CHAPTER-6 HEALTH EFFECTS AND RISK ASSESSMENT

6.1 INTRODUCTION

The present chapter reviews health effects and risk assessment from elemental mercury vapour and methylmercury at the exposure conditions commonly experienced by the general population. It was largely based on previous reviews by the World Health Organization (WHO 1990, 1991), the International Agency for Research on Cancer (IARC 1993), the US Environmental Protection Agency (EPA 1997, 2001), and the US National Research Council (NCR 2000).

Mercury exists in various physical and chemical forms. The most important from a toxicological point of view are the metallic form, also called the elemental form, and methylmercury. In this review only the two most important forms of mercury in relation to human exposure, i.e. elemental mercury vapour and methylmercury, will be dealt with in detail.

6.2 TOXICOKINETICS DATA

6.2.1 Elemental Mercury

Vapour of elemental mercury is rapidly absorbed via the lungs. In humans 75-85% of an inhaled dose is absorbed (Hursh et al., 1985, WHO 1991). Elemental mercury in liquid or vapour form is not well absorbed from the gastro-intestinal tract (possibly less than 0.01%) (Bornmann et al. 1970).

Elemental mercury is lipid soluble and its diffusion into the lungs and dissolution in blood lipids is rapid (Berlin 1986, WHO 1991). It is distributed throughout the body, and readily crosses the placental barrier and the blood-brain barrier (Vimy et al. 1990, Drasch et al. 1994).

Elemental mercury is oxidised to mercuric mercury in the erythrocytes and other tissue by a peroxidatic reaction with catalase- complex I; the formation of H_20_2 is rate limiting for the process. Glutathione peroxidase inhibits the reaction by competing with catalase for hydrogen peroxide. (Halbach and Clarkson 1978). Mercuric mercury only slowly crosses the blood brain barrier and the placental barrier. Ethanol inhibits catalase and therefore inhibits the oxidation of elemental mercury as has been demonstrated in both experimental animals and in humans (Hursh et al. 1980). Likewise, alcohol intake increases the amount of vapour lost by expiration (Sallsten et al. 2000), and consequently reduces the retention. On the other hand an extended retention-time in the blood will allow more mercury to pass the blood-brain barrier and placenta. Yoshida et al. (1997) found that pre-treatment of pregnant guinea pigs with ethanol increased the fetal exposure to elemental mercury compared to non ethanol pre-treated animals.

After uptake of elemental Hg, mercury is distributed to all kinds of tissue, but accumulates more in certain organs, including in particular the kidneys.

Elemental mercury is, after oxidation, excreted through the feces, urine, and to some extent also by exhalation and sweat. At occupational exposure, about 50 % is believed to be excreted

by urine and feces respectively. The rate of urinary excretion is probably dose-dependent (Barregard et al., 1996), and a considerable species difference has been observed.

Mercury concentrations in the blood decrease rapidly with an initial half-life of approximately two to four days, and a slower phase of a couple of weeks (Cherian et al. 1978, Barregard et al. 1992, Sallsten et al. 1993). In urine the half-life is 40-90 days (Roels et al. 1991, Barregard et al. 1992, Sallsten et al. 1994). These results therefore reflect the existence of compartments with elimination half-lives of about 2 months, presumably in the kidney.

In workers chronically exposed to mercury vapour, a good correlation has been observed between the current intensity of exposure and blood and urine mercury concentrations at the end of a work shift (Roels et al. 1987). Occupational exposure limits in Europe are given in the table below. For biomonitoring, blood and urine Hg levels may be used, see Chapter 5.

Table 6.1 - Occupational exposure limits (mg m^{-3})

	Countries	**Exposure Limit**
In Europe	Austria, Denmark, Finland, Switzerland	0.05
	Belgium	0.1
	Hungary	0.02
	Poland	0.025
	Sweden	0.030
Outside Europe	U.S.A, Japan	0.025

6.2.2 Methylmercury

As a lipophilic substance methylmercury easily passes biological membranes. About 95% of the methylmercury in fish ingested by humans was found to be absorbed from the gastrointestinal tract (Åberg et al., 1969, WHO 1990). Although the oral exposure route is most important for humans, it should be noted that methylmercury is also readily absorbed through the skin and the lungs.

Once absorbed into the blood stream, methylmercury enters the red blood cells bound to haemoglobin. A smaller fraction is found in the plasma. The cell to plasma ratio is 10-20 (Åberg et al., 1969, Kershaw et al., 1980, WHO 1990).

Less than 1% of the blood mercury is diffusible, and this fraction is believed to play an important role for the organ distribution of methylmercury. Experimental data suggest that the reduced glutathione (GHS) molecule is important for binding of mercury, as a reduction of available GSH decreases the concentration of mercury in the brain and kidneys (Richardson and Murphy 1975), and intracellular GHS seems to play a significant role in the metabolism of mercury.

The essential element selenium also is associated with the binding and transport of mercury, see below. However, it is still uncertain to which degree normal dietary intake of selenium affects the mercury toxicokinetics in humans.

The demethylation of methylmercury takes place in many organs including the brain (WHO 1990). The latter has been shown in humans (Tsubaki and Takahashi 1986, Pedersen et al. 1999), in monkeys (Lind et al. 1988), and in dogs (Hansen et al. 1989, Hansen and Danscher 1995). In skeletal muscles little or no demethylation activity has been observed in dogs (Hansen et al. 1989, Hansen and Danscher 1995), marine mammals and birds (Dietz et al. 1990), fish (AMAP 1998). Therefore fish muscle is a major source for dietary methylmercury exposure.

Demethylation is thought to occur via reactive oxygen intermediates (Suda et al. 1991). Macrophages may be important for demethylation of organic mercury (Suda and Tagahashi 1986). The liver, spleen, and lymph nodes are the most important organs for transforming methylmercury (Suda and Tagahashi 1990, Hansen et al. 1989, Hansen and Danscher 1995). The hypothetical, mechanistic model is presented in Figure 6.1

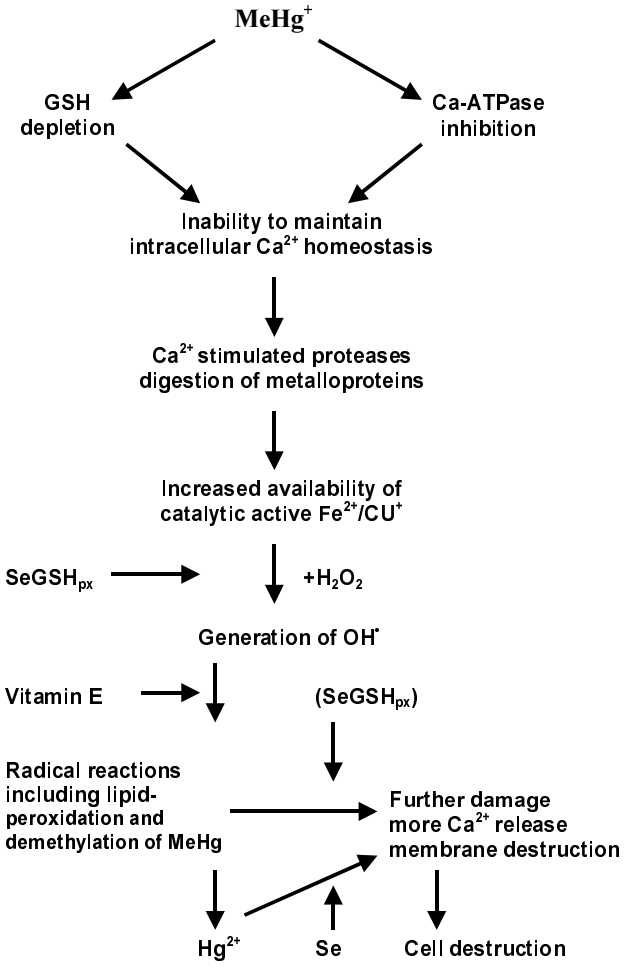

Figure 6.1 - Theoretical model for biochemical reactions of methylmercury (Hansen and Danscher 1997)

Methylmercury is mainly excreted via faeces. In humans, approximately 90% of the absorbed dose of methylmercury is excreted in the feces as mercuric mercury. Excretion via the urine is minor but slowly increases with time; at 100 days after dosing, urinary excretion of mercury accounted for 20% of the daily amount excreted. Methylmercury is also excreted in breast milk. The ratio of mercury in breast milk to mercury in whole blood was approximately 1:20 in women exposed to methylmercury via contaminated grain in Iraq between 1971 and 1972 (Bakir et al. 1973), and about 1:6 in Swedish women having a normal diet (Oskarsson et al. 1996).

