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MERCURY
(addendum)
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MERCURY (addendum)

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1. EXPLANATION

Mercury occurs naturally in the earth's crust, usually in the form of the mineral cinnabar (mercury(II) sulfide). It can be released into the global environment through a number of processes, both natural and anthropogenic. Global natural emissions of mercury have been estimated at up to 2400 tonnes per year (Bergan & Rohde, 2001), whereas mercury emissions from anthropogenic sources in the year 2000

were approximately 2200 tonnes (Pacyna et al., 2006). While relatively chemically inert, mercury occurs in three valence states: elemental mercury (also known as metallic mercury), monovalent mercurous ion and divalent mercuric ion (Horvat, 2005), elemental mercury and the divalent ion being the most important in nature. Inorganic mercury salts are usually found in the forms of mercury(II) sulfide (HgS), mercury(II) oxide (HgO) and mercury(II) chloride ($HgCl_2$) (Table 1). There are several organic mercury compounds; by far the most common in the environment and in the aquatic food-chain is methylmercury.

Table 1. Elemental and inorganic mercury compounds

Molecular formula	Relative molecular mass	Synonyms	CAS No.
Hg	200.59	Elemental	7439-97-6
		Quicksilver	
		Colloidal mercury	
$HgCl_2$	271.52	Mercuric chloride	7487-94-7
		Mercury(II) chloride	
		Corrosive sublimate	
		Mercuric bichloride	
		Mercury perchloride	
$Hg_2(NO_3)_2$	525.19	Mercurous nitrate	10415-75-5
		Mercury(I) nitrate	
		Mercury protonitrate	
$Hg(NO_3)_2$	324.66	Mercuric nitrate	10045-94-0
		Mercury(II) nitrate	
		Mercury pernitrate	
Hg_2O	417.18	Mercurous oxide	15829-53-5
		Mercury(I) oxide	
		Mercury oxide	
HgO	216.59	Mercuric oxide	21908-53-2
		Mercury(II) oxide	
		Santar	
$HgSO_4$	296.68	Mercuric sulfate	7783-35-9
		Mercury(II) sulfate	
		Mercury bisulfate	
HgS	232.66	Mercuric sulfide	1344-48-5
		Mercury(II) sulfide	

Table 1 (contd)

Molecular formula	Relative molecular mass	Synonyms	CAS No.
Hg_2Cl_2	472.08	Vermillion Cinnabar Mercurous chloride Mercury(I) chloride Calomel Calogreen	10112-91-1

CAS, Chemical Abstracts Service

Mercury was previously evaluated by the Committee at its tenth, fourteenth, sixteenth and twenty-second meetings (Annex 1, references *13*, *22*, *30* and *47*). At its sixteenth meeting, the Committee allocated a provisional tolerable weekly intake (PTWI) of 0.3 mg of total mercury (5 µg/kg body weight [bw]), of which no more than 0.2 mg (3.3 µg/kg bw) should be in the form of methylmercury, based primarily on the relationship between the intake of mercury from fish and mercury levels in blood and hair associated with the onset of clinical disease. The sixteenth meeting of the Committee noted that almost all dietary exposure to methylmercury is from fish and seafood and that methylmercury is probably by far the most toxic form of mercury in food; therefore, other forms of mercury could be given less weight when establishing a tolerable intake for mercury. The original PTWI for methylmercury (3.3 µg/kg bw) was revised at the sixty-first meeting (Annex 1, reference *166*) to 1.6 µg/kg bw, based on an assessment of results from various epidemiological studies involving fish-eating populations and developmental neurotoxicity. At the sixty-seventh meeting (Annex 1, reference *184*), the Committee provided further clarifications as to the relevance of the new methylmercury PTWI for different subgroups of the population.

At the sixty-first meeting, the Committee recommended that the total mercury PTWI be reviewed.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

During its original assessment of mercury (Annex 1, reference *31*), the Committee considered that the chemical form of mercury defined the nature of its biological and toxic effects, based on known differences in absorption, biotransformation, retention and excretion. Absorption and bioavailability of inorganic mercury salts in food were reported to be less than 15% in experimental animals, whereas human volunteers who ingested an oral tracer dose (approximately 6 µg

of mercury) of mercury(II) nitrate given either in an aqueous solution or protein bound (calf liver protein) absorbed an average of 5–10% of the dose (Rahola et al., 1973). After 58 days of monitoring, no significant radioactivity was found in the head region. In comparison, human volunteers ingesting an oral dose of 1.0 g of pulverized dental amalgam material (50% elemental mercury) absorbed only about 0.04% of the mercury dose (Sandborgh-Englund et al., 2004). Whereas distribution and rate of uptake of inorganic mercury compounds can vary, in general, the kidney, specifically the proximal renal tubules, has been shown to be the main organ for deposition and bioaccumulation (Yokel et al., 2006; Berlin, Zalups & Fowler, 2007).

(a) Mice

Various strains of mice (inbred, H-2-congenic A.SW, B10.S and F_1/F_2 hybrids) were exposed to mercury(II) chloride (mixed with ^{203}Hg) in drinking-water (2.7 mg/l) for 6 weeks and then sacrificed. Whole-body retention of mercury was on average 1.4% of the total mercury dose (252 µg), whereas kidney and liver were found to be the organs with the highest accumulated mercury levels in all strains (kidney on average had 13-fold higher mercury levels than liver) (Ekstrand et al., 2010).

Varying the oral dose of mercury(II) chloride given to mice has been shown to have only a minimal effect on estimated whole-body retention. Bom:NMRI strain male mice, 7–8 weeks of age, given a single oral dose of mercury(II) chloride ranging from 0.27 to 27 mg/kg bw retained from 1.2% to 2.8% of the initial dose as measured 14 days after dosing. Per cent retention was inversely related to magnitude of dose, which the authors suggested may have been related to renal damage and increased urinary excretion. The time required to eliminate 70% of the initial dose ranged from 19 to 37 h (Nielsen & Andersen, 1989).

Mice (male Albino) exposed to a single oral dose (gavage) of mercury(II) chloride (analytical reagent grade) (4 mg/kg bw) exhibited significant increases in both hepatic and renal metallothionein levels within 24 h (Tandon et al., 2001). Inhibition of γ-glutamylcysteine synthetase activity prior to exposure to inorganic mercury results in decreased renal mercury accumulation and increased urinary excretion (Berndt et al., 1985; Tanaka, Naganuma & Imura, 1990).

(b) Rats

Wistar strain female rats (n = 4) administered a single oral dose (gavage) of ^{203}Hg-labelled mercury(II) chloride at 0.2–20.0 mg/kg bw were reported to retain between 3.0% and 8.7% of the dose when assessed up to 120 h post-dosing (Piotrowski et al., 1992). Earlier studies have shown that age can be an important factor in metal absorption from the gastrointestinal tract. Suckling rats (1 week old, Albino strain) given an oral dose of ^{203}Hg (4 µg of mercury) in cows' milk retained on average 38% of the dose, compared with older animals (18 weeks of age), which retained only 6.7% of the dose (Kostial et al., 1978). In a similar experiment by the same authors, it was suggested that the higher whole-body retention in animals dosed with mercury in a milk vehicle may be related to higher gut retention, longer residency times and decreased mercury elimination (Kostial et al., 1981).

When female mice were dosed on lactation day 10 with 0.5 mg of ^{203}Hg-labelled mercury(II) chloride intravenously and the mercury transfer was followed in their offspring until the end of lactation (day 21), it was estimated that approximately 8% of the total dose was excreted in milk, and 15% of this was absorbed by the offspring (Sundberg, Oskarsson & Bergman, 1991).

Age dependency of mercury absorption by the duodenum has also been seen in rats. Following a dose of mercury(II) chloride of 16 µg/kg bw, absorption specifically by the duodenum measured 1 h later was highest in 6-day-old SD male rats (18.1%), compared with only 7.3% in 23-day-old weanlings or 3.6% in 7-week-old animals (Walsh, 1982).

Absorption of monovalent mercurous compounds has been reported to be less than the absorption of mercuric or divalent forms, likely due to solubility (Friberg & Nordberg, 1973). Mercuric salts (halides, sulfates, nitrates) are relatively water soluble, and Hg^{2+} ions in biological systems can form stable complexes with various moieties containing sulfhydryl groups (glutathione [GSH], cysteine, albumin, metallothionein, etc.) (Berlin, Zalups & Fowler, 2007). Mercury in the form of mercury(I) sulfide, a relatively water-insoluble inorganic mercury compound, has a much lower bioavailability compared with water-soluble mercury(II) chloride (Paustenbach, Bruce & Chrostowski, 1997; ATSDR, 1999). In contrast, organic mercurials are almost completely absorbed from the gastrointestinal tract. Organic mercury, specifically methylmercury, is readily bioavailable, with up to 94% of an oral dose, in the form of either methylmercury(II) chloride or methylmercury in fish tissue, absorbed by human volunteers (Magos & Clarkson, 2006).

Up to 50% of either a non-toxic dose (135 µg/kg bw) or a moderately nephrotoxic dose (500 µg/kg bw) of mercury(II) chloride administered intravenously to male SD rats (n = 4) was found in the kidneys within 3 h (Zalups, 1993).

In pregnant SD rats (n = 12) provided drinking-water containing mercury(II) chloride at 0.2 µg/ml from gestation day 0 to postnatal day 20, the majority of the accumulated mercury was found in the kidneys (52.7%); the organ with the next highest content was the liver (38.7%) (Feng et al., 2004). Although organ mercury concentrations were considerably lower in the offspring, the highest mercury levels were also found in the kidneys and liver, as well as in the spleen. In a similar experiment, following exposure of female SD rats (n = 3–4) to a single oral dose of ^{203}Hg-labelled mercury(II) acetate on lactation day 11 (0.1–5.8 mg/kg bw), increasing concentrations of mercury could be detected in milk; the concentrations were positively related to both dose and whole blood levels (Sundberg, Oskarsson & Bergman, 1991). At the highest dose, milk mercury levels were approximately 15.6% of whole blood mercury concentrations 24 h after dosing. By 72 h after dosing, mercury levels in milk had decreased to 8.6% of whole blood levels.

Excretion of inorganic mercury (mercuric) compounds in rats has been described as biphasic. In the first phase, approximately 35% of a non-toxic dose will be excreted within a few days, whereas the second phase involves a slower excretion rate, with a total half-life of 30 days (Nielsen, 1992).

SD rats (five of each sex per dose group), 45–50 days old, were dosed by gavage with mercury(II) chloride at 0, 2, 4, 6, 8 or 10 mg/kg bw per day for 14 consecutive days. Body weights were recorded on dosing days 0, 4, 7, 10 and 14 (sacrifice). At sacrifice, various organs were collected for mercury analysis. Significant mortality occurred in the three highest dose groups, with only one female rat surviving per group until termination. For males, two rats died in the 4 and 6 mg/kg bw per day dose groups, three in the 8 mg/kg bw per day dose group and all rats in the highest dose group. Kidney had the highest mercury content of all organs (three highest dose groups not analysed), with similar values obtained for both the 2 and 4 mg/kg bw per day doses (Khan et al., 2001).

Male Wistar rats, approximately 4 months old, were continually exposed to mercury(II) chloride at 15 mg/l in drinking-water for 6 months ($n = 5$) or 10 months ($n = 6$) (estimated dose 2.3 mg/kg bw per day). In a separate experiment, a similar group of rats was treated with the same dose of mercury(II) chloride for 1 year, and then kidneys were removed and analysed for dry weights and water content. At the end of the 4- and 6-month dosing periods, the rats were sacrificed and kidney tissues prepared for light and electron microscopic analysis. Relative kidney weights were increased by 27–30% in the groups exposed to mercury(II) chloride for 6 and 10 months. The 1-year study determined that the increase in relative kidney weight was not related to water retention. After 6 months of mercury exposure, there was an increase in the relative amount of interstitial tissue; after 10 months, the absolute volume of proximal tubule lumina and glomeruli in the mercury-treated rats had increased by approximately 50%, and the absolute volume of the proximal tubule cells had increased by 30% (Madsen & Maunsbach, 1981).

Groups of male SD rats (six per dose group) were exposed to mercury(II) chloride (^{203}Hg-labelled) in drinking-water at concentrations of 0, 5, 50 or 500 µmol/l for 8 weeks. Mercury intakes were reported as approximately 0, 21, 212 and 1526 µg/day or 0, 0.1, 1.0 and 7.3 mg/kg bw per day, respectively. Urine and faeces were collected on a daily basis, whereas body weights were measured weekly. Maximum blood mercury levels were achieved relatively quickly in all dose groups within the first 2 weeks. It was further estimated that steady-state mercury levels in the kidney were reached by 15 days in the 5 µmol/l dose group and by 30 days in the 50 µmol/l dose group (Morcillo & Santamaria, 1981).

Accumulation of mercury in the kidney has been related to both induction of, and binding to, metallothionein species and mercuric conjugates of GSH (Zalups, 2000; Zalups & Koropatnick, 2000; Berlin, Zalups & Fowler, 2007; Holmes, James & Levy, 2009). Exposure of male SD rats to a single subtoxic dose of mercury(II) chloride (135 µg/kg bw intraperitoneally) caused significant increases in γ-glutamylcysteine synthetase activity and related GSH-dependent enzymes in renal proximal tubule cells (Lash & Zalups, 1996).

Genetic polymorphisms in humans associated with reduced GSH production and mercury–GSH conjugation activities have been reported to influence the retention and excretion of mercury (Custodio et al., 2005; Gundacker et al., 2007; Ekstrand et al., 2010). Additional details on renal and hepatic accumulation and transport of inorganic mercury can be found in Berlin et al. (2007).

The major portion of absorbed inorganic mercury is excreted by the kidney (urine) and, to a lesser extent, through bile and faeces. The latter route involves formation of low molecular weight conjugates of mercury and GSH prior to secretion into bile (Ballatori & Clarkson, 1984). Lower rates of mercury secretion into bile by weanling rats, compared with adult animals, are thought to be related to their decreased ability to secrete sulfhydryl groups into bile. Available information suggests that excretory routes for both metallic mercury and inorganic mercury compounds are similar in humans and experimental animals (ATSDR, 1999).

Following parturition, SD rat pups (five of each sex) were injected subcutaneously with ^{203}Hg-labelled mercury(II) chloride at 5 mg/kg bw on postnatal day 22 or 29, and mercury elimination rates were followed for 5 days post-treatment by whole-body gamma counting. Mercury elimination curves were similar between the two groups, within almost 50% of the original dose excreted by 120 h post-injection (Daston et al., 1986). Mortality was observed in the treated rats, with those rats excreting 20–22% of the initial dose surviving until the end of the observation period.

Overall estimates of inorganic mercury half-lives in both experimental animals and humans range from 1 to 2 months (IPCS, 2003; Holmes, James & Levy, 2009).

2.1.2 Biotransformation

Following absorption from the gastrointestinal tract, inorganic mercury compounds in blood are bound, to a large extent, to sulfhydryl groups of haemoglobin and plasma proteins. Based on limited lipophilicity, neither mercury(I) nor mercury(II) is able to effectively cross the blood–brain barrier, in contrast to methylmercury. It has been hypothesized that thiol-conjugated mercury uptake and distribution may involve amino acid transporters, which may ultimately play a role in organ toxicity (Wei et al., 1999; Foulkes, 2000; Bridges et al., 2004; Lash et al., 2005; Bridges, Battle & Zalups, 2007; Rooney, 2007).

Actual metabolism of inorganic mercury compounds is limited, other than the previously described thiol and sulfhydryl conjugation reactions. Mercuric ions tend to be non-diffusible, and therefore binding facilitates transport. There is some limited evidence suggesting that mercury(II) (Hg^{2+}) can be reduced to metallic mercury and eliminated as metallic mercury vapour. Although not regarded as a major route of mercury elimination, reduction of mercury(II) to elemental mercury vapour was detected following exposure of adult CBA/J strain male mice to ^{203}Hg-labelled mercury(II) chloride at a single intraperitoneal dose of 0.5 mg/kg bw, as mercury (Dunn, Clarkson & Magos, 1981). Within 30 min after dosing, the mice had exhaled less than 5 ng of mercury. In comparison, elemental mercury can be readily oxidized by catalase and hydrogen peroxide to inorganic mercury (Hg^{2+}) (Clarkson, 1989; Rooney, 2007), whereas methylmercury can be demethylated through the action of reduced nicotinamide adenine dinucleotide phosphate (NADPH)–cytochrome P450 reductase to inorganic mercury (Clarkson & Magos, 2006). Generally, inorganic mercury present in the brain is the result of either in situ oxidation of elemental mercury or demethylation of organic mercury, both of which are lipid soluble and can cross the blood–brain barrier.

2.2 Toxicological studies

2.2.1 Acute toxicity

The oral median lethal dose (LD_{50}) of various inorganic mercury compounds ranges from 25 to 205 mg/kg bw (ATSDR, 1999; IPCS, 2003) (Table 2). The features of acute toxicity usually consist of shock, cardiovascular collapse, acute renal failure and severe gastrointestinal damage (IPCS, 1991). In female Sprague-Dawley rats, mercury(II) chloride at a single mercury dose of 7.4 or 9.2 mg/kg bw by gavage in water caused significant decreases in haemoglobin, erythrocytes and haematocrit at autopsy. A significant decrease in lactate dehydrogenase activity and mild to moderate renal effects, consisting of protein casts, cellular casts and interstitial sclerosis, were also observed. In female Bom:NMR mice, no effects were seen with mercury(II) chloride at a single mercury dose of 5 mg/kg bw by gavage (IPCS, 2003).

Table 2. Oral LD_{50} values for mercury compounds

Mercury compound	Species	LD_{50} (mg/kg)
Mercury(I) chloride (Hg_2Cl_2)	Rat	166
	Mouse	180
Mercury(II) chloride ($HgCl_2$)	Rat	37
	Human (LD_{lo})	10–42
Mercury(II) cyanide ($Hg(CN)_2$)	Rat	25
	Mouse	33
Mercury(I) sulfate (Hg_2SO_4)	Rat	205
	Mouse	152
Mercury(II) sulfate ($HgSO_4$)	Rat	57
	Mouse	25

LD_{lo}, lowest reported lethal dose
Source: Adapted from Von Burg (1995)

2.2.2 Short-term studies of toxicity

Four short-term studies in rodents (Druet et al., 1978; Bernaudin et al., 1981; Andres, 1984; NTP, 1993) were identified by several agencies from available toxicology databases and used to derive toxicological reference values: lowest-observed-adverse-effect level (LOAEL), reference dose (RfD), minimal risk level (MRL) and tolerable daily intake (TDI). Of these four studies, the first was used to estimate an oral LOAEL of 15.8 mg of mercury per person per day, based on the LOAEL of 0.05 mg/kg bw per day, as mercury(II) chloride, administered subcutaneously in rats (IPCS, 1991); the remaining three studies were used to derive the United States Environmental Protection Agency (USEPA) oral RfD for mercury of 0.3 µg/kg bw per day, based on LOAELs in rats ranging from 0.226 to 0.633 mg/kg bw per day, as mercury, with the application of an uncertainty factor of 1000 (IRIS, 1995). The last study was used to derive an intermediate MRL

for mercury of 2.0 µg/kg bw per day (ATSDR, 1999) and a TDI for mercury of 2.0 µg/kg bw per day (IPCS, 2003), based on the no-observed-adverse-effect level (NOAEL) of 0.23 mg/kg bw per day, as mercury(II) chloride, in rats following 5 days/week administration by gavage.

(a) Mice

In the NTP (1993) study, B6C3F1 mice (10 animals of each sex per group) were administered mercury(II) chloride (>99.5% purity) by gavage at 0, 1.25, 2.5, 5, 10 or 20 mg/kg bw per day, 5 days/week, for 6 months (0, 0.92, 1.9, 3.7, 7.4 or 14.8 mg/kg bw per day, as mercury). A decrease in body weight gain was reported only in the males of the highest dose group. Significant increases in absolute and relative kidney weights of male mice occurred at 3.7 mg/kg bw per day or greater and at 7.4 and 14.8 mg/kg bw per day, respectively. The kidney weight changes corresponded to an increased incidence of cytoplasmic vacuolation of renal tubule epithelium in males exposed to at least 3.7 mg/kg bw per day. The exposed female mice did not exhibit any histopathological changes in the kidneys.

