SCIENCE DOSSIER | *Jon Efskind, MD*

METALLIC MERCURY (Hg^0) THE BIOLOGICAL EFFECTS OF LONG-TIME, LOW TO MODERATE EXPOSURES

FEBRUARY 2009

Euro Chlor Science Dossier 13

January 2009

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Metallic mercury (Hg^0)

The biological effects of long-time, low to moderate exposures

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Metallic mercury (Hg^0)

The biological effects of long-time, low to moderate exposures

1. Introduction

1.1. Scope

The aim of this dossier is to present current knowledge of the effects of exposure to low levels of metallic mercury vapour and to define gaps in knowledge which could/should be covered by more research. What constitutes a low level of exposure is open to discussion. However, for the purposes of this report, a “low level” of exposure is considered to be one that gives a kidney burden producing a mercury urine concentration below 30 µg Hg/g creatinine. The science literature on the toxicity of inorganic mercury is huge. This report sometimes also refers to articles about the toxicity of organic mercury, especially that of methyl mercury. This has been done to try to fill gaps in knowledge on inorganic mercury. In these cases there is an uncertainty about the applicability or relevance of the additional information. (Organic mercury compounds contain mercury covalently bound to hydrocarbons (aliphatic or aromatic), while inorganic mercury compounds are oxides or salts of mercury (ionic mercury).

1.2. Toxicity of high and relatively high exposures

The public opinion of mercury toxicity is influenced by the potentially dramatic effects seen at high exposures. Such effects are most typically expressed in a small range of organs which are also the ones most extensively studied for more subtle effects at lower exposures.

High acute exposure may lead to interstitial pneumonitis with chest pains, dyspnoea, coughing and haemoptysis which might be lethal. Sub-acute high exposure has given rise to psychotic reactions with delirium and suicidal tendencies.

In high chronic exposure, the brain is also the critical organ causing broad functional disturbances (e.g. irritability, concentration loss and tremor). No data exist to produce a dose-response curve relating erethism (abnormal irritability or sensitivity of an organ or body part) to low level mercury exposure. However, mild symptoms of erethism are thought to occur at exposure levels giving urine values of more than 100 µg Hg/g creatinine.

A study of historical exposure to inorganic Hg has been made in a group of former workers with a mean age of 71 years. Several parameters from the peripheral and central nervous system of exposed individuals were significantly different from those of the control group more than 30 years after cessation of exposure (Letz et al 2000). Reduction in hand-eye coordination was significantly associated with the cumulative Hg exposure. So also were several peripheral nerve function outcomes like reduced motor nerve conduction velocities. While not significantly different, there was an apparent increase in resting tremor associated with exposure. Cumulative exposure was not associated with dementia or several cognitive neurobehavioral outcomes.

In the kidneys, higher exposures have been associated with proteinuria. Less commonly, the development of a nephrotic syndrome has been reported (WHO 1991).

2. Properties

Mercury (Hg) is the only metal liquid at room temperatures and then with a rather high vapour pressure. At 20 oC saturated air has a concentration two orders of magnitude (>10^2) higher than the occupational exposure limits used in most industrialised countries. Its melting point is -38.87 oC, and its boiling point is +356.58 oC. Hg is the 80^{th} element in the Periodic Table and is a transition metal. As such, it also exists as a cation. The oxidation states are Hg^0 (metallic), Hg^+ (mercurous) or Hg^{++} (mercuric).

Hg has a higher solubility in plasma than in water and passes relatively easily through lipid cell membranes and through the placenta. Hence, Hg as a transition metal should have a capacity for participating in chemical redox reactions within living cells and organisms (*WHO 1991, Kraus 2003, Seppänen et al 2004*).

2.1. Absorption

Hg vapour is readily absorbed from the lungs. Several studies in healthy volunteers showed that around 80% of what was inhaled was retained in the body. This amount was fairly independent of the ambient air concentration in the range 1 to 350 µg Hg/m^3 (*Kudsk 1969, Oikawa 1988*). The breathing state also had a minimal influence. Deep breathing gave only a marginally increased retention (*Oikawa 1988*). Mouth breathing as opposed to nose breathing reduced absorption by 20% (*Kudsk 1965*). Cigarette smoking had no influence on the rate of absorption (*Oikawa 1988),* while alcohol consumption markedly decreased it by 30-40% *(Kudsk 1965, Hursch et al 1980*).

