4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

The absorption, distribution, metabolism and excretion of inorganic mercury (Nordberg & Skerfving, 1972; WHO, 1976; Berlin, 1986; Clarkson *et al.*, 1988a; WHO, 1991; Clarkson, 1992), methylmercury compounds (Nordberg & Skerfving, 1972; WHO, 1976; Berlin, 1986; Magos, 1987; Clarkson *et al.*, 1988a; WHO, 1990) and phenylmercury acetate (Nordberg & Skerfving, 1972; WHO, 1976; Berlin, 1986; Clarkson *et al.*, 1988a) have been reviewed.

4.1.1 *Humans*

(*a*) *Metallic mercury and inorganic mercury compounds*

In five human volunteers who inhaled radioactive metallic mercury-197 or mercury-203 vapour for 14–24 min, an average of 74% was absorbed in the respiratory tract (Hursh *et al.*, 1976). The half-time for whole-body elimination averaged 58 days; however, elimination rates varied for different parts of the body: lung, 1.7 days; head, 21 days; kidney region, 64 days; chest, 43 days.

Absorbed metallic mercury is dissolved in the blood. Addition of metallic mercury-203 vapour to blood *in vitro* resulted in oxidation to mercuric mercury, but rather slowly (Hursh *et al.*, 1988). The authors concluded that metallic mercury may pass the blood–brain barrier. A man who accidentally ingested 135 g of liquid metallic mercury had raised mercury concentrations in blood, but to an extent indicating only minimal absorption (Suzuki & Tanaka, 1971). The average ratio of mercury in erythrocytes:plasma was about 2 during the first few days after a 14–24-min exposure of five volunteers by inhalation of metallic mercury-197 and mercury-203 vapour (Cherian *et al.*, 1978).

Studies in five volunteers who exposed their forearms to metallic mercury-203 vapour for 27–43 min indicated absorption of mercury through the skin of 0.01–0.04 ng/cm^2 per min per ng Hg/cm^3 air (Hursh *et al.*, 1989).

In five human volunteers who inhaled metallic mercury-197 vapour for 11–21 min, the kidney region accumulated the highest levels of mercury (Hursh *et al.*, 1980). In autopsy samples from seven dentists and one dental assistant, particularly high levels of mercury were found in the renal cortex (average, 8.6 μmol [1.7 mg]/kg wet weight) and pituitary glands (average, 9.8 μmol [2 g]/kg wet weight). In 24 controls, the values were 1.4 μmol [280 μg]/kg wet weight in renal cortex and 0.12 μmol [24 μg]/kg wet weight in pituitary (Nylander & Weiner, 1991). High levels have also been recorded in the thyroid glands of deceased mercury miners (average, 35 mg/kg fresh weight) (Kosta *et al.*, 1975).

Equimolar ratios of mercury:selenium were found in pituitary and thyroid glands, kidney and brain in subjects with occupational exposure to metallic mercury vapour (Kosta *et al.*, 1975; Nylander & Weiner, 1991). Renal biopsy samples from two patients with inorganic mercury poisoning had inclusion bodies which contained mercury and selenium (Aoi *et al.*, 1985).

The blood mercury concentration of nine men who had been exposed to high levels (> 100 μg/m^3) of metallic mercury vapour for three days decreased with a half-time of three days for a fast phase and 18 days for a slow phase; the half-times in the urine were 28 and 141 days, respectively (Barregård *et al.*, 1992).

Analysis of brain samples from a deceased subject who had been exposed to metallic mercury vapour for 18 months 16 years before death showed high levels of mercury, indicating that the brain has a compartment with very slow turnover of mercury. Most of the deposited mercury was in colloidal form (Hargreaves *et al.*, 1988).

The concentration of mercury in the blood of the infants of two women who had been exposed accidentally to metallic mercury vapour during pregnancy was similar to that in maternal blood at the time of delivery, indicating transplacental passage (WHO, 1991).

An average urinary mercury concentration of about 50 μg/g creatinine was seen in 10 workers exposed to 40 μg/m^3 of air in a dry alkaline battery factory; the concentration in blood was about 18 μg/L (Roels *et al.*, 1987).

In 10 volunteers who received single oral doses of either ^{203}Hg-mercuric nitrate as such or added to calf-liver protein, 75–92% of the dose was excreted in the faeces during the first four to five days. The average whole-body half-time for mercury (slow component) was 42 days. No difference was seen between the two forms of administration. The ratio of mercury in red blood cells to that in plasma was 0.4 over at least the first 50 days of the experiment. At that time, approximately equal amounts of mercury were excreted in faeces and urine (Rahola *et al.*, 1973).

In a study of two men who had accidentally inhaled aerosols of neutron-activated ^{203}Hg-mercuric oxide, the lung clearance pattern displayed two phases, with biological half-times of two and 24 days, respectively, in one man; in the second, lung clearance appears to have been more rapid. The authors stated that absorption may have occurred from the lung, gastrointestinal tract or both. The major site of systemic deposition was the kidney, the content of which decreased with half-times of 60 and 37 days, respectively, in the two subjects. After 40 days, excretion was mainly urinary (Newton & Fry, 1978).

In five human volunteers who inhaled metallic mercury-197 vapour for 11–21 min, mercury was excreted by exhalation of metallic mercury and excretion of mercury in faeces and urine (Hursh *et al.*, 1980).

(*b*) *Methylmercury compounds*

After a single oral dose of ^{203}Hg-methylmercury nitrate was given to three volunteers, methylmercury was almost completely absorbed. A maximum of 10% of the dose was deposited in the head region, presumably in the brain. Whole-body radiolabel decline followed a first-order process, with half-times of 70–74 days. The decline in radiolabel in the head was less rapid than in the rest of the body. In two of the subjects, faecal excretion accounted for about 87 and 90% of the total elimination during the 49 days that followed

administration (Åberg *et al.*, 1969). Gastrointestinal absorption was similarly high, whether methylmercury was given as the nitrate or bound to protein (Åberg *et al.*, 1969; Miettinen, 1973).

In six volunteers who ate a single meal of fish containing methylmercury, the ratio of the concentration of mercury in erythrocytes and plasma was 21. Incorporation of methylmercury into hair was proportional to the concentration in blood at the time of formation of the hair strand; the ratio hair:blood was 292. The average half-times in blood were 7.6 h and 52 days (Kershaw *et al.*, 1980).

In a study of 162 subjects who had been exposed to methylmercury through consumption of contaminated fish in Sweden in 1967–72, intake was associated with concentrations of mercury in blood and hair. After cessation of eating the contaminated fish, the concentration of mercury in the blood cells of four subjects decreased with a half-time of 58–87 days; in one subject, the half-time was 164 days (Skerfving, 1974). The ratio of mercury in blood cells and in plasma was 2–12 (Skerfving, 1988).

Individuals with long-term intake of around 200 μg methylmercury per day were estimated to have blood mercury concentrations of about 200 μg/L and hair concentrations of about 50 μg/g (WHO, 1990).

After consumption of bread contaminated with methylmercury for two months in Iraq, the molar fraction of total mercury as inorganic mercury in several people was 7% in whole blood, 22% in plasma, 39% in breast milk, 73% in urine and 16–40% in liver (WHO, 1990).

The average ratio of methylmercury in cord blood and in maternal blood was 1.66 (Suzuki *et al.*, 1984). The infants of 10 fishermen's wives who were exposed to methylmercury through consumption of fish in Sweden had about 47% higher mercury levels in erythrocytes and similar levels in plasma in comparison with their mothers. The concentration of total mercury in breast milk from 15 women was similar to that in plasma; only about 20% of the total mercury in the milk was methylmercury (Skerfving, 1988).

(*c*) *Phenylmercury compounds*

In 509 infants in Buenos Aires, Argentina, who were exposed to phenylmercury fungicide through contaminated diapers, the average urinary excretion of total mercury was about 20 times higher than in 166 matched controls; over 90% of the mercury was inorganic (Gotelli *et al.*, 1985).

4.1.2 *Experimental systems*

(*a*) *Metallic mercury and inorganic mercury compounds*

Kostial *et al.* (1983) observed that the retention of orally administered ^{203}Hg-mercuric chloride in the carcass, gut and whole body was higher in newborn rats (60–70%) than in weaned rats (14–15%).

Absorption of an aqueous solution of ^{203}Hg-mercuric chloride applied under occlusion onto about 3 cm^2 of the shaved skin of guinea-pigs was dependent on the mercury concentration. A maximal rate of about 0.02% per min was recorded during 5 h after application of 16 mg/ml (as mercury) (Friberg *et al.*, 1961).

In rats, rabbits and monkeys exposed for 4 h to 1 mg/m^3 of metallic mercury vapour or injected intravenously with an equivalent dose of mercuric nitrate, the main accumulation

was in the kidney, but 10 times more mercury entered the brain after exposure to mercury vapour than after injection of mercuric nitrate (Berlin *et al.*, 1969).

In rats exposed to metallic mercury vapour, mercury deposits were found by a histochemical technique in the nerve cells in the cerebellum and hypothalamus (Møller-Madsen, 1992). In frog nerve–muscle preparations treated with mercuric chloride (3 μM [600 μg]), mercuric ions penetrated the nerve-cell membrane through sodium and calcium channels (Miyamoto, 1983).

Khayat and Dencker (1982) found four-fold higher fetal mercury concentrations in mice after exposure to metallic mercury vapour by inhalation than after intravenous injection of mercuric chloride. The passage of metallic mercury through the blood–brain barrier is usually ascribed to its lipophilicity.

In studies of cell suspensions of erythrocytes from humans, ducks and mice exposed *in vitro* to mercury vapour, uptake was proportional to catalase activity, which shows that this enzyme is involved in oxidation of mercury vapour in the erythrocyte (Halbach & Clarkson, 1978). Catalase-mediated oxidation of the vapour has also been demonstrated in other tissues, e.g. liver (Magos *et al.*, 1978).

Intravenous injection of rats with mercuric chloride at 0.7 mg/kg bw induced metallothionein in kidney tissue, which resulted in the binding of mercury (Nishiyama *et al.*, 1987).

After administration of 12 or 25 daily doses of mercury at 1 mg/kg bw as ^{203}Hg-mercuric chloride, the mitochondria in the proximal convoluted tubules were found to be enlarged and there were many very fine, dense, small particles. After fragmentation of the renal tissue and centrifugation at high speed, the radiolabel was found in two fractions, corresponding to mitochondria and microsomes (Bergstrand *et al.*, 1959).

Mice given parenteral doses of mercuric chloride exhaled metallic mercury vapour; exhalation was proportional to the body burden of mercury (Dunn *et al.*, 1978). Following intravenous treatment of rats with mercuric chloride, mercury was excreted into bile as a low-molecular-weight complex which had gel filtration properties similar to those of a mercury–glutathione complex (Ballatori & Clarkson, 1984).