(b) Rats

In the Druet et al. (1978) study, both male and female Brown Norway rats were divided into groups of 6–20 animals each and received subcutaneous injections of mercury(II) chloride 3 times weekly for 8 weeks, at 0, 100, 250, 500, 1000 or 2000 µg/kg bw. An additional group was injected with a 50 µg/kg bw dose (15.8 µg/kg bw per day, as mercury) for 12 weeks. Antibody formation was measured by the use of kidney cryostat sections stained with a fluoresceinated sheep anti-rat immunoglobulin G (IgG) antiserum. In the first phase, rats developed antiglomerular basement membrane antibodies. During the second phase, the patterns of fixation of antisera changed from linear to granular. The immune response was accompanied by proteinuria and, in some cases, by a nephrotic syndrome. Tubular lesions were observed at the higher dose levels. Proteinuria was reported at doses of 100 µg/kg bw and above, but not at 50 µg/kg bw. Proteinuria was considered a highly deleterious effect, given that affected animals developed hypoalbuminaemia and many died. IgG antiserum was detected in all groups (including the 50 µg/kg bw dose level), except controls.

The Bernaudin et al. (1981) study involved the ingestion (gavage) of mercury(II) chloride at either 0 or 3000 µg/kg bw per week by male and female Brown Norway rats for up to 60 days. No organ abnormalities were reported using standard histological techniques in either experimental or control rats. Immunofluorescence histology revealed that 80% (4/5) of the mercury(II) chloride–exposed rats were observed to have a linear IgG deposition in the glomeruli after 15 days of exposure. After 60 days of mercury(II) chloride exposure, 100% (5/5) of the rats were seen with a mixed linear and granular pattern of IgG deposition in the glomeruli and granular IgG deposition in the renal arteries. Weak proteinuria was observed in 60% (3/5) of the rats fed mercury(II) chloride for 60 days. The control rats were observed to have no deposition of IgG in the glomeruli or arteries, as well as normal urinary protein concentrations.

In the Andres (1984) study, mercury(II) chloride (3 mg/kg bw in 1 ml of water) was given by gavage to five Brown Norway rats and two Lewis rats (sex not specified) twice a week for 60 days. A sixth Brown Norway rat was given only 1 ml of water by gavage twice a week for 60 days. After 2–3 weeks of exposure, the mercury(II) chloride–treated rats started to lose weight, and two rats died 30–40 days after dosing. No rats were observed to develop detectable proteinuria during the 60-day period. The kidneys appeared normal in all animals when evaluated using standard histological techniques, but examination by immunofluorescence showed deposits of IgG present in the renal glomeruli of only the mercury-treated Brown Norway rats. The treated Brown Norway rats were also observed with mercury-induced morphological lesions of the ileum and colon with abnormal deposits of immunoglobulin A (IgA) in the basement membranes of the intestinal glands and of IgG in the basement membranes of the lamina propria. All observations in the Lewis rats and the control Brown Norway rat appeared normal.

Adult male Wistar rats (n = 6) were given mercury(II) chloride at a dose of 0.25 mg/kg bw per day for periods of 15, 30, 45 or 60 days by gavage. At termination, blood and liver samples were collected for analysis of various biochemical parameters. Plasma glucose (>30 days), cholesterol, triglycerides and total protein (>15 days) were all reduced (by up to 50%), whereas urea concentrations were increased (>15 days). Hepatic GSH levels were slightly reduced by approximately 10% (>30 days) (Merzoug et al., 2009).

Exposure of weanling Wistar rats (five per dose group) to mercury(II) chloride in the diet (0, 75, 150 or 300 µg/g) for 4 weeks induced significant increases in relative kidney weights in both males and females at all doses but had no effect on relative liver weights (Jonker et al., 1993).

In the NTP (1993) study, Fischer rats (10 animals of each sex per group) were administered mercury(II) chloride in deionized water by gavage at 0, 0.312, 0.625, 1.25, 2.5 or 5 mg/kg bw per day, 5 days/week, for 6 months (0, 0.23, 0.46, 0.92, 1.9 or 3.7 mg/kg bw per day, as mercury). Survival was not affected, although body weight gains were decreased in males at the highest dose and in females at or above 0.46 mg/kg bw per day. Absolute and relative kidney weights were significantly increased in both sexes with exposure to at least 0.46 mg/kg bw per day. In males, the incidence of nephropathy (characterized by foci of tubular regeneration, thickened tubular basement membrane and diluted tubules with hyaline casts) was 80% in the controls and 100% for all treated groups; however, severity was assessed to be minimal in the controls and two lowest dose groups and minimal to mild in the 0.92 mg/kg bw per day group and higher. In females, there was a significant increased incidence of nephropathy only in the high dose group (4/10 with minimal severity). No treatment-related effects were observed in the other organs; however, histopathology on the other organs was performed only on control and high-dose rats. A NOAEL from this study was identified as 0.23 mg/kg bw per day, as mercury(II) chloride (0.20 mg/kg bw per day, as mercury) (NTP, 1993). A concurrent study was conducted in which groups of Fischer rats (30 per group) were dosed with the low, middle and high dose groups from the main 6-month study and sacrificed at 2-, 4- and 6-month intervals (10 per time point) for tissue residue analysis. As expected, kidneys were the main organ bioaccumulating

mercury, with maximum kidney mercury concentrations reached in all dose groups by 4 months (Dieter et al., 1992).

Groups of male Wistar rats (44 animals per group) were exposed to mercury(II) chloride at 0, 50 or 100 mg/l for 90 days through oral administration in the drinking-water (calculated intakes were approximately 0, 4 and 8 mg/kg bw per day, respectively). Mercury exposure for 90 days resulted in an increase in the absolute and relative wet weights of the testis and a decrease in the absolute and relative wet weights of the accessory sex glands in both treated groups, compared with the matched control. Marked perturbation in testosterone levels in serum was also detected in treated groups during the study. Cauda epididymal sperm count or motility decreased significantly in both treated groups, and qualitative examination of testicular sections revealed fewer mature luminal spermatozoa in comparison with the control. When the mercury-treated males were mated with normal cyclic females, mercury exposure resulted in a decline of the reproductive performance of male rats. These effects were associated with a significant increase in mercury content of testes and blood in a time-dependent and dose-dependent fashion, respectively. Evidence of induction of oxidative stress was reflected in terms of perturbations in antioxidant defence as measured by the activities of antioxidant enzymes (superoxide dismutase and catalase) and a significant dose-dependent increase in the testicular lipid peroxidation as a consequence of pro-oxidant exposure. These results suggest that an increase in free radical formation relative to loss of the antioxidant defence system after mercury exposure may render the testis more susceptible to oxidative damage, leading to their functional inactivation (Boujbiha et al., 2009). In comparison, adult rats (n = 20) and guinea-pigs (n = 12) (strain not identified) given mercury(II) chloride at a dose of 1 mg/kg bw per day intraperitoneally for 30 consecutive days did not exhibit changes in testicular weight (Chowdhury & Arora, 1982).

Adult Sprague-Dawley rats were used to investigate the effect of mercury(II) chloride on testicular and epididymal morphological alterations and interferon-gamma (IFN-γ) and interleukin-4 (IL-4) levels in serum. Groups of males (five per group) were given drinking-water containing mercury(II) chloride at 0 (control), 0.01, 0.05 or 0.1 µg/ml in deionized water (approximately equivalent to 0, 1.5, 7.5 and 15.0 µg/kg bw per day, as mercury) for 1, 2 or 3 months. Some rats also received mercury(II) chloride for 7 months. No effects on body weight, testis weight or epididymis weight were observed. Morphological alterations, however, were found in the epididymis and testis. After 1 month of mercury administration, degenerative changes, such as peritubular cell dissociation, were noted at 0.1 µg/ml. Seminiferous tubules in testis tissue sections from rats receiving mercury for 1 month showed epithelium disorganization, loss of cohesion and germ cell shedding independent of the dose. After 2 months of exposure to either 0.05 or 0.1 µg/ml, progressive degeneration with spermatogenic arrest at the spermatocyte stage and reduction in sperm density and hypospermatogenesis were observed in seminiferous epithelium by light and electron microscopy. Leydig cells showed cytoplasmic vacuolation and nuclear signs of cell death. Loss of peritubular cell aggregation was observed in the epididymis. Mercury accumulation was detected in both organs by mass spectroscopy. Rats showed increased IFN-γ levels in serum

compared with controls, but they reached significance only after 7 months of mercury administration (no information on whether there was a dose-dependent response within the test dose range). The results demonstrate that sublethal concentrations of mercury(II) chloride can induce morphological and ultrastructural modifications in male reproductive organs of rats. These contribute to functional alterations of spermatogenesis, with arrest at the spermatocyte stage, hypospermatogenesis and possibly impaired steroidogenesis, which together could affect male fertility (Penna et al., 2009). Note that this study suggested the 0.01 µg/ml dose, or approximately 1.5 µg/kg bw per day, as mercury, to be an effect level; however, based on the results presented, effects at this dose appear to be equivocal, due to inconsistent dose-dependent responses.

Adult male Wistar rats (five per dose group) were dosed by gavage with mercury(II) chloride at 0, 1 or 2 mg/kg bw per day for 30 consecutive days. At sacrifice, the right testis was removed and weighed, and the testicular plasma membrane was isolated. Whereas the highest dose caused an approximate 10% decrease in relative testis weight, no weight change was noted in the low dose group.

The activities of various membrane-bound enzymes were either increased (alkaline phosphatase, γ-glutamyl transferase) or decreased (5′ nucleotidase, Ca^{2+}-adenosine triphosphatase [ATPase], Mg^{2+}-ATPase, Na^{+}-K^{+}-ATPase) by the mercury treatments (Ramalingam & Vimaladevi, 2002).

2.2.3 Long-term studies of toxicity and carcinogenicity

An evaluation by IPCS (2003) based on various long-term studies in rodents indicated that the 2-year NTP (1993) bioassay in rats and mice appeared to be the most relevant and appropriate for assessing the carcinogenicity of mercury(II) chloride.

(a) Mice

In the 2-year NTP (1993) study, B6C3F1 mice (60 animals of each sex per group) were administered mercury(II) chloride (>99.5% purity) by gavage at 0, 5 or 10 mg/kg bw per day, 5 days/week, for 104 weeks (0, 3.7 and 7.4 mg/kg bw per day, as mercury). An interim sacrifice (10 animals of each sex per dose) was conducted after 15 months of exposure. The survival rates in the controls, low dose group and high dose group were, respectively, 72%, 72% and 62% for males and 82%, 70% and 62% for females. Female mice exhibited a significant increase in the incidence of nephropathy (21/49, 43/50 and 42/50 in control, low-dose and high-dose females, respectively). Nephropathy was observed in 80–90% of the males in all groups, with the severity increasing as the dosage increased. The incidences of renal tubular hyperplasia were 1/50, 0/50 and 2/49 in control, low-dose and high-dose males, respectively. The combined incidences of renal tubular adenomas and adenocarcinomas were 0/50, 0/50 and 3/49 in control, low-dose and high-dose males, respectively. Although no tumours were seen in the low-dose males, a statistically significant positive trend for increased incidence of renal tubular malignancies with increased dose was observed. These observations were

considered important, because renal tubular hyperplasia and tumours in mice are rare (IRIS, 1995). It was considered that there was equivocal evidence of carcinogenic activity in male mice (renal tubular adenomas and adenocarcinomas) and no evidence in female mice (NTP, 1993).

(b) Rats

Fischer 344 rats (60 animals of each sex per group) were administered mercury(II) chloride (>99.5% purity) by gavage in water at doses of 0, 2.5 or 5 mg/kg bw per day, 5 days/week, for 104 weeks (0, 1.9 and 3.7 mg/kg bw per day, as mercury) (NTP, 1993). An interim sacrifice (10 animals of each sex per group) was conducted after 15 months of exposure. Survival after 24 months was 43%, 17% and 8% in control, low-dose and high-dose males, respectively, and 58%, 47% and 50% in control, low-dose and high-dose females, respectively. During the second year of the study, body weight gains of low-dose and high-dose males were 91% and 85% of those of controls, respectively, and body weight gains of low-dose and high-dose females were 90% and 86% of those of controls, respectively. At study termination, nephropathy was evident in both treated and control rats, with incidences of 6/50, 29/50 and 29/50 in control, low-dose and high-dose males, respectively. The incidences of forestomach squamous cell papillomas in the control, low-dose and high-dose groups were 0/50, 3/50 and 12/50 in males and 0/50, 0/49 and 2/50 in females, respectively. The incidence of papillary hyperplasia of the stratified squamous epithelium lining of the forestomach was elevated at a statistically significant rate in all dosed males (3/49, 16/50 and 35/50 in control, low-dose and high-dose males, respectively) and in high-dose females (5/50, 5/49 and 20/50 in control, low-dose and high-dose females, respectively). The incidence of thyroid follicular cell carcinomas, adjusted for survival, showed a significant positive trend in males; the incidence was 1/50, 2/50 and 6/50 in control, low-dose and high-dose groups, respectively. The combined incidence of thyroid follicular cell neoplasms (adenoma and/or carcinoma) was not significantly increased (2/50, 6/50 and 6/50 in control, low-dose and high-dose males, respectively). In female rats, a significant decrease in the incidence of mammary gland fibroadenomas was observed (15/50, 5/48 and 2/50 in control, low-dose and high-dose females, respectively). The high mortality in both groups of treated males indicates that the maximum tolerated dose (MTD) was exceeded in these groups and limits the value of the study for assessment of carcinogenic risk. NTP (1993) considered the forestomach tumours to be of limited relevance to humans because of structural considerations and the observation that the tumours did not appear to progress to malignancy. The relevance of the thyroid carcinomas was also questioned, because these neoplasms are usually seen in conjunction with increased incidences of hyperplasia and adenomas, which were not observed in this study (IRIS, 1995).

2.2.4 Genotoxicity

Several in vitro studies have shown that mercury(II) chloride induces single-strand breaks in the deoxyribonucleic acid (DNA) of Chinese hamster ovary cells (Cantoni, Evans & Costa, 1982; Cantoni & Costa, 1983; Cantoni et al., 1984a,b; Christie et al., 1984, 1986), spindle disturbance in human lymphocytes, cell

transformation in Syrian hamster cells (Casto, Myers & DiPaolo, 1979) and sister chromatid exchanges (Howard et al., 1991) and chromosomal aberrations in both Chinese hamster ovary cells and human lymphocytes (Morimoto, Iijima & Koizumi, 1982; Verschaeve, Kirsch-Volders & Susanne, 1984). In vitro studies with human lymphocyte cultures detected various genotoxic effects by inorganic mercury (mercury(II) chloride or mercury(II) nitrate), including micronuclei, sister chromatid exchange, increased levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), decreased proliferation rate index and decreased mitotic index (Ogura, Takeuchi & Morimoto, 1996; Lee et al., 1997; Rao et al., 2001; Silva-Pereira et al., 2005).

(a) In vitro

Lymphocyte cultures prepared from blood samples collected from 10 healthy adults were incubated with mercury(II) chloride at concentrations ranging from 0.1 to 1000 µg/l for 9 h. Following cytogenetic analysis, the relative frequency of cells with gaps, breaks and gaps plus breaks was determined. There was an approximate 2-fold increase in cells with chromosomal alterations (mainly gaps), but only in the lowest and highest dose groups. In comparison, at the same doses, methylmercury(II) chloride produced a stronger response in all dose groups (up to an 18-fold increase). Also, no cells with polyploidy aberrations were seen in the mercury(II) chloride lymphocyte cultures (Silva-Pereira et al., 2005).

Cytokine-activated peripheral blood mononuclear cell cultures from three human volunteers were incubated in the presence of mercury(II) chloride at 0.1–50 µmol/l (0.27–13.6 mg/l) for 24 h before collection and analysis for 8-OHdG, micronuclei and chromosomal aberrations. Significant cytotoxicity was observed at the highest dose. Aberrations (excluding gaps) were increased at the two highest doses of mercury(II) chloride that were scored (10 and 20 µmol/l) and were significantly related to loss in cell viability. At lower doses, the main chromosomal aberration was described as single chromatid breaks. Micronuclei were also increased, but only at doses of mercury(II) chloride at which significant toxicity was observed (20 µmol/l). A significant increase in 8-OHdG (approximately 2- to 3-fold) was also observed at higher doses of mercury(II) chloride (10 and 20 µmol/l). The authors suggested that the observed increase in chromosomal aberrations was likely due to mercury(II) chloride acting as an inhibitor of the mitotic spindle, whereas an increase in 8-OHdG arose due to oxidative DNA damage (Ogura, Takeuchi & Morimoto, 1996).

Although culturing human peripheral blood lymphocyte cultures with mercury(II) chloride at concentrations ranging from approximately 1 to 10.5 µmol/l (0.27–2.8 mg/l) for 72 h produced a slight increase in sister chromatid exchanges (less than 2-fold compared with controls), simultaneous exposure to ascorbic acid completely blocked the effect (Rao et al., 2001).

Mercury(II) chloride and mercury(I) chloride were also positive in the *Bacillus subtilis* rec-assay (Kanematsu, Hara & Kada, 1980). In general, Ames assay results with *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and mercury(II) chloride have been negative, both with and without S9 metabolic activation (NTP, 1993).

Chinese hamster ovary cells were cultured in the presence of mercury(II) chloride (up to 100 µmol/l or 27 mg/ml) for 60 min and then assessed for cellular GSH content and DNA single-strand breaks by alkaline elution. Concentrations of mercury(II) chloride greater than 25–50 µmol/l were very cytotoxic, whereas lower concentrations produced single-strand DNA breaks similar to X-ray treatment (1.5–6.0 Gy) (Cantoni, Evans & Costa, 1982).

(b) In vivo

In an in vivo study with male Swiss albino mice (five animals per group) administered a single oral dose of mercury(II) chloride (analytical grade) (0, 3, 6 or 12 mg/kg bw), a dose-related increase in bone marrow chromosomal aberrations was observed 24 h following dosing (Ghosh et al., 1991). Chromatid breaks and gaps were the most commonly observed aberrations. The mitotic index was also significantly reduced by all mercury doses. As simultaneous exposure to chlorophyllin significantly reduced the incidence of chromosomal aberrations, it was suggested that a possible mechanism of mercury(II) chloride DNA damage may involve generation of free radicals.

In other studies, no increase in chromosomal aberrations was observed in bone marrow or spermatogonia of mice (Poma, Kirsch-Volders & Susanne, 1981).

Male Swiss albino mice, 12 weeks of age, were treated intraperitoneally with a single dose of mercury(II) chloride at 0, 2, 4 or 6 mg/kg bw, with bone marrow cells and spermatogonia sampled 12, 24, 36 and 48 h after injection for cytogenetic analysis. The frequencies of gaps, breaks and exchanges in either bone marrow cells or spermatogonia were not significantly increased compared with the controls (Poma, Kirsch-Volders & Susanne, 1981).

In a similar experiment using the same model, 30 min following a single intraperitoneal dose of mercury(II) chloride at 6 mg/kg bw, the mice were also treated with an intraperitoneal dose of ethyl methanesulfonate (EMS) at 200 mg/kg bw. Whereas EMS caused a significant increase in bone marrow chromosomal aberrations, no effect was observed when mercury(II) chloride was administered either alone or in combination with EMS (Poma, Kirsch-Volders & Susanne, 1984).

In rats, a weak, but dose-related, increase in dominant lethal mutations was reported after repeated oral administration of mercury(II) chloride (Zasukhina et al., 1983).

Groups of male Wistar rats (five per group) were treated by gavage with a single dose of mercury(II) chloride (0.0054, 0.0108, 0.0216, 0.0432 or 0.0864 mg/kg bw). At various times after mercury treatment, beginning at 24 h and extending to 2 weeks, blood samples were collected, and leukocyte DNA damage was determined by the comet assay (Grover et al., 2001). All mercury doses were shown to produce an increase in DNA comet tail lengths by 24 h, which gradually decreased to near control levels by 72 h, without significantly affecting cell viability.

Adult female Wistar rats (eight per dose group) were administered mercury(II) chloride orally at a dose of 0.068, 0.136 or 0.272 mg/kg bw for 5 consecutive days. Three days after the last dose, the animals were killed, and

mercury levels in the liver and the kidney were measured. A significant increase in mercury concentration in the kidney was observed in the two highest dose groups, whereas liver mercury burden showed a significant increase only in the highest dose group, compared with controls. Blood samples were analysed using the comet assay and the acridine orange supravital staining micronucleus test. Mean tail length and tail moment in lymphocytes and micronucleated reticulocytes were significantly higher or more frequent in the treated rats than in control rats, regardless of the dose of mercury(II) chloride, whereas the difference between the treated groups for both comet assay and micronucleus parameters was not statistically significant (Rozgaj, Kasuba & Blanusa, 2005).

(c) Summary

In vitro data show that mercury(II) chloride has genotoxic potential. Despite mixed results, chromosomal aberrations have been observed in an in vivo study using the oral exposure route (Ghosh et al., 1991). Although direct interaction with DNA has not been conclusively seen, indirect DNA damage induced by inorganic mercury may involve free radicals and oxidative stress, disruption of microtubules and inhibition of DNA repair mechanisms (Crespo-López et al., 2009).

