (Gotelli et al., 1985) the urinary excretion of mercury has been used as a measure of the exposure or of the absorbed dose. The rational is that phenylmercury compounds are rapidly broken down in the body to inorganic mercury. The latter is accumulated in the kidneys and excreted in the urine.

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