Methylmercury bound to cysteine is excreted into the bile. Subsequent gastrointestinal absorption leads to an enterohepatic circulation. However, some of the methylmercury may get demethylated by gut bacteria and is then poorly absorbed and therefore eliminated via the faeces. Breast-fed infants may not harbour the demethylating bacteria in the gut, and faecal elimination may therefore be less efficient.

Biological half-lives published in four studies of controlled exposures to methylmercury range from 32 to 70 days (Miettinen et al. 1971, Sherlock et al. 1984, Smith et al. 1994, Kershaw et al. 1980) with a pooled mean of approximate 49 days (SD 7.5) (Clewell et al. 1999). Half-lives obtained from patients during the Iraqi grain poisoning incident averaged 72 days (Al-Sharistani and Shihab 1974).

For biomonitoring, scalp hair or blood is often used to assess methylmercury exposure, see Chapter 5.

6.3 BIOLOGICAL EFFECTS OF MERCURY IN DIFFERENT PHYSICAL AND CHEMICAL FORMS

6.3.1 Elemental Mercury

Signs and symptoms observed in mercury vapour poisoning differ depending on the level and duration of exposure. Most studies have been performed in occupationally exposed subjects, but there are also some data from accidents in the general population, and on low level exposure from dental amalgams. The latter subject has been widely discussed and reviewed (US Public Health Service 1993).

In the following, information is given first on neoplastic effects and then on non-neoplastic effects from various organs/systems.

6.3.1.1 Neoplastic Effects

Data on the carcinogenicity of metallic mercury and its inorganic compounds mainly come from studies on cancer occurrence in occupational populations, including dentists, nuclear weapon manufacturers, chloralkali workers and miners. Previous data are summarized in reviews (IARC 1993, Boffetta 1993).

Cohorts of 3454 male and 1125 female dentists and 4662 dental nurses identified from the Swedish census in 1960 were followed for cancer development in the period 1961-79 by linkage with cancer register data (Ahlbom *et al.*, 1986). The overall standardized incidence ratio (SIR) was 2.1 (95% CI, 1.3-3.4) for glioblastoma (astrocytoma III-IV) in comparison with national incidence rates. The SIR of glioma (astrocytoma I-II) was 1.8 (95% CI, 0.5-4.7), that of meningioma was 1.3 (95% CI, 0.5-2.8). For comparison, physicians and female nurses were also studied; no indication was found of an excess of glioblastomas. In another analysis of intracranial gliomas from the same population, the increased incidence among dentists was confirmed, and an increased incidence was reported also among other medical professions (McLaughlin *et al.*, 1987).

A cohort of 2133 white men from Oak Ridge, TN, USA, who were exposed to metallic mercury was studied with regard to mortality in comparison with national rates for white men (Cragle *et al.*, 1984). Exposure to mercury occurred in the context of lithium production in a nuclear weapons plant. The cohorts were followed-up from 1953 until 1979. There was an excess of lung cancer (SMR, 1.34; 95% CI 0.97-1.81). A similar excess, however, was present in a group of workers from the same plant but unexposed to mercury. The SMR was 1.22 for cancers of the brain and central nervous system (95% CI 0.33-3.12) and 1.65 for kidney cancer (95% CI 0.45-4.23). It was, however, recently reported that these workers were exposed to high levels of radiation through routine chest X-ray, also with high-dose techniques, some of them as often as once a month (Cardarelli 2001).

Mortality and cancer incidence were reported for a group of 1190 male Swedish chloralkali workers in whom mercury had been measured in the blood or urine for at least one year between 1946 and 1984 (Barregard *et al.*, 1990). Their mortality and cancer incidence were compared with those of the general male population. The mean level of mercury excreted in the urine had been about 200 μg/L in the 1950s, 150 μg/L in the 1960s and less than 50 μg/L in the 1980s. Lung cancer was the only type of tumour in clear excess (SIR with a latency of 10 years 2.0; 95% CI, 1.0-3.8). There were slight excesses of some other cancers with a latency of 10 years or more, namely brain tumours (SIR, 2.7; 95% CI, 0.5-7.7), kidney cancer (1.6; 0.3-4.7). One case of mesothelioma was observed, suggesting exposure to asbestos.

A cohort study included 674 male Norwegian chloralkali workers exposed to inorganic mercury for more than one year prior to 1980, who had a mean cumulative urinary concentration of 740 μg/L (Ellingsen *et al.*, 1993). During the follow-up period, there were 19 incident cases of lung cancer, with 11.5 expected (SIR, 1.66; 95% CI, 1.00-2.59) on the basis of national rates. There was no correlation with cumulative mercury dose, employment or latency; a somewhat increased frequency of smoking and exposure to asbestos (one mesothelioma was found) were considered to explain the excess of lung cancer. Three kidney cancers and two brain tumours were observed *versus* 3.2 and 2.5 expected, respectively.

A multicentric study included 6784 male and 265 female workers of four mercury mines and mills in Spain, Slovenia, Italy and the Ukraine (Boffetta *et al.*, 1998). Workers were employed between the beginning of the century and 1990; the follow-up period lasted from the 1950s to the 1990s. An increase was observed in mortality from lung cancer (SMR 1.19, 95% CI 1.03-1.38) and liver cancer (SMR 1.64, 95% CI 1.18-2.22). There was no relationship between lung cancer and employment or estimated mercury exposure. For liver cancer, there was a trend with estimated cumulative exposure but not with duration of employment, however the

excess was not present in a parallel analysis of cancer incidence among workers from Slovenia. No increase was observed for other types of cancer, including brain and kidney tumours.

A cohort study included 326 men and 820 women compensated since 1974 for mercury intoxication in an area of Italy in which felt hat manufacture was the main source of occupational exposure to mercury, who were followed up for mortality during 1950-1992 (Merler *et al.*, 1994). Lung cancer mortality was increased among women (SMR 2.10, 95% CI 1.05-3.76), but not among men (SMR 1.16, 95% CI 0.68-1.86). Heavy exposure to mercury but also to arsenic and other chemicals occurred in the Italian hat-making industry.

Lung cancer is the only neoplasm, which seems to be consistently increased among various groups of workers exposed to metallic and inorganic mercury. The main difficulty in the interpretation of the data on lung cancer is the possible co-exposure to other lung carcinogens, in particular arsenic (in the fur industry), radon and silica (among miners). An additional limitation is the almost universal lack of data on tobacco smoking. The fact that no increase was found in a large group of European mercury miners not exposed to quartz (Boffetta et al. 1998) argues against the hypothesis that mercury vapour should cause lung cancer. There is no suggestion of a consistent increase of any other neoplasm, including brain and kidney cancers, in these populations.

The International Agency for Research on Cancer (IARC, 1993) evaluated metallic mercury and inorganic mercury compounds and found them not classifiable (group 3) with respect to carcinogenicity in humans.