2.2.5 Reproductive and developmental toxicity

A number of studies on the reproductive effects of mercury(II) chloride or mercury(II) acetate in rodents were evaluated in IPCS (2003). Most of these were conducted with non-oral exposure, and the lowest effect levels were observed following a single intraperitoneal injection of mercury(II) chloride at 1 mg/kg bw, as mercury, in male mice, with decreased conceptions in females, and at 0.74 mg/kg bw, as mercury, in rats, with seminiferous tubular degeneration (Lee & Dixon, 1975). Only one study was carried out by oral exposure, in which pregnant hamsters receiving mercury(II) acetate at a single dose of 22 mg/kg bw, as mercury, on gestation day 8 showed an increase in the incidence of resorptions and small and oedematous embryos in the presence of histological damage in the liver and kidney in the dams (Gale, 1974).

(a) Mice

Groups of male and female C57BL/6 mice (25 of each sex per group) were exposed by gavage to mercury(II) chloride (>99.5% purity) at doses of 0.00, 0.25, 0.50 or 1.00 mg/kg bw per day, and males were paired with females receiving the same dose. Dosing continued for males throughout mating, whereas dosing in females continued throughout mating, gestation and lactation. The males were necropsied at the end of mating, following 81 days of exposure, and the females were necropsied at the conclusion of lactation (total exposure duration 79 days). Fertility indices, parturition, gestation, live birth litter size, survival indices and implantation efficiency were recorded and statistically analysed. Fertility indices were significantly ($P < 0.05$) reduced in all treated groups. The fertility indices, however, for all dose groups were 16% compared with 44% in the control group and showed no dose-dependent response. Exposure of parental mice to mercury(II) chloride did not affect the litter size, and only the high dose showed a decrease in

the live birth index. No evidence of mercury-induced target organ toxicity was seen in either the clinical pathology parameters or the histomorphological evaluations. It was indicated that oral exposure to mercury(II) chloride at 0.25–1.00 mg/kg bw per day produced adverse effects on the reproductive performance of mice, in the absence of overt mercury toxicity, with a LOAEL of 0.25 mg/kg bw per day, as mercury(II) chloride, or 0.185 mg/kg bw per day, as mercury (Khan et al., 2004).

CD-1 male mice, 3 months of age, were exposed to mercury(II) chloride in drinking-water (4 mg/l) for 12 weeks. The estimated daily intake was 0.65 mg/kg bw per day. At sacrifice, significant decreases in both body weight gain (28%) and relative testes weight (46%) were observed. Histopathological testes examination revealed that testicular necrosis, focal hyperplasia and interstitial oedema were evident in the mercury-treated animals. Average epididymal sperm numbers were also significantly reduced (55%) (Orisakwe et al., 2001).

Adult male albino Swiss strain mice dosed with mercury(II) chloride by gavage at 1.25 mg/kg bw per day for 45 days showed no change in body weight gain, but relative testis and cauda epididymal weights were significantly reduced (29% and 44%, respectively) compared with controls (Rao & Sharma, 2001). After a 45-day recovery period, testes weights had recovered to 90% of control values, whereas cauda epididymal weights were no longer reduced. Caudate epididymis sperm count, sperm motility and sperm viability were significantly reduced by the mercury treatment (63%, 49% and 39%, respectively), with some recovery also observed following termination of treatment (21%, 26% and 0% reduced compared with controls). Serum testosterone levels were reduced by mercury treatment (61%), with the inhibition persisting after the recovery period (48% reduced compared with controls). When the mercury-treated male mice were mated with control estrous females, mating success (per cent sperm-positive females per mated male) was reduced to 0%. However, after the recovery period, mating success had increased to 50%, compared with 92.5% for control males.

(b) Rats

Several short-term studies in rats in which reproductive effects were observed are discussed in section 2.2.2.

White female rats (strain not identified) were mated with the same variety of male rats that had been chronically exposed to mercury(II) chloride (0, 0.025, 0.25 or 2.5 mg/kg bw per day) by gavage for 12 months. At day 20 of gestation, the female rats were sacrificed, and embryonic material was analysed. No effects were observed in the total number of implantation sites or number of live embryos, but the two highest mercury(II) chloride doses produced an increase in the number of resorptions compared with controls (Zasukhina et al., 1983).

Sprague-Dawley rats (20 per group), approximately 7 weeks of age, were treated daily with mercury(II) chloride by gavage in a two-generation reproduction study (Atkinson et al., 2001). Males (F_0) were treated with mercury(II) chloride at 0.00, 0.50, 1.00 or 2.00 mg/kg bw per day and mated with females dosed with mercury(II) chloride at 0.00, 0.75, 1.50 or 3.00 mg/kg bw per day. F_0 males were dosed throughout the pre-cohabitation (60 days) and cohabitation period (21 days).

As clinical signs of toxicity were observed on day 43 (males) and day 27 (females), the high doses were reduced from 2.00 to 1.5 mg/kg bw per day in males and from 3.00 to 2.5 mg/kg bw per day in females. F_0 females were dosed throughout the pre-cohabitation (16 days), cohabitation (21 days), gestation and lactation periods. Following lactation, selected F_1 males and females were exposed to the same doses received by their parents (F_0).

Body weight gain was significantly reduced beginning in treatment week 7 in the high-dose males, whereas body weights were reduced in both the mid- and high-dose females from weeks 5 to 10. Following a 21-day cohabitation period, pregnant females were allowed to deliver their litters, with sufficient F_1 offspring selected for the F_1 breeding. Dosing of F_1 animals was the same as for the F_0 generation, except only the low and middle dose groups were used (insufficient offspring from high dose group). Significant dose-dependent reductions were seen in the F_0 generation fertility index (95%, 63.2%, 36.8% and 11.8% for controls, low dose group, middle dose group and high dose group, respectively), but not in the F_1 generation animals. Implant efficiency was also reduced in all dose groups for the F_0 generation (23.2%, 11.3%, 7.2% and 3.6% for controls, low dose group, middle dose group and high dose group, respectively), but only in the middle dose group for F_1 animals. Body weight gain for F_1 pups was decreased during lactation for all dose groups, an effect that persisted in adulthood. However, body weight gain of the F_2 pups was not significantly changed compared with controls. With the exception of a slightly reduced relative seminal vesicle weight in the mid- and high-dose F_0 males, no additional male sex organ (testis, prostate, epididymis) changes were noted in low-dose F_0 or low- and mid-dose F_1 animals.

Further details noted from the Atkinson et al. (2001) study included the following: the male and female dosages were not administered consistently throughout the study; no subsequent decreases in fertility index were observed in the F_1 generation, even with continued exposure to mercury(II) chloride at 1.0–1.5 mg/kg bw per day; there were variable sex ratios in the F_1 offspring (0.75–2.00) that were not related to dose; and the total number of reported live F_1 males equalled the total number of live offspring surviving until the end of the experiment (which contradicts the reported sex ratio).

Groups of pregnant female Wistar rats (two per group) were treated, by gavage, with mercury(II) chloride (99.5% purity), dissolved in distilled water, at 0.4, 0.8 or 1.6 mg/kg bw, as mercury, 1) from day 5 to day 15 during pregnancy (P protocol); 2) from day 5 to day 15 of pregnancy plus for 4 weeks of lactation (P+L protocol); and 3) from day 5 to day 15 of pregnancy plus for 4 weeks of lactation, with the offspring being further treated for 8 weeks post-weaning (P+L+P protocol). The authors reported that there were minor body weight decreases in the pups from dams treated in the two highest dose groups (data not provided), but the treatment, in general, failed to cause major signs of general intoxication in the rats (Papp, Nagymajtenyi & Vezer, 2005).

Weanling female SD rats (20 per dose group) were treated with mercury(II) chloride (0, 1 or 2 mg/kg bw per day) by gavage beginning on postnatal day 21 for 60 days prior to mating with unexposed males. Mating (10 per dose group)

proceeded with proven fertile males, and the females were euthanized on gestation day 13. By 30 days of mercury(II) chloride exposure, body weight gain in exposed females was slightly reduced in both dose groups (<10%); however, no indication as to whether the fertility index was affected was provided. Total implantations were slightly decreased in only the high dose group compared with controls, whereas non-viable implantations (i.e. sites on the uterus that showed signs of resorption in addition to sites that had not developed into a live fetus) were increased in the same dose group only. A slight decrease was also noted in plasma progesterone levels, whereas luteinizing hormone, expressed on a microgram per pituitary gland basis, was increased, both only in the high dose group (Heath et al., 2009).

2.2.6 Special studies

(a) Immunological effects

(i) Mice

Administration of mercury(II) chloride in drinking-water at a dose of 0.7 mg/kg bw per day, as mercury, for 2 weeks resulted in an increased lymphoproliferative response after stimulation with T-cell mitogens in a strain of mice particularly sensitive to the autoimmune effects of mercury (SJL/N). In the same experiment, a strain of mice that is not predisposed to the autoimmune effects of mercury (DBA/2) showed no increase in lymphocyte proliferation (Hultman & Johansson, 1991).

Male BALB/c mice (four per group) were exposed to mercury(II) chloride continuously at 0, 0.3, 1.5, 7.5 or 37.5 mg/l, as mercury, in drinking-water for 14 days. Body weight was reduced in the highest dose group, whereas the relative kidney and spleen weights were significantly increased. The dose range of mercury used did not cause hepatotoxicity, as indicated by circulating alanine aminotransferase and aspartate aminotransferase levels. Circulating blood leukocytes were elevated in the highest dose group. Exposures from 1.5 to 37.5 mg/l dose-dependently decreased CD3+ T lymphocytes and both CD4+ and CD8+ single-positive lymphocyte populations in the spleen (Table 3). Exposure to 7.5 and 37.5 mg/l, as mercury, decreased the CD8+ T lymphocyte population in the thymus, whereas double-positive CD4+/CD8+ and CD4+ thymocytes were not altered. Mercury altered the expression of inflammatory cytokines (tumour necrosis factor-alpha, IFN-γ, IL-12, c-myc and major histocompatibility complex II) in various organs, especially at 1.5 mg/l (300 µg/kg bw per day, as mercury) and higher. Results indicated that a decrease in T lymphocyte populations in immune organs and altered cytokine gene expression may contribute to the immunotoxic effects of inorganic mercury. A no-observed-effect level (NOEL) of 0.3 mg/l in water (60 µg/kg bw per day, as mercury) was indicated (Kim, Johnson & Sharma, 2003).

In three independent experiments, pregnant BALB/c strain mice ($n = 6$, 3 and 3 for experiments 1, 2 and 3, respectively) were exposed to mercury(II) chloride in drinking-water (10 mg/l) throughout the entire gestation period, with mercury dosing stopped at parturition. Offspring ($n = 6$) from the mercury-exposed females were weaned and at 10 weeks of age examined for immunophenotype and function of

Table 3. Effects of mercury on lymphocyte populations in the spleen of male BALB/c mice

Mercury concentration in water (mg/l)	Splenocytes ($\times 10^{5}$) per spleen expressing receptor		
	CD3+	CD4+	CD8+
0	47.69 ± 0.93	31.29 ± 0.64	14.08 ± 0.27
0.3	43.59 ± 0.64	28.75 ± 0.55	12.93 ± 0.53
1.5	41.01 ± 1.65*	26.92 ± 1.20*	12.02 ± 0.53*
7.5	37.91 ± 1.81*	25.04 ± 1.30*	10.89 ± 0.58*
37.5	35.56 ± 0.94*	23.14 ± 0.61*	10.69 ± 0.35*

* $P < 0.05$, significantly different from the control

spleen and thymus cells (Pilones, Tatum & Gavalchin, 2009). Mercury(II) chloride intake by the dams, although not provided, could be estimated as approximately 2 mg/kg bw per day (reported 5 ml water consumption per day). No body weight changes were seen in maternal animals or offspring as a result of mercury exposure, and thymus cellularity and spleen cellularity were not affected. Proliferative responses of splenocytes to both T cell (concanavalin A) and B cell (lipopolysaccharide) mitogens were slightly increased in the mercury-exposed mice compared with controls, along with increased production of some cytokines (IFN-γ and IL-4). In the female mice, an increase was also seen in the number of splenic CD4+ IdLNF1-specific T cells, which have been associated with the development of nephritis in susceptible mice strains.

(b) Neurological effects

(i) Rats

A study with rats exposed to mercury(II) chloride at a mercury dose of 0.74 mg/kg bw per day for up to 11 weeks resulted in neurological disturbances consisting of severe ataxia and sensory loss (Chang & Hartmann, 1972), but there was no indication as to whether the route of exposure was subcutaneous or oral (IPCS, 2003).

Electrophysiological parameters (electrocorticogram, cortical evoked potentials, conduction velocity and refractory periods of peripheral nerve) of the male offspring at the age of 12 weeks (eight per group per treatment protocol) from dams in the groups treated according to the protocols described in the Papp, Nagymajtenyi & Vezer (2005) study (see section 2.2.5) were investigated. The rats' spontaneous and evoked electrophysiological activity underwent dose- and treatment-dependent changes (increased frequency of spontaneous activity, lengthened latencies and duration of evoked potentials, lower conduction velocity of the peripheral nerve, etc.). However, compared with the controls, the effects were not significant ($P < 0.05$) at mercury(II) chloride doses up to 1.6 mg/kg bw per day

(high dose), with the exception that the auditory evoked potential was decreased and the refractory periods were longer in the 0.4 mg/kg bw dose group treatment protocol (P+L+P).

(ii) In vitro

In a study to characterize the effects that chelators have on the cytotoxic effects of metals, mixed cortical cell cultures containing both neuronal and glial cells were prepared from fetal mice. The cells were cultured with inorganic mercury (mercury(II) chloride), methylmercury, thimerosal, lead chloride or iron citrate in media and plated. After 24 h, the level of cell death was determined by the lactate dehydrogenase release assay. The cell deaths caused by mercury(II) chloride at 0.1, 1 and 5 µmol/l were 5%, 40% and 100%, respectively, compared with 0.4% in the controls (Rush, Hjelmhaug & Lobner, 2009). In a study to elucidate the role of intracellular GSH in protecting against mercury toxicity, mercury(II) chloride was shown to reduce the viability of Neuro-2A neuroblastoma cells (derived from the brain of an albino strain A mouse) grown in Dulbecco's Modified Eagle's Medium and reduced cellular GSH and oxidized GSH (recycled by GSH reductase back to GSH) concentrations at 1 µmol/l and higher (Becker & Soliman, 2009).

2.3 Observations in humans

2.3.1 Sources and pathways of exposure

In addition to the consumption of food containing inorganic mercury, exposure occurs through use of mercury-containing medicinal and ethnic or folk products. These include skin-lightening creams, topical creams for acne and skin cleansing, impetigo, syphilis and psoriasis, laxatives, ear drops, worming powders, teething powders, diaper powders, diuretics and cathartics, antimicrobial, antifungal and antihelminthic preparations, and traditional, ethnic or herbal medicines and folk remedies, such as Ayurvedic medicines from India and traditional medicines from China (Kang-Yum & Oransky, 1992; Hardy et al., 1995; CDC, 1996; Cooper et al., 2007; Risher & De Rosa, 2007; Liu et al., 2008; Martena et al., 2010).

2.3.2 Biomarkers of exposure

The absorption of ingested inorganic mercury from the gastrointestinal tract is estimated to vary from 2% to 38% in adults, depending on the mercury species, with most estimates being in the range of 10–15% (Holmes, James & Levy, 2009). Different mercury salts vary in solubility, with mercury(II) salts being more soluble than mercury(I) salts, which likely influences their relative absorption and toxicity. Inorganic mercury does not easily cross cell membranes such as the blood–brain barrier and placenta, although it may do so more easily by forming complexes with selenium. Moreover, metallic and organic species of mercury can undergo metabolic interconversion to inorganic forms in situ by means of oxidation and demethylation, respectively, resulting in an accumulation in tissues such as the brain and the fetus.

Excretion of inorganic mercury occurs primarily via the urine and, to a lesser extent, the bile and faeces. The half-life of inorganic mercury in the blood is only a few days, making it useful as a biomarker of recent exposure only. Moreover, total blood mercury reflects both organic and inorganic forms, limiting the information it provides solely about exposure to inorganic forms. Organic forms account for 80% of the total mercury concentration in hair, making it of limited use as a biomarker of inorganic mercury (George et al., 2010). The greatest tissue accumulation of inorganic mercury occurs in the kidneys, where it is bound to metallothionein and filtered from the blood. Accordingly, the preferred biomarker for estimating chronic exposure to inorganic mercury is considered to be the concentration in urine. A recent study in which a variety of mercury biomarkers, including total mercury, inorganic mercury and methylmercury in whole blood, red cells, plasma, hair and urine, were measured in adults confirmed total urinary mercury as the best biomarker for inorganic mercury exposure (Berglund et al., 2005). The concentration of mercury in the first morning void correlates well with the concentration in a 24 h urine sample (R^2 = 0.85), and correction for creatinine did not improve the correlation (Cianciola et al., 1997). The concentration in a spot sample, adjusted for creatinine, is more weakly correlated with the concentration in a 24 h sample (R^2 = 0.37) (Martin et al., 1996).

There are limited data on genetic influences on the metabolism of inorganic mercury. In one study of polymorphisms in glutamyl-cysteine ligase and glutathione *S*-transferase genes, conducted in gold miners (Custodio et al., 2005), individuals with genotypes associated with decreased GSH had greater retention, as reflected in higher total mercury concentrations in whole blood, plasma and urine. In studies of dental professionals exposed to elemental mercury, polymorphisms in brain-derived neurotrophic factor and coproporphyrinogen oxidase have been reported to modify the expressions of mercury neurotoxicity (Echeverria et al., 2005, 2006; Heyer et al., 2006). However, polymorphisms in the serotonin transporter gene (*5-HTTLPR*) or the catechol-*O*-methyltransferase gene do not modify the expressions of mercury neurotoxicity (Heyer et al., 2008, 2009).

There does not appear to be a consensus regarding the normal reference range for urinary mercury concentration, with levels ranging from 5 to 20 µg/l (Abbaslou & Zaman, 2006; Mahajan, 2007; Risher & De Rosa, 2007).

2.3.3 Clinical observations

Case reports of inorganic mercury intoxication suggest that acute ingestion of large amounts of mercury(II) oxide, mercury(II) chloride or mercury(II) bromide can be fatal (Ly, Williams & Clark, 2002; Triunfante et al., 2009). Although the lethal dose is uncertain, the cases of intentional ingestion suggest a mercury range of 10–>50 mg/kg bw. In two cases, the postmortem blood mercury levels were 11.7 µg/ml and 2.95 µg/ml (Triunfante et al., 2009). In other reports, individuals survived the ingestion of approximately 40 g of mercury(II) oxide (Ly, Williams & Clark, 2002), 0.9 g of mercury(II) chloride (Yoshida et al., 1997) and 100 g of mercury(II) chloride (Boscolo et al., 2009).

Acute exposure via ingestion can result in corrosive gastroenteritis, oropharyngeal burns, nausea, haematemesis, severe abdominal pain, anaemia, liver enzyme elevations, gingivitis, haematochezia, acute tubular necrosis, immunological glomerulonephritis, acute renal failure, pulmonary oedema and cardiovascular collapse (Mahajan, 2007).

The effects of chronic exposure differ somewhat depending on the form of inorganic mercury (e.g. mercury(I) versus mercury(II) chloride). In general, inorganic mercury exposure is associated with a classic triad of signs, including tremor, neuropsychiatric disturbances and gingivostomatitis. A fine intention tremor, which usually begins with the hands, can progress to choreoathetosis and spasmodic ballismus. Sensorimotor neuropathy (e.g. paraesthesias, particularly of glove and stocking sensory loss) and visual disturbances might also be present. The central nervous system signs and symptoms include emotional lability (particularly irritability and excessive shyness), delirium, headache, memory loss, insomnia, anorexia and fatigue. Renal dysfunction is also prominent and can be manifested in forms ranging from asymptomatic proteinuria (e.g. elevation of β_2-microglobulin or *N*-acetyl-D-glucosaminidase) to oliguria or anuria and to nephrotic syndrome. The dose–effect relationships are uncertain. Holmes, James & Levy (2009) stated that detectable changes in renal function begin to occur at urinary mercury concentrations of >5–10 µg/g creatinine, with "clear toxicity" at 35 µg/g creatinine.

Some data suggest that occupational exposure to mercury (mining, refining) is associated with increased risk of overall mortality, death from hypertension, non-ischaemic heart disease, pneumoconiosis, and nephritis and nephrosis (Boffetta et al., 2001). However, limited exposure information was available.