In guinea-pigs exposed for a short time to metallic mercury vapour after parturition, the mercury concentration in milk was slightly lower than that in plasma. Neonates had increased concentrations of mercury in tissues and particularly in the kidney (Yoshida *et al.*, 1992). In rats given mercuric acetate orally, a linear relationship was observed between mercury concentrations in plasma and in milk (Sundberg *et al.*, 1991).

Selenium affects the tissue distribution and excretion of mercuric mercury. For example, three weeks' administration of sodium selenite or seleno-L-methionine (7.5, 37.5 or 75 μmol/L in drinking water) to BOM:NMRI mice increased the whole-body retention of a single oral dose (5 or 25 μmol [1 or 5 mg]/kg bw of ^{203}Hg-mercuric chloride. The effect on organ distribution varied with the dose of mercury and the type and dose of selenium compound (Nielsen & Andersen, 1991).

Human oral bacteria caused some methylation of mercuric chloride *in vitro* (Heintze *et al.*, 1983).

(*b*) *Methylmercury compounds*

Exposure of rats to ^{203}Hg-methylmercury chloride vapour at 10–28 mg/m^3 for 6–24 h was followed by efficient uptake of methylmercury through the lungs (0.6–7 nmol/g fresh tissue) [no data on absorbed fraction given]. Rats given a single oral dose of 0.75–2.3 mg/kg bw had several times higher mercury concentrations in organs (18.6–107.6 nmol/g fresh tissue). In liver and kidney, 42–50% of the mercury was in the soluble fraction, 32–43% in the crude nuclear fraction, 6–9% in the mitochondria and 9–11% in the microsomal fraction. In brain, 29% was in the soluble and 27% in the nuclear fraction, 31% was in mitochondria and 10% in microsomes (Fang, 1980).

Absorption of an aqueous solution of ^{203}Hg-methylmercury dicyandiamide applied under occlusion onto about 3 cm^2 of the shaved skin of guinea-pigs was dependent on concentration. A maximal disappearance of 5.9% was recorded during 5 h after application of 16 mg/ml (as mercury) (Friberg *et al.*, 1961).

Significant species differences have been observed in the distribution of methylmercury compounds in the body: The ratio between mercury concentrations in erythrocytes and plasma is about 20 in monkeys (17 in squirrel, 25 in rhesus), 25 in guinea-pigs, 7 in mice and more than 100 in rats (for review, see Magos, 1987). After prolonged administration of methylmercury compounds, the brain:blood ratios are 3–6 in squirrel monkeys (for review, see Berlin, 1986), 3.3 in pigs, 1.2 in guinea-pigs, 1.2 in mice and 0.06 in rats (for review, see Magos, 1987).

Following intraperitoneal injection of 1 mg/kg bw methylmercury chloride into four strains of mice, a significant difference in mercury concentrations was observed among strains, particularly in the blood. The rate of elimination from organs also differed: the biological half-time in blood (days) was 5.03 in BALB/c, 5.52 in C3H, 7.79 in C57Bl and 3.81 in CD-1 mice; that in kidneys was 8.73, 7.73, 7.47 and 4.54, respectively (Doi & Kobayashi, 1982). Eight days after intraperitoneal administration of ^{203}Hg-methylmercury chloride (0.4 mg/kg bw as Hg) to two strains of mice, males had significantly higher mercury concentrations in kidney than had females (C129F$_1$ strain: 5.33 and 3.34%; 129 strain: 7.47 and 3.57% of the dose in males and females, respectively). There was no sex difference in whole-body mercury retention (Doherty *et al.*, 1978).

The percentage of inorganic mercury in total mercury in tissues of squirrel monkeys that received single or repeated weekly doses of methylmercury nitrate by stomach tube at about 0.8 mg/kg bw Hg, was about 20% in liver, about 50% in kidney, 30–85% in bile and < 5% in brain, showing that methylmercury is demethylated (Berlin *et al.*, 1975). Similarly, inorganic mercury was demonstrated in the kidney and to a lesser extent in the liver of rats given daily doses of methylmercury dicyandiamide (Magos & Butler, 1972).

The relative concentration of inorganic mercury in mice increased gradually after a single intravenous injection of 25 μg methylmercury chloride and was about 30% after 22 days; the author concluded that mice obtain a lower fraction of inorganic mercury in the kidney than rats (Norseth, 1971). Cats fed either methylmercury-contaminated fish or methylmercury hydroxide added to fish accumulated inorganic mercury in the liver and kidney; 62% was recovered as methylmercury in kidney and 80% in liver. The metabolism of the methylmercury in the contaminated fish and of the added hydroxide was similar (Albanus

et al., 1972). Similar results were found in cats fed methylmercury-contaminated fish or methylmercury chloride (Charbonneau *et al.*, 1976), and no difference in metabolism was seen in rats given four different salts of methylmercury orally or subcutaneously (Ulfvarson, 1962).

Methylmercury added as the chloride *in vitro* to erythrocytes from humans, rabbits and mice was complexed to a low-molecular-weight compound—probably glutathione. In rats, such binding was minimal (Naganuma *et al.*, 1980). Following the addition of methylmercury chloride to erythrocytes from mice, rats and humans in another study, mercury was found to be bound to haemoglobin—probably cysteinyl residues (Doi & Tagawa, 1983). In rats, L-cysteine enhanced the uptake of mercury by the brain after administration of methylmercury chloride by intracarotid injection. There were indications of a transport system carrying methylmercury over the brain capillary endothelial cell membrane (Aschner & Clarkson, 1988).

In rats injected intravenously with methylmercury chloride, methylmercury was present in the bile as a low-molecular-weight compound complex, which was identified as methylmercury glutathione on the basis of thin-layer chromatography, gel filtration and ionic exchange (Refsvik & Norseth, 1975).

After intravenous injection into rats, methylmercury was excreted into the bile, predominantly as methylmercury cysteine, which is largely reabsorbed from the intestine. There is thus enterohepatic circulation of methylmercury (Norseth & Clarkson, 1971). In rat gut, however, a fraction of methylmercury is converted to inorganic mercury, which is then excreted mainly in the faeces (Rowland *et al.*, 1980).

Hamsters administered a single oral dose of 10 mg/kg bw methylmercury chloride excreted about 50% of the mercury (only about 10% of which was inorganic mercury) in the urine within one week. In rabbits given 0.4 mg/kg bw intravenously, < 2% was excreted in the urine (Petersson *et al.*, 1989).

After addition of 250 ng methylmercury chloride to three hydroxyl radical producing systems, copper ascorbate, xanthine oxidase hypoxanthine–ferric monosodium ethylenediaminetetraacetate and hydrogen peroxide–ultraviolet B light, analysis of inorganic mercury revealed significant dealkylation, which appeared to be unrelated to either superoxide or hydrogen peroxide production alone (Suda *et al.*, 1991). In rat liver microsomes treated with 500 ng methylmercury chloride, both inorganic mercury and hydroxy radical contents increased after addition of NADPH and were further increased by KCN (Suda & Hirayama, 1992).

Selenium affects the tissue distribution and excretion of methylmercury. For example, selenite increased the brain levels of mercury in rats treated with methylmercury (Magos & Webb, 1977).

(*c*) *Phenylmercury and methoxyethylmercury compounds*

Faecal excretion of 0.120 mg/kg bw Hg as phenylmercury acetate in rats was 65% during 48 h after a single oral dose and 30% after intravenous administration of the same dose, indicating that more than half of the phenylmercury salt was absorbed (Prickett *et al.*, 1950).

In rats given an intraperitoneal injection of phenylmercury acetate, the compound was metabolized rapidly to mercuric mercury (Magos *et al.*, 1982).

Daniel *et al.* (1971) administered a single subcutaneous dose of methoxy-^{14}C-ethylmercury chloride to rats. Within three days, about half of the radiolabel appeared in exhaled air, with 44% in ethylene and 5% in carbon dioxide (44% after pyrolysis of air). Mercury was accumulated in kidney: A few hours after dosing, inorganic mercury constituted about one-half of the total mercury in that organ; after one day, all of the mercury was inorganic. About 25% of the radiolabel was excreted in urine over 4 days and about 10% after 8 days.

4.2 Toxic effects

The toxic effects of inorganic mercury (WHO, 1976; Kark, 1979; Berlin, 1986; Clarkson *et al.*, 1988a; Dayan *et al.*, 1990; WHO, 1991; Clarkson, 1992), methylmercury compounds (WHO, 1976; Berlin, 1986; Clarkson *et al.*, 1988a; Dayan *et al.*, 1990; WHO, 1990) and phenylmercuric acetate (Skerfving & Vostal, 1972; WHO, 1976; Berlin, 1986; Clarkson *et al.*, 1988a) have been reviewed.

4.2.1 *Humans*

(*a*) *Inorganic mercury*

Workers accidentally exposed for 4–8 h to metallic mercury at levels estimated to have ranged from 1 to 44 mg/m^3 developed chest pain, dyspnoeic cough, haemoptysis, impairment of pulmonary function and interstitial pneumonitis (McFarland & Reigel, 1978). Acute massive exposure to metallic mercury vapour can result in psychotic reactions with delirium (for review, see Kark, 1979).

Troen *et al.* (1951) reported 18 cases of human poisoning by ingestion of single doses of mercuric chloride. In nine fatal cases, the lowest estimated dose was 2 g. Gastrointestinal and renal lesions were observed at autopsy.

Roels *et al.* (1985) examined 131 male and 54 female workers exposed to metallic mercury vapour in several factories in Belgium and 114 and 48 unexposed control male and female workers. In responses to a questionnaire, several symptoms of central nervous system disorder (memory disturbances, depressive feelings, fatigue and irritability) were more prevalent among exposed subjects than controls. A significantly increased prevalence of hand tremor was recorded in the group of exposed men, as compared to male controls (15 *versus* 5%). The average concentrations of mercury in urine were 52 μg/g creatinine in exposed men and 37 μg/g creatinine in women; the corresponding levels in controls were 0.9 and 1.7 μg/g creatinine.

In a study of 89 chloralkali workers with a median urinary mercury concentration of 25 μg/g creatinine (range up to 83) and a control group of 75 workers from other industries (median concentration, 2 μg/g creatinine), an association was observed between urinary mercury concentration, self-reported symptoms—tiredness, confusion and degree of neuroticism (Langworth *et al.*, 1992a)—and urinary excretion of *N*-acetyl-β-glucosaminidase, a lysosomal enzyme originating from tubular epithelial cells. No significant effect on serum titres of autoantibodies (including antiglomerular basement membrane and antilaminin) was observed (Langworth *et al.*, 1992b). Elevated excretion of *N*-acetyl-β-glucosaminidase was also reported by Barregård *et al.* (1988) in chloralkali workers.