6.3.1.2 Neurological Effects

As reviewed by the US EPA (1997), the reports from accidental exposures to high concentrations of mercury vapors (Aronow et al. 1990; Fagala and Wigg 1992; Taueg et al. 1992), as well as studies of populations chronically exposed to potentially high concentrations (Ehrenberg et al. 1991; Roels et al. 1982; Sexton et al. 1978) have shown effects on a wide variety of cognitive, sensory, personality and motor functions. In general, symptoms have been observed to subside after removal from exposure. However, persistent effects (tremor, cognitive deficits) have been observed in occupationally exposed subjects 10-30 years after cessation of exposure (Albers et al. 1988, Kishi et al. 1993, Mathiesen et al. 1999, Letz et al. 2000). Studies of workers exposed to elemental mercury vapor have reported a clear increase in symptoms from the CNS at exposure levels greater than 0.1 mg/m^3 (Smith et al. 1970) and clear symptoms of mercury poisoning at levels resulting in urinary mercury greater than 300 µg in a 24-hour urine sample (Bidstrup et al. 1951). Several studies, however, have shown evidence of neurotoxicity at approximately 2- to 4-fold lower concentrations. Self-reported memory disturbances, sleep disorders, anger, fatigue, and/or hand tremors were increased in workers chronically exposed to an estimated 0.025 mg/m^3 (urinary and blood Hg levels of about 25 µg/g and 10 µg/L) (Langworth et al. 1992), but not in a recent study with somewhat lower exposure levels, U-Hg 10-15 µg/g (Ellingsen et al. 2001). Also, objective measures of cognitive and/or motor function in exposed populations have shown significant differences from unexposed controls (Ehrenberg et al. 1991; Liang et al. 1993; Roels et al. 1982). In the study by Langworth et al. (1992), there were, however, no objective findings in neuropsychological tests or tremor recordings. This was also mainly the case in the study by

Ellingsen et al. (2001), although there were possibly some exposure-related effects. Tremor was reported at long-term exposure to relatively low concentrations of mercury vapour (Fawer et al., 1983; Chapman et al., 1990), and mild tremor may constitute an early adverse effect (Biernat et al., 1999, Netterstrøm et al., 1996). Several studies failed, however, to show an increase of tremor at low level exposure (Roels et al. 1989, Langworth et al. 1992, Ellingsen et al. 2001).

6.3.1.3 Renal Effects

The kidney is, together with the CNS, a critical organ for exposure to mercury vapour. The kidney accumulates Hg to a larger extent than most other tissue with concentrations in occupationally unexposed groups typically of 0.1 – 0.3 µg/g (Drasch 1996, Barregard 1999, Hac 2000, Falnoga 2000). The critical kidney mercury concentration is not known, but levels in subjects with ongoing occupationally exposure may be in the order of 25 µg/g (Kazantzis 1962, Borjesson 1995, Barregard 1999). Some of the mercury in the kidney is, however, bound to selenium, decreasing the toxicity, see section on interactions below.

High exposure may cause (immune-complex mediated) glomerulonephritis with proteinuria and nephritic syndrome. This has been shown at occupational exposure (Kazantzis 1962, Tubbs et al., 1982), as well as after use of mercury-containing ointment or skin-lightening creams (Becker et al. 1962, Kibukamusoke et al. 1974), but the reported cases are relatively few. Therefore, a specific genetic susceptibility is probably needed for a frank nephritis to develop, for a review see Enestrom and Hultman (1995).

More common at high exposure is proteinuria, glomerular (albumin) as well as tubular (low molecular weight proteins). Albuminuria is, however, generally not seen at exposure levels resulting in urinary mercury below 100 µg/g creatinine (Buchet et al., 1980, Roels et al., 1982, 1985, Langworth et al. 1992, Barregard et al. 1997, Ellingsen et al. 2000).

Effects on the renal tubules, as demonstrated by increased excretion of low molecular proteins like the proximal tubular enzyme NAG (N-acetyl-beta-D-glucosaminidase) have been shown at low-level exposure, and may constitute the earliest biological effect. This effect was previously shown at occupational exposure with urinary mercury of about 35 µg/g creatinine, equivalent to long term exposure to air levels of 25-30 µg/m^3 (Barregard et al., 1988, Langworth et al., 1992, Cardenas et al., 1993). In a recent report by Ellingsen et al. (2000), such an effect was, however, shown also in workers with urinary mercury of about 10 µg/g creatinine. Ongoing research (Wastensson G, personal communication, 2001) confirmed this in Swedish chloralkali workers, at levels above about 5 µg/g creatinine. This is only slightly higher than that found in the general population. The reversibility of these findings and the possible long term implications of tubular proteinuria are still open for discussion (Jarup et al. 1998).

Among male European mercury miners an increased mortality was observed from nephritis and nephrosis (SMR 1.55, 95 % CI 1.13-2.06) (Boffetta et al., 2001), whereas this was not shown among chloralkali workers (Barregard et al., 1990, Ellingsen et al., 1993).

6.3.1.4 Respiratory Effects

Respiratory toxicity in humans following exposure to elemental mercury vapors has been characterized by pulmonary edema and congestion, coughing, interstitial pneumonitis, and respiratory failure (Bluhm et al. 1992a, Taueg et al. 1992, WHO 1991). Barregard et al. (1990) and Ellingsen et al. (1993) found no associations between mortality from respiratory disease and mercury exposure among workers exposed to mercury in the chloralkali industry, although the power of the studies were low. Merler et al. (1994) found no excess mortality of respiratory disease in men (SMR, 0.67; 95% CI, 0.35 – 1.14) exposed to mercury in the fur hat industry. This was also true for mercury miners, except for pneumoconiosis (Boffetta et al., 2001).

6.3.1.5 Cardiovascular Effects

Signs of cardiovascular toxicity in humans after acute exposure to elemental mercury include tachycardia, elevated blood pressure and heart palpitations (Bluhm et al. 1992a; Snodgrass et al. 1981; Soni et al. 1992, Wõssmann et al. 1999). Intermediate-duration exposure to elemental mercury vapors produced similar effects (i.e., tachycardia and elevated blood pressure) (Fagala and Wigg 1992; Foulds et al. 1987). Piikivi (1989) demonstrated a positive correlation between heart palpitations and urinary mercury concentrations in workers from a chloralkali plant. It is unclear from the available scientific literature, however, whether the effects on cardiovascular function are due to direct cardiac toxicity or to indirect toxicity (e.g., due to effects on neural control of cardiac function) of elemental mercury. Barregard et al. (1990) showed that Swedish chloralkali workers had increased mortality of ischemic heart disease and cerebrovascular disease. There were, however, no such findings in Norwegian chloralkali workers (Ellingsen 1993).

Among European mercury miners, increased mortality from hypertension (SR 1.46, 95 % CI 1.08-1.93) and from heart diseases other than ischemic (SMR 1.36, 95 % CI 1.20-1.53), and increased with time since first employment and with estimated cumulative mercury exposure. Findings were not conbsistent among countries. No increase was shown for ischemic heart disease or cerebrovascular diseases (Boffetta et al., 2001).

6.3.1.6 Gastrointestinal and Hepatic Effects

The most common sign of frank mercury poisoning is stomatitis, which is usually reported following acute, high concentration exposure to elemental mercury vapors (Bluhm et al. 1992a; Snodgrass et al. 1981). Other commonly reported gastrointestinal effects include nausea, vomiting, diarrhea and abdominal cramps (Bluhm et al. 1992a; Lilis et al. 1985; Sexton et al. 1978, Snodgrass et al. 1981, Vroom and Greer 1972). No increased mortality from the digestive system was observed in European mercury miners (Boffetta et al. 2001)

6.3.1.7 Effects on the Thyroid

The thyroid may accumulate mercury at exposure to Hg^0 (Kosta et al. 1975, WHO 1991, Falnoga 2000). It has recently been shown (Barregard et al. 1994, Ellingsen et al. 2000b) that moderate occupational exposure affects the deiodinase responsible for the deiodination of thyroxine (T4) to triiodothyronine (T3), a seleno-enzyme. This tends to increase T4 and reverse T3 levels, and increase the T4/T3 ratio. The effects were seen at current urinary Hg levels of 15-30 µg/g creatinine (Barregard 1994, Ellingsen 2000b), thus at levels as low as the those where the first minor effects on the CNS and the kidneys were reported.

6.3.1.8 Immunological Effects

The ability of mercury to induce immune-mediated disease has been thoroughly investigated in mice and rats experimentally exposed to inorganic mercury compounds, in most studies divalent mercury, but also mercury vapour. The type of response depends on the strains, some of them being susceptible to autoimmune disease and some being resistant. It is therefore assumed that the genotype is probably important also for the potential immunological effects in humans, for a review see Enestrom and Hultman (1995).