A very small amount of mercury (about 2% of what is taken up by the lungs) enters the body through the skin. However, half of this is eliminated by desquamation (*Hursch et al 1989*).

The absorption of metallic Hg from the intestine is very slight. Around 0.01% of the total amount enters by this route.

2.2. Metabolism

Within the body at low blood concentrations, mercury is transformed/oxidised by first order kinetics, to the mercuric ion. This passes the blood-brain barrier, the placenta and lipid cell membranes only very slowly. Hence, within the tissues, the ionic mercury may become trapped thus increasing the half-life in different organs. However, even though the oxidation in the blood is fairly rapid, it is calculated that about 97% of the mercury absorbed through the lungs will reach the brain un-oxidised (Hursch et al 1988). The reverse reduction process also occurs making a redistribution within the body and some elimination from the lungs possible. The kidneys are the organs storing most of the body burden, around 50-90% (WHO 1991), with a half-life of approximately 60 days (Barregård et al 1991, Ellingsen et al 1993).

In a study of dentists exposed to metallic mercury vapour there was no evidence for this to be transformed into organic mercury within the human body (Chang et al 1987).

2.3. Elimination

The main elimination pathways of mercury from the body are by the urine and faeces. The urinary pathway dominates when the exposure is high (WHO 1991). A small elimination pathway is by exhalation (Dunn et al 1978). After a single short exposure 7% of the body burden was exhaled during 3 days post-exposure (Hursch et al 1976).

2.4. General toxicology, a brief overview

2.4.1. Molecular interactions

The majority of the mercury in blood plasma is bound to albumin and other large proteins. Mercuric ions have a great affinity to bond to reduced sulphur atoms, especially those on endogenous thiol-containing molecules, such as glutathione, cysteine, metallothionein, homocysteine, N-acetylcysteine and albumin. The affinity constant for mercury bonding to thiolate anions is of the order of 1015 - 1020. For comparison, the affinity constants for Hg bonding to oxygen- (e.g. carbonyl) or nitrogen- (e.g. amino) containing ligands are about 12 orders of magnitude lower. Hence, the biological effects of inorganic and organic Hg are related to their interactions with residues containing the sulfhydryl (thiol) group.

By breaking R_S_S_R bonds in proteins, the tertiary structure of the proteins might be changed to such a degree that antigenicity, and probably also sometimes enzyme activity, are changed. This has potentially important toxicological consequences (Moszczynski 1999).

Molecular interactions of mercury with sulfhydryl groups in albumin, metallothionein, glutathione and cysteine have been implicated in mechanisms involved in the kidney proximal tubular uptake, accumulation, transport and toxicity of mercuric ions (McGoldrick 2003).

One of the main intracellular effects of mercury is the induction of and binding to metallothioneins (Piotrowski et al 1974). These are small intracellular proteins with an approximate molecular weight of 6 to 7 kDa. They contain numerous cysteine residues. They have the capacity to bind various metals, including inorganic mercury, cadmium, zinc, copper, silver and platinum. Production of metallothioneins can thus serve as a storage mechanism for these metals. This is physiologically most important in providing a reservoir for zinc. The formation of these compounds may also act as a detoxifying mechanism in relation to mercury, protecting other macromolecules from a harmful exposure (Valko et al 2005).