Acrodynia is a primarily cutaneous disease that can result from inorganic or elemental mercury poisoning, either from dermal exposure or from gastrointestinal absorption. It is most often observed in infants but has also been reported in children and adolescents. It is characterized by a painful, pink oedematous swelling of the feet and/or hands, with skin desquamation, morbilliform rashes, severe muscle cramping in the legs, arterial hypertension and tachycardia. Other signs can include photophobia, ulceration of mucosa, alopecia, nail loss, excessive perspiration and salivation, irritability, sleep disturbance and muscle weakness. The dose–response relationship for acrodynia is not well established. In case reports of children presenting with acrodynia, the urinary mercury levels, prior to chelation, range widely, often as high as several hundred micrograms per litre, but rarely below 30 µg/l (Torres, Rai & Hardiek, 2000; Horowitz et al., 2002; Weinstein & Bernstein, 2003; Beck et al., 2004; Abbaslou & Zaman, 2006; Michaeli-Yossef, Berkovitch & Goldman, 2007; Koh, Kwong & Wong, 2009).

In children, a variety of neurological effects have also been reported following inorganic mercury intoxication, including developmental delay and regression, poor sleep, affective disturbance and self-directed aggression (Chrysochoou et al., 2003). In two randomized trials of dental amalgam involving elemental mercury exposure, children in the amalgam groups, in which the peak mean urinary mercury concentrations were <1 and 3.2 µg/g creatinine, respectively, followed over the

5- to 7-year follow-up periods, had neuropsychological and behavioural outcomes that were not significantly different from those of children in the control group (Bellinger et al., 2006, 2007a,b, 2008; DeRouen et al., 2006; Lauterbach et al., 2008). A meta-analysis of 12 studies involving adults occupationally exposed to mercury concluded that adverse neuropsychological effects on the adult central nervous system, expressed as deficits in memory, attention and psychomotor functions, are consistently found at urinary mercury concentrations greater than 35 µg/g creatinine (Meyer-Baron, Schaeper & Seeber, 2002). However, in a study involving dentists (mean urinary mercury concentration 3.3 µg/l, standard deviation [SD] = 4.9 µg/l) and dental assistants (mean urinary mercury concentration 2.0 µg/l, SD = 2.3 µg/l), significant associations were found between urinary mercury level and scores on a variety of tests of attention, concentration, visual memory, executive functions, fine motor function and sensory function (Echeverria et al., 2006). Mercury exposure in the dental profession is mainly from elemental mercury used in amalgam materials and/or mercury.

Although some studies of occupational exposure have suggested associations between inorganic mercury exposure and some cancers, the data are limited, and IARC (1993) has classified inorganic mercury compounds in Group 3 (not classifiable as to their carcinogenicity to humans).

Evidence that exposure to inorganic mercury causes immunotoxicity in humans is very limited. In a study of chloralkali workers, despite higher urinary mercury levels than in the referent group, no differences were found in IgG levels, antinuclear autoantibodies or circulating immune complexes (Barregard et al., 1997). In another group of workers with a median urinary mercury concentration of 25.4 µg/g creatinine, no differences were seen, compared with the control group, in terms of several markers of immune function (IgA, IgG, immunoglobulin M [IgM], autoantibodies [antiglomerular basement membrane and antilaminin autobodies]) (Langworth et al., 1992). Some work has suggested a role for mercury as a cofactor in autoimmune disease, interacting with genetic predisposition or some other triggering event (e.g. an acquired event, such as malaria infection) (Silbergeld, Silva & Nyland, 2005; Vas & Monestier, 2008). Increased expression of antinuclear and antinucleolar autoantibodies was reported in people living in a gold mining area in Amazonian Brazil (Silva et al., 2004), as well as a positive interaction between mercury exposure and malaria (Silbergeld, Silva & Nyland, 2005). Other reports, usually in occupational cohorts, have linked increased mercury exposure to increased T cell proliferation (Moszczynski et al., 1995), antilaminin antibodies (Lauwerys et al., 1983) and antifibrillarin antibodies (scleroderma patients) (Arnett et al., 2000). In a recent in vitro study using human peripheral mononuclear cells, mercury(II) chloride disrupted cytokine signalling pathways, stimulating the production of pro-inflammatory cytokines and reducing the release of anti-inflammatory cytokines (Gardner et al., 2009).

Case series and case reports have demonstrated an association between the topical use of mercury(II) ammonium chloride–containing creams and nephrotic syndrome. Among 60 nephrotic syndrome patients, 15–56 years of age, in Kenya, 53% admitted using a skin-lightening cream for a mean duration of 13 months before the onset of symptoms (range 1–36 months) (Barr et al., 1972). The urinary mercury

level ranged from 0 to 250 µg/l, with the highest levels among patients who were using the cream at the time (mean concentration of 150 µg/l, compared with mean concentrations of 29 µg/l among patients who had discontinued use and 6 µg/l among patients who had never used the cream). Percutaneous renal biopsy material was available for 34 patients, 50% of whom showed a "minimal change glomerular lesion", 38% a proliferative glomerulonephritis and 12% a membranous glomerulonephritis. Follow-up of 26 patients after 6 months to 2 years showed complete remission in 50% of the patients, with most (77%) occurring spontaneously. Remission occurred 3–11 months (mean of 6 months) after a patient stopped using the cream.

3. ANALYTICAL METHODS

3.1 Chemistry

The chemistry of mercury relevant to its presence in foods has been recently well described (EFSA, 2008).

3.2 Description of analytical methods

Sample handling is generally critical only for water samples. The best materials for water sample storage and processing are polytetrafluoroethylene and fluorinated ethylene-propylene. Fresh samples are usually stored deep-frozen, lyophilized in darkness or sometimes sterilized. It has been reported that methylmercury may be decomposed in some food matrices with repeated freezing and unfreezing (particularly in bivalves). However, relatively little is known about the effect of storage on the stability of methylmercury in food samples (Leermakers et al., 2005).

Whatever the method used, it should be noted that it must be used in accordance with Good Laboratory Practice, including analytical quality assurance, the use of fully validated methods (acceptable performance criteria for limit of detection [LOD], limit of quantification [LOQ], specificity, fidelity, accuracy and precision), the use of suitable reference materials (if possible, certified reference materials) and external quality assurance (Jorhem, 2008). Participation in proficiency testing programmes and intercomparison exercises of appropriate sample matrices is highly recommended for laboratories producing results for mercury in food as an integral part of their quality control schemes.

3.2.1 Methods of analysis for determining total mercury content of foods

Most data regarding mercury in food relate to total mercury. Following acidic digestion of samples, cold vapour atomic absorption spectrometry (CV-AAS) or cold vapour atomic fluorescence spectrometry (CV-AFS) has been widely used for the determination of total mercury in several food matrices (Sánchez Uria & Sanz-Medel, 1998). An LOQ of about 30 µg/kg dry mass in foods may be obtained by CV-AAS. Further sensitivity enhancement may be obtained by CV-AFS. The main advantages of the cold vapour technique are the separation of the analyte from the

potentially interfering sample matrix and its comparatively low cost. The most frequently occurring interference in CV-AAS or CV-AFS is that of nitrites and nitric oxides reducing the signal of mercury, requiring either stripping the sample digest with inert gas or treating it with reducing agents (Nunes et al., 2005). With an LOQ of about 10 µg/kg dry mass and greater selectivity, inductively coupled plasma mass spectrometry (ICP-MS) is increasingly being used with an addition of gold chloride to mercury standard solutions to avoid the mercury memory effects (Noël et al., 2005; Julshamn et al., 2007). Although the instrumentation is expensive to purchase and to operate, the ability of ICP-MS to provide low LOQs, to provide a wide dynamic linear range and to measure many elements simultaneously can offset these cost factors.

In conclusion, CV-AAS or CV-AFS and increasingly ICP-MS have been used for a wide variety of food samples with good results, although some modifications or care may be required for certain types of matrix.

3.2.2 Methods of analysis for determining organic mercury levels in food

The implementation of a technical analysis of mercury compounds requires procedures that typically involve the following steps: extraction and/or enrichment of the matrix, derivation of non-volatile ionic species during gas chromatographic (GC) separation, cleaning (if necessary), chromatographic separation and selective detection. Each step is critical to the viability and comparability of final results. Immediately after the sampling stage, it is recommended that the samples be placed in a freezer to reduce the risk of degradation during storage, until the analysis is made (Yu & Yan, 2003; Leermakers et al., 2005; Parker & Bloom, 2005).

Basically, all the speciation methodology is generally targeted on the separation and determination of methylmercury, and there has been no conclusive identification of other species. Numerous separation and detection techniques that have been coupled to perform the speciation analysis of mercury have been recently reviewed (Carro & Mejuto, 2000; Szpunar et al., 2000; Cornelis et al., 2003, 2005; Siepak & Boszke, 2004; Leermakers et al., 2005; Stoichev et al., 2006; Björn et al., 2007; Krystek & Ritsema, 2007; EFSA, 2008). However, no standardized method of mercury speciation exists, and consequently there is a real need for the development of a fully validated method according to standardized international criteria for the determination of reliable organic mercury levels in foods.

GC with both packed and capillary columns has been the most widely used technique for the separation of mercury species, whereas high-performance liquid chromatography (HPLC) is increasingly being applied (Sánchez Uria & Sanz-Medel, 1998; Carro & Mejuto, 2000; Harrington, 2000). The detection of mercury species by GC has mainly been carried out by electron capture detector, which is, however, not specific to mercury. CV-AAS and CV-AFS are therefore more appropriate for detection, together with microwave-induced plasma atomic emission spectrometry (MIP-AES), inductively coupled plasma atomic emission spectrometry (ICP-AES), mass spectrometry (MS) and, increasingly, ICP-MS (Sánchez Uria & Sanz-Medel, 1998; Carro & Mejuto, 2000; Willoud et al., 2004; Monperrus et al., 2008). All these techniques provide sufficiently good sensitivity to be used for the analysis of certain

food samples: LODs were about 40 µg/kg for MS, 10 µg/kg for CV-AAS, 1 µg/kg for CV-AFS, 5 µg/kg for MIP-AES or ICP-AES and less than 3 µg/kg for ICP-MS. Besides its high sensitivity and selectivity, ICP-MS yields more accurate and precise results by speciated isotope dilution mass spectrometry (SID-MS) (Leermakers et al., 2005; Monperrus et al., 2008). In recent years, the use of ICP-MS in speciation analysis has increased tremendously; this is evident from the large number of publications devoted to the use of ICP-MS in the speciation of mercury (Leermakers et al., 2005). However, such advanced instrumentation is not always available in the laboratories of some countries with important fish catches, and the most commonly used method reported was CV-AAS, which is a well-established and proven method for determining the mercury content of foods. Extraction of the mercury species from its matrix is one of the most critical steps, because two conflicting issues need to be addressed: obtaining high extraction efficiency and preventing losses.

Extraction procedures vary, but most are based on the initial work of Westöö (1966, 1967, 1968), where the sample is treated with hydrochloric acid to release methylmercury from sulfhydryl groups and sodium chloride to enable its recovery into the organic phase (benzene or toluene). Inorganic mercury remains in the aqueous phase. The organic phase is further back-extracted to aqueous cysteine solutions to purify the extract. Modifications have included other organic phases, thiosulfate instead of cysteine, application of copper(II) to release methylmercury from proteins, use of bromide or chelating agents to improve extraction, further purification by back-extraction into the organic phase and defatting the samples prior to digestion to prevent emulsifications (Sánchez Uria & Sanz-Medel, 1998; Carro & Mejuto, 2000). Some workers have analysed the extracted mercury species as for total mercury, denoting it as organic mercury, and the aqueous phase of the sample for Hg^{2+}. Other workers differentiate between inorganic and organic mercury compounds by selective reduction, where the samples are treated with tin(II) chloride, reducing Hg^{2+} to Hg^0 and leaving mercury–carbon bonds intact. After complete purging of Hg^0, it is analysed by CV-AAS or CV-AFS, while the remaining sample, assumed to contain only organic mercury, is analysed as for total mercury. Instead of extraction, biological samples treated with sulfuric and iodoacetic acids have been subjected to steam distillation, where volatile methylmercury iodide is distilled off. The distillate is usually derivatized with sodium tetraethylborate (forming methylethylmercury) to improve sensitivity and performance of the GC analysis. However, the steam distillation may produce methylmercury from Hg^{2+} as an artefact (Bloom, Colman & Barber, 1997). Alkaline digestions, usually in the presence of cysteine to avoid losses of methylmercury hydroxides and to stabilize the mercury–carbon bond, with subsequent acidification and extraction of methylmercury as above, have also been used. The hydroxide releases methylmercury quantitatively from proteins. This procedure is often followed by derivatization with sodium tetraethylborate prior to GC analysis. However, in the presence of high levels of inorganic mercury, Hg^{2+} may be converted to methylmercury during derivatization (Delgado et al., 2007).

By using HPLC instead of GC for separation, the derivatization procedure may be omitted, and the cleanup becomes less critical, with LODs similar to those

for GC methods (Sánchez Uria & Sanz-Medel, 1998; Carro & Mejuto, 2000; Harrington, 2000; Leermakers et al., 2005; Hight & Cheng, 2006). Digestion may be carried out in aqueous cysteine hydrochloride directly at 60 °C and the solution analysed for methylmercury and Hg^{2+} with reversed-phase HPLC after simple filtration (Chiou, Jiang & Daadurai, 2001; Hight & Cheng, 2006; Percy et al., 2007). Precision and accuracy in single-laboratory validations have been shown to be satisfactory, but validation by way of intercomparison and/or interlaboratory studies is required. Although these methods appear promising, they have only recently been introduced and are therefore currently not in widespread use.

3.2.3 General conclusions

The techniques most frequently used to release mercury species from solid samples are acid leaching or alkaline digestion, with the option of applying ultrasonic or microwave energy to assist in the procedure. In alkaline media, methylmercury appears to be more stable than in acid media, and proteins are easily hydrolysed.

GC has been the most widely used technique for the separation of mercury species, whereas HPLC is increasingly being applied (Leermakers et al., 2005; Hight & Cheng, 2006). The methods of detection (LOD in parentheses) of CV-AAS (10 µg/kg), CV-AFS (1 µg/kg), MIP-AES or ICP-AES (5 µg/kg), MS (40 µg/kg) and ICP-MS (<3 µg/kg) all have sufficient sensitivity for food samples. The advantage of MS and ICP-MS is their multielement and multi-isotope capabilities that can yield more accurate and precise results by SID-MS, which can also check for species transformations and extraction recoveries (Krata & Bulska, 2005; Leermakers et al., 2005; Monperrus et al., 2008). Once in solution, methylmercury may decompose when exposed to light, low pH and high storage temperatures (Devai et al., 2001; Yu & Yan, 2003; Hight & Cheng, 2006; Delgado et al., 2007). Other factors, such as the type of storage container, may also affect the stability. Dimethylmercury is, for several reasons, not reliably determined by most of the methods above (Leermakers et al., 2005).

Available certified reference materials and proficiency testing schemes or intercomparison exercises exist for both total mercury and methylmercury to demonstrate and maintain analytical quality assurance. However, there is a current need for fully validated, standardized methods for determination of methylmercury and inorganic mercury.

4. SAMPLING PROTOCOLS

Some authorities have regulations with regard to specific sampling protocols for mercury and other contaminants. For example, the European Commission has regulated the number and size of incremental samples, size of the aggregate sample and precautions to be taken for control purposes (EC, 2007).

5. LEVELS AND PATTERNS OF CONTAMINATION IN FOOD COMMODITIES

At its present meeting, the Committee reviewed data from eight countries on the occurrence of mercury in different food commodities analysed between 1997 and 2009. The total number of analytical results for total mercury was more than 106 740, with 93% coming from Europe (Finland, France, Spain), 5% from Asia (China, Japan), 1% from the Americas (Brazil, Canada) and 1% from Oceania (Australia), for water (85%), fish (6%), shellfish (2%) and other food groups (6%). The 2128 samples analysed for methylmercury came from France, China and Japan for fish (94%), shellfish (2%) and other products (4%). Each of the dossiers contained analyses on individual samples, except for the dossier from China. The Committee obtained additional analytical data from the published literature. However, the Committee did not receive any occurrence data on inorganic mercury levels in foods or water. Finally, owing to the lack of required information, such as LOD, LOQ, analytical quality assurance, analytical methods, results below LOD and no results given, the data submitted to the Committee from the Netherlands and Singapore could not be used for this report.

5.1 Australia

The dossier from Australia (20th Australian Total Diet Study [TDS]) contained aggregated analytical results on total mercury from 1076 samples comprising 51 finfish collected in 2002: 21 fish fillets, raw, unfrozen (mean 0.018 mg/kg, median 0.016 mg/kg and range 0.005–0.050 mg/kg) and 21 fish portions and 9 canned tuna (mean 0.742 mg/kg, median 0.250 mg/kg and range 0.042–3.50 mg/kg) (FSANZ, 2003). No more indication of the fish species analysed was given. No indication was given of the number of species found to contain total mercury at concentrations greater than 0.5 or 1 mg/kg.

The maximum concentration found in foods other than fish was 0.048 mg/kg for prawns (mean 0.021 mg/kg and median 0.016 mg/kg).

Very low levels of organic mercury were detected in fish portions (mean 0.808 µg/kg and range not detected [ND] to 2.7 µg/kg) and in tuna, canned (mean 0.918 µg/kg and range ND–2.2 µg/kg). Surprisingly, using the maximum concentration found in fish portions or canned tuna, the percentages of methylmercury were estimated to be only 0.077% and 0.71%, respectively.

Updated data from the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) database indicated a mean total mercury concentration of 0.37 mg/kg (range 0.1–0.71 mg/kg) in 24 samples of shark sampled in 2007. No indication was given of the number of species found to contain total mercury at concentrations greater than 0.5 mg/kg.

5.2 Brazil

The dossier from Brazil (Morgano et al., 2005, 2007) contained analytical results on total mercury from 550 samples of 45 non-predatory fish species (covering 6 species of freshwater fish from pisciculture establishments) collected in

2001–2006. Fifty-one per cent of the data were below the LOQ of 0.0007 mg/kg, and the maximum concentration found was 0.878 mg/kg (mean 0.095 mg/kg, median 0.06 mg/kg). Only two species—meca (*Xiphias gladius*) and bagre (*Genidens barbus*)—contained total mercury at concentrations greater than 0.5 mg/kg.

5.3 Canada

Data from Canada (Dabeka, McKenzie & Bradley, 2003) indicated that total mercury was measured in 259 total diet food composites from two Canadian cities: Whitehorse and Ottawa. Levels were generally low, with 46% of the composites having concentrations below the LOD, which ranged from 0.026 to 0.506 µg/kg. The fish category ($n = 8$) contained the highest mercury concentrations, which averaged 67 µg/g and ranged from 24 to 148 µg/kg, with the highest level being in the canned fish from Whitehorse. All composites were below the Canadian guideline for total mercury in fish of 0.5 mg/kg.

The methylmercury levels in predatory fish species marketed in Canada were also estimated (Forsyth et al., 2004). Mercury was detected in all analysed samples of swordfish, marlin, shark and tuna purchased from major supermarket outlets and fish retailers in three cities across Canada. Total mercury and methylmercury levels ranged up to 3.85 mg/kg and 2.35 mg/kg, respectively. Swordfish contained the highest levels, followed by shark, fresh/frozen tuna and marlin. Levels in canned tuna were considerably less than those in the other examined samples (Table 4).

Table 4. Summary of total mercury and methylmercury concentrations in predatory fish from Canada

Species	*n*	Mean concentration (µg/kg)		Concentration range (µg/kg)	
		MeHg	HgT	MeHg	HgT
Marlin	3	489	842	212–881	336–1743
Shark	12	849	1360	285–1538	390–2729
Swordfish	10	1080	1822	300–2346	399–3845
Tuna, all canned	37	98	157	9–411	20–587
Tuna, canned, unidentified	5	25	47	10–43	25–69
Tuna, canned, albacore	16	166	260	105–229	193–384
Tuna, canned, skipjack	5	47	93	23–98	36–174
Tuna, canned, yellowfin	11	57	85	9–411	20–587
Tuna, fresh, frozen	13	662	929	61–1319	77–2121

HgT, total mercury; MeHg, methylmercury

It was estimated that methylmercury represents between 30% (in tuna skipjack, canned) and 94% (in fresh shark and tuna) of total mercury. Canned tuna products contained significantly less mercury and methylmercury than did retail fresh/frozen tuna, swordfish, marlin and shark samples. Canned albacore tuna, however, did contain significantly more mercury and methylmercury than did other canned tuna products sold in Canada. The percentage of mercury present in tuna as methylmercury was positively correlated with the total mercury levels, so that older, larger tuna tend to have most of the mercury present as methylmercury. This trend was not found in retail samples of shark or swordfish.