Some studies in subjects occupationally exposed to moderate levels of Hg^0 reported increase of antilaminin autoantibodies (Lauwerys 1983), alterations of certain T-lymphocyte populations, or increased immunoglobulin E (IgE) levels (Queiroz and Dantas 1997a,b, Dantas and Queiroz 1997, Moszczynski et al. 1995). One study showed increased autoantibodies to myeloperoxidase (Ellingsen et al. 2000a). In several studies no immunological effects were shown (Bernard 1987, Langworth 1992, 1993, Ellingsen 1994, Barregard 1997), and in particular anti-DNA or anti-nucleolar antibodies have not been reported except in one study (Cardenas et al. 1993). Cross-sectional studies of unselected populations may have low power to detect such effects if only a limited part of the populations is sensitive.

6.3.1.9 Dermal Effects

Exposure to elemental mercury vapors for acute or intermediate duration may elicit a response known as acrodynia or "pink disease", which is characterized by peeling palms of hands and soles of feet, excessive perspiration, itching, rash, joint pain and weakness, elevated blood pressure and tachycardia (Fagala and Wigg 1992, Karpathios et al. 1991, Schwartz et al. 1992). Rash and stomatitis have been reported after high inhalation exposures (Bluhm et al. 1992, Barregard 1996).

6.3.1.10 Developmental Effects

A study of the pregnancies of Polish dental professionals showed a high frequency of malformations of a nonspecified nature (Sikorski et al. 1987). In contrast, a study of Swedish dental professionals found no increases in malformations, abortions, or stillbirths (Ericson and Kallen 1989). An increase in low birth weight infants was noted in the offspring of female

dental nurses (Ericson and Kallen 1989); however, in this same study similar effects were not observed for either dentists or dental technicians, and socioeconomic factors may have contributed to the effects observed.

6.3.1.11 Reproductive Effects

In occupational exposure studies, paternal exposure to metallic Hg does not appear to cause infertility or malformations (Alcser et al. 1989, Lauwerys et al. 1985). However, a study of pregnancy outcomes among the wives of 152 Hg-exposed men revealed an increased incidence of spontaneous abortions (Cordier et al. 1991). Preconception paternal urinary Hg concentrations above 50 µg/L were associated with a doubling of the spontaneous abortion risk. Elghancy et al. (1997) compared the pregnancy outcomes of 46 Hg- exposed workers to those of 19 women who worked in nonproduction areas of the same factory. Women exposed to inorganic Hg had a higher rate of congenital anomalies. Concentrations were up 0.6 mg/m^3. No significant differences in stillbirths or miscarriage rates were noted between the two groups of women. Rowland et al. (1994) found that the probability of conception among female dental hygienists who prepared more than 30 amalgams per week and had at least five poor hygiene practices when handling Hg was only 63% of that among unexposed controls. Women with lower exposures, however, were more fertile than unexposed controls. A large study conducted in Norway compared reproductive success rates among 558 female dental surgeons with those of 450 high-school teachers (Dahl et al. 1999). They concluded that exposure to Hg, benzene, and chloroform was not associated with decreased fertility except for a possible Hg effect on the last pregnancy of multiparous dental surgeons.

6.3.1.12 Genotoxicity

Two occupational studies (Anwar and Gabal 1991, Popescu et al. 1979) reported on workers inhaling inorganic mercury; the data were inconclusive regarding the clastogenic activity of inorganic mercury. Workers involved in the manufacture of mercury fulminate ($Hg[OCN]_2$) had a significant increase in the incidence of chromosomal aberrations and micronuclei in peripheral lymphocytes when compared to unexposed controls (Anwar and Gabal 1991). There was no correlation between urinary mercury levels or duration of exposure to the increased frequency of effects; the study authors concluded that mercury may not have been the clastogen in the manufacturing process. In a study by Popescu et al. (1979), 18 workers exposed to a mixture of mercuric chloride, methylmercuric chloride and ethylmercuric chloride had significant increases in the frequency of acentric fragments. Barregard et al. (1991) demonstrated a correlation between cumulative mercury exposure and induction of micronuclei among a group of chloralkali workers, suggesting a possible genotoxic effect.

6.3.2 Organic Mercury Compounds

Intoxications with alkoxialkyl or aryl compounds are similar to intoxications with inorganic mercury compounds, because these organomercurials are relatively unstable and are broken down to form mercuric mercury. Alkyl mercury compounds, such as methylmercury are more stable and result in entirely different adverse effects. As originally described by Hunter et al.

(1940), the poisoned adult patient develops paresthasia in the fingers, the tongue and the face, particularly around the mouth. Later on, disturbances occur in the motor functions, resulting in ataxia and dysphasia. The visual field is decreased, and in severe cases the result may be total blindness, and impaired hearing may progress to complete deafness. Such cases were seen in Minamata, where fish was severely contaminated by effluents from a local chemical factory (Harada 1995).

In the following information is given on first on neoplastic effects and then on non-neoplastic effects from various organs/systems.

6.3.2.1 Neoplastic Effects

Direct SMRs for biliary tract cancer in the Japanese prefectures in 1975 were correlated with an environmental pollution index related to use of agricultural chemical products for the years 1962-66 (Yamamoto et al. 1986). In both men and women, only weak, non-significant correlations were found for exposure to mercuric compounds (such as phenylmercury acetate, used as a fungicide in Japan until 1971) converted to the dose of inorganic mercury.

The mortality pattern was studied in the population of a small area of the city of Minamata, Kumamoto Prefecture, Japan, which consisted mainly of fishermen and their families (Tamashiro et al. 1986) and where 70% of the 1612 confirmed cases (including 527 deaths) of Minamata disease in the Prefecture through 1983 were known to have occurred (see below for a description of Minamata disease). SMRs were computed for different causes of death in 1970-81 by using age-specific rates for the entire city for 1972-78. The SMR for cancer of the oesophagus was 2.05 (95% CI, 0.67-4.78), that of liver cancer was 2.07 (95% CI, 1.16-3.42) and that of lung cancer was 1.52 (95% CI, 0.79-2.65). An elevated SMR was also seen for chronic liver disease and cirrhosis (2.16; 95% CI, 1.41-3.17; based on 26 cases). There was some evidence that alcohol consumption in the area was above the Japanese average, which might have contributed to the increased mortality from oesophageal and liver cancer.

Methylmercury chloride caused renal tumours in several studies in mice but not in rats, and the IARC judged that there is sufficient evidence for carcinogenicity of methylmercury chloride in experimental animals (IARC 1993). The overall evaluation for methylmercury compounds was that they are possibly carcinogenic to humans (group 2B).

6.3.2.2 Neurological and Developmental Effects

The original epidemiological report of methylmercury poisoning involved 628 human cases that occurred in Minamata, Japan, between 1953 and 1960. The overall prevalence rate for the Minamata region for neurologic and mental disorders was 59%. Among this group 78 deaths occurred, and hair concentrations of mercury ranged from 50–700 ppm. The most common clinical signs observed in adults were paresthesia, ataxia, sensory disturbances, tremors, impairment of hearing and difficulty in walking. Examination of the brains of severely affected patients that died revealed marked atrophy of the brain (55% normal volume and weight) with cystic cavities and spongy foci. Microscopically, entire regions were devoid of neurons, granular cells in the cerebellum, Golgi cells and Purkinje cells. Extensive

investigations of congenital Minamata disease were undertaken, and 20 cases that occurred over a 4-year period were documented. In all instances the congenital cases showed a higher incidence of symptoms than did the cases wherein exposure occurred as an adult. Severe disturbances of nervous function were described, and the affected offspring were very late in reaching developmental milestones. Hair concentrations of mercury in affected infants ranged from 10 to 100 ppm (Harada 1995, Harada 1997, Tsubaki and Takahashi 1986, WHO 1990).