2.4.2. Oxidative stress

Exposure of experimental animals to inorganic or organic Hg is accompanied by the induction of oxidative stress. This has been suggested by several in vivo and in vitro studies. Rat kidney mitochondria have been shown to be a principal intracellular target of Hg++. Hg++ increased the H_2O_2 formation approximately four-fold at the uniquinone-cytochrome-b region (antimycin A (AA)-inhibited) and two-fold at the NADH dehydrogenase region (rotenone-inhibited) in mitochondria in an in vitro study (Lund et al 1991, 1993). The increased H_2O_2 formation resulting from Hg++ exposure may lead to oxidative tissue damage, such as lipid peroxidation. This might be observed in mercury-induced nephro-toxicity. In these studies the mitochondria were supplemented with a respiratory chain substrate (succinate or malate/glutamate) and an electron transport inhibitor (AA or rotenone). Hg++ at low concentrations seemed to deplete mitochondrial glutathione (GSH) (an antioxidant) and enhance H_2O_2 (an oxidant) formation in kidney mitochondria under conditions of impaired respiratory chain electron transport.

Mercury-induced H_2O_2 production and lipid peroxidation were investigated in vitro in rat kidney (Miller et al 1991). Depending upon the supply of mercury and coupling site specificity, variable increases in the production of H_2O_2 were observed. Concentrations of 12 nmol mercury/mg protein completely depleted the content of GSH in 30 minutes. This suggests that depletion of mitochondrial GSH is responsible for the intra-mitochondrial oxidative stress.

Some ambiguities have been addressed concerning the mechanism of mercury-induced oxidative injury (Gstraunthaler et al 1983). Several studies have been undertaken to determine whether mercury itself causes oxidative damage, or if it makes cells more sensitive to other species producing oxidative stress. It has been found that inorganic mercury can enhance the ability of other reagents to induce lipid peroxidation.

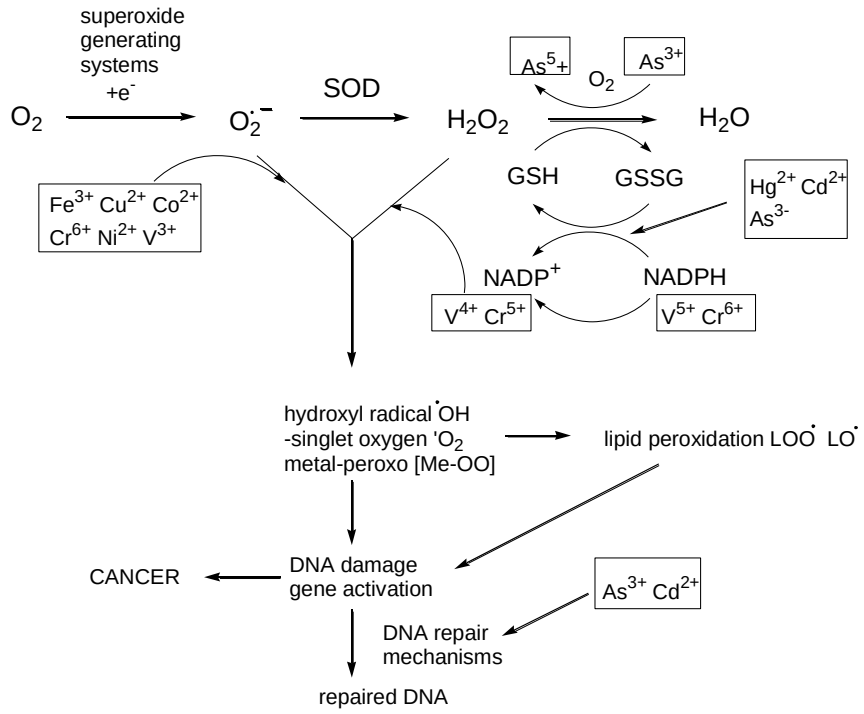

Figure 1. Pathways of metal-induced oxidative stress.

The principal influence on various points of action of a number of heavy metals is schematically depicted. The only influence of inorganic mercury in this process is limiting the availability of reduced glutathione (GSH). (After Valko et al 2005.)

The effects of $HgCl_2$ on lipid peroxidation (LPO), glutathione reductase (GR), glutathione peroxidase (GSH-px), superoxide dismutase (SOD) and glutathione (GSH) levels in different organs of mice (CD-1) were evaluated (Mahboob et al 2001). The results indicated that Hg-treatment enhanced lipid peroxidation in all tissues, but this was only significant in kidney, testis and epididymis tissues, suggesting that these organs were more susceptible to Hg toxicity. An observed increase in antioxidant enzyme levels in testis under this exposure could be a mechanism for protecting the cells against reactive oxygen species.