5.4 China

The dossier from China (2007 Chinese TDS) contained analytical results on total mercury and methylmercury in aquatic food and 11 other food groups (cereals, legumes, potatoes, meat, eggs, milk, vegetables, fruits, sugar, beverages and waters, alcoholic beverages) of 12 provinces of four regions (Table 5). Methylmercury was found only in aquatic food groups from all 12 provinces. The ranges for total mercury and methylmercury contents of aquatic foods were 4.77–46.0 µg/kg and 3.29–31.6 µg/kg, respectively, with means of 18.5 µg/g and 12.6 µg/kg, respectively, all well below the national maximum levels for aquatic food (500 µg/kg for non-predatory fish, 1000 µg/kg for predatory fish). The highest total mercury content (46.0 µg/kg) and methylmercury content (31.6 µg/kg) were found for Ningxia, an interior province in North 2 region. The second highest total mercury content (41.4 µg/kg) and methylmercury content (29.8 µg/kg) were found in Guangxi province, a coastal province in South 2 region. The mean total mercury and methylmercury contents for coastal provinces (Liaoning, Hebei, Shanghai, Fujian, Guangxi) were 21.0 µg/g and 14.0 µg/kg, respectively, and those for the interior provinces were 18.2 µg/g and 11.5 µg/kg, respectively. The orders of mean total mercury and methylmercury contents of four regions are both South 2 > North 2 > South 1 > North 1. The percentages of methylmercury in total mercury in aquatic foods ranged from 50% to 87%, with a mean of 68%. The total mercury mean results for the other food groups were less than 5 µg/kg (compared with 18.5 µg/kg for aquatic foods), and the per cent contributions of food composites to the corresponding national maximum levels were generally low, except in Shanghai province (more than 25% for cereals, legumes, potatoes, meat, eggs, vegetables and fruits).

In another study, in Zhoushan, China, the average total mercury and methylmercury concentrations in all species of fish (n = 148) were 0.26 mg/kg and 0.18 mg/kg, respectively (Cheng et al., 2009). Total mercury and methylmercury levels measured in all fish samples ranged from lows of 0.01 mg/kg and 0.0004 mg/kg wet mass, respectively, found in a specimen of *Monopterus albus* (mean concentrations of 0.13 mg/kg and 0.09 mg/kg, respectively). Approximately 15% and 19% of the samples, respectively, showed total mercury and methylmercury levels that exceeded the limits established by the Chinese National Standards Agency (CNSA) (0.3 mg/kg and 0.2 mg/kg, respectively; CNSA, 1994). The proportion of methylmercury relative to total mercury in the fish ranged from 59% to 84%, with a mean of 74%, which indicated, according to the authors, that fish accumulate more organic mercury (methylmercury) than inorganic mercury.

Table 5. Total mercury and methylmercury in foods from 12 provinces of China in 2007 Chinese TDS

Food groups	Total mercury concentrations (μg/kg)[a]													1990 Chinese TDS[b]	2000 Chinese TDS[c]
	Heilong-jiang	Liaoning	Hebei	Shanxi	Ningxia	Henan	Shanghai	Hubei	Fujian	Sichuan	Guangxi	Jiangxi	Mean	Mean	Mean
Cereals	0.13 (0.7)	0.35 (1.8)	0.10 (0.5)	0.26 (1.3)	0.51 (2.6)	ND	12.03 (60.2)	0.36 (1.8)	0.78 (3.9)	0.58 (2.9)	0.10 (0.5)	0.10 (0.5)	1.39	13	9.0
Legumes	0.31 (3.1)	0.43 (4.3)	ND	0.13 (1.3)	0.12 (1.2)	ND	13.05 (130.5)	0.20 (2.0)	0.11 (1.1)	ND	0.92 (9.2)	ND	1.91	10	4.0
Potatoes	0.10 (1.0)	ND	ND	0.13 (1.3)	0.12 (1.2)	ND	10.45 (104.5)	ND	0.13 (1.3)	0.14 (1.4)	0.14 (1.4)	ND	1.60	7	4.9
Meat	1.46 (2.9)	0.43 (0.9)	0.41 (0.8)	0.38 (0.8)	0.45 (0.9)	0.19 (0.4)	13.51 (27.0)	1.01 (2.0)	0.39 (0.8)	0.42 (0.8)	1.45 (2.9)	1.30 (2.6)	1.78	13	5.6
Eggs	0.20 (0.4)	0.32 (0.6)	0.24 (0.5)	6.08 (12.2)	0.32 (0.6)	0.15 (0.3)	38.10 (76.2)	8.30 (16.6)	1.11 (2.2)	1.15 (2.3)	0.41 (0.8)	0.45 (0.9)	4.74	29	5.0
Aquatic foods	4.77 (1.0)	13.35 (2.7)	10.71 (2.1)	8.47 (1.7)	46.04 (9.2)	10.45 (2.1)	28.06 (5.6)	10.30 (2.1)	11.54 (2.3)	14.49 (2.9)	41.41 (8.3)	22.22 (4.4)	18.48	40	24.4
MeHg	**3.29**	**11.7**	**8.03**	**5.31**	**31.6**	**7.46**	**14.0**	**7.34**	**6.39**	**9.76**	**29.8**	**15.9**	**12.55**		
Milk	ND	ND	ND	0.15 (1.5)	0.17 (1.7)	ND	1.77 (17.7)	2.23 (22.3)	ND	ND	ND	0.46 (4.6)	0.96	0	1.8
Vegetables	0.10 (1.0)	0.30 (3.0)	0.18 (1.8)	0.24 (2.4)	ND	ND	13.49 (134.9)	0.25 (2.5)	0.46 (4.6)	0.45 (4.5)	1.82 (18.2)	0.48 (4.8)	1.78	3	3.2

Table 5 (contd)

Food groups	Total mercury concentrations (μg/kg)[a]													1990 Chinese TDS[b]	2000 Chinese TDS[c]
	Heilong-jiang	Liaoning	Hebei	Shanxi	Ningxia	Henan	Shanghai	Hubei	Fujian	Sichuan	Guangxi	Jiangxi	Mean	Mean	Mean
Fruits	ND	0.26 (2.6)	0.20 (2.0)	0.13 (1.3)	0.18 (1.8)	0.17 (1.7)	3.84 (38.4)	ND	0.11 (1.1)	0.14 (1.4)	0.24 (2.4)	ND	0.59	4	2.7
Sugar	ND	0.16	0.22	0.15	0.19	0.45	0.95	ND	0.22	0.10	0.25	ND	0.30	0	5.0
Beverages and water	ND	ND	ND	ND	ND	ND	0.73	ND	ND	ND	ND	ND	0.06	0	0.3
Alcoholic beverages	ND	ND	ND	0.51	ND	ND	4.12	ND	0.11	ND	0.21	0.10	0.42	2	1.3

MeHg, methylmercury; ND, not detected

[a] Figures in parentheses are the per cent contributions (%) of food composites to the corresponding national maximum levels. There were no national maximum levels for sugar, beverages and water, or alcoholic beverages.

[b] Data from Chen & Gao (1993).

[c] Data from Li, Gao & Chen (2006).

Table 6. Summary of total mercury and methylmercury concentrations in fish from Hong Kong SAR

Species	*n*	Mean concentration (µg/kg)		Concentration range (µg/kg)	
		MeHg	HgT	MeHg	HgT
Local whole fish	224	55	69	3–349	3–374
Imported whole fish	42	140	179	21–1010	29–1370
Tuna, canned	14	144	181	27–430	37–469
All samples	280	72	91	3–1010	3–1370

HgT, total mercury; MeHg, methylmercury

Total mercury and methylmercury levels in crops, poultry, milk, drinking-water, food oil and salt samples (n = 88) were all below the corresponding CNSA limits (total mercury: 0.02 mg/kg for grain, 0.01 mg/kg for vegetables, 0.05 mg/kg for both egg and meat, 0.01 mg/kg for milk and 1000 ng/l for drinking-water; CNSA, 1994).

Another study from Hong Kong Special Administrative Region (SAR) estimated the total mercury and methylmercury concentrations in 280 samples of fish purchased from different commercial outlets (covering 89 species of whole fish and three types of canned tuna) (Tang et al., 2009). For total mercury, the median concentration was 63 µg/kg (range 3–1370 µg/kg), and for methylmercury, the median concentration was 48 µg/kg (range 3–1010 µg/kg) (Table 6). Total mercury and methylmercury were detectable in all 280 samples, of which 277 fish samples (99%) contained total mercury (range 3–469 µg/kg) and methylmercury (range 3–430 µg/kg) at levels below the regulatory limit of 500 µg/kg. Only three samples of imported alfonsino (*Beryx splendens*) had mercury levels higher than 500 µg/kg (total mercury: mean 1053 µg/kg, range 609–1370 µg/kg; methylmercury: mean 827 µg/kg, range 509–1010 µg/kg).

5.5 Finland

The dossier from Finland (Finland, 2010) contained analytical results on total mercury from 74 samples, comprising 31 milk and milk products (31% of samples above the LOQ) and 43 finfish (all above the LOQ), collected in 2006–2009. The only fish species found to contain total mercury at concentrations greater than 1.0 mg/kg was pike (1.53 mg/kg). One additional species, tuna, was found to contain total mercury at concentrations greater than 0.5 mg/kg (Table 7).

5.6 France

The dossier submitted by France (France, 2010) contained individual analytical results on total mercury from 999 samples of foods other than fish products, 90 545 aggregated samples of water, 3499 finfish and 1892 shellfish, collected in 1997–2007.

Table 7. Data on mercury concentrations in finfish from Finland

Mean concentrations of total mercury:				
Samples	No. of samples	Mean (mg/kg)		
All samples	43	0.108		
<1 mg/kg	42	0.074		
<0.5 mg/kg	41	0.060		
Mean concentrations of total mercury by species:				
Species	No. of samples	Mean (mg/kg)		% reduction
		All samples	Violative samples removed	
>1 mg/kg				
Pike	1/2	0.94	0.35	63

Of the 999 samples of foods other than fish products, 86% were less than the LOQs (range 0.003–0.011 mg/kg), and the maximum concentration found was 0.050 mg/kg in fungi (mean 0.022 mg/kg, median 0.018 mg/kg). Ninety-eight per cent of water samples were below the LOQ of 0.02 µg/l, with a maximum of 4.3 µg/l (mean lower bound 0.005 µg/l; mean upper bound 0.339 µg/l).

The only shellfish species found to contain total mercury at concentrations greater than 0.5 mg/kg was common scallop, with a concentration of 0.86 mg/kg, and 20% of the data were below the LOQ (maximum 0.1 mg/kg). Only 1.4% of the data were below the LOQ (maximum 0.1 mg/kg) for the fish species. The fish species found to contain total mercury at concentrations greater than 1.0 mg/kg (with the percentage within a species that exceeded either of the two limits in parentheses) were lamprey (100%), Portuguese dogfish (92%), swordfish (89%), shark (83%), marlin (50%), picked dogfish (35%), tuna (24%), catshark (22%), scabbardfish (14%), ling (14%), pike (2.2%) and ray (2.9%). The maximum content of 4.3 mg/kg was found in tuna. Additional species were found to contain mercury at concentrations greater than 0.5 mg/kg: emperor (39%), Atlantic bonito (25%), smooth hounds (20%), megrim (18%), barbell (9.1%), sheatfish (6.3%), mullet (6.3%), pike perch (8.1%), grenadier (5.4%), gurnard (2.7%), seabass (2.7%), eel (2.4%), anglerfish (2.3%), pout (2.2%), European perch (1.9%), whiting (1.1%), cod (1.0%) and trout (0.1%) (Table 8).

The dossier also contained analytical results on methylmercury from 153 samples of fish products (105 finfish, 44 shellfish and 4 seafood-based dishes) collected in 2005 (Sirot et al., 2008). No shellfish species contained methylmercury at concentrations greater than 0.5 mg/kg (range 0.002–0.451 mg/kg), with the maximum concentration found in edible crab. Of the 105 individual finfish samples analysed, all were quantified, and only 7 (6.7%) were found to contain methylmercury at concentrations greater than 0.5 mg/kg, with one (1%)

Table 8. Data on mercury concentrations in finfish from France

Mean concentrations of total mercury:				
Samples	No. of samples	Mean (mg/kg)		
All samples	4480	0.185		
<1 mg/kg	4398	0.144		
<0.5 mg/kg	4228	0.115		
Mean concentrations of total mercury by species:				
Species	No. of samples	Mean (mg/kg)		% reduction
		All samples	Violative samples removed	
>1 mg/kg				
Lamprey	7/11	1.13	0.75	34
Portuguese dogfish	23/26	1.99	0.27	86
Swordfish	21/36	1.14	0.66	42
Shark	4/6	1.76	0.57	68
Marlin	2/4	1.95	0.19	90
Picked dogfish	1/21	0.39	0.30	23
Tuna	17/411	0.37	0.32	14
Catshark	2/59	0.43	0.39	9
Scabbardfish	1/21	0.36	0.33	8
Ling	1/50	0.31	0.29	6
Pike	1/46	0.14	0.11	21
Ray	1/69	0.12	0.10	17
Mean concentrations of methylmercury:				
Samples	No. of samples	Mean (mg/kg)		
All samples	105	0.167		
<1 mg/kg	104	0.154		
<0.5 mg/kg	98	0.121		
Mean concentrations of methylmercury by species:				
Species	No. of samples	Mean (mg/kg)		% reduction
		All samples	Violative samples removed	
>1 mg/kg				
Swordfish	1/4	0.94	0.79	16

concentration greater than 1 mg/kg. The only species found to contain methylmercury at concentrations greater than 1.0 mg/kg (the percentage within a species that exceeded either of the two limits shown in parentheses) was swordfish (100%), with a concentration of 1.42 mg/kg (Table 8). Two additional species were found to contain mercury at concentrations greater than 0.5 mg/kg: emperor fish (67%) and tuna (20%).

It was estimated that methylmercury is between 90% and 105% of total mercury.

5.7 Japan

The dossier submitted by Japan (Japan, 2010) contained individual analytical results on total mercury from 3877 samples of foods other than fish products (cereals, fruits and vegetables) collected in 2002–2009 and on total mercury and methylmercury from 1275 finfish collected in 2007–2009.

The maximum concentration found in foods other than fish products (20% of samples above the LOQ; mean 0.003 mg/kg, median 0.002 mg/kg) was 0.013 mg/kg in rice.

The fish species (100% above the LOQ) found to contain total mercury at concentrations greater than 1.0 mg/kg (with the percentage within a species that exceeded the limit in parentheses) were swordfish (66%), splendid alfonsino (31%), blue shark (14%), marlin (11%) and tuna (2.6%). The maximum content of 11.4 mg/kg was found in striped marlin. No additional species were found to contain total mercury at concentrations greater than 0.5 mg/kg (Table 9).

Of the 1275 individual finfish samples analysed (100% above the LOQ), 408 (32%) were found to contain methylmercury at concentrations greater than 0.5 mg/kg, with 111 (8.7%) containing methylmercury at concentrations greater than 1 mg/kg. The species found to contain methylmercury at concentrations greater than 1.0 mg/kg (with the percentage within a species that exceeded either of the two limits in parentheses) were swordfish (54%), splendid alfonsino (17%), blue shark (13%), tuna (2.0%) and marlin (1.0%) (Table 9). The maximum methylmercury content of 2.8 mg/kg was found in swordfish. No additional species were found to contain methylmercury at concentrations greater than 0.5 mg/kg.

Of the 1275 individual finfish samples analysed, methylmercury ranged between 38% and 100% of total mercury, except for 6 samples of the same species, blue marlin (n = 50), where methylmercury was only 8–11% of total mercury. It should be noted that these six samples contained the highest levels of total mercury (range 2.0–11.4 mg/kg). The other blue marlin species had methylmercury contents ranging from 38% to 75% of total mercury.

5.8 Spain

The mercury contents of 25 samples of fish and shellfish products most frequently consumed in Spain were determined (Sahuquillo et al., 2007). There was wide variability, not only among the mercury levels of different fish species, but also for different samples of the same species—with the methylmercury content ranging

Table 9. Data on mercury concentrations in finfish from Japan

Mean concentrations of total mercury:				
Samples	No. of samples	Mean (mg/kg)		
All samples	1275	0.546		
<1 mg/kg	1117	0.390		
<0.5 mg/kg	736	0.249		
Mean concentrations of total mercury by species:				
Species	No. of samples	Mean (mg/kg)		% reduction
		All samples	Violative samples removed	
>1 mg/kg				
Swordfish	79/120	1.30	0.65	50
Splendid alfonsino	37/120	0.77	0.54	30
Blue shark	13/90	0.66	0.54	18
Marlin	12/110	0.70	0.37	47
Tuna	17/655	0.45	0.42	7
Mean concentrations of methylmercury:				
Samples	No. of samples	Mean (mg/kg)		
All samples	1275	0.444		
<1 mg/kg	1164	0.353		
<0.5 mg/kg	867	0.248		
Mean concentrations of methylmercury by species:				
Species	No. of samples	Mean (mg/kg)		% reduction
		All samples	Violative samples removed	
>1 mg/kg				
Swordfish	65/120	1.10	0.64	42
Splendid alfonsino	20/120	0.65	0.53	18
Blue shark	12/90	0.59	0.49	17
Marlin	1/110	0.30	0.29	3
Tuna	13/655	0.39	0.37	5

from below 54 to 596 µg/kg wet mass (Table 10). Total mercury contents in fish analysed in this study did not exceed the maximum levels established by the European Union. The highest mercury levels corresponded to predatory fish species located at the highest level of the food-chain (tuna, swordfish). According to the authors, despite their small size compared with tuna, salmon and swordfish, the relatively high mercury contents in mollera (poor cod, *Trisopterus minutes*) and pagre (common sea bream, *Pagrus pagrus*) could be explained by the fact that they came from Valencian coastal waters at the mouth of the Albufera lake. The comparison of methylmercury contents in fresh and canned tuna shows a mean difference of 17%. In samples of the same brand of canned tuna, differences between batches, perhaps ascribable to differences in the origin of the tuna, were found. Methylmercury contents were higher in canned natural tuna than in tuna in vegetable oil. The differences cannot be ascribed to the brine; considering the high variability in methylmercury content in the same fish species, the possibility that the difference could be due to the fish origin cannot be ruled out. However, the fact that the lower content corresponded to tuna in oil (with values even lower than those corresponding to the analysed fresh tuna) could also be explained by partial dissolution of organic mercury in the oil.

Table 10. Summary of methylmercury concentrations in fresh or canned fish and shellfish from Spain

Fish species	Mean methylmercury concentration (mg/kg)
Fresh species	
Tuna, fresh	0.596
Swordfish	0.479
Mollera	0.199
Pagre	0.153
Hake	0.143
Serrano	0.131
Perch	0.070
Mackerel	0.064
Chucla	0.059
Anchovy	0.058
Salmon	<0.054
Salmon, smoked	<0.054
Sardine	<0.054
Sole	<0.054
Prawn, cooked	<0.054

Table 10 (contd)

Fish species	Mean methylmercury concentration (mg/kg)
Prawn, fresh	<0.054
Llisa	<0.054
Mussel	<0.054
Canned species	
Tuna, natural	0.609
Tuna, in vegetable oil A	0.455
Tuna, in vegetable oil B	0.207
Tuna, in vegetable oil C	0.423
Mackerel	0.094
Mussel	<0.054
Octopus	<0.054

Finally, according to the network Rapid Alert System for Food and Feed, the number of foods that exceeded regulatory limits on mercury in imported foods by the European community is 449 over the period 2002–2008.

5.9 General conclusions

Total mercury levels in foods other than fish products were generally low (range 0.0001–0.050 mg/kg), with about 80% of the 6183 samples containing levels below the LOQs. The highest levels were found in fungi. Mean methylmercury levels reported by China in non-fish samples ranged from 0.001 to 0.023 mg/kg, with a maximum concentration found in poultry. No other information on methylmercury in non-fish samples was received from other countries. In water, total mercury levels in 98% of 90 545 samples analysed in France were below the LOQ of 0.02 µg/l, with a maximum of 4.3 µg/l.

Total mercury levels in 1892 shellfish samples (80% above LOQ) ranged from 0.002 to 0.86 mg/kg. No shellfish species contained methylmercury at concentrations greater than 0.5 mg/kg (range 0.002–0.451 mg/kg), with the maximum concentration found in edible crab.

Total mercury levels in 6114 fish samples ranged from 0.001 to 11.4 mg/kg, with the maximum concentration found in marlin. About 5% exceeded 1 mg/kg, particularly for lamprey, Portuguese dogfish, swordfish, shark, marlin, splendid alfonsino, picked dogfish, tuna, catshark, scabbardfish, ling, pike and ray.