In 1971, an unknown number of people in Iraq were exposed to methylmercury-treated seed grain that was used in home-baked bread. Toxicity was observed in many adults and children who had consumed this bread over a three-month period, but the population that showed greatest sensitivity were offspring of pregnant women who ate contaminated bread during gestation. The predominant symptom noted in adults was paresthesia, and it usually occurred after a latent period of from 16 to 38 days. In adults symptoms were dose-dependent, and among the more severely affected individuals ataxia, blurred vision, slurred speech and hearing difficulties were observed. Signs noted in the infants exposed during fetal development included cerebral palsy, altered muscle tone and deep tendon reflexes, as well as delayed developmental milestones. Some information indicated that male offspring were more sensitive than females. The mothers experienced paresthesia and other sensory disturbances but at higher doses than those associated with their children exposed *in utero* (Bakir 1973, WHO 1990).

Thus, several neurological signs and symptoms are among the cardinal features of high-dose exposures to MeHg in adults. As no pathognomonic test is available to confirm the diagnosis of Minamata disease, cases were identified on the basis of a characteristic combination of symptoms (Harada 1997; Uchino et al. 1995). These included peripheral neurophathy, dysarthria, tremor, cerebellar ataxia, gait disturbance, visual-field constriction and disturbed ocular movements, hearing loss, disturbance of equilibrium, and subjective symptoms such as headache, muscle and joint pain, forgetfulness, and fatigue. The earliest effects due to methylmercury in adults, such as paresthesias, seem to appear at hair Hg concentrations above 50 µg/g or blood mercury concentrations above 200 µg/l (WHO 1990).

Later studies of patients with Minamata disease reported increased pain thresholds in the body and distal extremities (Yoshida et al. 1992). Lebel et al. (1998) found that abnormal performance on the Branches Alternate Movement Task (BAMT) was significantly associated with all measures of Hg exposure, in adults from an Amazonian village, and abnormal visual fields were associated with mean and peak hair Hg concentrations. The authors stress that the dose-related decrements in visual and motor funtions were associated with hair Hg concentrations below 50 ppm, a range in which clinical signs of Hg intoxication are not apparent.

The developing organism is much more susceptible to the toxic effects of methylmercury than the mature. Congenital methylmercury poisoning may result in a cerebral palsy syndrome, even though the mother remains healthy or suffers only minor symptoms due to the exposure (Davis et al. 1994).

Two large studies have been performed recently in order to assess the impact on the fetal brain from more moderate exposure to MeHg in pregnant women. The studies were performed in the Faroe Islands, and in the Seychelles Islands, comparing biomarkers of the

MeHg exposure of the mother and foetus with neuropsychological and other endpoints at pre-school age.

The Faroe Island population was exposed to methylmercury mainly from pilot whale meat with relatively high concentration of methylmercury, around 2 µg/g (EPA 2001). The study of about 900 Faroe children showed that prenatal exposure to methylmercury resulted in neuropsychological deficits at 7 years of age (Grandjean et al., 1997). The brain functions most vulnerable seems to be attention, memory, and language, while motor speed, visuospatial function, and executive function showed less robust decrements at increased mercury exposures. The mercury concentration in cord blood appeared to the best risk indicator for the adverse effects, which were apparently not due to a large number of covariates examined. Special concern was expressed with respect to the impact of PCB, which was present in the diet (in whale blubber) of these Faroe mothers. The results were roughly unchanged, however, when PCB levels were taken into account. Developmental delays were significantly associated with maternal hair mercury concentrations below 10 µg/g. Benchmark calculations based on the Faroe Islands data suggested that the lower 95 % confidence limit for a doubling of a 5% abnormality response occurred at maternal hair Hg levels around 10 µg/g and at cord blood levels corresponding to this hair level (Budtz-Jørgensen et al., 2000). However some models yielded much lower benchmark doses. Each doubling of the prenatal methylmercury exposure level was associated with a developmental delay of 1-2 months. Thus, on an individual basis the effects may seem innocuous, but they may have severe implications on a population basis.

Another prospective study was performed in the Seychelles islands, where the MeHg exposure was of similar extent. The fish consumption of pregnant women in the Seychelles is high, typically 10-15 meals per week (Shamlaye 1995), while the mercury concentrations in the ocean fish consumed is relatively low, with a mean of 0.2-0.3 µg/g (Cernichiari 1995). No effects on developmental tests up to 5.5 years of age were found to be associated with MeHg exposure, as measured by hair-Hg in the pregnant mothers (Davidson et al. 1998, Crump et al. 2000, Myers 2000, Axtell 2000, Palumbo 2000). The main longitudinal study was started in 1989-1990 and comprised about 700 mother-child pairs. Maternal hair (mean about 7 µg/g) and child hair, but not cord-blood levels were used as markers of MeHg exposure in this study. The benchmark calculations were similar to those used in the Faroe Island study. A reanalysis using raw scores rather than age standardized scores showed similar results. If anything, the results rather suggested a beneficial effect of mercury from the ocean fish consumption (Davidson 2001).

In addition there is a study from the New Zealand, suggesting an effect on the mental development of children at the age of 4 and 6-7 years. In a high-exposure group the average maternal hair-Hg was about 9 µg/g, and controls groups were selected with lower exposure levels. In total, about 200 children were examined at 6-7 years of age and a negative association was found between maternal hair-Hg and neuropsychological development of the children. This study was originally published as reports from the Swedish EPA (Kjellstrom 1986, Kjellstrom 1989), and was therefore less taken into account, but later the bench-mark analysis of the original data was published in a peer-reviewed journal (Crump 1998, EPA 2001).

Some cross-sectional studies using neuropsychological testing of older children in different settings, e.g. in the Amazonas and on the Madeira island also found significant associations with mercury exposure (for a review, see EPA 2001).

The studies mentioned, especially those in the Faroe Islands and the Seychelles are very important for the risk assessment of MeHg in humans and have been extensively evaluated, see section 6.7.

6.3.2.3 Renal Effects

Renal toxicity has rarely been reported following human exposure to organic forms of Hg. The only evidence of a renal effect following ingestion of Hg-contaminated fish comes from a death-certificate review conducted by Tamashiro et al. (1986). They evaluated causes of death among residents of a small area of Minamata City that had the highest prevalence of MD using age-specific rates for the entire city as a standard. Between 1970 and 1981, the number of deaths attributed to nephritic diseases was higher than expected among women who resided in that region (SMR, 2.77; 95% CI, 1.02 – 6.02) but was within the expected range (SMR, 0.80; 95% CI, 0.17 – 2.36) among men who resided in this region.

6.3.2.4 Cardiovascular Effects

Jalili and Abbasi's (1961) described EKG abnormalities in severely poisoned patients hospitalized during the Iraqi grain poisoning epidemic, and similar findings were reported in four family members who consumed ethylmercury-contaminated pork (Cinca et al. 1979). Salonen et al. (1995) compared dietary intake of fish and Hg concentrations in hair and urine with the prevalence of acute myocardial infarction (AMI) and death from coronary heart disease or cardiovascular disease in a cohort of 1,833 Finnish men. Dietary Hg intake ranged from 1.1 to 95.3 µg per day (mean 7.6 µg per day). Over a 7-year observation period, men in the highest tertile (at or more than 2 ppm) of hair Hg content had a 2.0-fold higher risk (1.2 – 3.1) of AMI than men in the two lowest tertiles. A later follow-up (Rissanen 2000) showed a protective effect of omega-3 fatty acids with respect to acute coronary disease, which was, however less evident in those with high hair Hg. The authors concluded that a high mercury content in fish could attenuate the protective effect of these fatty acids. A recent study by Sørensen et al. (1999) showed an association between prenatal exposure to MeHg and cardiovascular function at age 7 in the children from the Faroe Isands. Diastolic and systolic blood pressures increased by 13.9 and 14.6 mmHg, respectively, as cord-blood Hg concentrations rose from 1 to 10 µg/L. In boys, heart-rate variability, a marker of cardiac autonomic control, decreased by 47% as cord-blood Hg concentrations increased from 1 to 10 µg/L.

6.3.2.5 Genotoxicity

Skervfing et al. (1974) found limited support for an association between chromosomal abberations and mercury in red blood cells in subjects consuming large amounts of contaminated fresh-water fish. Wulf et al. (1986) reported an increased prevalence of sister chromatid exchange in humans who ate Hg-contaminated seal meat. However, information on

smoking status and exposure to other heavy metals was not provided for those individuals, making interpretation of the study difficult. More recently, Franchi et al. (1994) reported a correlation between the prevalence of micronuclei in peripheral lymphocytes and blood Hg concentrations in a population of fishermen who had eaten Hg-contaminated seafood.