The administration of $HgCl_2$ to male Sprague-Dawley rats was reported to cause marked decreases in the activity of SOD, catalase, GSH-px, and glutathione disulphide reductase in the renal cortex. Suppressed activities of these enzymes would be expected to enhance the susceptibility of renal epithelial cells to oxidative injury (Gstraunthaler et al 1983).

It has also been reported that in addition to reducing glutathione levels, the levels of other cellular antioxidants, including vitamin C and E, are depleted in the kidneys of rats treated with $HgCl_2$ (Fukino et al 1984).

The protective effect of vitamin E against the reproductive toxicity of $HgCl_2$ has been investigated in male mice (Rao and Sharma 2001). Animals given vitamin E with mercuric chloride had lower concentrations of mercury in the testis and epididymis. After mercuric chloride treatment the animals were permitted to recover for 45 days. That resulted in a partial recovery of sperm and biochemical parameters. Vitamin E co-treatment has been shown to have a protective role against mercury-induced male reproductive toxicity.

The chelating agents DMPS (2,3-dimercapto-1-propanesulfonic acid) or DMSA (meso-2,3-dimercaptosuccinic acid) are effective in reducing mercury concentrations in kidney. GSH, vitamin C, lipoic acid alone, or in combination have not such an effect. (A chelating agent is a chemical substance binding to toxic (and also other) metals. They can have the effect of significantly increasing the excretion of these metals and thereby reducing their toxic effects.)

Whether GSH, vitamin C, or lipoic acid, alone or in combination with DMPS or DMSA decreases brain mercury in young rats, has been tested (Aposhian et al 2003). The results showed that

chelating regimens (DMPS or DMSA) did not reduce the mercury content in the brain. It was also concluded that any possible palliative effect of GSH, vitamin C, or lipoic acid for the treatment of Hg toxicity resulting from mercury vapour exposure, did not involve Hg mobilisation from the brain or kidney.

2.4.3. Mercury and Zinc

Chronic exposure to zinc (Zn) induces enhanced synthesis of metallothioneins. Metallothioneins contain numerous cysteine residues and have the capacity to bind various metals. They serve as an important physiological reservoir for Zn (Bruce 2001). Unlike other trace elements, Zn does not accumulate in the body to form permanent stores. Hence, it seems likely that there might be competition between Hg and Zn for the sulfhydryl binding sites. This could reduce the availability of Zn.

Chronic deprivation of Zn results in increased sensitivity to oxidative stress.

Zn is, however, a redox-inert metal, but it functions as an antioxidant by protecting sulfhydryl groups of proteins and by reduction of the formation of the hydroxyl radical from H2O2 (Valko et al 2005).

The correlation between $HgCl_2$ toxicity and level of zinc has been investigated (Fukino et al 1984). The effect of the administration of $HgCl_2$ on Zn deficiency in rats was studied in the kidney of rats fed with either zinc-deficient (low-zinc, < 1.5 ppm) or zinc-supplemented (80 ppm) diet for 10 days. It was concluded that depletion of zinc affects various protective mechanisms of the organism and thus increases susceptibility to the toxic effects of $HgCl_2$.

2.4.4. Mercury and Selenium

It has been shown that one mechanism for Hg toxicity in animals is an increased lipid peroxidation and oxidative stress (Bulat et al 1998, Girardi and Alias 1995). Several enzymes participate in counteracting such mechanisms. Among them is glutathione peroxidase (GSH-Px). In the kidney, selenium-dependent GSH-px is produced in the proximal tubular cells (Whitin 1998). These cells are (normally) rich in selenium (Se) (Högberg 1986).

Individuals with a poor Se status might have a diminished capacity for the production of selenium-dependent GSH-Px from proximal tubular cells. Also, in the kidney, Hg forms complexes with Se in a relationship Hg-Se of 1:1 which are bound to proteins. The formation of these Hg-Se complexes might then further deplete the biologically available kidney Se below a critical level necessary for a sufficient production of the GSH-Px within the kidney. By such a mechanism the damaging effects of peroxidation might be increased due to a diminished the availability of selenium-dependent GSH-Px from proximal tubular cells.