Of 44 African women with nephrotic syndrome, 70% used or had used mercury-containing skin-lightening creams; the corresponding fraction among other general medical female in-patients was 11% (Barr *et al.*, 1972). In eight other cases of nephrotic syndrome, IgG and C3 complement deposits were observed in glomeruli (Lindqvist *et al.*, 1974). Proteinuria and the nephrotic syndrome have also been described in workers exposed to mercury compounds (Kazantzis *et al.*, 1962).

Lauwerys *et al.* (1983) studied 62 workers in a chloralkali plant and a zinc–mercury amalgam factory with a mean urinary mercury concentration of 56 μg/g creatinine. Eight exposed workers, but none of 60 control workers who were not occupationally exposed to heavy metals but were matched to the exposed group with respect to age and socioeconomic status, had serum antibodies towards laminin, a non-collagen glycoprotein found *inter alia* in the glomerular basal membrane. No alterations were seen in a large battery of renal function tests.

In studies of dentists and chloralkali workers exposed to metallic mercury vapour (mean urinary mercury concentration, 1.3 nmol/mmol [2.3 μg/g] creatinine in dentists and 26 nmol/mmol [46 μg/g] creatinine in chloralkali workers), no significant effect on endocrine function (pituitary, thyroid and adrenal glands, testis) was observed as compared to controls (0.4–0.6 nmol/mmol [0.7–1.06 mg/g] creatinine) without occupational exposure (Erfurth *et al.*, 1990). Similar results were reported by Langworth *et al.* (1990) in dental personnel.

In a study reported in detail on p. 271, Barregård *et al.* (1990) studied mortality among 1190 chloralkali workers who had been monitored biologically for exposure to metallic mercury vapour for at least one year in 1946–84. For workers with > 10 years of latency, mortality from all causes was not significantly increased (SMR, 1.1; 95% CI, 0.9–1.3), but mortality from circulatory disease was slightly increased (SMR, 1.3; 95% CI, 1.0–1.5). No such elevation was reported in another study of workers exposed to metallic mercury (Cragle *et al.*, 1984; see p. 269).

Contact dermatitis with sensitization against metallic mercury has been reported. For example, Ancona *et al.* (1982) reported such a case in a dentist who had a positive epicutaneous patch test. Finne *et al.* (1982) performed patch tests on 29 patients with amalgam fillings and oral lichen planus. Positive reactions to mercury were found in 62% as compared with 3% of controls (2300 eczema cases). After the amalgam fillings had been removed from four patients, an improvement in the oral changes was recorded.

In the 1940s, 'pink disease' (acrodynia), presenting as irritation, insomnia, sweating, photophobia and general rash in children, was reported to be associated with exposure mainly to calomel (mercurous chloride) in, e.g. teething powder and ointments (Warkany, 1966). Cases have also been associated with exposure to other chemical forms of mercury, e.g. metallic mercury vapour from broken fluorescent tubes (Tunnessen *et al.*, 1987). The mechanism by which the condition occurs has not been elucidated. Three of six children with mucocutaneous lymph node syndrome (Kawasaki disease), including increased serum IgE and eosinophilia, had urinary concentrations of mercury (16–25 μg/24 h) higher than established normal levels (< 10 μg/24 h). The syndrome may represent a hypersensitivity reaction to environmental pollution with mercury (Orlowski & Mercer, 1980).

(*b*) *Methylmercury compounds*

The first case of 'methylmercury poisoning' was described in a worker exposed to methylmercury phosphate and nitrate for a period of four months (Hunter & Russell, 1954). Since then, numerous descriptions have been published, mainly in connection with outbreaks of poisoning in subjects consuming contaminated fish in Japan (Minamata disease) (Igata, 1991) or seeds treated with methylmercury dicyandiamide, e.g. in Iraq (Bakir *et al.*, 1973). Its main features are that: (i) the target organ is the central nervous system; (ii) there is a latent period between exposure and onset of clinical disease; (iii) the symptoms and signs include paraesthesia in the hands, feet and lips, concentric constriction of visual fields and ataxia; and (iv) morphological changes occur in the visual and precentral cortical areas as well as in the cerebellum. There is also evidence of peripheral neuropathy (Rustam *et al.*, 1975).

In the cohort study in two administrative subunits in the vicinity of Minamata City, Japan (Tamashiro *et al.*, 1986; see pp. 275–276), significantly elevated SMRs were observed for cerebral haemorrhage (1.67, 95% CI; 1.24–2.24), liver disease (2.00; 1.33–2.89), senility (2.34; 1.67–3.26) and violent death (accident, poisoning, suicide) (1.48; 1.12–1.97).

(*c*) *Phenylmercury, ethylmercury and methoxyethylmercury compounds*

A study of 509 infants exposed to phenylmercury acetate from contaminated diapers showed a clear dose–response relationship between the concentration of organomercury compounds in urine and urinary excretion of γ-glutamyl transpeptidase, an enzyme in the brush borders of renal tubular cells. Children with the highest mercury excretion also had increased 24-h urine volumes. Some of the children also had 'pink disease' (Gotelli *et al.*, 1985).

A few cases of systemic poisoning by ethylmercury and methoxyethyl compounds have been reported (for review, see Skerfving & Vostal, 1972). Most patients showed symptoms and signs of disorders in the gastrointestinal tract and kidneys (albumin, red cells and casts in urine).

4.2.2 *Experimental systems*

(*a*) *Metallic mercury and inorganic mercury compounds*

Application of 2 ml of a solution containing 0.24 mol [65 g] mercuric chloride resulted in the death of 3/20 guinea-pigs after two days (Wahlberg, 1965).

Ashe *et al.* (1953) reported damage to brain, liver, kidney, heart and lungs of rabbits exposed to mercury vapour at a concentration of 29 mg/m^3 air. Damage was seen after exposure as short as 1 h. Microscopic changes were observed in mitochondria of the renal proximal tubule after 12 or 25 daily doses of 1 mg/kg bw Hg as mercuric chloride to rats (Bergstrand *et al.*, 1959).

In a susceptible strain of rats (Brown–Norway), subcutaneous injections of mercuric chloride caused a systemic autoimmune nephritis characterized by the production of various antibodies to self and non-self antigens and an increase in total serum IgE concentrations. A biphasic autoimmune glomerulonephritis occurred: initially, anti-glomerular basement membrane antibodies were produced, resulting in linear IgG deposition along the glomerular capillary walls. Later, granular IgG deposits appeared which are responsible for

an immune-complex type glomerulonephritis (Druet *et al.*, 1978). Mercuric chloride appears to induce a T cell-dependent polyclonal activation of B cells in Brown–Norway rats (Pelletier *et al.*, 1986); most animals develop proteinuria, which in some animals progresses to a nephrotic syndrome that is sometimes lethal (Druet *et al.*, 1978), while in other animals the condition is transient. There is a striking strain difference. By crossing susceptible rats with unsusceptible Lewis rats, susceptibility was shown to depend on three or four genes, one of which is located within the major histocompatibility complex (Druet *et al.*, 1982). Certain strains of mice may develop similar glomerular conditions after injection with mercuric chloride (Hultman & Enestrom, 1987).

In Lewis rats injected subcutaneously with mercuric chloride (1 mg/kg bw three times a week for up to 4 weeks), no autoimmune disorder was observed. Instead, animals showed proliferation of suppressor/cytotoxic T cells in the spleen and lymph nodes. As a consequence, they developed a non-antigen-specific immunosuppression and responded to neither classical mitogens nor alloantigens (Pelletier *et al.*, 1987a). Mercuric chloride could also inhibit the development of an organ-specific autoimmune disorder, Heymann's nephritis (Pelletier *et al.*, 1987b).

Micromolar concentrations of mercury have been shown to increase the release of acetylcholine in frog neuromuscular preparations (Manalis & Cooper, 1975) and that of dopamine in adult mouse brain homogenates (Bondy *et al.*, 1979).

Significant decreases in the activities of several enzymes of the glutathione (GSH) metabolic pathway in kidney—GSH disulfide reductase, GSH-peroxidase, γ-glutamyl-cysteine synthetase and γ-glutamyl transpeptidase—were seen 24 h after subcutaneous administration of 10 μmol[2.5 ml]/kg bw mercuric chloride to Sprague–Dawley rats; in the liver, only the activity of GSH disulfide reductase was decreased. After administration of 30 μmol [7.5 mg]/kg bw, the decreases in specific enzyme activities were accompanied by large losses of cellular protein and decreased GSH concentrations in both kidney and liver. The effects could be blocked by sodium selenite (Chung *et al.*, 1982). The mercuric ion binds to reduced sulfhydryl groups in proteins and inhibits a wide range of enzymes (for review, see Kark, 1979).

In Holtzman rats given a lethal intravenous dose of 3 mg/kg bw Hg as mercuric chloride and sacrificed after 4 h, there was extensive renal haemorrhage. Kidney mitochondria contained mercury at 4–5 nmol[0.8–1 μg]/mg protein and showed uncoupling of oxidative phosphorylation.

In mitochondrial preparations of kidney cortex from Sprague–Dawley rats, mercury at concentrations of 2 nmol/mg protein and above affected mitochondrial respiration: clear stimulation of state 4, mild stimulation of state 3 and inhibition of the 2,4-dinitrophenol-induced uncoupled respiration rate. These effects were both preventable and reversible by addition of albumin or dithioerythritol to the in-vitro system (Weinberg *et al.*, 1982a), but not in mitochondria isolated 3 h after subcutaneous administration of mercuric chloride at 5 mg/kg bw, when the concentration of mercury in mitochondrial protein was 0.72 ± 0.10 nmol/mg (Weinberg *et al.*, 1982b).

Addition of mercuric chloride at concentrations of 1–6 μm [0.2–1.2 mg] mercury to preparations of mitochondria from rat kidney cortex and heart in the presence of antimycin

A decreased the production of superoxide but increased hydrogen peroxide production. The authors concluded that mercuric ion caused dismutation of the superoxide, leading to increased hydrogen peroxide formation, which could lead to oxidative tissue damage. Addition of mercurous ions did not affect superoxide production (Miller *et al.*, 1991). [The Working Group noted that the mercury concentrations employed were high.]

Mercuric ions from mercuric chloride, added at 1 mM (270 mg), reacted *in vitro* with isolated DNA (Eichhorn & Clark, 1963). [The Working Group noted the very high concentration used.] No study has shown covalent binding to DNA, e.g. by isolating such an adduct from DNA after complete hydrolysis to nucleosides.

Inhibition of protein synthesis was observed in cell-free systems prepared from mouse glioma after addition of mercuric chloride at a concentration of 2×10^{-5} M [5 mg] (Nakada *et al.*, 1980). [The Working Group noted the high concentration used.] Mercuric chloride at a concentration of 10 μM [2.3 mg] reduced lipid synthesis in isolated mouse sciatic nerve (Clöez *et al.*, 1987).