6.3.3 Interactions

Nutritional status and dietary interactions can affect the outcome of mercury studies, either by influencing the toxicity of mercury or by having effects on the endpoints measures. Protective effects of nutrients like selenium, vitamin E and omega-3 fatty acids might attenuate potentially harmful effects of mercury. At the other extreme, mal-nourishment could affect study results either by directly reducing the sensitivity of an end point tested or by exacerbating the effects of mercury and thereby increasing the sensitivity to mercury toxicity. Nutritional factors such as iron or folate deficiencies that disrupt neuronal development might increase the impact of mercury. Conversely adequate dietary iron and folate levels might reduce the impact of mercury. Pathways through which diet and nutrition might affect the results of mercury toxicity studies include 1) the potential for attenuating a mercury effect 2) exacerbating a mercury effect or 3) as confounders by causing toxicity due to other food-components or contaminants.

Selenium

The essential micro-nutrient selenium is the best described food component that might protect against toxic effects from mercury. Numerous animal studies have documented a protective role of selenium against both inorganic and organic mercury (for reviews: Goyer 1997, Chapman and Chan 2000), however no protective effects have been confirmed in humans (NRC 2000). A few studies have addressed the human aspect. In workers occupationally exposed to elemental mercury Bulat et al. (1998) found significantly lower concentrations of the tow antioxidative defence enzymes glutathione peroxidase (GPX) and superoxide dismutase (SOD) in mercury exposed workers compared to non exposed. This was, however, not the case in a previous study by Barregard et al. (1990) in chloralkali workers. In such workers with low exposure to mercury vapour Ellingsen et al. (2000) found the highest activity in urine of N-acetyl-beta-D-glucosaminidase (U-NAG), a marker of effects on the renal tubules, in exposed workers with the lowest blood selenium concentrations.

Drasch et al. (1996) determined total mercury and selenium in kidney cortex samples of 195 deceased, non-occupationally exposed individuals. The molar ratio Se/Hg was high, up to 300 in cases with relatively low mercury concentrations, but decreased with increasing mercury to unity at mercury concentrations of 700-1000 ng/g, and remained constant at higher mercury concentrations. This means that mercury exposure decreases the reserve of free biologically active selenium. This is in accordance with observations by Falnoga et al. (2000) on autopsy samples of retired Idrija mercury mineworkers and non-exposed controls. In exposed individual a Se/Hg ratio of 1 in organs (thyroid, pituitary, kidney cortex, nucleus dentatus) was observed when the concentration of mercury was >1000 ng/g.

No observations on the Se/Hg ratio in humans with a documented intake of methylmercury have been reported, but will probably parallel what has been found in marine mammals. Dietz et al. (2000) found that selenium was present in molar surplus to mercury in molluscs, crustaceans, fish and seabirds. 1:1 molar ration was found in tissues of marine mammals with high mercury concentrations (above approx. 2000 n/g). This was most clearly demonstrated for liver and kidney. In liver samples from pilot whales Caurant et al. (1996) showed a molar ratio between selenium and mercury indicating the formation of an Hg-Se complex after demethylation of methylmercury. This is by the authors suggested as the major mechanism of detoxification.

Even if selenium protection of humans still is regarded controversial (WHO 1990), the experimental evidence in connection with existing data on molar ratios found in human tissues indicates that there is probably a protective effect. The amount of biological available selenium in the diet should be addressed in epidemiological studies of mercury exposure.

The mechanism by which mercury and selenium react has been studied by Sasakura and Suzuki (1998) who suggests that the interaction occurs through the general mechanism, i.e. mercury form the unit complex (Hg-Se)n, and then the complex binds to selenoprotein P to form the ternary complex (Hg-Se)nm-selenoprotein P in the blood stream.

In a study of Latvian fish consumers Hagmar et al. (1998) showed correlations between fish intake, plasma-levels of selenium, selenoprotein P, glutathione peroxidase, and organic mercury in erythrocytes.

Vitamin E

Kling et al. (1987) found that vitamin E was equal or superior to all synthetic antioxidants tested in alleviating the toxicity of organic mercury poisoning. The cause of observed antioxidant protection during organic mercury exposure is not known but the protection may result form the ability to scavenge free radicals generated by induction of *in-vivo* peroxidation by the mercury compound. The relevance of this to human exposure is unclear.

Omega-3 Fatty Acids

Chalon et al. (1998) demonstrated that fish oil affects monoaminergic neurotransmission and behavior in rats. Omega-3 fatty acids might enhance neurotoxicological function and their deficiency might contribute to lower test results, which would confound MeHg toxicological studies in human populations. Individuals consuming less fish might perform more poorly. Individuals on a diet high in fish might demonstrate the competing effects of enhanced function from these fatty acids and reduced function because of the presence of MeHg in the same food source. A case-control study in Greece concluded that low fish intake is associated with an increased risk of cerebral palsy (Petridou et al. 1998). Populations eating diets rich in fish might have enhanced neural development that could mask adverse effects on development caused by MeHg. However, there is no evidence to date that supplementation of omega-3 fatty acid to the diet of a well-nourished term infant further enhances neurological development or attenuates the toxic effects of Hg.

Alcohol

Ethanol has been shown to potentiate MeHg toxicity in mice and rats (Turner et al. 1981, Chapman and Chan 2000, Rumbeiha et al. 1992, Turner et al. 1981, McNeil et al. 1988). Ethanol administered to male rats in conjunction with daily injections of MeHg chloride has resulted in a dose-dependent increase in tissue concentrations of both total Hg and MeHg in the brain and kidneys and in the morbidity and mortality of the these animals. For effects of alcohol on the oxidation of Hg^0 to Hg^{2+}, please see section 6.2.1.

Garlic

Many compounds (or their metabolites) in garlic could act as metal chelating or complexing agents and increase methylmercury excretion. Such chemicals can be converted to thiols or include thiols, glutathione, vitamine C, and thiol amino acids, (Block 1985). Garlic also contains selenium (0.72-1.52 μg of selenium per gram of garlic), which might influence Hg toxicokinetics.

Other Dietary Factors

There are strong indications that wheat bran, but neither cellulose nor pectin, when consumed concurrently with MeHg administration, might reduce the Hg concentration in the brain. In a study of male BALB/c mice, a dose-response relationship between brain Hg concentrations and the percentage of wheat bran was seen across 0%, 5%, 15%, and 30% wheat bran in the diet. The highest dose of wheat bran decreased the half-time of Hg elimination by 43%, and decreased the brain Hg concentrations by 24%. Corresponding reductions were seen in the Hg concentrations in the blood of the bran-fed animals. Reductions of that magnitude have been associated with a lower incidence and severity of symptoms of neurotoxicity in rats. The effect has been attributed partially to binding of the Hg to bran, reducing its absorption from the gut and decreasing intestinal transit time. Using evidence of an increase in mercuric Hg in the large intestines of the bran-fed mice, it has also been hypothesized that wheat bran increased the rate of demethylation of organic Hg in the gut (Rowland et al. 1986). However, the possible mechanisms of these interactions have not yet been elucidated. For review se Chapman and Chan (2000).

6.4 CRITERIA FOR SELECTING CRITICAL POPULATION GROUPS FOR RISK ASSESSMENT

The purpose of risk assessment is to protect the subjects most vulnerable to adverse effects of the pollutant in question. This assessment should not take into regard specific risks of increased exposure, e.g., through occupational processes or dietary habits, but should be based solely on the degree of susceptibility to the toxicity at particular levels of exposure.

For inorganic mercury, none of the toxicokinetic evidence suggests any variations in absorption or retention of mercury vapour or inorganic mercury compounds related to age or

sex. Current information does not allow any judgment whether children or the fetus are any more vulnerable than is the adult human being. Risk assessment must there be carried out based upon the evidence available, although much of the information originates from occupational exposures of adult men. Appropriate consideration must be given to the concern that such working populations may exhibit a greater resistence to toxic effects than the general population. It should be noted, however, that immunological effects owing to exposure to inorganic mercury, e.g. glomerulonephritis, may affect preferentially humans with a certain genotype.