The proportion of total mercury contributed by methylmercury generally ranged between 30% and 100%, depending on species of fish, size, age and diet. Furthermore, in about 80% of these data, methylmercury accounted for more than 80% of total mercury. However, a few submitted data showed proportions of methylmercury of about 10% or less.

6. FOOD CONSUMPTION AND DIETARY EXPOSURE ESTIMATES

6.1 General considerations on exposure to mercury from food

From a food perspective, the predominant human exposure to mercury occurs through the consumption of fish and shellfish. Analysis of a wide variety of foodstuffs collected from 12 different countries has shown that mercury concentrations in fish and shellfish are approximately 10–100 times greater than those in other foods, including cereals, potatoes, vegetables, fruits, meat, poultry, eggs, milk and milk products (Toro et al., 1994) (see also section 5).

The contribution of methylmercury to total mercury in fish varies with respect to age, size and trophic level of the fish species. In general, the percentage of total mercury contributed by methylmercury is usually 80–100% in fish muscle, but it can be significantly lower in organs such as liver and kidney (Storelli & Marcotrigiano, 2000; Storelli, Stuffler & Marcotrigiano, 2002). Additional studies have confirmed that for the majority of fish species studied, organic mercury represents the major fraction of total mercury in muscle tissue (Bloom, 1992; Holsbeek, Das & Joiris, 1997; Krystek & Ritsema, 2005; Storelli, Busco & Marcotrigiano, 2005; Storelli et al., 2005; Houserova et al., 2007; Afonso et al., 2008). For herbivorous fish, methylmercury can account for up to 70% of total mercury, whereas for piscivorous species, a maximum 100% contribution is possible (Lasorsa & Allen-Gil, 1995; Mason, Reinfelder & More, 1995). In a 5-year survey of canned tuna, up to 89% of the total mercury was considered to be methylmercury (Burger & Gochfeld, 2004), whereas a wide range of percentages (30–79%; average 62.4%) was reported in 37 samples of canned tuna in another survey (Forsyth et al., 2004). Analysis of 89 different species of fish and 3 varieties of canned tuna collected from commercial markets in Hong Kong SAR (n = 280 samples) gave a median methylmercury to total mercury percentage of 76% (Tang et al., 2009).

For marine mammals, a significant fraction of total mercury detected in organs (liver and kidney) is in the form of inorganic mercury (Wagemann et al., 1998). In general, the concentration of total mercury for marine mammals is greatest in liver, followed by kidney and then muscle tissue. As with fish, total mercury in muscle tissue is dominated by methylmercury; for liver, while variable, depending on species and age of the animal, the average contribution of organic mercury to total mercury is only approximately 15% (range 9–40%) (Wagemann et al.,1998).

Analysis of various bivalve species has shown variability in the ratio of methylmercury to total mercury, ranging from approximately 20% to 89% (Liang et al., 2003).

While limited details were available at the time for mercury speciation in foods other than fish and shellfish, earlier reports (IPCS, 1976) indicated that total mercury levels usually did not exceed 60 µg/kg, with methylmercury predominating, except in some samples of organ meats (liver and kidney). In the IPCS (1991) report on inorganic mercury, it was reported that inorganic mercury levels in most foods were usually below the LOD (20 ng/g). Based on the results of various TDSs, it was estimated that the average dietary inorganic mercury exposure was approximately 4.2 µg/day (IPCS, 1990). In all food items not related to fish, total mercury was

presumed to be only inorganic mercury, whereas for fish, 20% of total mercury exposure (3.0 µg/day) was considered to be exposure to inorganic mercury.

Inorganic forms of mercury appear to have limited potential for uptake by terrestrial plants (Bloom, 1992).

An additional source of dietary inorganic mercury can be from human milk. In a study that investigated the total and inorganic mercury content of human milk and blood in relation to fish consumption and amalgam fillings, an average of 51% of total mercury detected in milk (0.6 ng/g) was in the inorganic form, compared with only 26% in blood (Oskarsson et al., 1996). Mercury levels in milk were correlated to the number of amalgam fillings, but not methylmercury intake. Other studies have reported that there is a significant correlation between the concentration of mercury in breast milk and the number of amalgam surfaces in mothers reporting low fish consumption (one fish meal per month) (Da Costa, Malm & Dórea, 2005).

6.2 National estimates

6.2.1 Total mercury

Most of the available dietary exposure assessments for mercury were from national TDSs. These include the following TDSs: Australia (2000–2001), Canada (1998–2000), China (2007), Czech Republic (2000), France (2001–2002), Japan (2008), New Zealand (2003–2004), the Republic of Korea (2005), the United Kingdom (2006) and the USA (1991–2005). Published data from other studies focusing on special subpopulations were also available. These include TDSs conducted in Chile (Santiago) and Spain (Catalonia), as well as studies of fishermen and their household members in Zhoushan Island (China), residents of Changchun city in north-east China, secondary-school students in Hong Kong SAR, frequent seafood consumers in France (the CALIPSO study, or the Fish and Seafood Consumption Study and Biomarkers of Exposure to Trace Elements, Pollutants and Omega-3), exposures from fish and shellfish in Spain and modelled exposure estimates for fish consumers in the USA.

In general, most studies available allowed for the estimation of dietary exposure to total mercury from fish and shellfish, as well as from other foods.

(a) Australia

Total diet total mercury exposures reported in the 2000–2001 TDS (FSANZ, 2003) ranged from 0.01–0.08 µg/kg bw per day for adult females and 12-year-old girls to 0.01–0.25 µg/kg bw per day for infants (9 months of age). Total mercury exposures from foods other than fish and shellfish ranged from 0–0.07 µg/kg bw per day for adult females and 12-year-old girls to 0–0.24 µg/kg bw per day for infants (Table 11). The lower limits of the exposure ranges correspond to estimates derived assuming that samples with non-detectable concentrations have total mercury concentrations of 0 µg/kg, whereas upper limits assume that samples with non-detectable concentrations have total mercury concentrations equal to the LOD. It should be noted that, except for bacon, the only foods with detectable total mercury concentrations were fish and shellfish foods. Further, in the case of bacon, the

Table 11. Total diet total mercury exposures from the 2000–2001 Australian TDS

Subpopulation	Exposure from total diet (µg/kg bw per day)	Exposure from fish and shellfish[a] (µg/kg bw per day)	Exposure from foods other than fish or shellfish[a] (µg/kg bw per day)
Adult males (25–34 years)	0.01–0.09	0.01	0–0.08
Adult females (25–34 years)	0.01–0.08	0.01	0–0.07
Boys (12 years)	0.01–0.10	0.01	0–0.09
Girls (12 years)	0.01–0.08	0.01	0–0.07
Toddlers (2 years)	0.01–0.20	0.01	0–0.19
Infants (9 months)	0.01–0.25	0.02	0–0.24

[a] Derived by combining food consumption data and median total mercury levels reported in the Food Standards Australia New Zealand 20th Australian TDS report (FSANZ, 2003).

majority of the samples (17 out of 21) had non-detectable concentrations. Hence, all lower limits for exposure values are zero, and all upper limits overestimate exposures; therefore, it is not possible to estimate the contribution of individual foods to the total exposure to total mercury.

(b) Canada

Total diet total mercury exposure estimates for the Canadian population varied from 0.010–0.012 µg/kg bw per day (females 65+ years) to 0.055–0.062 µg/kg bw per day (infants 0–1 month). Total mercury exposures from foods other than fish and shellfish ranged from 0.004–0.006 µg/kg bw per day (females 65+ years) to 0.019–0.026 µg/kg bw per day (infants 2–3 months) (Table 12). The lower limits of the ranges correspond to estimates derived assuming that samples with non-detectable concentrations have a total mercury concentration of 0 µg/kg, whereas upper limits assume that samples with non-detectable concentrations have concentrations equal to the LOD. Foods other than fish and shellfish contributing most to total mercury exposures are dairy products (in the case of the children and infant subpopulations) and meats (for the older subpopulations). However, these contributions were based on the upper limit estimates that assumed that samples with non-detectable concentrations have concentrations equal to the LOD; hence, the contributions of highly consumed foods may be artificially inflated.

(c) Chile

Total diet total mercury exposure estimates for the population of Santiago, Chile, available from a TDS conducted between 2001 and 2002, ranged from 0.059

Table 12. Total diet total mercury exposures from the 1998–2000 Canadian TDS

Subpopulation	Total diet exposure[a] (µg/kg bw per day)	Exposure from fish and shellfish (µg/kg bw per day)	Exposure from foods other than fish or shellfish[a] (µg/kg bw per day)	Food other than fish and shellfish contributing most to total exposure[b]
M & F 0–1 month	0.055–0.062	0.038	0.017–0.024	Dairy products (44%)
M & F 2–3 months	0.019–0.026	0	0.019–0.026	Baby foods (52%)
M & F 4–6 months	0.018–0.027	0	0.018–0.027	Dairy products (58%)
M & F 7–9 months	0.021–0.028	0.003	0.018–0.025	Dairy products (44%)
M & F 10–12 months	0.015–0.023	0	0.015–0.023	Dairy products (49%)
M & F 1–4 years	0.033–0.042	0.017	0.016–0.025	Dairy products (36%)
M & F 5–11 years	0.032–0.038	0.020	0.011–0.018	Dairy products (28%)
M 12–19 years	0.022–0.026	0.014	0.008–0.012	Meat and meat products (24%)
M 20–39 years	0.027–0.030	0.019	0.008–0.011	Meat and meat products (30%)
M 40–64 years	0.018–0.021	0.012	0.006–0.009	Meat and meat products (26%)
M 65+ years	0.017–0.019	0.012	0.005–0.007	Meat and meat products (23%)
F 12–19 years	0.023–0.026	0.017	0.006–0.009	Dairy products (22%)
F 20–39 years	0.017–0.019	0.011	0.007–0.008	Meat and meat products (24%)
F 40–64 years	0.026–0.028	0.021	0.005–0.007	Meat and meat products (21%)
F 65+ years	0.010–0.012	0.006	0.004–0.006	Meat and meat products (19%)

F, female; M, male

[a] Lower limits of the ranges assume a zero concentration when the mercury concentration for individual composites fell below the LOD, whereas the upper limits assume a concentration equal to the LOD for these composites.

[b] Contributions based on exposure estimates derived assuming a concentration equal to the LOD when the mercury concentration for individual composites fell below the LOD.

Source: Dabeka, McKenzie & Bradley (2003)

Table 13. Total diet total mercury exposures for the population of Santiago, Chile, using a TDS

	Total mercury exposure[a] (µg/kg bw per day)
Exposure from fish and shellfish	0.024
Exposure from foods other than fish and shellfish	0.035–0.055
Total exposure	0.059–0.079

[a] Estimates were derived by combining consumption estimates and mean total mercury concentrations reported in Muñoz et al. (2005) and assume a body weight of 65 kg. Lower limits of the ranges assume a zero concentration for foods where the mean mercury concentration was below the LOD, whereas the upper limits assume a concentration equal to the LOD for these foods.

Source: Muñoz et al. (2005)

to 0.079 µg/kg bw per day. Total mercury exposures from foods other than fish and shellfish ranged from 0.035 to 0.055 µg/kg bw per day (Table 13). Lower limits of the ranges assume a zero concentration for foods where the mean mercury concentration was below the LOD, whereas the upper limits assume a concentration equal to the LOD for these foods. Bread is the highest contributor to the total mercury exposure from food other than shellfish. Its contribution ranged from 27% when the LOD is used for foods with mean concentration below the LOD to 43% when a zero concentration is assumed for these foods.

(d) China

Total diet total mercury exposure for an average adult Chinese male was estimated to be 0.08 µg/kg bw per day in the 2007 TDS. Other estimates from Changchun city for adults 18–77 years old are comparable (0.10 µg/kg bw per day); however, estimates for a subpopulation of fishermen and their families were much higher (0.92 and 0.47 µg/kg bw per day for adult males and females, respectively, and 0.67 µg/kg bw per day for children). Total mercury exposures from fish and shellfish for the general population are generally low: 0.01 µg/kg bw per day (2007 TDS) and 0.08 µg/kg bw per day among secondary-school children in Hong Kong SAR. However, exposures from fish and shellfish were well above these levels for the subpopulation of fishermen and their families (Table 14).

(e) Czech Republic

Total diet total mercury exposure estimates for the general population of the Czech Republic were 0.008 µg/kg bw per day in 2000 and 0.009 µg/kg bw per day in 2001. No estimates were submitted for the contribution of the various foods to total exposure to total mercury (Table 15).

Table 14. Total diet total mercury exposures from the 2007 China TDS and published studies

Study	Population	Total dietary exposure (µg/kg bw per day)	Exposure from fish and shellfish (µg/kg bw per day)	Exposure from foods other than fish and shellfish (µg/kg bw per day)
2007 TDS[a]	Adult male (average)	0.08	0.01	0.07
	Adult male (97.5th percentile)	0.51	NA	NA
Changchun city[b]	Adults 18–77 years	0.10	0.01	0.09
Fishermen and families in Zhoushan Island[c]	Adult males	0.92	0.87	0.05
	Adult females	0.47	0.41	0.06
	Children	0.67	0.57	0.10
Hong Kong SAR[d] (2000)	Secondary-school children (consumers with average exposure)	NA	0.08	NA
	Secondary-school children (consumers with high exposure)	NA	0.25	NA

NA, not available
[a] China (2010).
[b] Li, Wang & Luo (2006).
[c] Cheng et al. (2009).
[d] Tang et al. (2009).

(f) France

Total diet total mercury mean exposure estimates for the average French 3- to 4-year-old child and adult as estimated by the TDS were 0.26 µg/kg bw per day and 0.16 µg/kg bw per day, respectively. The corresponding estimates of total mercury exposures from foods other than fish and shellfish were 0.24 µg/kg bw per day and 0.15 µg/kg bw per day (Table 16). These estimates were derived assuming ½ LOD for foods with non-detectable concentrations and ½ LOQ for foods with concentrations below the LOQ. Hence, exposure estimates for foods with non-detectable concentrations are artificially overinflated and cannot be used to estimate contributions of individual foods. Total mercury exposures from fish for frequent fish consumers were much higher (Table 17), ranging from 0.87 µg/kg bw per week (0.12 µg/kg bw per day) to 1.75 µg/kg bw per week (0.25 µg/kg bw per day).

Table 15. Total diet total mercury exposures from the 2000 and 2001 Czech TDS

Country	Total diet study	Population	Mean total mercury exposure (µg/kg bw per day)[a]
Czech Republic	Czech TDS 2000	General population (0–88 years)	0.008
Czech Republic	Czech TDS 2001	General population (0–88 years)	0.009

[a] From TDS studies submitted to the GEMS/Food database.

Table 16. Total diet total mercury exposures from the first French TDS (2000–2001)

	Exposure (µg/kg bw per day)			
	Child 3–4 years		Adult 15+ years	
	Mean	95th percentile	Mean	95th percentile
Fish and shellfish	0.02	NA	0.02	NA
Foods other than fish and shellfish	0.24	NA	0.15	NA
Total diet	0.26	0.41	0.16	0.25

NA, not applicable
Source: Leblanc et al. (2005)

(g) Japan

Total mercury exposure estimates for the Japanese population for the period ranging from 1977 to 2008 were submitted to the Committee. The data from the period 2000–2008 are presented in Table 18. Total dietary total mercury exposures were estimated to be 0.14 µg/kg bw per day in 2000 and increased to 0.17 µg/kg bw per day in 2008. Exposures from foods other than fish and shellfish are low (0.02 µg/kg bw per day in 2000 and 0.01 µg/kg bw per day in 2008). The meats and eggs group is the highest contributor to total mercury exposures from foods other than fish and shellfish.

(h) New Zealand

Total diet total mercury exposures for New Zealand range from 0.066 µg/kg bw per day for female adolescents (11–14 years) to 0.16 µg/kg bw per day for infants (6–12 months). The 2003–2004 New Zealand TDS report (NZFSA, 2005) indicates that fish products contributed 74% of the dietary mercury exposure for young males and 65% for toddlers (Table 19).

Table 17. Total mercury and methylmercury exposures from fish foods for frequent seafood consumers in France (the CALIPSO study)

Location	Age/sex group	Total mercury exposure (µg/kg bw per week)			Methylmercury exposure (µg/kg bw per week)		
		Mean	SD	95th	Mean	SD	95th
LeHavre[a]	M 18–64 years	0.87	0.55	1.94	0.88	0.57	1.93
	F 18–64 years	1.17	1.15	2.69	1.17	1.17	2.69
	M & F 65+ years	1.25	1.22	3.45	1.26	1.31	3.45
	F 18–44 years	1.04	0.96	2.28	1.07	1.02	2.27
	All	1.12	1.08		1.13	1.11	
Lorient[a]	M 18–64 years	1.4	0.21	3.11	1.44	0.34	3.1
	F 18–64 years	1.63	1.13	3.75	1.67	1.15	3.67
	M & F 65+ years	1.74	0.89	3.32	1.75	0.89	3.3
	F 18–44 years	1.5	1.15	2.79	1.54	1.16	2.8
	All	1.6	1.04		1.63	1.05	
La Rochelle[a]	M 18–64 years	1.39	1.29	3.01	1.42	1.27	3.08
	F 18–64 years	1.59	1.15	3.52	1.65	1.19	3.62
	M & F 65+ years	1.75	1.06	3.58	1.79	1.09	3.81
	F 18–44 years	1.39	0.92	3.03	1.43	0.96	3.09
	All	1.55	1.19		1.59	1.21	
Toulon[a]	M 18–64 years	1.54	1.31	4.73	1.5	1.29	4.09
	F 18–64 years	1.71	1.44	4.11	1.69	1.42	4.43
	M & F 65+ years	1.54	1.13	3.05	1.5	0.8	2.87
	F 18–44 years	1.61	1.27	3.87	1.6	1.29	4.26
	All	1.66	1.38		1.63	1.35	
All[a]	All	1.48	1.2		1.49	1.2	
All[b,c]	M 18–64 years				1.33	1.19	2.83
	F 18–64 years				1.33	1.19	3.86
	M & F 65+ years				1.58	0.98	3.48
	F 18–44 years				1.33	0.92	2.86
	All				1.51	1.17	3.52

F, female; M, male; SD, standard deviation

[a] Leblanc (2006).

[b] Sirot et al. (2008).

[c] Data from a subset of the CALIPSO survey participants.

Table 18. Total mercury exposure from the 2000–2008 Japan TDS[a]

Year	Total diet total mercury exposure (µg/kg bw per day)	Total mercury exposure from fish (µg/kg bw per day)	Total mercury exposure from foods other than fish and shellfish (µg/kg bw per day)
2000	0.14	0.12	0.02
2001	0.14	0.12	0.02
2002	0.18	0.15	0.02
2003	0.16	0.14	0.03
2004	0.17	0.15	0.02
2005	0.19	0.17	0.02
2006	0.15	0.14	0.01
2007	0.15	0.13	0.01
2008	0.17	0.16	0.01

Table 19. Total diet total mercury exposure from the 2003–2004 New Zealand TDS

Population	Mean mercury exposure (µg/kg bw per day)[a]	% from fish and shellfish
Adult males 25–99 years	0.1	NA
Adult males 19–24 years	0.11	74[b]
Males 11–14 years	0.088	NA
Adult females 25–99 years	0.09	NA
Females 11–14 years	0.066	NA
Children 5–6 years	0.1	NA
Children 1–3 years	0.15	65[b]
Infants 6–12 months	0.16	NA

NA, not available

[a] From 2003–2004 New Zealand TDS studies submitted to the GEMS/Food database.

[b] As reported in the 2003–2004 New Zealand TDS.

(i) Republic of Korea

The average total diet total mercury exposure estimate for the population of the Republic of Korea was 0.04 µg/kg bw per day, ranging from 0.03 µg/kg bw per day for adults 65+ years of age to 0.06 µg/kg bw per day for children 3–6 years of age. Total mercury exposure from foods other than fish and shellfish was estimated to be 0.01 µg/kg bw per day (Table 20). The food group contributing most to total

Table 20. Total diet total mercury exposures from the 2005 Republic of Korea TDS

Subpopulation	Total mercury exposure	
	µg/day	µg/kg bw per day
3–6 years	1.2	0.06
7–12 years	1.8	0.05
13–19 years	2.2	0.04
20–29 years	2.7	0.04
30–49 years	2.9	0.05
50–64 years	2.5	0.04
65+ years	1.5	0.03
Males	2.7	0.04
Females	2.1	0.04
All: Total diet	2.4	0.04
All age groups: Foods other than fish and shellfish	0.6	0.01
All age groups: Fish and shellfish	1.8	0.03

Source: Kwon et al. (2009)

mercury exposure from foods other than fish and shellfish is the "vegetables" food group, with a contribution of 36%. However, Kwon et al. (2009) do not clarify how samples with non-detectable total mercury concentrations were treated, so it is not possible to tell if this estimate reflects the true contribution of the "vegetable" food group to total mercury exposure or is an artefact of the high consumption of vegetables in the Republic of Korea and the use of LOD values for samples with non-detectable total mercury concentrations.