Sodium selenite dramatically decreased the acute nephrotoxicity of mercuric chloride in rats, when given simultaneously or even 1 h after mercury (Pařízek & Oštádalová, 1967).

(*b*) *Methylmercury compounds*

There are clear species differences in symptoms and signs of poisoning by methylmercury compounds. Blindness has been reported in man, rats, monkeys and pigs, but not in cats (for reviews, see WHO, 1976, 1990). Man, monkeys and cats develop ataxia; but in rats dosed orally with methylmercury chloride reduced conduction velocities and histopathological changes occurred in peripheral nerves, while the central nervous system was not affected (Fehling *et al.*, 1975).

Renal damage is a typical finding in rats. Male Wistar rats fed mercury at 0.250 mg/kg bw per day as methylmercury chloride for up to 26 months developed severe renal tubular damage. The estimated mercury level in kidney was 30.2 mg/kg in males and 60 mg/kg in females (Munro *et al.*, 1980). Nuclear swelling and vacuolar degeneration of the cytoplasm were seen in SPF ICR mice fed a diet containing 10 ppm methylmercury chloride for 26 weeks (Hirano *et al.*, 1986). Treatment of monkeys with daily oral doses of 80–125 μg/kg bw methylmercury hydroxide for 3–12 months did not appear to affect the general well-being of the animals, but ultrastructural changes occurred in the kidneys, with intracytoplasmic vacuoles and electron-dense inclusion bodies in the proximal tubuli (Chen *et al.*, 1983).

In mice fed mercury at 3.9 mg/kg diet as methylmercury chloride for 12 weeks, thymus weight and cell number were decreased, the lymphoproliferative response to T and B mitogens was increased in thymus and spleen, and natural killer cell activity was decreased in the spleen and blood (Ilbäck, 1991). Mice fed methylmercury chloride at doses of 1–10 mg/kg diet for 84 days had significantly higher mortality rates when inoculated with encephalomyelitis virus (nononcogenic) than did animals not given methylmercury (Koller, 1975).

Impairment of adrenal and testicular function occurred in rats given 23 intraperitoneal injections of 0.26 mg methylmercury chloride over six weeks (Burton & Meikle, 1980); thyroid function was impaired in mice given two intraperitoneal doses of 5 mg/kg bw (Kawada *et al.*, 1980).

Female Charles River CD rats were given 3–10 mg/L methylmercury hydroxide in drinking-water four weeks prior to mating and through day 19 of pregnancy. With concentrations of 3–5 mg/L, there was decreased synthesis of mitochondrial structural proteins in the livers of the fetuses and inhibition of several mitochondrial enzymes (Fowler & Woods, 1977a). In male rats of the same strain treated similarly for six weeks, electron microscopy revealed swelling of the renal proximal tubule cell mitochondria at a dose of 5 mg/L. The respiratory control ratios were decreased (mitochondrial respiratory dysfunction), and effects were seen on enzyme activities, including decreased monoamine oxidase and cytochrome oxidase and increased δ-aminolaevulinic acid synthetase. The rats had increased urinary excretion of porphyrins but no deterioration in standard renal function tests (Fowler & Woods, 1977b).

Mouse glioma cell cultures treated with methylmercury chloride (5×10^{-6} M for 4 h) showed inhibition of cell mitosis, by blockage of the polymerization of tubulin to microtubuli, with accumulation of cells during mitosis. Electron microscopy showed an absence of microtubuli as mitotic spindle fibres and disorganization of chromosomes (Miura *et al.*, 1978).

Sodium selenite, and possibly also the chemically unknown form of selenium found in marine foods, delayed the onset of the toxic effects of methylmercury chloride in rats and reduced the severity of its effects (Chang & Suber, 1982).

Methylmercury hydroxide added to fish homogenate and methylmercury-contaminated fish were equally neurotoxic to cats (Albanus *et al.*, 1972). Similar results (ataxia, loss of balance or motor incoordination, loss of nerve cells) were found in cats fed either methylmercury chloride or methylmercury-contaminated fish (Charbonneau *et al.*, 1976).

(*c*) *Phenylmercury, ethylmercury and methoxyethylmercury*

Renal damage was observed in mice, rats and rabbits given phenylmercury nitrate and chloride intraperitoneally or intravenously (Weed & Ecker, 1933). Ethylmercury poisoning has been described in rats, rabbits, cats, sheep, swine and calves. The symptoms are similar to those of methylmercury poisoning (Skerfving & Vostal, 1972). In rats administered the fungicide methoxyethyl mercury chloride (2 mg/kg bw for 50 days or 0.2 mg/kg bw for 80 days) intraperitoneally, impaired weight gain, renal damage and signs of nervous system damage (e.g. tremor, ataxia) were seen (Lehotzy & Bordas, 1968).

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

The effects of inorganic and organomercury compounds on human reproduction and development have been reviewed (Khera, 1979; Inskip & Piotrowski, 1985; Schardein, 1985; Burbacher *et al.*, 1990; Roeleveld *et al.*, 1990; Shepard, 1992).

(*a*) *Metallic mercury and inorganic mercury compounds*

(i) *Exposure of women*

Adverse pregnancy outcomes have been reported following exposure to mercuric chloride tablets (Afonso & de Alvarez, 1960), to mercuric iodide-containing soap (Lauwerys

et al., 1987) and in a dental surgery unit where the concentration of mercury exceeded the threshold limit value of 0.05 mg/m^3 (Gelbier & Ingram, 1989). After exposure of a woman prior to week 17 of pregnancy to metallic mercury in a contaminated carpet (24-h urinary concentration of mercury, 230 μg/L), no adverse effect was seen on birth weight, growth or on acquisition of developmental milestones in the child at the age of two (Thorp *et al.*, 1992).

Heidam (1984) conducted a historical prospective study of pregnancy outcomes in women in 12 selected occupations in the Danish county of Funen. Controls were employed in occupations with less exposure to chemicals. Dental assistants returned 94% of the 772 mailed questionnaires on pregnancy history. The incidence of spontaneous abortions in dental assistants in private clinics was 11.2% in 259 pregnancies, yielding a crude odds ratio of 1.1 (95% CI, 0.7–1.8). After control for confounding variables, including age at gravidity, pregnancy order and maternal age at pregnancy, the odds ratio was 1.0 (0.6–1.6). Dichotomization of dental assistants according to whether they reported exposure to inorganic mercury compounds also showed no increase in the spontaneous abortion rate in the exposed subgroup.

Brodsky *et al.* (1985) conducted a postal survey of 30 272 female dental assistants in California (USA) regarding the use of anaesthetic agents and mercury amalgams and health and pregnancy histories for the years 1968–78. The response rate was 70%. Exposure was categorized on the basis of the number of amalgam restorations prepared per week into no, low (0–40) or high (> 40) . Outcomes were adjusted for maternal age and cigarette smoking. No relationship was observed between exposure and spontaneous abortion or congenital abnormalities.

Sikorski *et al.* (1987) evaluated reproductive function and outcome in 81 women (45 dentists, 36 dental assistants) exposed occupationally to metallic mercury and in 34 unexposed women [occupational details not given] recruited at random in the Lublin region of Poland. Exposure was ascertained by determination of mercury in samples of scalp and pubic hair; the mercury content in hair was related to duration of employment and to the number of amalgams used per week. A total of 57 exposed women had 117 pregnancies, 24% of which ended in spontaneous abortion, stillbirth or congenital malformations (including five cases of spina bifida). Thirty unexposed women had 63 pregnancies, 11% of which ended in an adverse outcome. Reproductive failure was associated with the mercury content of the hair. The frequency of menstrual disorders was also high in exposed women and was related to the number of years employed and to the mercury content of scalp hair. [The Working Group noted that temporal matching of exposure and pregnancy was not carried out, that no mention of potential confounders was made and that hair mercury levels poorly reflect exposure to metallic mercury.]

Ericson and Källén (1989) evaluated 8157 infants born to dentists, dental assistants and dental technicians in Sweden between 1976 and 1986. Outcomes were standardized for maternal age and parity, year of birth and sex of the infant. There was no suggestion of an increased rate of stillbirths or congenital malformations. The risk ratio for low birth weight (< 2500 g) was 0.9 (95% CI, 0.7–1.2) for dentists, 1.2 (1.0–1.3) for dental assistants and 0.8 (0.5–1.4) for dental technicians. Data on spontaneous abortions were available only for 1980–81, and the rates for dentists, dental assistants and dental technicians corresponded to expected figures. The authors also reported no increase in the rates of spontaneous abortion

or neural tube defects among women working in dentistry, as ascertained in a small prospective study in Malmö in 1964–65. [The Working Group noted that no marker of exposure to mercury was used.]

De Rosis *et al.* (1985) studied the possible effects on reproductive function and outcome in women of exposure to mercury vapour in two mercury vapour lamp factories in Italy. Workers were exposed to mercury in only one plant, where time-weighted averages exceeded 0.05 mg/m^3 in 1972–76; they were subsequently reduced to < 0.01 mg/m^3. Workers in a second plant were used as the reference group. Participation was 79% (153 women) in the exposed plant and 88% (293) in the reference plant. Past health events were ascertained by interview. The prevalence and incidence of menstrual cycle disorders were higher in the exposed group, with an age-standardized ratio of abnormal cycles of 1.4. Exposed married women also had a higher prevalence of primary subfecundity. No difference in the rates of spontaneous abortion was found, but the malformation rate, particularly of dislocations of the hip, was higher in the exposed group (6/106 births) than in the unexposed group (0/218 births); however, the authors noted that the prevalence of the condition differed between northern and southern Italy.

(ii) *Exposure of men*

A questionnaire on fertility was distributed to the total male work force of three factories in which workers were exposed to mercury vapour and of two control plants with comparable work characteristics in Belgium (Lauwerys *et al.*, 1985). Blood and urine mercury concentrations were used as indices of exposure. The mercury-exposed group consisted of 17 workers in a zinc–mercury amalgam factory, 35 workers in a chloralkali plant and 51 workers in plants for the manufacture of electrical equipment. The 50th and 95th percentiles of mercury in the urine were 36.9 and 147.1 μg/g creatinine. No difference was noted between the observed and expected numbers of children in the mercury-exposed group.

Alcser *et al.* (1989) conducted a retrospective study of reproductive function in 247 white male workers who had been employed for at least four months between 1953 and 1966 at a US Department of Energy plant where large quantities of metallic mercury were used in 1953–1963. Intermittent periods of potentially high exposure occurred, especially between 1955 and 1956, and a quarterly programme of urine analysis charted worker exposure from 1953 onwards. A control group was selected from unexposed workers at the same plant. Most measures of reproductive health (fertility rates, incidence of major malformations and childhood illnesses) did not differ between the two groups. The wives of the exposed men had a higher rate of miscarriages; however, this effect was also present prior to exposure to mercury.