However, by themselves these Hg-Se complexes have been found to be of low toxicity (Bjørkman 1994, Drasch et al 1996). Hence, this Hg-Se complex formation might also represent an alternative pathway for the detoxification of Hg provided the level of bio-available Se is above the critical level for the production of sufficient GSH-Px.

2.4.5. Mercury and Calcium

The intracellular concentrations of calcium are important for maintaining appropriate cellular functions. They are well documented. Inorganic mercury has been found to affect calcium homeostasis. Hg toxicity at the cellular level is accompanied by changes in the permeability of the plasma membrane. Treatment of renal cells with inorganic Hg produces a rapid increase in intracellular Ca++ levels. These returned quickly after cessation of exposure to about twice the original level. The cytosolic content of calcium increased mainly from non-mitochondrial intracellular stores, presumably derived from the endoplasmic reticulum. Cytotoxicity was associated with the phase of rapid increase in intracellular calcium, and was dependent on the presence of extracellular calcium (Chavez and Holguin 1988).

The toxicological influence of inorganic Hg (as $HgCl_2$) on intracellular cytosolic free calcium has also been studied in immune cells where Hg has been shown to have toxic effects.

Changes in Ca++ in T lymphocytes were investigated for two mercury compounds, methyl mercury (MeHg) and inorganic mercury ($HgCl_2$). It has been suggested that an intracellular redistribution of the stores of Ca++ is a result of cellular exposure to Hg. However, in contrast to MeHg, $HgCl_2$ caused only a Ca++ influx from the extracellular medium (Tan et al 1993).

Studies of the effect of $HgCl_2$ on neutrophil granulocytes have shown a dose-dependent impairment of the chemo-attractant-stimulated motility in these cells. The Hg++-induced fluxes of Ca++ could be prevented by small sized polyethylene glycols but these glycols showed a similar dose-dependent effect on the neutrophil cell motility (Loitto and Magnusson 2004).

In neutrophils exposed to MeHg, in parallel with the increase in Ca++, there has been shown to be a marked reduction in nitric oxide (NO) production and also in the production of NO-synthetase and its mRNA. A calcium channel blocker used in human medicine, verapamil, can antagonise this inhibitory effect of MeHg, suggesting that MeHg inhibits the NO production at least in part through the Ca++ -activated adenyl cyclase-cAMP-protein-kinase pathway (Kuo 2002).

3. Exposure estimates

Most specialists in occupational health and researchers studying Hg toxicity agree that for chronic health effects urinary Hg (U-Hg) is the most appropriate surrogate for tissue/organ exposure in living humans. For the acute health effects blood Hg (B-Hg) is more appropriate. For U-Hg the best method would be a 24 hour composite sampling, but this is not practical except for laboratory experiments. Since the water excretion by the kidney varies according to the state of hydration of the person, the concentration of any particular component varies as well. Hence, a correction for the general concentration of the urine would improve the precision of a spot sample.