(j) Spain

Total diet total mercury exposures in a TDS study conducted in Catalonia, Spain, ranged from 0.26 µg/kg bw per day for adult females and seniors to 0.83 µg/kg bw per day for children. Total mercury exposures from foods other than fish and shellfish ranged from 0.14 to 0.58 µg/kg bw per day (Table 21). The food group contributing most to this exposure is the cereals group. It contributed from 46% (adult females) to 53% (adolescents) of this exposure.

(k) United Kingdom

Average total diet total mercury exposures in the 2006 United Kingdom TDS ranged from 0.02 to 0.04 µg/kg bw per day (free-living elderly) to 0.04–0.12 µg/kg bw per day (children 1.5–4.5 years) (Table 22). Foods other than fish and shellfish contributed 25–92% to the average total mercury exposure (Table 23). The food

Table 21. Total mercury exposure in Catalonia, Spain[a]

Subpopulation	Total diet total mercury exposure (µg/kg bw per day)	Total mercury exposure from fish and shellfish (µg/kg bw per day)	Total mercury exposure from foods other than fish and shellfish (µg/kg bw per day)
Children	0.83	0.25	0.58
Adolescents	0.37	0.12	0.25
Adult males	0.30	0.13	0.18
Adult females	0.26	0.12	0.14
Seniors	0.26	0.12	0.14

[a] Estimates were derived from the µg/day exposure estimates in Llobet et al. (2003) by assuming the following body weight values for children, adolescents, adult males, adult females and seniors: 20 kg, 50 kg, 70 kg, 65 kg and 65 kg, respectively.
Source: Adapted from Llobet et al. (2003)

Table 22. Total diet total mercury exposure in the 2006 United Kingdom TDS

Population	Total mercury exposure (µg/kg bw per day)	
	Mean	High level
Adults	0.01–0.05	0.10–0.13
Toddlers (1.5–4.5 years)	0.04–0.12	0.17–0.26
Young people (4–18 years)	0.03–0.08	0.11–0.18
Elderly (free living)	0.02–0.05	0.09–0.12
Elderly (institutionalized)	0.02–0.04	0.07–0.12
Vegetarians	0.02–0.05	0.12–0.15

Source: UKFSA (2009)

groups contributing most to this exposure are the "beverage" group (when non-detectable concentrations are set at the LOD) and the "other vegetable" group (when non-detectable concentrations are set at zero).

(l) United States of America

Total average total mercury exposures in the USA, based on total mercury concentrations collected between 1991 and 2005, ranged from 0.008 µg/kg bw per day (males 14–16 years) to 0.021 µg/kg bw per day (females 60–65 years) (Table 24). Fish and shellfish contributed more than 96% to total diet total mercury exposures for all subpopulations considered.

Table 23. Contributions to total diet total mercury exposure in the 2006 United Kingdom TDS[a]

Food source	Total mercury exposures (µg/kg bw per day)	
	Assuming LOD for ND concentrations	Assuming 0 for ND concentrations
Fish and shellfish	0.04	0.01
Foods other than fish and shellfish	0.01	<0.01
Total diet	0.05	0.01

ND, non-detected

[a] Estimates derived by combining TDS consumption estimates with concentrations reported in the 2006 United Kingdom TDS (UKFSA, 2009).

Table 24. Total mercury exposure in the 1991–2005 USA TDS[a]

Subpopulation	Total diet total mercury exposure (µg/kg bw per day)
M & F 6–11 months	0.005
M & F 2 years	0.019
M & F 6 years	0.013
M & F 10 years	0.019
F 14–16 years	0.013
M 14–16 years	0.008
F 25–30 years	0.013
M 25–30 years	0.010
F 40–45 years	0.012
M 40–45 years	0.011
F 60–65 years	0.021
M 60–65 years	0.016
F 70 years	0.014
M 70 years	0.017
Total USA	0.016

[a] Estimates derived by combining TDS Version 3 consumption estimates (http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/TotalDietStudy/ucm184232.htm) with concentrations reported in the 1991–2005 TDS (USFDA, 2007).

6.2.2 Methylmercury

Estimates of methylmercury exposures for foods other than fish and shellfish were available from only one study. All other available methylmercury exposure estimates were for fish and shellfish.

(a) Australia

Only fish and shellfish were analysed for methylmercury levels. Table 25 summarizes estimates of total mercury and methylmercury exposures from fish and shellfish. Estimated methylmercury exposures from fish and shellfish are much lower than the estimated total mercury exposures from these foods. It is not clear if there is an error in the concentrations reported for methylmercury in fish and shellfish.

Table 25. Total mercury and methylmercury exposures from fish and shellfish consumption in the 2000–2001 Australian TDS

Subpopulation	Total mercury exposures (ng/kg bw per day)[a]	Methylmercury exposures (ng/kg bw per day)[a]
Adult males (25–34 years)	12	0–0.122
Adult females (25–34 years)	13	0–0.106
Boys (12 years)	11	0–0.112
Girls (12 years)	11	0–0.077
Toddlers (2 years)	15	0–0.143
Infants (9 months)	21	0–0.163

[a] Derived by combining food consumption data and median mercury levels reported in the Food Standards Australia New Zealand 20th Australian TDS report, available at: http://www.foodstandards.gov.au/scienceandeducation/publications/20thaustraliantotaldietsurveyjanuary2003/

(b) China

Estimates of total mercury and methylmercury exposures were available for a subpopulation of fishermen in Zhoushan Island in China and their families (Table 26). Methylmercury constituted most of the mercury exposures for this subpopulation.

(c) France

Estimates of total mercury and methylmercury exposures from fish and shellfish for frequent seafood consumers in France (the CALIPSO study) are summarized in Table 17 above. However, only fish and shellfish were analysed in this study; hence, it is not possible to estimate the fraction of total mercury exposures from total diet that is attributable to methylmercury. However, the results confirm that almost all of the mercury exposure from fish is in the methylmercury form.

Table 26. Total mercury and methylmercury exposures for fishermen and their families in Zhoushan Island, China

Food	Exposure (μg/kg bw per day)		
	Adult males	Adult females	Children
Total mercury[a]			
Total diet	0.92	0.47	0.67
Fish and shellfish	0.87	0.41	0.57
Other foods	0.05	0.06	0.10
Methylmercury			
Total diet	0.88	0.44	0.63
Fish and shellfish	0.84	0.39	0.55
Other foods	0.04	0.05	0.08

[a] Data taken from Table 14.

(d) Spain

Exposures to methylmercury for the Spanish population were estimated by Sahuquillo et al. (2007) to be 46.2 μg/week (6.6 μg/day). However, only fish and shellfish were analysed in this study, and no estimates of exposure to total mercury were provided. Hence, it is not possible to estimate the fraction of total mercury exposure that is attributable to methylmercury or the fraction of methylmercury exposure that is attributable to fish and shellfish.

(e) United States of America

Table 27 summarizes modelled methylmercury exposure estimates for the population of the USA. However, the estimates are for methylmercury exposures from fish only, and no estimates of total mercury exposures were provided. Hence, it is not possible to estimate the fraction of total mercury exposures that is attributable to methylmercury or the fraction of methylmercury exposure that is attributable to fish and shellfish.

6.2.3 Inorganic mercury

IPCS (2003) estimated dietary exposure to inorganic mercury to be approximately 4.3 μg/day—that is, 0.067 μg/kg bw per day for a 64 kg adult.

It is possible to estimate the dietary exposure to inorganic mercury from studies that have provided both total mercury and methylmercury exposure estimates as the difference between total mercury and methylmercury exposures. For countries that have provided only total mercury exposures but have provided separate exposure estimates for fish and shellfish and foods other than fish and

Table 27. Estimated exposure to methylmercury from fish in the USA

Population percentile	Exposure to methylmercury from fish (µg/day)[a]	
	Women of childbearing age	Men aged 16–45 years
Average	1.4 (1.3–1.4)	1.8 (1.7–1.9)
10th percentile	0.0 (0.0–0.1)	0.0 (0.0–0.1)
25th percentile	0.2 (0.1–0.3)	0.3 (0.2–0.4)
Median (50th percentile)	0.7 (0.6–0.7)	0.9 (0.7–1.0)
75th percentile	1.6 (1.5–1.8)	2.1 (1.9–2.3)
90th percentile	3.4 (3.1–3.6)	4.3 (3.9–4.7)
95th percentile	4.9 (4.5–5.5)	6.4 (5.6–7.5)
99th percentile	10.3 (8.1–12.8)	13.4 (10.9–17.3)

[a] Numbers in parentheses are the 5th to 95th uncertainty percentiles.
Source: USFDA (2009)

shellfish, it may be possible to estimate inorganic mercury exposures by applying some default assumptions on the fraction of methylmercury in fish and shellfish and other foods. Based on results summarized above, methylmercury can constitute up to 70–100% of total mercury in fish and up to 50% of total mercury in shellfish. The fraction of total mercury consisting of methylmercury shows a wide variability in bivalve species (20–89%) and canned tuna (30–79%). These percentages can be applied to total mercury exposure estimates associated with these fish and shellfish groups, if available, thus allowing for the estimation of inorganic mercury exposures from fish and shellfish.

6.3 International estimates

Mercury occurrence data were submitted by France and Japan, but were deemed to be not sufficiently representative for use in deriving international estimates of dietary exposure in combination with food consumption data from the GEMS/Food consumption cluster diets. No international estimates of dietary exposure were prepared.

7. DOSE–RESPONSE ANALYSIS AND ESTIMATION OF CARCINOGENIC/ TOXIC RISK

7.1 Identification of key data for risk assessment

In the majority of species tested to date with inorganic mercury compounds, kidney effects (weight changes, histopathology) appear to be consistently observed at relatively similar doses. In the 6-month NTP (1993) study, groups of rats and mice (both sexes, 10 animals per dose group) were treated by gavage with six different

doses of mercury(II) chloride over a 16-fold range. There was no mortality observed, but kidney mercury concentrations and relative kidney weights did increase in a dose-dependent manner. Although minimal nephropathy was a common finding in all male rats, including controls, there was a dose-dependent increase in the incidence of mild nephropathy (defined as dilated tubules with hyaline casts, foci of tubular degeneration and thickened tubular basement membranes) beginning in the second lowest dose group. Relative kidney weight increases were observed in both sexes of rats, with a NOAEL estimated at 0.23 µg/kg bw per day, as mercury (lowest dose tested). While kidney effects were also observed in mice (more prevalent in males), they generally occurred at higher doses (NOAEL of 1.9 mg/kg bw per day, as mercury).

7.1.1 Pivotal data from biochemical and toxicological studies

Toxicological studies in experimental animals available prior to 2000 indicated that the most sensitive adverse effect of exposure to inorganic mercury (mercury(II) chloride) was autoimmune glomerulonephritis. The lowest NOAEL with respect to this end-point was 200 µg/kg bw per day, as mercury, based on short- or medium-term exposure in rats. In the last several years, since the evaluation by the World Health Organization (IPCS, 2003), there have been limited oral toxicological studies in experimental animals with inorganic mercury. Among them, one reproductive study (Khan et al., 2004) and one short-term study (Penna et al., 2009) indicate that at doses lower than the NOAEL of 200 µg/kg bw per day, adverse effects on reproductive performance and on testis can be induced, with LOAELs of 185 µg/kg bw per day, as mercury, in mice and 1.5 µg/kg bw per day, as mercury, in rats, respectively. Another short-term (14-day) study (Kim, Johnson & Sharma, 2003) showed that for the immune system in mice, the LOAEL was 300 µg/kg bw per day, as mercury, but the NOEL was 60 µg/kg bw per day, as mercury (respectively higher and lower than the 200 µg/kg bw per day NOAEL).

Since both the LOAEL of 1.5 µg/kg bw per day, as mercury, in rats and the NOEL of 60 µg/kg bw per day, as mercury, in mice are lower than the current lowest NOAEL of 200 µg/kg bw per day, as mercury, they may be considered relevant for updating the hazard characterizations of inorganic mercury. For the former LOAEL (1.5 µg/kg bw per day, as mercury), although based on morphological alterations in the testis, such as progressive degeneration with spermatogenic arrest at the spermatocyte stage, hypospermatogenesis in seminiferous epithelium and cytoplasmic vacuolation in Leydig cells, no clear dose-dependent response/effects are provided in the original report (Penna et al., 2009). Additional experiments that assessed reproductive performance with significantly higher doses of inorganic mercury (Atkinson et al., 2001; Rao & Sharma, 2001) do support testicular/fertility effects, but the effects seem to be transient, even with continued dosing. In addition, in the NTP (1993) subacute, subchronic and chronic assays, no testicular histopathological effects were reported in rats or mice at mercury(II) chloride doses up to 5 mg/kg bw per day.

In the case of the NOEL of 60 µg/kg bw per day, as mercury, in mice, the data presented appear to demonstrate a pattern of dose-dependent decreases in CD3+, CD4+ and CD8+ lymphocyte populations following 14 days of exposure in

mice, and the effects at the dose of 60 µg/kg bw per day, as mercury, were not statistically different from the controls (Kim, Johnson & Sharma, 2003). In comparison, developmental exposure to a considerably higher dose of mercury(II) chloride (2 mg/kg bw per day) in the same strain of mice for a longer duration produced no effects on thymus or spleen cellularity (Pilones, Tatum & Gavalchin, 2009). Considering the minimal decrease observed in the indicated lymphocyte populations, the toxicological significance of these findings would require additional investigation.

7.1.2 Pivotal data from human clinical/epidemiological studies

While toxicological effects in humans have been induced following exposure through various routes to inorganic mercury compounds, the available data from epidemiological investigations and studies that include biomarkers of exposure or effect were not considered suitable for assessing overall risk.

7.2 General modelling considerations

For the risk assessment of mercury(II) chloride, critical effects observed in the toxicological database included increased relative kidney weight in male and female rats (NTP, 1993). In general, dose–response modelling of toxicological data is used to determine a point of departure for further risk assessment. Dose–response data were used to derive the 95% lower confidence limit of the benchmark dose (BMDL) for the observed increases in relative kidney weights.

7.2.1 Selection of data

The NTP (1993) bioassay in the rat was considered to be the pivotal study for risk assessment because it employed low-dose exposures to mercury(II) chloride by the oral route. The most prominent dose–response effect of mercury(II) chloride in the 6-month NTP (1993) study was increased relative kidney weight in rats. Other end-points from this study were considered (i.e. terminal body weight, alkaline phosphatase, cholinesterase, nephropathy) for benchmark dose (BMD) modelling (data not shown); however, the BMDLs generated were greater than those estimated for increased relative kidney weight. In support of this data set, shorter-term exposure of weanling rats to higher doses of mercury(II) chloride via the diet produced similar effects.

7.2.2 Measure of exposure

Mercury(II) chloride was administered by gavage, 5 days/week, for 6 months to rats in the NTP (1993) bioassay.

7.2.3 Selection of mathematical model

(a) Modelling procedure for continuous data

BMD modelling was conducted using the USEPA's BMD software (BMDS version 2.1.1) with all available continuous models (i.e. exponential, Hill, linear,

polynomial, power). Benchmark responses (BMRs) of one standard deviation of the control mean or 10% extra risk were modelled for comparison purposes. An adequate fit was judged based on the goodness of fit *P*-value ($P > 0.1$), scaled residual closest to the BMR and visual inspection of the model fit. In addition to the three criteria for judging adequate model fit, whether the variance needed to be modelled and, if so, how it was modelled also determined final use of the model results. If a homogenous variance model was recommended based on statistics provided from the BMD model runs, the final BMD results would be estimated from a homogenous variance model. If the test for homogenous variance was negative ($P < 0.1$), the model was run again while applying the power model integrated into the BMDS to account for non-homogenous variance (known as non-homogenous model). If the non-homogenous variance model did not provide an adequate fit to the variance data, the data set would be considered unsuitable for BMD modelling. Models that passed the goodness of fit test ($P > 0.1$) were considered to be acceptable; from these models, the lowest BMDL was selected.

7.3 Potency estimates

7.3.1 BMD analyses for kidney weight

All available continuous models in the BMDS (version 2.1.1) were fit to the relative kidney weight data for male and female F344 rats exposed to mercury(II) chloride by gavage for 6 months in the NTP (1993) bioassay (Table 28). For comparison purposes, BMRs of one standard deviation and 10% of the control mean were used for the BMD modelling. As assessed by the chi-squared goodness-of-fit statistic, the Hill and some exponential models in the BMDS provided adequate fit to the data using a homogenous variance model (Table 29).

Table 28. Relative kidney weights for male and female rats gavaged with mercury(II) chloride for 6 months

Dose (mg/kg bw per day)	*n*	Relative (to body weight) kidney weights ± SE (g)	
		Males	Females
0	10	3.67 ± 0.07	3.80 ± 0.07
0.312	10	4.05 ± 0.06	4.09 ± 0.10
0.625	10	4.34 ± 0.06*	4.29 ± 0.05*
1.25	10	4.34 ± 0.12*	4.46 ± 0.09*
2.5	10	4.38 ± 0.08*	4.57 ± 0.11*
5.0	10	4.17 ± 0.09*	4.62 ± 0.11*

SE, standard error
* $P < 0.01$
Source: NTP (1993)

Table 29. Dose–response modelling of relative kidney weights in male and female F344 rats gavaged with mercury(II) chloride for 6 months[a]

Model	*P*-value	AIC	BMD_{1SD}	$BMDL_{1SD}$	BMD_{10}	$BMDL_{10}$
Males						
Exponential 4[b]	0.1189	93.481	0.136	0.072	0.220	0.112
Exponential 4[c]	0.123	265.555	0.119	0.063	0.221	0.115
Exponential 5[b]	0.127	93.2075	0.275	0.094	0.308	0.148
Exponential 5[c]	0.131	265.271	0.267	0.082	0.307	0.152
Hill[b,d]	0.248	95.2074	0.299	0.131	0.311	0.184
Females						
Exponential 4[b]	0.9926	87.6721	0.291	0.169	0.449	0.250
Exponential 4[c]	0.9898	264.2337	0.253	0.149	0.444	0.258
Exponential 5[b]	0.9926	87.6721	0.291	0.169	0.449	0.250
Hill[b]	0.9993	85.7644	0.291	0.125	0.430	0.193

AIC, Akaike's information criterion; BMD_{1SD}, benchmark dose for a one standard deviation response; BMD_{10}, benchmark dose for a 10% response; $BMDL_{1SD}$, lower limit on the benchmark dose for a one standard deviation response; $BMDL_{10}$, lower limit on the benchmark dose for a 10% response; SD, standard deviation

[a] BMD(L)s have not been corrected for dosing schedule.

[b] Assumes normal distribution.

[c] Assumes lognormal distribution.

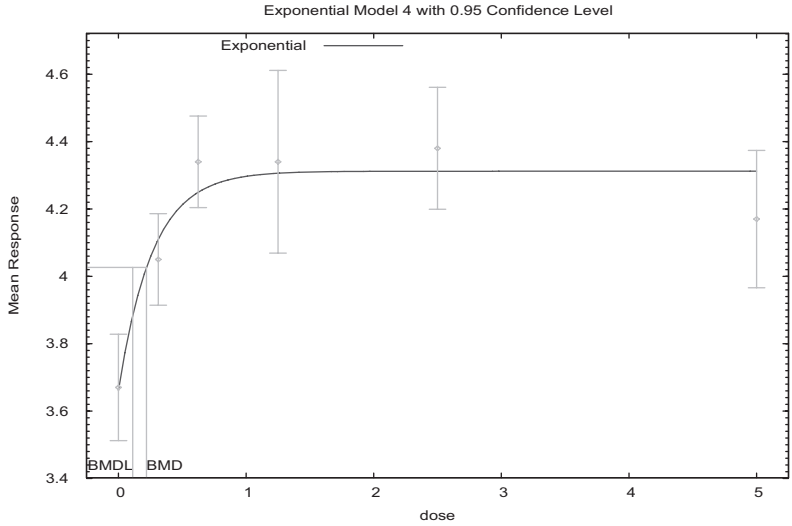
[d] Power parameter was unrestricted.

For both males and females, the BMDLs estimated by the acceptable models were similar; therefore, the more conservative lowest BMD may be selected. As kidney weight changes in male rats appear to be more sensitive than those in female rats, the lowest estimated BMD_{10} and $BMDL_{10}$ for reduced relative kidney weight are 0.220 and 0.112 mg/kg bw per day, respectively. Figure 1 shows the relative kidney weights of male rats fitted to the four-parameter exponential model.