Cordier *et al.* (1991) studied the rate of spontaneous abortions in wives of workers exposed to mercury vapour at a chloralkali plant in France and compared it with that of the wives of controls from the same plant. Reproductive history was ascertained by questionnaire in 1984, and exposure history was provided by a plant physician. Urinary mercury levels were measured in most potentially exposed workers at least once a year from 1968. The response to the questionnaire was about 75%, resulting in the inclusion of 118 exposed and 283 unexposed workers. Results were adjusted for maternal age, gravidity, tobacco use and

alcohol consumption. The risk for spontaneous abortion increased significantly with increasing urinary mercury concentration in the three months preceding pregnancy. For example, for urinary mercury concentrations in excess of 50 μg/ml in the three-month period prior to the initiation of pregnancy, the spontaneous abortion rate was 18.4/100 pregnancies, as compared with 8.6/100 in the wives of unexposed men [RR, 2.1; 95% CI, 1.1–4.1]. No relationship between exposure to mercury and birth weight or the frequency of malformations was found.

(*b*) *Methylmercury compounds*

In a review of the literature, Inskip and Piotrowski (1985) found no evidence that miscarriage, stillbirth, major deficits in birthweight, chromosomal damage or hormonal imbalances in infants were associated with exposure to methylmercury compounds; microcephaly appeared to be the only congenital malformation associated with exposure. Most effects were expressed as clinical symptoms, such as delay in disappearance of primitive reflexes, mental disturbances, retardation of physical development, retardation in emergence of behaviour patterns, disturbances in chewing and swallowing, motility disturbances, impairment of voluntary movements and coordination (ataxia) and constriction of the visual field. Manifestations of toxicity were not always evident at birth but sometimes developed later in childhood.

In a review of autopsy reports on children exposed *in utero* to methylmercury compounds, Burbacher *et al.* (1990) concluded that high concentrations in the brain (12–20 ppm [mg/kg]) decreased brain size, damaged the cortex, basal ganglia and cerebellum and resulted in ventricular dilatation, ectopic cells, gliosis, disorganized layers, misorientated cells and loss of cells. At those tissue concentrations, the neurobehavioural effects included blindness, deafness, cerebral palsy, spasticity, mental deficiency and seizures. At concentrations of 3–11 ppm (mg/kg), mental deficiency, abnormal reflexes and muscle tone and retarded motor development occurred [no data were presented on neuropathological effects]. At low levels (< 3 ppm), delayed psychomotor development was reported.

Foldspang and Hansen (1990) studied reproductive outcomes in Godthaab (365 infants; 45.9% of total births in the period) and Thule (11 infants; 100% of total births), Greenland, between January 1983 and December 1986. Women were invited to participate in the study when they entered maternity clinics at the beginning of labour; nonparticipation was attributed to the difficulty of collecting data in the Arctic. Socioeconomic data, cigarette consumption, consumption of traditional Greenlandic foods (eating whale and seal meat was widespread) and other data were obtained by interview or from hospital records. Maternal and umbilical blood samples were collected at delivery and assayed for total mercury. The average blood mercury concentration of the infants was 21 μg/ml (range, 2–136 μg/L), while maternal levels averaged 14.9 μg/ml (range, 2–128 μg/L). Birth weights were inversely proportional to maternal and offspring blood mercury concentrations. [The Working Group noted that maternal height was not taken into account although birthplace was.]

Grandjean and Weihe (1993) studied birthweight in relation to fish consumption in residents of the Faroe Islands. A total of 1024 births that occurred between March 1986 and December 1987 were included. The average birthweights of the infants of nonsmoking

mothers were 3400 g in 13 infants whose mothers consumed no fish and 3600 g (n = 83), 3850 (n = 220), 3800 (n = 183) and 3750 g (n = 114) in women who consumed 1, 2, 3 and > 4 fish dinners per week, respectively. The average total mercury concentrations in cord blood were 20, 118, 105, 133 and 138 nmol/L [4, 23.6, 21, 26.6 and 27.6 μg/L] in the same groups. Thus, elevated cord blood mercury concentrations were associated with increased birth-weight, but the authors attributed this correlation to the content of (n-3)-poly-unsaturated fatty acids in fish.

4.3.2 *Experimental systems*

(*a*) *Metallic mercury and inorganic mercury compounds*

The reproductive and developmental effects of metallic mercury and its salts in laboratory animals have been reviewed (Khera, 1979; Barlow & Sullivan, 1982; Léonard *et al.*, 1983; Schardein, 1985; Shepard, 1992). In a review, Barlow and Sullivan (1982) concluded that exposure to metallic mercury (e.g. inhalation of 0.3 ppm [2.5 mg/m^3] by rats for 6 h per day for three weeks prior to pregnancy and again on gestation days 7–20) and to inorganic mercury (e.g. intravenous injections of 2–4 mg/kg bw mercury into hamsters on gestation day 8) can cause fetal growth retardation and prenatal and postnatal mortality.

Altered oocyte maturation was reported after exposure of hamsters to mercuric chloride on day 1 of the oestrous cycle. The effective dose levels were as low as 1 mg/kg bw per day (Lamperti & Printz, 1973; Watanabe *et al.*, 1982).

When mercuric chloride (at 5–80 μM [1.2–18.8 mg]) was added to freshly prepared human semen samples *in vitro*, a dose- and time-dependent decrease in sperm motility was observed. In addition, morphological changes and silver-enhanced mercury deposits in the sperm were noted (Ernst & Lauritsen, 1991).

Exposure of mice to mercuric chloride as 1.5 or 2.0 mg/kg bw Hg by intravenous injection on day 0 resulted in a high incidence of abnormal blastocysts when cells were examined on day 3.5 of gestation (Kajiwara & Inouye, 1986). The minimal effective doses of mercuric acetate that reduced embryonic viability and increased the incidence of malformations and growth retardation in hamsters treated on day 1 of gestation were 35, 25 and 8 mg/kg by oral administration, 8 mg/kg by subcutaneous injection, 4 mg/kg by intravenous injection and 2 mg/kg by intraperitoneal injection (Gale, 1974). Subcutaneous injection of 15 mg/kg bw mercuric acetate to six strains of hamsters on gestation day 8 caused increased resorptions and abnormal and growth retarded fetuses, the incidence of which varied slightly from strain to strain (Gale, 1981).

Exposure of mice to doses of 7.5–25 mg/kg mercuric chloride by subcutaneous injection on day 16 of gestation resulted in a 40% reduction in fetal accumulation of vitamin B_{12} and α-aminobutyric acid within 4 h; no overt fetal toxicity was observed with doses up to 15 mg/kg, although some fetal deaths occurred with 20 mg/kg and maternal lethality was seen at 25 mg/kg (Danielsson *et al.*, 1984). Reduced levels of fetal zinc, copper and iron were reported 4–24 h after exposure of pregnant rats to 0.79 mg/kg mercury by intravenous injection on gestation day 12 (Holt & Webb, 1986a). Intravenous injections of 0.5–0.6 mg/kg mercuric chloride on gestation day 7 were reported to cause fetal malformations. A slightly higher dose (0.79 mg/kg) caused resorptions when given on day 12 and growth retardation

when given on days 8, 10, 12, 14 or 16. The authors attributed the fetal effects to alterations in maternal renal function resulting from exposure to mercury (Holt & Webb, 1986b).

Subcutaneous administration of 1 mg/kg bw mercuric chloride to Sprague–Dawley rats on the last eight days of gestation induced a transient increase in urinary excretion of β_2-microglobulin and albumin in both mothers and offspring. In male offspring, these effects reappeared at 180 days of age. A follow-up experiment in which females were dosed throughout pregnancy showed an effect on male offspring renal function at 3–4 months of age, but not at 10 months (Bernard *et al.*, 1992).

Decreased embryonic growth was observed after exposure *in vitro* of day-10 rat embryos to 4 μM mercuric chloride for 48 h or to 20 μM for 24 h; the concentrations required to affect morphogenesis were 1 μM and 20 μM, respectively (Kitchin *et al.*, 1984; Saillenfait *et al.*, 1990).

(*b*) *Methylmercury compounds*

The literature on the effects of exposure to methylmercury compounds on prenatal development in experimental animals, including effects on the function of several organ systems in postnatal animals following exposure *in utero*, is extensive (for reviews, see Khera, 1979; Reuhl & Chang, 1979; Léonard *et al.*, 1983; Inskip & Piotrowski, 1985; Mottet *et al.*, 1985; Schardein, 1985; Burbacher *et al.*, 1990; Shepard, 1992).

Subcutaneous injection of hamsters with 6.4 or 12.8 mg/kg bw mercury as methylmercury chloride on day 1 of the oestrous cycle did not affect the number of oocytes released (Watanabe *et al.*, 1982).

Albino male rats received 0, 5 or 10 μg/kg bw per day methylmercury chloride by intraperitoneal injection for 15–90 days. Time- and dose-dependent decrease in seminiferous tubular diameter, numbers of Sertoli cells per tubular cross section, and numbers of spermatogonia, preleptotene spermatocytes, pachytene spermatocytes and step-7 spermatids were found. Zygotenes at stages XII through XIII and pachytenes at stages XII through early XIV of seminiferous tubules were most affected. The Sertoli cell was suggested as the target of toxicity (Vachhrajani *et al.*, 1992). [The Working Group noted that the testes were immersed, fixed and embedded in paraffin.] In freshly prepared human semen samples to which methylmercury chloride was added *in vitro*, similar, but less rapid and pronounced changes than those seen with mercuric chloride (see above) were present; however, no silver-enhanced mercury deposition was seen in sperm (Ernst & Lauritsen, 1991).

No effect on the ability to inseminate females or produce viable young was noted in male mice exposed by oral intubation for five to seven days to up to 5 mg/kg mercury as methylmercury chloride in seven consecutive five-day breeding trials. When male Wistar rats were exposed by oral intubation at the same dose regimen for seven days and followed over 14 consecutive five-day breeding trials, a reduced incidence of pregnancy was seen at 5 mg/kg on days 0–15 after treatment and reduced numbers of viable implants were seen on days 5–20 after 2.5 or 5 mg/kg. With longer-term exposures (up to 125 days), reduced numbers of viable implants were seen 25–30 days after exposure to 1 mg/kg and 85–90 days after exposure to 0.5 mg/kg (Khera, 1973a).

A three-generation study showed a lower viability index in F_1 and F_2 generations of rats following dietary exposure to 2.5 but not to 0.5 ppm (mg/kg) methylmercury chloride.