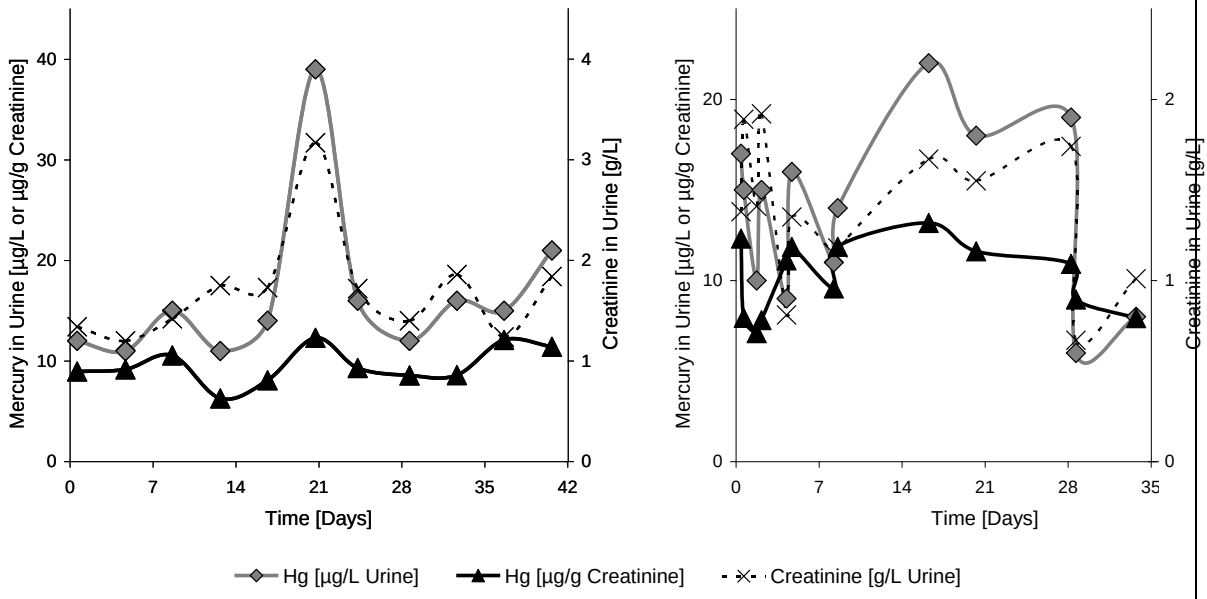

Figure 2: Day to day variation in the uncorrected Hg concentration of two persons occupationally exposed to metallic mercury vapour. It also shows the relative stability of the creatinine corrected concentration. (Will et al 2008)

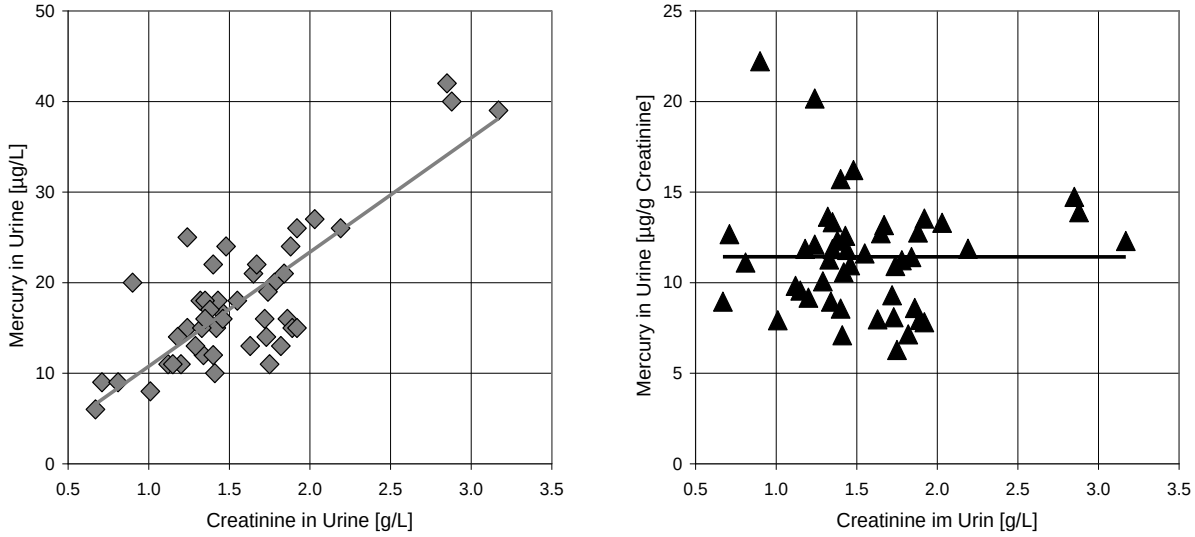

Figure 3: The association between the uncorrected concentrations of Hg in urine as well as the creatinine corrected Hg content in urine relative to the creatinine concentration of four occupationally exposed persons. (Will et al 2008)

As illustrated in Figure 3 the creatinine correction also fits well on a group level. Hence, the creatinine correction is considered by most experts to be the best practical way for eliminating imprecision in this kind of exposure estimate. An imprecision in the measure of exposure can often obscure real biological effects in statistical analysis. Under rare and special circumstances such a lack of correction might, however, also lead to bias.