7.3.2 Conclusions from dose–response analysis

In the dose–response analysis, statistical models were fitted to the experimental data that were considered relevant for further consideration. Those resulting in acceptable fits based on biological and statistical considerations were selected to derive the BMD_{10} and $BMDL_{10}$ values. This procedure results in a range of BMD_{10} and $BMDL_{10}$ values for each end-point considered (Table 30). The results summarized in Table 30 show that the $BMDL_{10}$s are moderately lower than the BMD_{10}s, indicating that the confidence intervals are fairly narrow.

Figure 1. Exponential four-parameter model of relative kidney weight data in male F344 rats from 6-month NTP (1993) study



Note: BMD(L)s have not been corrected for dosing schedule.

Table 30. Summary of the results of dose–response modelling of relative kidney weights in male rats administered mercury(II) chloride by gavage

End-point	Response	Range of BMD_{10}[a] (mg/kg bw per day)	Range of $BMDL_{10}$[a] (mg/kg bw per day)
Male relative kidney weight	10% extra risk	0.220–0.311	0.112–0.184

[a] Doses have not been adjusted for contribution of Hg^{2+} to mercury(II) chloride or for study dosing schedule.

The range of $BMDL_{10}$s calculated based on the reduction in relative kidney weight in male rats is 59.1–97.1 µg/kg bw per day (adjusted to account for 5 days/week dosing rather than 7 days/week dosing and the fact that Hg^{2+} represents approximately 73.9% of the administered dose of mercury(II) chloride). The more conservative lower end of this range of values is recommended for use in the evaluation.

8. COMMENTS

8.1 Absorption, distribution, metabolism and excretion

Following oral exposure, inorganic mercury salts show limited absorption, which is related to their water solubility. In human volunteers, the average

absorption of a tracer dose of inorganic mercury given as mercury(II) nitrate was 5–10%, whether delivered in a protein-bound matrix or as a solution.

Inorganic mercury compounds are not lipid soluble and do not readily cross the blood–brain barrier or placenta membranes. Ionic species of inorganic mercury readily bind to sulfhydryl groups of various thiol-containing compounds, such as GSH, cysteine and metallothionein. Kidneys exhibit the greatest concentration of mercury following exposure to inorganic mercury compounds. The main pathways of excretion of absorbed inorganic mercury are via the urine and, to a lesser extent, in the faeces. Owing to the poor absorption of orally administered inorganic mercury, the majority of the ingested dose in humans is excreted in the faeces. Inorganic mercury can also be excreted via the breast milk. The half-life for inorganic forms of mercury in humans has been estimated at 1–2 months.

8.2 Toxicological data

Haematological, hepatic and renal effects have been reported in rats or mice administered sublethal single oral doses of mercury(II) chloride. Renal effects usually observed with mercury(II) chloride at doses above 5 mg/kg bw per day include interstitial sclerosis, renal tubular damage and proximal tubular necrosis. Severe gastrointestinal damage, including inflammation and necrosis of the fore-stomach and necrosis of the glandular stomach, can also be induced with high doses of inorganic mercury, in particular for mercury(II) compounds, which are relatively more corrosive than mercury(I) compounds.

Longer-term exposure (subchronic to chronic) to inorganic mercury at doses above 1–5 mg/kg bw per day can induce a variety of effects related to general toxicity (decrease in body weight gain, changes in clinical and haematological parameters), as well as organ-specific effects (increased kidney and adrenal weights, testicular atrophy). Effects associated with relative kidney weight changes include marked thickening of glomerular and tubular basement membranes, degeneration and atrophy of the tubular epithelium and increased severity of nephropathy. Treatment of mice and rats by gavage with mercury(II) chloride at doses ranging from 1.25 to 20 mg/kg bw per day and from 0.312 to 5.0 mg/kg bw per day, respectively, for 6 months produced a variety of renal effects, which occurred with greater frequency and severity in male animals. Unlike organic mercury compounds, neurotoxicity is not usually observed, even at exposure levels that produce frank toxicological effects in other organs.

Reproductive effects induced by inorganic mercury include decreased fertility, reduced implantation efficiency and decreases in both live births and litter sizes. The observed effects seem to involve male-specific end-points (testicular atrophy, androgen decreases, spermatogenesis disruption) more than effects in females. However, inconsistencies have been noted in some experimental responses. A consistent observation in most reproduction studies includes increased relative kidney weights in the offspring.

Inorganic mercury compounds have produced some genotoxic effects in vitro and in vivo, with stronger evidence from in vitro experiments, including single-strand DNA breaks, sister chromatid exchanges and chromosomal aberrations.

However, the mechanisms appear to involve primarily induction of oxidative stress (reactive oxygen species) or disruption of microtubules rather than direct interaction with DNA, including adduct formation, which has not been demonstrated.

Chronic exposure of mice and rats to mercury(II) chloride at doses ranging from 2.5 to 10 mg/kg bw per day has produced some indications of carcinogenicity. The main findings included an increased incidence of forestomach hyperplasia, forestomach squamous cell papillomas and a marginal increase in thyroid follicular cell carcinomas in male rats. In mice, renal tubule tumours were seen only in high-dose males, but the incidence was not statistically significant compared with historical controls. It was concluded by NTP (1993) that there was some evidence of carcinogenic activity of mercury(II) chloride in male F344 rats, based on the increased incidences of squamous cell papillomas of the forestomach and the marginally increased incidence of thyroid follicular cell neoplasias, equivocal evidence in both female rats and male mice, and no evidence in female rats. However, NTP (1993) considered that the forestomach lesions in male rats may have limited relevance, as they did not progress to malignancy (direct tissue irritation effect). Also, as follicular cell carcinomas in rats usually result from increased incidences of hyperplasia and adenomas, it was further noted that the combined incidence of thyroid follicular cell neoplasms (adenomas and carcinomas) was not significantly increased. IARC (1993) considered that there is limited evidence in experimental animals for the carcinogenicity of mercury(II) chloride, based on results from the NTP (1993) bioassay.

8.3 Observations in humans

Human data on the adverse health effects of exposure to inorganic mercury, including renal effects, consist of case reports or case series that do not allow the identification of dose–response relationships. Therefore, they do not provide an adequate basis for deriving a health-based guidance value. They do, however, provide evidence that supports the use of adverse renal effects observed in experimental species as the basis for such a derivation. Nephrotic syndrome, including proliferative or membranous glomerulonephritis, has been associated with the topical use of mercury(II) ammonium chloride creams. Based on the limited number of studies of cancer and the absence of consistent findings, IARC (1993) concluded that there is inadequate evidence in humans for the carcinogenicity of mercury and mercury compounds. As a result, inorganic mercury compounds were not classifiable as to their carcinogenicity in humans (Group 3).

8.4 Analytical methods

Sample handling is generally critical only for water samples. The best materials for water sample storage and processing are polytetrafluoroethylene and fluorinated ethylene-propylene. Fresh samples are usually stored deep-frozen, lyophilized in darkness or sometimes sterilized. It has been reported that methylmercury may be decomposed in some food matrices with repeated freezing and unfreezing (particularly in bivalves). However, relatively little is known about the effect of storage on the stability of methylmercury in food samples.

Following acidic digestion of samples, CV-AAS or CV-AFS has been widely used for the determination of total mercury in several food matrices. An LOQ of about 30 µg/kg dry mass in foods may be obtained by CV-AAS. Further sensitivity enhancement may be obtained by CV-AFS. The main advantage of the cold vapour technique is the separation of the analyte from the potentially interfering sample matrix and its comparatively low cost. However, to avoid interference by CV-AFS, special precautions must be taken to completely remove vapours when nitric acid is used for digestion. With an LOQ of about 10 µg/kg dry mass and greater selectivity, ICP-MS is increasingly being used with an addition of gold chloride to mercury standard solutions to avoid the mercury memory effects. Although the instrumentation is expensive to purchase and to operate, the ability of ICP-MS to provide low LOQs, to provide a wide dynamic linear range and to measure many elements simultaneously can offset these cost factors.

Basically, all the speciation methodology is generally targeted on the separation and determination of methylmercury, and there has been no conclusive identification of other species of mercury.

Extraction of the mercury species from its matrix requires an aggressive treatment, such as acid digestion, distillation or alkaline extraction, with the option of applying ultrasonic or microwave energy to assist in the procedure. Extraction is one of the most critical steps, because two conflicting issues need to be addressed: obtaining high extraction efficiency and preventing losses. In alkaline media, methylmercury appears to be more stable than in acid media, the proteins being easily hydrolysed.

GC has been the most widely used technique for the separation of mercury species, whereas HPLC is increasingly being applied. The detection methods (LOD in parentheses) of CV-AAS (10 µg/kg), CV-AFS (1 µg/kg), MIP-AES or ICP-AES (5 µg/kg), MS (40 µg/kg) and ICP-MS (<3 µg/kg) all have sufficient sensitivity for food samples. The advantage of MS and ICP-MS is their multielement and multi-isotope capabilities that allow for more accurate and precise results by SID-MS, which can also check for species transformations and extraction recoveries. Once in solution, methylmercury may decompose when exposed to light, low pH and high storage temperatures. Other factors, such as the type of storage container, may also affect the stability.

Available certified reference materials and proficiency testing schemes or intercomparison exercises exist for both total mercury and methylmercury to demonstrate and maintain analytical quality assurance. However, there is a current need for fully validated, standardized methods for determination of methylmercury and inorganic mercury.

8.5 Sampling protocols

Some authorities have regulations with regards to specific sampling protocols for mercury and other contaminants. For example, the European Commission has regulated the number and size of incremental samples, size of the aggregate sample and precautions to be taken for control purposes.

8.6 Levels and patterns of contamination in food commodities

At its present meeting, the Committee reviewed data from eight countries on the occurrence of mercury in different food commodities analysed between 1997 and 2009. The total number of analytical results for total mercury was more than 106 740, with 93% coming from Europe (Finland, France, Spain), 5% from Asia (China, Japan), 1% from the Americas (Brazil, Canada) and 1% from Oceania (Australia), for water (85%), fish (6%), shellfish (2%) and other food groups (6%). The 2128 samples analysed for methylmercury were from fish (94%), shellfish (2%) and other products (4%). However, the Committee did not receive any occurrence data on inorganic mercury in foods or water.

Total mercury levels in 98% of 90 545 water samples analysed in France were below the LOQ of 0.02 µg/l, with a maximum of 4.3 µg/l.

Total mercury levels in foods other than fish products were generally low (range 0.0001–0.050 mg/kg), with about 80% of the 6183 samples containing levels below the LOQs. The highest levels were found in fungi. Mean methylmercury levels reported by China in non-fish samples ranged from 0.001 to 0.023 mg/kg, with a maximum concentration found in poultry. No other information on methylmercury in non-fish samples was received from other countries.

Total mercury levels in 1892 shellfish samples (80% above LOQ) ranged from 0.002 to 0.86 mg/kg. No shellfish species contained methylmercury at concentrations greater than 0.5 mg/kg (range 0.002–0.451 mg/kg), with the maximum concentration found in edible crab.

Total mercury levels in 6114 fish samples ranged from 0.001 to 11.4 mg/kg, with the maximum concentration found in marlin.

The proportion of total mercury contributed by methylmercury generally ranged between 30% and 100%, depending on species of fish, size, age and diet. Furthermore, in about 80% of these data, methylmercury accounted for more than 80% of total mercury. However, a few submitted data showed proportions of methylmercury of about 10% or less.

8.7 Food consumption and dietary exposure assessment

8.7.1 National estimates

Most of the available dietary exposure assessments for mercury were from national TDSs. These include the following TDSs: Australia (2000–2001), Canada (1998–2000), China (2007), Czech Republic (2000), France (2001–2002), Japan (2008), New Zealand (2003–2004), the Republic of Korea (2005), the United Kingdom (2006) and the USA (1991–2005). Published data from other studies focusing on special subpopulations were also available. These include TDSs conducted in Chile (Santiago) and Spain (Catalonia) and studies of fishermen and their household members in Zhoushan Island (China), residents of Changchun city in north-east China, secondary-school students in Hong Kong SAR, frequent seafood consumers in France (the CALIPSO study), exposures from fish and shellfish in Spain and modelled exposure estimates for fish consumers in the USA.

In general, most studies available allowed for the estimation of dietary exposure to total mercury from fish and shellfish as well as from other foods. Table 31 summarizes the estimates of mean dietary exposure to total mercury from the total diet, from fish and shellfish, and from other foods extracted from the studies listed above. Estimated mean dietary exposure to total mercury ranged from 0.07 to 5.81 µg/kg bw per week, while the estimated mean dietary exposure to total mercury from fish and shellfish ranged from 0.07 to 1.75 µg/kg bw per week. The estimated mean dietary exposure to total mercury from foods other than fish and shellfish ranged from 0 to 4.06 µg/kg bw per week. The upper limit of that range corresponds to a subpopulation of children. When only total population or subpopulations of adults were considered, the estimated mean dietary exposure to total mercury from foods other than fish and shellfish ranged from <0.01 to 1.01 µg/kg bw per week. The main contributors to this average dietary exposure were breads and cereals.

The studies did not provide 90th-percentile estimates of the dietary exposure to total mercury from foods other than fish and shellfish; hence, the 90th-percentile exposure estimates were derived by multiplying the mean exposure estimates by 2 (WHO, 1985). The resulting 90th-percentile exposure to total mercury from foods other than fish and shellfish was estimated to range from <0.02 to 2.03 µg/kg bw per week for the general population or adult subpopulations and from <0.02 to 8.12 µg/kg bw per week when children subpopulations are included.

The contribution of fish and shellfish to the total dietary exposure ranged from 40% to 100% when samples with non-detectable concentrations were assigned a zero concentration. Estimates of per cent contribution for foods other than fish and shellfish based on dietary exposure estimates derived from concentration data using the LOR or LOR/2 for non-detects are not reliable because they artificially inflate the contribution of these foods, particularly when the LOR is high. Only studies from which it was possible to separately estimate the contribution of fish and shellfish and other foods to total dietary exposure to mercury are presented in Table 31.

It was assumed that the predominant source of inorganic mercury in the diet is foods other than fish and shellfish.

8.7.2 International estimates

The available mercury occurrence data were deemed to be not sufficiently representative for use in deriving international estimates of dietary exposures in combination with food consumption from the GEMS/Food consumption cluster diets. No international estimates of dietary exposure were prepared.

8.8 Dose–response analysis and estimation of carcinogenic/toxic risk

Kidney effects are consistently observed in various experimental species (weight changes, proximal tubule damage and progressive nephropathy). Relative kidney weight increases observed in rats following exposure to mercury(II) chloride are also associated with a dose-dependent increase in renal mercury accumulation and with significant changes in the renal cortex, including increases in both proximal

Table 31. Contribution of fish and shellfish to total dietary exposure to mercury (national estimates)

Country	Average total dietary exposure to mercury (µg/kg bw per day)			% from fish and shellfish
	Total diet	Fish and shellfish	Other foods	
Estimates derived by assigning a zero value to samples with concentrations below the LOD or LOQ				
Australia TDS	0.01–0.02	0.01–0.02	0–0	100–100
Canada TDS (excluding infants)	0.01–0.03	0.01–0.02	<0.01–0.02	51–80
Chile (Santiago)	0.06	0.02	0.03	41
China (Zhoushan Island)	0.47–0.92	0.41–0.87	0.05–0.10	87–95
Japan TDS	0.17	0.16	0.01	92
Republic of Korea TDS	0.04	0.03	0.01	76
United Kingdom TDS[a]	0.02–0.04	—	—	—
USA TDS	0.01–0.02	0.01–0.02	<0.01–<0.01	96–100
Estimates derived by assigning a non-zero value (LOD or LOQ) to samples with concentrations below the LOD or LOQ				
Australia TDS	0.08–0.26	0.01–0.02	0.06–0.24	7–17
Canada TDS (excluding infants)	0.01–0.04	<0.01–0.04	0.01–0.03	40–74
Chile (Santiago)	0.08	0.02	0.06	31
United Kingdom TDS[b]	0.04–0.12	—	—	25
Estimates derived by assigning a non-zero value (LOD/2 or LOQ/2) to samples with concentrations below the LOD or LOQ				
China TDS	0.08	0.01	0.07	13
China (Changchun city)	0.10	0.01	0.09	13
France TDS[c]	0.16–0.26	0.02–0.02	0.15–0.24	9–10
New Zealand TDS	0.11–0.16	0.08–0.10	0.03–0.06	65–74
Spain (Catalonia)	0.28 –0.83	0.12–0.25	0.14–0.58	30–46
Total range (µg/kg bw per day)[d]	**0.01–0.83**	**0.01–0.25**	**0–0.58**	
Total range (µg/kg bw per week)[d]	**0.07–5.81**	**0.07–1.75**	**0–4.06**	

[a] High exposures (97.5th percentile) ranged from 0.07 to 0.17 µg/kg bw per day.
[b] High exposures (97.5th percentile) ranged from 0.12 to 0.26 µg/kg bw per day.
[c] High exposures (95th percentile) ranged from 0.25 to 0.41 µg/kg bw per day.
[d] Excluding the study of fishermen and their families in Zhoushan Island, China.

tubule and glomerular volumes. The Committee therefore considered it appropriate to model kidney weight changes, which generally occurred at doses similar to or lower than other renal effects. Data on relative kidney weight increases were taken from the NTP (1993) study, in which rats and mice of both sexes were exposed by gavage to mercury(II) chloride, 5 days/week for 6 months. Other end-points from this study were considered (i.e. terminal body weight, serum alkaline phosphatase and cholinesterase, incidence of mild nephropathy) for BMD modelling (data not shown); however, the BMDLs generated were greater than those estimated for increased relative kidney weight. Models that passed the goodness-of-fit test ($P > 0.10$) were considered to be acceptable, and the lowest BMDL was selected from these models (Table 32). The 6-month exposure was deemed sufficient to establish a health-based guidance value, because the half-life of mercury(II) chloride in rats is estimated at less than 30 days, steady-state renal mercury concentrations were reached by 4–6 months and exposures in the same dose range for longer durations produced early mortality. The Committee further considered that a 10% change for increased relative kidney weight was appropriate as a BMR to establish a health-based guidance value. This decision was based on the following: the kidney weight data were modelled based on reported mean values, animals in the lowest experimental dose (0.325 mg/kg bw per day) already exhibited a 10% increase in mean relative kidney weight and the severity of nephropathy was significantly increased only at doses greater than or equal to 1.25 mg/kg bw per day.

Table 32. Dose–response modelling[a] for a 10% increase in relative kidney weight for male and female F344 rats gavaged with mercury(II) chloride for 6 months[b]

Sex	BMD_{10} (mg/kg bw per day as mercury(II) chloride)	$BMDL_{10}$ (mg/kg bw per day as mercury (II) chloride)
Males	0.22–0.31	0.11–0.18
Females	0.430–0.45	0.19–0.25

[a] BMDS version 2.1.1.

[b] BMD(L)s have not been adjusted for the dosing schedule of 5 days/week.

9. EVALUATION

The Committee noted that there was a lack of quantitative data on methylmercury in non-fish products and on inorganic mercury in general.

The Committee assumed that the predominant form of mercury in foods other than fish and shellfish is inorganic mercury. While data on speciation of inorganic mercury in foods are limited, the Committee agreed that the toxicological database for mercury(II) chloride was relevant for assessing the health risk of foodborne inorganic mercury. The NTP (1993) bioassay provided limited evidence for carcinogenicity; however, direct reaction of mercury(II) chloride with DNA has

not been demonstrated. Therefore, setting a health-based guidance value was considered appropriate.

The lowest $BMDL_{10}$ for relative kidney weight increase in male rats was calculated to be 0.11 mg/kg bw per day as mercury(II) chloride. This corresponds to 0.06 mg/kg bw per day as mercury, adjusted from a 5 days/week dosing schedule to an average daily dose and for the per cent contribution of inorganic mercury to mercury(II) chloride dose. After application of a 100-fold uncertainty factor, the Committee established a PTWI for inorganic mercury of 4 µg/kg bw (rounded to one significant number).

The previous PTWI of 5 µg/kg bw for total mercury, established at the sixteenth meeting, was withdrawn.

In the absence of evidence to the contrary, the new PTWI for inorganic mercury was considered applicable to dietary exposure to total mercury from foods other than fish and shellfish. The upper limits of estimates of average dietary exposure to total mercury from foods other than fish and shellfish for adults (1 µg/kg bw per week) and for children (4 µg/kg bw per week) were at or below the PTWI.

9.1 Recommendations

There is a need for:

- validated analytical methods for both inorganic mercury and methylmercury applicable in several food matrices;
- more information on the inorganic mercury and methylmercury content of foods as consumed that mainly contribute to overall dietary exposure.

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