Growth retardation was observed in F_{2a} females at 0.1, 0.5 and 2.5 ppm and in F_{2a} males at 2.5 ppm; and increased relative kidney weights were observed in P males and females, F_{1a} males and F_{2a} females and males at 2.5 ppm, in F_{2a} males and females at 0.5 ppm and in F_{2a} females and F_{1a} males at 0.1 ppm (Verschuuren *et al.*, 1976c).

The development of mouse blastocysts *in vitro* was affected by exposure to methylmercury chloride at 1 μM (Matsumoto & Spindle, 1982) and 0.3 μM (Müller *et al.*, 1990). Intravenous injections of 2 mg/kg bw mercury or more as methylmercury chloride to mice on day 0 of gestation (Kajiwara & Inouye, 1986) or intraperitoneal injection of 5 mg/kg bw to rats during the pre-implantation period (Giavini *et al.*, 1985) affected embryonic development and/or viability.

Prenatal effects on offspring were reported following exposure of mice *in vivo* to methylmercuric chloride during pregnancy at doses as low as 5 mg/kg (fetal weight, Fuyuta *et al.*, 1978; embryonic death, Curle *et al.*, 1983, 1987); malformations commonly seen at this dose or at slightly higher doses included cleft palate and hydronephrosis. The effective dose levels in the rat fetus appear to be similar to those that cause effects in mice (Fuyuta *et al.*, 1979); however, the manifestations vary somewhat (e.g. cleft palate is observed less frequently at 7.5 mg/kg). Reductions in embryonic growth and viability were seen in rat embryos taken on gestation day 10 and exposed *in vitro* to 30 μM methylmercury chloride for 48 h; effects on morphogenesis were reported at the lowest dose tested (3 μM) (Kitchin *et al.*, 1984). Histological damage to the developing brain was observed following exposure *in utero* to methylmercury chloride of hamsters (10 mg/kg on gestation day 10; 2 mg/kg on gestation days 10–15; Reuhl *et al.*, 1981), guinea-pigs (7.5 mg/kg on day 21, 28, 35, 42 or 49; Inouye & Kajiwara, 1988) and cats (0.25 mg/kg on gestation days 10–58; Khera, 1973b). Monkeys (*Macacca fascicularis*) exposed to 50 or 90 μg/kg bw methylmercury hydroxide by oral intubation for 124 days before mating appeared to have lower conception rates and smaller offspring, but small sample sizes precluded statistical significance (Burbacher *et al.*, 1984).

Exposure of mice *in utero* to doses as low as 8 mg/kg of methylmercury compounds on single days of gestation resulted in neonatal mortality and growth impairment (Gates *et al.*, 1986, using the chloride), hydrocephaly (Choi *et al.*, 1988, chloride), changes in activity in the open field (Spyker *et al.*, 1972, dicyandiamide [$CH_3HgNHC(NH)NHCN$]; Su & Okita, 1976, hydroxide) and altered swimming behaviour (Spyker *et al.*, 1972, dicyandiamide). Snell *et al.* (1977, chloride) reported lower concentrations of liver glycogen in fetal (two-day prenatal) rats but higher concentrations in six-day-old rats exposed to 4 or 8 mg/kg on gestation day 9. Robbins *et al.* (1978, chloride) found decreased levels of cytochrome P450 and certain xenobiotic metabolizing enzymes in 26–36-week-old male but not female offspring exposed by oral intubation to 5 mg/kg on gestation day 0 or to 2.75 mg/kg on gestation day 7. Chang and Sprecher (1976a,b) reported morphological evidence of renal tubular damage (degenerative changes in epithelial cells of the proximal tubule, hyperplastic changes in distal convoluted tubules and thickening of the tubular epithelial linings) in neonatal rats after injection of dams with 1 or 4 mg/kg methylmercury chloride on day 8 of gestation. Smith *et al.* (1983) exposed rats to 4 mg/kg methylmercury chloride on days 8, 10 and 12 of gestation and observed reduced uptake of an organic anion (*para*-aminohippurate) by renal slices on postnatal day 42, but not on days 1 or 7, and reduced ability to eliminate sodium and water in volume-loaded rats when measured on postnatal day 42. Slotkin *et al.* (1986) reported slightly

reduced growth rates after weaning, alteration of renal function, an increased level of liver ornithine decarboxylase and altered renal ornithine decarboxylase response to isoproterenol and vasopressin in rats injected subcutaneously with 0.5 or 1.0 mg/kg methylmercury hydroxide on days 8–21 of gestation.

Bornhausen *et al.* (1980) reported impaired operant behaviour performance in the offspring of rats exposed to daily doses as low as 0.01 mg/kg methylmercury chloride on days 6–9 of gestation. A large, multilaboratory evaluation of behavioural effects in rat offspring exposed to 2 or 6 mg/kg methylmercury chloride on days 6–9 of gestation found increased auditory startle response at the high dose in young offspring and dose-related effects on figure-8 maze exploratory activity and in the pharmacological response to d-amphetamine challenge in older animals (Buelke-Sam *et al.*, 1985). In rats exposed to mercury at 3.9 mg/kg diet as methylmercury chloride *via* their dams during gestation and lactation and *via* the diet up to the age of 50 days, no histological change occurred in the brain, but increased noradrenaline levels were observed in the cerebellum (Lindström *et al.*, 1991). Rice (1992) found deficits in fixed interval performance, but not in discrimination reversal or activity patterns, in offspring of *Macaca fascicularis* exposed to steady-state levels during gestation and subsequently of 0, 10, 25 or 50 μg/kg per day mercury as methylmercury chloride. One infant in the high-dose group showed overt signs of methylmercury toxicity.

4.4 Genetic and related effects

4.4.1 *Humans* (see also Table 15, pp. 304–305 and Appendices 1 and 2)

(*a*) *Dietary exposures*

Skerfving *et al.* (1970) examined nine Swedish subjects who ate methylmercury-contaminated fish (containing 1–7 ppm [mg/kg] mercury) at least three times a week for more than five years and four controls who ate uncontaminated fish (containing $\leq$ 0.05 ppm [mg/kg] mercury] less than once a week. There was no significant difference in the frequency of chromosomal aberrations between exposed subjects and controls nor any correlation between aneuploidy or polyploidy rates and concentrations of mercury in red blood cells, which were in the range of 5–17 ng/g in controls and 21–370 ng/g in exposed subjects. Mercury concentrations were, however, significantly correlated with the frequency of structural rearrangements. The study was expanded to include a total of 23 exposed subjects (including the nine subjects already reported in 1970) and 16 controls (Skerfving *et al.*, 1974). Small differences were observed in the frequency of chromosomal aberrations in the exposed groups, and a significant correlation was seen between chromatid-type aberrations, 'unstable' chromosome-type aberrations or aneuploidy and mercury concentrations in red blood cells, which were in the range of 3–17 ng/g in controls and 12–1100 ng/g in exposed subjects. There was no correlation between the frequency of chromosomal aberrations and variations in mercury concentrations in repeated samplings.

Wulf *et al.* (1986) investigated the frequency of sister chromatid exchange in the lymphocytes of 147 Eskimos living in Greenland or Denmark, who were divided into three groups according to their intake of seal meat: subjects who ate seal meat at least six times a week and had an average concentration of mercury in the blood of 62.5 μg/L (range, 41.9–

65.4); subjects who ate seal meat two to five times per week and had mercury concentrations of 23.2–51.0 μg/L; subjects who ate seal meat once a week or less and had an average mercury concentration in the blood of 22.2 μg/L (range, 5.4–26.4). The mean frequency of sister chromatid exchange was 1.7-fold higher in the group that ate seal meat at least six times a week than in those who ate it less than once a week and 10.7-fold higher in the intermediary group. An increase of 10 μg/L in blood mercury corresponded to an increase of 0.2–0.3 sister chromatid exchanges per cell. [The Working Group noted that the reported results are difficult to interpret because only a series of statistical analyses is provided in the article, with limited original cytogenetic data.]

No increase in the frequency of sister chromatid exchange or numerical chromosomal alterations was detected in 16 subjects who ate fish caught from a methylmercury-contaminated area in Colombia as compared to 14 controls who ate fish from an uncontaminated area. The blood mercury ranges were 2.2–25.8 μg/L in unexposed and 10.2–97.3 μg/L in the exposed people. The frequency of structural chromosomal aberrations was increased only when achromatic lesions (chromatid and chromosome gaps) were included (Monsalve & Chiappe, 1987).

(*b*) *Occupational exposures*

Verschaeve *et al.* (1976) examined seven control subjects (blood mercury concentration, 2.5–8.4 μg/L) and 28 mercury-exposed subjects (blood mercury concentration, < 0.1–13 μg/L; urinary mercury concentration, < 0.1–114.12 μg/L) in Belgium, 10 of whom were under medical supervision for mercury intoxication, and 18 subjects working with mercury at Brussels University. An increased frequency of aneuploidy was seen in those subjects exposed to metallic mercury (14), amalgams (3), phenylmercury (8) and ethylmercury (3), while the incidence of structural chromosomal aberrations was increased only in the last (small) group. An increased rate of hyperploidy was also reported by Verschaeve *et al.* (1978) in 16 workers exposed to phenylmercury acetate (blood mercury concentration, 0–5.6 μg/L), compared to 12 unexposed controls (0–3.5 μg/L). In an abstract, Verschaeve and Susanne (1979) reported an increased rate of aneuploidy, with no increase in the frequency of structural chromosomal aberrations in 10 subjects exposed to mercury-containing amalgams in a dental practice and compared with 10 controls, but they could not rule out other factors such as X-rays. Verschaeve *et al.* (1979) failed to detect any chromosomal effect in 28 workers exposed to metallic mercury (urinary mercury concentration, 7–175 μg/L) in a chloralkali plant, as compared with 20 unexposed controls (eight from the same plant [< 5–15 μg/L] and 12 from the general population). In the discussion of the report, the authors questioned the positive findings in their previous three studies, stating that they might have been due to lack of information on exposures to agents other than mercury in the subjects. Negative results were also reported in a more recent Belgian study (Mabille *et al.*, 1984) involving cytogenetic analyses of 25 unexposed subjects (urinary mercury, < 5 μg/g creatinine, and blood mercury, < 6 μg/L) and of 22 workers exposed to metallic mercury in a chloralkali plant and in a plant in which mercury is amalgamated with zinc (urinary mercury, 8.2–286 μg/g creatinine, and blood mercury, 7.5–105.2 μg/L).

Popescu *et al.* (1979) examined peripheral blood lymphocytes from 22 workers in two departments of a chemical plant in Romania, four of whom were exposed to vapours of

metallic mercury and 18 to a mixture of mercuric chloride, methylmercury chloride and ethylmercury chloride. During the year before the study, atmospheric mercury concentrations ranged from 0.15 to 0.44 mg/m3. Urinary analysis demonstrated high concentrations of mercury (100–896 μg/L). When compared with 10 unexposed controls, neither exposed group had an increased rate of aneuploidy or of total structural chromosomal aberrations; however, both had a significant increase in the frequency of acentric fragments.