When considering research on chronic effects of Hg exposure, it is necessary to consider what kind of exposure parameter would be the most preferable. Several options are available. One could be a sum of "exposure" over the years, e.g. measured as the cumulative U-Hg. That could in practice be calculated as the sum of the timed (e.g. yearly) averages of U-Hg (∑U-Hg), another might be the timed (e.g. yearly) average of that sum divided by the time (e.g. sum of years) under exposure ($\sum U\text{-}Hg_{corr}/\sum year_{expo}=\sum U\text{-}Hg_{corr} \cdot \sum year_{expo}^{-1}$). This has been termed the "intensity of exposure" by some authors.

The average concentration over the last year could also be used. The least satisfactory of all would be the U-Hg spot value at the time of investigation. For acute or sub-acute effects the spot B-Hg or spot U-Hg could *a priori* be considered as satisfactory.

When doing epidemiological research on Hg toxicity, the studies are bound to use the best exposure indicators available. These indicators might be urine Hg concentrations from a series of individuals or all individuals, taken at regular or irregular intervals more or less representative for the general exposure situation.

Very often the exposure estimate is based on only one spot sample for each individual taken at the time of the study. Even today, some studies are reported with no correction for the general urine concentration, the creatinine correction.

To make it easier for the reader to compare results from different studies, this report gives estimates of what corrected U-Hg values might have been in some of the studies quoted when such information is lacking. Experience has shown that a correction factor of 1.3 should be used. This is in line with some other authors (e.g. *Kraus 2003*). If such a correction has been done by other authors in published meta-analysis, their data are used (e.g. *Meyer-Baron et al 2002, 2004*).

4. Clinical neurotoxicology

4.1. Basal neurotoxicology

The effect of methyl mercuric (MeHg) chloride has been studied in purified brain cell cultures. The rate of oxygen uptake in oligodendrocytes, astrocytes and cerebral cortical and cerebellar granular neurons from embryonic and neonatal rat brains has been determined. The results revealed that mitochondria may be the earliest target of MeHg neurotoxicity. The electron transport chain in the mitochondria is the most likely site where an excess of reactive oxygen species are generated in the brain. The oxidative stress thus induced, is important in MeHg poisoning (*Yee 1996*). From what is generally known of Hg toxicology, this might also be the case for inorganic Hg that has passed through the blood/brain barrier and entered into the different types of nerve cells.

As noted above (section 2.4.3) Hg^{++} has a high affinity for sulfhydryl groups. It might replace Zn^{++} at the binding sites in metallothioneins (probably the most important physiological reservoir for zinc). This may, in turn, result in relative depletion in organs or tissues with a high zinc demand. Zinc deficiency has been shown to result in several electrophysiological, gross and ultrastructural changes in the eyes in a variety of species. For example, in the retina, zinc is believed to serve as an antioxidant, to interact with taurine and vitamin A, to modify photoreceptor plasma membranes, to regulate the light-rhodopsin reaction and to modulate synaptic transmission. Sub-optimal zinc status resulting from exposure to Hg may thus influence the development and progression of several chronic eye diseases (*Bruce 2001*).

4.2. Clinical neurotoxicology of inorganic Hg / industrial exposure

4.2.1. Current exposure

Several epidemiological studies on neurotoxicity have been performed in industrial cohorts. Some of the most informative ones are referred to in Table 1.