Organic mercury compounds are known to be particularly toxic during development, and they pass freely over the placental barrier. Children and the fetus therefore constitute the critical population group. Observations of severe congenital methylmercury poisoning cases revealed that the mother suffered limited clinical symptoms of the exposure, while permanent damage occurred to the fetal nervous system. Risk assessment should therefore appropriately focus on exposures during pregnancy.

6.5. RISK ASSESSMENT

The risk assessment done by the U.S. environmental Protection Agency (U.S. EPA) follows the paradigm established by the National Academy of Sciences (National Research Council. Risk Assessment in the federal Government: Managing the Process. Washington: National Academy Press 1983). This entails a series of interconnected steps including

Hazard identification uses available data on biological end points related to a material to determine if that material is likely to pose a hazard to human health. These data are also used to define the type of potential hazard.

Dose-response assessment uses data from human and animal studies to estimate the amount of material that is expected to produce a given effect in humans. In this step it is generally necessary to apply mathematical models to the data to calculate a quantitative risk estimate usable for low dose exposure.

Exposure assessment seeks to determine the extent to which a population is exposed to the material. Data limitations on the populations of interest often necessitate the use of models to provide relevant estimates of exposure.

Risk characterisation is the last step of the risk assessment process. This step evaluates assessments of human health and ecological effects, identifies human subpopulations or ecological species potentially at risk, and delineates areas of uncertainty, limitations, and assumptions made in the risk assessment.

6.5.1 Background

This evaluation is properly based on national and international risk assessments carried out for this priority pollutant. The U.S. Environmental Protection Agency prepared a detailed

Mercury Report to Congress (1997), which focussed on methylmercury. An extensive review was prepared by the U.S. Agency for Toxic Substances and Disease Registry (1999), which concluded in proposed exposure limits for mercury vapour, inorganic mercury, and methylmercury. Because of new information on the developmental effects of methylmercury, the U.S. National Institutes of Health convened an international group of experts, including several from the EU, to discuss the evidence in November, 1998. The report of the five working groups is available on the NIH server. This report was then followed by a comprehensive review carried out by an expert group under the auspices of the National Academy of Sciences in Washington (NRC 2000). The monograph was published in 2000 and then led to a revision of the U.S. EPA risk assessment for methylmercury, as documented in the 2001 report (EPA 2001).

6.5.2 Hazard Identification

Mercury vapour easily penetrates the blood-brain barrier and is a well documented neurotoxicant. In addition there are effects on the kidney and thyroid. The evidence on possible carcinogenicity is limited. A critical effect on which risk assessment should be based is therefore the neurotoxic effects, e.g. the induction of tremor. The effects on the renal tubule should also be considered, even if this effect is probably less serious. The effect may well be reversible, but if the the exposure to the general population is chronic, the effect is still relevant. For the thyroid, two studies suggested effects on the deiodination of thyroid hormones, but the impact on the thyroid is still less well described than that on the nervous system and the kidneys.

Methylmercury is a well documented neurotoxicant which may in particular cause adverse effects on the developing brain. This compound passes both the placental barrier and the blood-brain barrier, and exposures during pregnancy therefore constitute the main concern.

6.5.3 Methodology for Dose-Response Assessment

Quantitative risk assessments for non-cancer effects are commonly based on determination of a NOAEL (No Observed Adverse Effect Level) from controlled studies in animals. In this context NOAEL is defined as the highest experimental dose that does not produce a statistically or biologically significant increase in adverse effects over those of controls. An “acceptable safe” daily dose for humans is then derived by dividing the NOAEL by a safety factor, usually 10 to 1000, to account for sensitive subgroups of the population, data insufficiency, and extrapolation from animals to humans. Depending on its exact definition and application this value is referred to as the reference dose (RfD) by US-EPA, ADI by The Food and Drug Administration or MRL (minimum risk level) by Agency for Toxic Substances and Disease Registry (ATSDR).

In the event that that the lowest experimental dose shows a significant difference from the control it is termed a LOAEL (lowest observed adverse effect level, and an extra factor of (usually 10) is used in the determination of the RfD, ADI; or MRL (EPA 1998).

Various reports have provided RfDs for methylmercury derived from animal studies (Rice 1992, Gilbert et al. 1993, Zelikoff et al. 1995, Rice 1996). Nonhuman primate studies indicate that adverse developmental effects in several outcomes occur at 50 microgram/kg per day maternal dose. Uncertainty factors of 10 were used for LOAEL to NOAEL and a safety factor of 100 to account for species and interindividual differences give a RfD of 0.05 µg/kg per day.

In recent years the use of the NOAEL has become controversial because of inherent uncertainties and there has been an increasing interest in new approaches based on dose-response modelling techniques. Crump (1984) suggested application of the lower 95% confidence limit of the dose corresponding to a predefined increase (usually 5%, or 10%) over the background rate. Crump (1995) defined the benchmark dose (BMD) as the estimated dose that corresponds to the specified risk above the background risk, while the BMDL is the corresponding lower 95% limit. This notation has now become standard usage in risk assessment. However, the benchmark calculations depend on the assumed dose-response model, and the results therefore should only be taken as indicative of approximate orders of magnitude (Budtz-Jørgensen et al. 2000).

Another obstacle is that in practice it is often difficult to differentiate the contributions of variability and uncertainty to the observed variation in the reported measurements of a particular parameter (Allen et al. 1996). Distinction between variability and uncertainty is important. Uncertainty can be defined as the possible error in estimating the "true" value of a parameter for a representative "average" person. Variability, on the other hand, should only be considered to represent true inter-individual differences. Understood in these terms, uncertainty is a defect (lack of certainty) which can typically be reduced by experimentation, and variability is a fact of life, which must be considered regardless of risk assessment method used. (Clewell et al. 1999)

6.5.4 Dose-Response Assessment Scenarios in Europe

The major source of exposure to mercury vapour for the general population of Europe is from dental amalgam fillings (WHO 1991). In addition, exposure may occur at inappropriate handling of mercury waste, and close to mercury mines and industrial plants using mercury, see Chapter 5. Some of these exposures are likely to be of a lasting nature, i.e., occurring over a protracted period of time. Evidence from long-term industrial exposure to mercury vapour is therefore highly relevant to exposure situations involving the general population.

Methylmercury exposure originates almost entirely from freshwater fish, marine fish and other seafood. Some population groups have a high exposure from marine mammals, but the most common source is fish, especially species high up in the food chain, see Chapter 5. Some governmental authorities have already taken action and recommended that pregnant women abstain from eating certain types of fish known to contain increased methylmercury concentrations.

While governmental monitoring deals with concentrations of mercury in food items, the main concern from a public-health viewpoint is the dose, i.e. amount ingested from a fish meal. The dose depends on the size of the fish meal and the frequency of such meals. Efforts to reduce

the risks of methylmercury exposure must therefore not overlook the situation, however rare, of the pregnant woman who eats fish every day.

6.6 COMPARISON BETWEEN EUROPE AND IN OTHER PARTS OF THE WORLD

Mercury vapour is a risk of decreasing importance in Europe, as mercury-containing thermometers and other instruments are being phased-out, and the emissions from the chloralkali industry have decreased. In addition, only one mercury mine remains in operation in Europe today. New developments in dental technology have resulted in filling materials that can substitute amalgam for many purposes. However, the information on safety and durability of these new materials is still rather limited, and amalgam fillings are therefore likely to remain in use.

The methylmercury risk will depend on the dietary habits and local sources of contaminated fish and seafood. The increased exposures documented in the Faroe Islands, Greenland and other northern populations are mainly due to ingestion of marine mammals. The extent of this problem within Europe is therefore limited. However, a study from the island of Madeira showed that the consumption of local black scabbard resulted in average methylmercury exposures that were even higher than on the Faroe Islands. Similarly, evidence on mercury in seafood from the Tyrrhenian Sea have shown concentration levels which overlap with those present in pilot whale meat. Thus, excess exposures occur in Europe, see Chapter 5, and may reach or even exceed levels observed in populations in which adverse effects on brain development have been documented.