In an abstract, Mottironi *et al.* (1986) reported an increased rate of sister chromatid exchange in somatic cells [presumably lymphocytes] from 29 workers exposed to metallic mercury and inorganic mercury in two plants [presumably in the USA] (blood mercury, 6.7–103.9 μg/L) over that in 26 unexposed controls (blood mercury, 1.5–6.1 μg/L). The increase was significant in 'exposed workers with high mercury levels' in the blood [no detail given] and was enhanced by cigarette smoking. An increased rate of sister chromatid exchange, related to time since exposure, was detected in Argentina in the lymphocytes of 38 children, aged one month to five years, who had been intoxicated by the use of phenylmercury acetate to disinfect diapers. Nineteen unexposed children served as controls. The increased rate of sister chromatid exchange disappeared nine months after exposure ceased (Mudry de Pargament *et al.*, 1987).

Barregård *et al.* (1991) compared the incidence of lymphocytic micronuclei in 26 chloralkali workers (air mercury concentration, 25–50 μg/m^3) and 26 unexposed controls in Sweden. No difference was found between the two groups, and no correlation was found with current mercury concentrations in erythrocytes, plasma or urine (exposed, 4.8–64.6 μg/L, 2.8–40 μg/L and 5–186 μg/L; controls, 2.4–20.2 μg/L, 0.8–2.6 μg/L and 0.2–9.6 μg/L, respectively). A significant association was found between the number of micronuclei in phytohaemagglutinin-stimulated blood and previous exposure to mercury (measured either by a cumulative exposure index, i.e. integrated yearly mean blood mercury over employment time, or number of occasions when blood mercury peaks were > 150 nmol/L [30 μg/L]), suggesting an accumulation of cytogenetic effects in T lymphocytes. No such association was seen in pokeweed mitogen-stimulated blood. Anwar and Gabal (1991) examined 29 workers in an explosives factory in Egypt (mean urinary mercury concentration, 123 μg/L) who were exposed to mercury fulminate [$Hg(CNO)_2$] (which results from the reaction between mercuric nitrate, ethanol and nitric acid) and 29 controls (mean urinary mercury concentration, 39 μg/L). The frequencies of both micronuclei and chromosomal aberrations (gaps, breaks and fragments) were increased in the exposed group. The authors reported, however, that the increases were not correlated with urinary concentration of mercury or duration of exposure, putting in question the role of mercury in the observed clastogenic effect. In addition, there was no increased incidence of either aneuploidy or polyploidy.

4.4.2 *Experimental systems*

The genetic and related effects of mercury compounds have been reviewed (Ramel, 1972; Léonard *et al.*, 1983; Kazantzis & Lilly, 1986).

(*a*) *Inorganic mercury compounds* (see also Table 16, pp. 307–309 and Appendices 1 and 2)

Almost all of the studies reported were carried out with mercuric chloride.

Table 15. Genetic and related effects of mercury in humans

Test system	Result without exogenous metabolic system	Dose[a] (LED/HID)	Reference
Food contaminated with organomercury compounds[b]			
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	(+)[c]	0.042	Wulf *et al.* (1986)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	-	0.040	Monsalve & Chiappe (1987)
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	(+)	0.126	Skerfving *et al.* (1970)
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	(+)	0.12	Skerfving *et al.* (1974)
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	-[d]	0.040	Monsalve & Chiappe (1987)
AVH, Aneuploidy, human lymphocytes *in vivo*	-	0.126	Skerfving *et al.* (1970)
AVH, Aneuploidy, human lymphocytes *in vivo*	(+)	0.12	Skerfving *et al.* (1974)
Occupational and environmental exposures to mercury			
Metallic mercury			
SLH, Sister chromatid exchange, human lymphocytes *in vivo*[e]	+	0.027	Mottironi *et al.* (1986) abstract
MVH, Micronucleus induction, phytohaemaglutinin-stimulated human (T) lymphocytes *in vivo*	(+)	0.025	Barregård *et al.* (1991)
MVH, Micronucleus induction, pokeweed mitogen-stimulated human (T/B) lymphocytes *in vivo*	-	0.025	Barregård *et al.* (1991)
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	-	NR	Verschaeve *et al.* (1976)
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	+	NR	Popescu *et al.* (1979)
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	-	NR	Verschaeve *et al.* (1979)
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	-	0.031	Mabille *et al.* (1984)
AVH, Aneuploidy, human lymphocytes *in vivo*	?	NR	Verschaeve *et al.* (1976)
AVH, Aneuploidy, human lymphocytes *in vivo*	-	NR	Popescu *et al.* (1979)
AVH, Aneuploidy, human lymphocytes *in vivo*	-	NR	Verschaeve *et al.* (1979)
Amalgams			
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	-	NR	Verschaeve *et al.* (1976)
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	-	NR	Verschaeve & Susanne (1979) abstract
AVH, Aneuploidy, human lymphocytes *in vivo*	?	NR	Verschaeve *et al.* (1976)
AVH, Aneuploidy, human lymphocytes *in vivo*	?	NR	Verschaeve & Susanne (1979) abstract

Table 15 (contd)

Test system	Result without exogenous metabolic system	Dose[a] (LED/HID)	Reference
Ethylmercury compounds [unspecified]			
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	?	NR	Verschaeve *et al.* (1976)
AVH, Aneuploidy, human lymphocytes *in vivo*	?	NR	Verschaeve *et al.* (1976)
Mercury fulminate [$Hg(CNO)_2$]			
MVH, Micronuclei, human lymphocytes *in vivo*	?	NR	Anwar & Gabal (1991)
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	?	NR	Anwar & Gabal (1991)
AVH, Aneuploidy, human lymphocytes *in vivo*	-	NR	Anwar & Gabal (1991)
Methylmercury chloride/ethylmercury chloride/mercuric chloride mixture			
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	+	NR	Popescu *et al.* (1979)
AVH, Aneuploidy, human lymphocytes *in vivo*	-	NR	Popescu *et al.* (1979)
Phenylmercury compounds [unspecified]			
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	-	0.008	Verschaeve *et al.* (1976)
AVH, Aneuploidy, human lymphocytes *in vivo*	(+)	0.008	Verschaeve *et al.* (1976)
Phenylmercury acetate			
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	-	0.002	Verschaeve *et al.* (1978)
AVH, Aneuploidy, human lymphocytes *in vivo*	?	0.002	Verschaeve *et al.* (1978)
SCE, Sister chromatid exchange, human lymphocytes *in vivo*	+	NR	Mudry de Pargament *et al.* (1987)

+, positive; (+), weakly positive; +?, positively questioned by the authors themselves; -, negative; ?, inconclusive

[a]Mean concentration of Hg in blood; μg/ml. NR, not reported

[b]Organic mercury of alimentary source (seal or fish meat)

[c]Also contaminated with lead, cadmium and selenium

[d]Excluding gaps; positive if gaps are included

[e]Exposure to metallic mercury mixed with organic mercury

In bacteria, assays for differential toxicity suggest that mercuric chloride induces DNA damage very weakly in *Bacillus subtilis* and not in *Escherichia coli*. No studies of bacterial mutation were available to the Working Group.

Mercuric chloride weakly induced mitotic recombination and induced mitochondrial mutations in *Saccharomyces cerevisiae*. It induced various types of mutations in the plant, *Anacharis canadensis*.

Mercuric chloride did not increase the frequency of micronuclei in cultured fish cells, even when tested at doses 10 times higher than those that were effective for some organomercury compounds.

In cultured mammalian cells, mercuric chloride inhibited DNA repair induced by X-rays but not that induced by ultraviolet radiation. Gene mutations at the *tk* locus were induced in a single study with mouse lymphoma L5178Y cells, but only in the presence of an exogenous metabolic system from rat liver. Sister chromatid exchange was induced in cultured Chinese hamster ovary cells and in human lymphocytes. Spindle disturbance and chromosomal aberrations were induced by mercuric chloride in most studies with cultured mammalian cells, including human lymphocytes, and by mercuric acetate in mouse oocytes *in vitro*.

Mercuric chloride enhanced cell transformation produced by simian adenovirus SA7 in Syrian hamster embryo primary cell cultures. Mercuric acetate did not induce anchorage-dependent growth of human fibroblasts.

In larvae of the newt, *Pleurodeles waltl*, mercuric chloride induced chromosomal aberrations and micronuclei in erythrocytes. Studies of mammals exposed to mercuric chloride *in vivo* have given negative or conflicting results. Neither chromosomal aberrations nor aneuploidy were observed in Syrian hamster bone marrow or oocytes following a single subcutaneous dose of 12.8 mg/kg mercuric chloride. In mice, the frequency of chromosomal aberrations in bone-marrow cells was increased in one study after a single oral dose of 3 mg/kg but not in another study by intraperitoneal injection of a dose of 6 mg/kg. In a second study in mice, chromosomal aberrations were not induced in spermatogonia. Weak dominant lethal effects have been described in rats and mice. Mercuric acetate did not induce chromosomal aberrations in mouse oocytes after subcutaneous or intravenous dosing.

(*b*) *Organomercury compounds* (see also Table 17, pp. 312–320 and Appendices 1 and 2)

Very weak differential toxicity was induced in *B. subtilis* by methylmercury chloride, but negative results were obtained with phenylmercury chloride and bis(ethylmercury)hydrogen phosphate. Mutations were not induced in *Salmonella typhimurium* by methylmercury chloride or methylmercury acetate.

Methylmercury chloride did not induce gene conversion or mitotic recombination in *S. cerevisiae*, but conflicting results were obtained for induction of mutations, mitochondrial mutations and aneuploidy.

Phenylmercury nitrate induced mutations in seedlings of *Zea mays*, and phenylmercury hydroxide induced mutations in seedlings of a number of different plants. Chromosomal aberrations and/or spindle disturbances were induced in *Allium cepa* roots by all of eight compounds tested.