6.7 LIMITS VALUES FOR MERCURY EXPOSURE SUGGESTED BY WHO AND OTHER ORGANISATIONS

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) decided at a meeting in 1999 (WHO 1999) to maintain the provisional tolerable weekly intake (PTWI) of 3.3 µg/kg body weight. The committee considered data on intake, the quantitative relationship between daily intake of methylmercury and concentrations in blood and hair, and ongoing epidemiological studies. The information available was by the committee considered to be insufficient for evaluating the neurodevelopmental effects on offspring of mothers with low intakes of methylmercury. A clear indication of consistent risk was not detected in the ongoing epidemiological studies. The committee noted that fish (the major source of methylmercury in the diet) contribute importantly to nutrition, especially in certain regional and ethnic diets, and recommended that the nutritional benefits are weighed against the possibility of harm, when limits on the methylmercury concentration in fish or on fish consumption are under consideration. The committee intends to reevaluate methylmercury in 2002 when the 96-month evaluation of the Seychelles cohort and other relevant data have become available.

The National Research Council (NRC 2000) recently reviewed the studies for the US EPA. The NRC found the basis for the US EPA reference dose (RfD) of 0.1 µg/kg body weight to

be scientifically justifiable for the protection of public health. The NCR argued that the RfD should be derived from an analysis of data from the Faroe Islands study, instead of data from Iraq which was the case previously. The NRC review and the studies were again reviwed by an external expert panel, and then the EPA evaluation was finally presented in 2001 (EPA 2001), as part of a water quality criterion.

The EPA evaluation (EPA 2001) includes a thorough analysis of the above-mentioned studies, especially those conducted on children from the Faroe islands and the Sechelles islands. Since the results from these two studies disagree, the merits and weaknesses of the studies were discussed, as well as possible reasons for the conflicting results. Both studies were considered being of high scientific quality, and no serious flaws could be detected. In this situation, the EPA decided to rely on the Faroe island study, showing a negative effect of the exposure levels to methylmercury. The similar results from the smaller, and less well peer reviewed study from the New Zealand, as well as some later cross-sectional studies from other parts of the world, contributed to this conclusion.

The benchmark dose analysis used was based on the lower 95 % confidcence limit for a 5 % effect level (above background) applying a linear model to dose-response data based on cord blood Hg. The cord blood data were converted to maternal intakes. Several of the neuropsychological tests used, and also an integrated analysis gave similar results with respect to benchmark doses. One test (the Boston Naming Test) was chosen for the formal calculatins of the RfD. Other models for the benchmark analyses are possible (Budtz-Jørgensen et al. 2000) and resulted in lower benchmark dose limits, but the linear model was considered the most appropriate one. The EPA chose an uncertainty factor of 10 accounting for pharmacokinetic inter-individual variability, gaps of knowledge on possible long term effects, and uncertainty concerning the relationships between cord and maternal blood mercury concentration, and as mentioned, the EPAs final RfD was set at 0.1 µg/kg body weight and day (EPA 2001) .

In contrast to the EPA´s use of the Iraqi study, the Agency for Toxic Substances and Disease Registry (ATSDR) developed its proposed minimum risk level (MRL) of 0.3 µg/kg/day for methylmercury from the Seychelles Child Development data.

Based on an average daily intake of 17.5 gram of fish, the U.S EPA also calculated a Tissue Residue Criterion of 0.3 mg methylmercury per kg of fish. This limit is weighted on all fish and shellfish consumed. For higher intakes, a lower limit would be needed.

For mercury vapor, studies of occupationally exposed humans have shown slight adverse effects on the CNS and kidneys at long-term air levels of 25-30 $\mu g/m^3$ or equivalent urinary Hg levels of 30-35 µg/g creatinine. Based on the LOAEL for effect on the CNS, the US EPA determined a reference concentration (RfC) for mecury vapor of 0.3 $\mu g/m^3$ for the general population (EPA 1997). The RfC took into account a conversion from occupational exposure to continuous exposure for the general population, lack of data on reproductive effects, the use of a LOAEL instead of a NOAEL, and susceptible subgroups. The US ATSDR established a minimum risk level (MRL) of 0.2 $\mu g/m^3$ based on the same occupational data.

6.8 CONCLUSIONS AND RECOMMENDATIONS

6.8.1 Elemental Mercury

For mercury vapor, studies of occupationally exposed humans have shown slight adverse effects on the CNS and kidneys, and probably also on the thyroid, at long-term air levels of 25-30 µg/m^3 or equivalent urinary Hg levels of 30-35 µg/g creatinine. The US EPA determined a reference concentration (RfC) for mecury vapor of 0.3 µg/m^3 for the general population (EPA 1997). Recent studies suggested that the limit for adverse effects (LOAEL) in occupationally exposed subjects may be lower than indicated above. There is no universal agreement on which uncertainty factors to use. In ongoing work on a EU position paper on arsenic, cadmium, and nickel, factors of 5-10 were used for similar conversion from occupational exposure to continuous exposure, factors of 5-10 for the use of a LOAEL, and a factor of 10 for variation of susceptibility. The total factor was 500. A similar procedure would result in a limit value for elemental mercury of 0.05 µg/m^3. We propose the use of 25 µg/m^3 as starting point, a factor of 10 for continuous exposure of the general population during a whole life-time, and uncertainty factors of 5 for the use of a LOAEL and 10 for individual susceptibility. The proposed limit value will then be 0.05 µg/m^3, as an annual average. This air level is rarely exceeded in ambient air in Europe, however. A typical daily absorbed dose would be 0.6-0.8 µg of Hg for adults. Exposure to Hg^0 from dental amalgam in most cases represents a much higher daily uptake than this level would give rise to (WHO 1991).

6.8.2 Methylmercury

The developing brain is considered the most sensitive target organ for methylmercury toxicity, and data are available for assessment of exposure-response analyses. Effects on the adult population in general, e.g. increased risks of cardiovascular disease, cannot be excluded, but have yet to be substantiated. Therefore the risk assessment is based on neuro-developmental effects.

The basis for the US EPAs reference dose (RfD) has recently been re-evaluated and found scientifically justifiable for the protection of public health (NRC 2000). It is now derived from a benchmark dose analysis using the lower 95 % confidcence limit for a 5 % effect level applying a linear model to dose-response data based on cord blood Hg from the Faroe islands study (EPA 2001). The cord blood data were converted to maternal intakes. Several neuropsychological tests were evaluated with similar results, and the formal calculations were based on one of them (the Boston Naming Test). Several models for the the benchmark analyses are possible (Budtz-Jorgensen et al. 2000), but the linear model was considered the most appropriate one. The EPA chose an uncertainty factor of 10 accounting for pharmacokinetic inter-individual variability, gaps of knowledge on possible long term effects, and uncertainty concerning the relationships between cord and maternal blood mercury concentration.

We share the view of the recent evaluations by the US EPA and NRC. No new information has emerged that would change the risk assessment. Moreover, the considerations made for the US will be valid also for the European population.

We therefore consider the US EPA RfD of 0.1 µg per kg body weight to be appropriate for Europe. It should be noted that it is mainly relevant for fertile women, and that it includes an uncertainty factor.

The reference dose will be exceeded if a substantial amount of fish, contaminated with mercury, is ingested. As an example, if the weekly intake is about 100 g (one typical fish meal per week) of fish with > 0.4 mg/kg, the RfD will be exceeded. This suggests that fish Hg levels should be kept below this limit.

Fish is, however, a valuable part of the diet, in adults as well as in children, and a source of e.g. protein, vitamin E, selenium, and omega 3 fatty acids, see above. At high consumption of fish with low levels of mercury, like in the Seychelles Islands, the advantages and disadvantages may counterbalance each other. Because of the beneficial effects of fish consumption, the long-term aim is not to replace fish in the diet by other foods, but to reduce the MeHg concentrations in fish. If this is not possible, dietary restrictions with respect to fish with high levels of MeHg should be advised for pregnant women.

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