Table 16. Genetic and related effects of inorganic mercury compounds in experimental systems

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Mercuric chloride (74% Hg)				
PRB, Lambda-prophage induction, *E. coli*	-	0	0.036	Rossman *et al.* (1991)
BSD, *Bacillus subtilis rec* strains, differential toxicity	(+)	0	10 000	Kanematsu *et al.* (1980)
ERD, *Escherichia coli rec* strains, differential toxicity	-	0	0.5	Brandi *et al.* (1990)
SCH, *Saccharomyces cerevisiae* D7, mitotic crossing-over	(+)	0	60	Fukunaga *et al.* (1981)
SCR, *Saccharomyces cerevisiae* N123, petite mutation	(+)	0	20	Fukunaga *et al.* (1981)
PLM, *Anacharis canadensis*, mutation	+	0	740	MacFarlane & Messing (1953)
***, Micronuclei, fish (*Lepomis macrochirus*)[b] cells *in vitro*	-	0	0.8	Babich *et al.* (1990)
***, Inhibition of X-ray-induced DNA repair, Chinese hamster ovary cells *in vitro*	+	0	0.2	Christie *et al.* (1986)
***, Inhibition of UV-induced DNA repair, Chinese hamster ovary cells *in vitro*	-	0	10	Christie *et al.* (1986)
DIA, DNA strand breaks, Chinese hamster ovary cells *in vitro*	+	0	5.0	Cantoni *et al.* (1982)
DIA, DNA strand breaks, Chinese hamster ovary cells *in vitro*	+	0	2.0	Robison *et al.* (1982)
DIA, DNA strand breaks, Chinese hamster ovary cells *in vitro*	+	0	5.0	Cantoni & Costa (1983)
DIA, DNA strand breaks, Chinese hamster ovary cells *in vitro*	+	0	5.0	Cantoni *et al.* (1984a)
DIA, DNA strand breaks, Chinese hamster ovary cells *in vitro*	+	0	1.0	Cantoni *et al.* (1984b)
DIA, DNA strand breaks, Chinese hamster ovary cells *in vitro*	+	0	10	Robison *et al.* (1984)
DIA, DNA strand breaks, mouse embryo fibroblasts *in vitro*	+	0	0.02	Zasukhina *et al.* (1983)
DIA, DNA strand breaks, rat embryo fibroblasts *in vitro*	+	0	0.02	Zasukhina *et al.* (1983)
RIA, DNA repair, Syrian hamster cells *in vitro*	(+)	0	2.0	Robison *et al.* (1984)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus, *in vitro*	-	(+)	4.44	Oberly *et al.* (1982)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	(+)	0	2.0	Howard *et al.* (1991)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	0	0.2	Howard *et al.* (1991)

Table 16 (contd)

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Mercuric chloride (contd)				
***, Spindle disturbances, Indian muntjac fibroblasts *in vitro*	+	0	0.1	Verschaeve *et al.* (1984)
***, Spindle disturbances, human lymphocytes *in vitro*	+	0	0.2	Verschaeve *et al.* (1984)
T7S, Cell transformation, SA7/Syrian hamster embryo cells *in vitro*	+	0	10	Casto *et al.* (1979)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	0	0.08	Morimoto *et al.* (1982)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	0	4.0	Verschaeve *et al.* (1985)
CHT, Chromosomal aberrations, human HeLa cells *in vitro*	-	0	7.4	Umeda *et al.* (1969)
***, Chromosomal condensation, human lymphocytes *in vitro*	+	0	2.0	Andersen *et al.* (1983)
***, Micronuclei in red blood cells, newt larvae *in vivo*	+		0.012	Zoll *et al.* (1988)
CBA, Chromosomal aberrations, mouse bone-marrow cells *in vivo*	-		4.44, ip × 1	Poma *et al.* (1981)
CBA, Chromosomal aberrations, Syrian hamster bone-marrow cells *in vivo*	(+)		4.74, sc × 1	Watanabe *et al.* (1982)
CBA, Chromosomal aberrations, mouse bone-marrow cells *in vivo*	+		2.22, po × 1	Ghosh *et al.* (1991)
CGG, Chromosomal aberrations, mouse spermatogonia *in vivo*	-		4.44, ip × 1	Poma *et al.* (1981)
COE, Chromosomal aberrations, Syrian hamster oocytes *in vivo*	-		9.47, sc × 1	Watanabe *et al.* (1982)
***, Chromosomal aberrations, newt larvae and embryos *in vivo*	+		0.06, water × 4 days	Zoll *et al.* (1988)
AVA, Aneuploidy, Syrian hamster bone-marrow cells *in vivo*	-		9.47, sc × 1	Watanabe *et al.* (1982)
AVA, Aneuploidy, Syrian hamster oocytes *in vivo*	-		9.47, sc × 1	Watanabe *et al.* (1982)
DLM, Dominant lethal mutation, mice	(+)		1.48, ip × 1	Suter (1975)
DLR, Dominant lethal mutation, rats	(+)		0.0003, po/day × 12 months	Zasukhina *et al.* (1983)
Mercurous chloride				
BSD, *Bacillus subtilis rec* strains, differential toxicity	+	0	10 000	Kanematsu *et al.* (1980)

Table 16 (contd)

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Mercuric acetate (63% Hg)				
CIA, Chromosomal aberrations, mouse oocytes *in vitro*	+	0	35	Jagiello & Lin (1973)
TIH, Anchorage-independent growth, human foreskin fibroblasts *in vitro*	-	0	2.0	Biedermann & Landolph (1987)
COE, Chromosomal aberrations, mouse oocytes *in vivo*	-		2, iv × 1	Jagiello & Lin (1973)
COE, Chromosomal aberrations, mouse oocytes *in vivo*	-		10, sc × 1	Jagiello & Lin (1973)

+, considered to be positive; (+), considered to be weakly positive in an inadequate study; -, considered to be negative; ?, considered to be inconclusive (variable responses in several experiments within an adequate study); 0, not tested

[a]LED, lowest effective dose; HID, highest ineffective dose. In-vitro tests, μg/ml; in-vivo tests, mg/kg bw. Doses given as concentration of element, not concentration of compound; ip, intraperitoneally; sc, subcutaneously; iv, intravenously; po, orally by gavage

[b]Bluegill sunfish

***Not displayed on profile

Methylmercury hydroxide induced sex-linked recessive lethal mutations, but not chromosomal aberrations or meiotic crossing-over, in *Drosophila melanogaster*. It induced chromosomal aberrations in *Stethophyma grossum*. Methylmercury chloride, methylmercury hydroxide, phenylmercury hydroxide and phenylmercury acetate consistently induce aneuploidy in *D. melanogaster*. Methylmercury chloride did not induce aneuploidy in silkworms.

Studies of gene mutations in cultured mammalian cells have yielded varying responses. In one study, both methylmercury chloride and methoxyethylmercury chloride induced ouabain-resistance and *hprt* locus mutations in Chinese hamster V79 cells, whereas in another study with the same cells methylmercury hydroxide did not induce mutations at the *hprt* locus.

The induction of spindle disturbances and chromosomal aberrations has been studied extensively in cultured mammalian cells, including human lymphocytes. Significant responses were obtained consistently with methylmercury chloride in a number of studies of both end-points. In addition, chromosomal aberrations and/or spindle disturbances have been induced by methylmercury hydroxide, methoxyethylmercury chloride, dimethyl mercury, ethylmercury chloride and phenylmercury chloride in independent studies using Chinese hamster V79 cells, human HeLa cells and lymphocytes *in vitro*. The frequency of micronuclei, which may be an expression of either spindle disturbances or chromosomal breakage, was increased in cultured cells from *Lepomis macrochinus* (bluegill sunfish) after treatment with methylmercury chloride, ethylmercury chloride or phenylmercury chloride.

Methylmercury chloride induced chromosomal aberrations in larvae and embryos of the newt, *Pleurodeles waltl*, and micronuclei in peripheral erythrocytes of the larvae. Treatment of pregnant rats with methylmercury chloride induced chromosomal aberrations in the livers of the fetuses, but it did not induce chromosomal aberrations in the bone marrow of Syrian hamsters and rats or in oocytes of Syrian hamsters. Intraperitoneal injection of methylmercury acetate did not induce micronuclei in mouse bone-marrow cells. Spindle disturbances were induced by methylmercury chloride in fetal lung and liver cells after treatment of mice *in vivo* in two studies and in killifish (*Fundulus heteroclitus*) embryo cells. Aneuploidy was seen in bone-marrow cells and oocytes of Syrian hamsters in one study but not in another. Methylmercury chloride appears therefore to be more active as a clastogen in fetal than in adult tissues of mice and more active as a spindle poison in Syrian hamster bone marrow than in Syrian hamster oocytes.

Dominant lethal mutation has been demonstrated in male rats and female mice, but not in male mice, treated with methylmercury chloride. Methylmercury hydroxide induced either weak or no dominant lethal effect in male mice. Methylmercury acetate did not induce sperm-head abnormalities in mice.

Few studies have been conducted on nonionized organomercury compounds; those that have been performed reflect the properties described above. Thus, dimethylmercury induced DNA fragmentation in the slime mould *Physarum polycephalum* and chromosomal aberrations and aneuploidy in cultured human lymphocytes; it induced chromosomal aberrations in mouse oocytes *in vitro* but not *in vivo*.

Several fungicides containing organomercury compounds have been tested for genotoxic activity in various plant systems. Spindle disturbances were induced by Panogen 5, 8 and 15, while chromosomal aberrations were induced by Agrimax M, Granosan, Ceresan M, Betoxin and New Improved Ceresan. [The Working Group noted that the different results may not reflect different properties, as various authors were involved.] In *D. melanogaster*, sex-linked recessive lethal mutation was induced by Ceresan and Ceresan M, but not by Agallol 3, and neither Ceresan nor Agallol 3 induced dominant lethal effects.

The azo dye, mercury orange, was not mutagenic to strains of *S. typhimurium*.

Considerations with regard to genotoxic mechanisms

Mercury has not only a direct effect on chromosomes, resulting in clastogenic effects in eukaryotes, but also causes disturbance of the spindle mechanism, owing to its high affinity for the sulfhydryl groups contained in spindle fibre proteins. Organomercury compounds inhibit the spindle mechanism even more strongly than colchicine, but, in contrast to colchicine, produce a gradual transition to c-mitosis at sub-lethal doses, which may result in aneuploidy and/or polyploidy.

In general, inorganic mercury compounds are less effective than ionizable organomercury compounds in inducing genetic effects *in vitro*. Similarities in the effects of different mercury compounds may suggest similar modes of action, while differences may be due to variations in solubility and bioavailability and in the rate of formation of a common toxic entity.

The possible contribution of reactive oxygen species to the genotoxicity of inorganic mercury compounds has also been addressed. ^{203}Hg-Mercuric chloride is taken up by Chinese hamster ovary cells and was reported to bind to the DNA in these cells in a temperature-dependent manner; however, DNA strand breaks were induced at 37 °C, but not at 4 °C, even though there was uptake and DNA binding of mercury at the lower temperature. It therefore appears that DNA strand breaks induced by Hg^{2+} require cellular metabolic processes. Mercuric chloride induced single-strand breaks in DNA of Chinese hamster ovary cells, and the effect was related linearly to leakage of superoxide into the medium. DNA damage induced by mercuric chloride can be increased by the addition of diethyldithiocarbamate, which inhibits superoxide dismutase, as well as by diethyl maleate, which depletes glutathione. Single strand breakage was inhibited by superoxide dismutase, catalase, glycerol and ascorbate (Cantoni *et al.*, 1984b).