Table 1. Epidemiological studies on neurotoxicity of mercury in industry

Reference	N (Cases/control)	Mean exposure	Effect
Günther et al 1996	Low: 34/37 High: 21/37	Low: 26 µg/g High: 122 µg/g	No below 38 µg/g Yes above 38 µg/g
Liang et al 1993	88/70	25 µg/l (≈ 19.2 µg/g)	Yes (by the most exposed)
Piikivi et al 1984	36/36 sub-group 20/36	58 µg/l (≈ 45 µg/g) > 110 µg/l (≈ 85 µg/g)	No (Yes, if mean > 85 µg/g)
Piikivi and Hänninen 1989	60/60	17 µg/g	No
Roels et al 1985	131/114	52 µg/g	No below 50 µg/g
Ellingsen et al 2001	46/46	16.6 µg/g	Slight

Exposures are given as current spot U-Hg at the time of the investigation. Concentrations are given either corrected for by U-creatinine or uncorrected. µg/g means µg Hg/g creatinine. If uncorrected values are given, an indication of what the corrected values might have been is given in brackets: (≈ µg/g).

The study by *Liang et al (1993)* was done in a fluorescent tube factory. Air concentrations varied at worksite from 8-85 µg/m^3 with an average of 33 µg/m^3.Two departments had a really high exposure with air concentration means at 85 µg/m^3 in the lamp vacuumis department and 78 µg/m^3 in the Hg-filling department. The exposures are given as the current air and urine concentrations.

The exposure pattern in the cohort (19 men, 69 women) was not described with respect to placement in the factory nor as a yearly average or cumulative doses. The average time of exposure was 10.4 years. Correcting for confounding by age, the authors found a trend towards poorer performance in the neurobehavioral tests with increasing time of exposure. The alteration of neuro-behavioural patterns was most likely connected to impairment of the cognitive function according to the authors. This was manifested as poorer performance in mental arithmetic, two-digit search, and switching attention. These results might have been un-duly weighted by the observations on workers with the highest exposures.

In the sub-group with the higher exposure (as measure by urinary Hg (U-Hg) in the *Piikivi et al* (1984) study there were statistically significant deficits on the verbal concept formation test (similarities) and the memory tests. These differences were found both for the sub-groups with the high time-weighted U-Hg average exceeding 110 µg/l (≈ 80 µg/g) and with an instantaneous U-Hg exceeding 51 µg/l (≈ 45 µg/g). For the lower exposed group, no deviant results were found.

In the study by *Ellingsen et al (2001),* no statistically significant differences in the neuropsychological test results between the exposed and the control populations were detected. Statistically significant associations were, however, found between inorganic Hg in blood (the variable reflecting the most recent exposure) and the results of WAIS Digit Symbol Test and Benton Visual Retention Test among the exposed workers. This could indicate a small effect of exposure to Hg on visuo-motor/psychomotor speed and attention, and immediate visual memory.

No effect was shown between any measures of past or recent exposure upon tremor. Current smoking was, however, associated with tremor. This puts the results of the tremor study of *Fawer et al (1983)* from a mixed occupational setting into question. No account was taken of the confounding influence of smoking in that study.

4.2.2. Historical exposure

There have been relatively few studies done on past (historical) exposure. The most important ones are summarised in Table 2.

Table 2. Studies of historical exposures to mercury

Reference	N (Cases/control)	Mean exposure	Effect
Mathiesen et al 1999	75/52	3 µg/g (58 µg/g yearly mean)	Yes, slight
Frumkin et al 2001	147/132	2.8 µg/g 56 µg/g exposed	No considering multiple tests
Bast-Pettersen et al 2005	49/49	3.0 µg/g	No
Ellingsen et al 1993 Andersen et al 1993	77/53	3.2 µg/g (58 µg/g yearly mean)	Yes, slight

Exposures are given as current spot U-Hg at the time of the investigation unless otherwise indicated. Concentrations are given corrected for by U-creatinine (µg/g means µg Hg/g creatinine).

In the study by *Mathiesen et al (1999),* the Benton Visual Retention Test (correct) and Grooved Pegboard Time for both hands showed a statistically significant poorer performance in the

historically exposed group compared with the reference group. The magnitude of exposure was important. The lowest exposed sub-group, <550 (nmol/l/ year (≈ 85 µg/g) during the time under exposure, did not perform any worse than the controls in this sub-set of the test battery applied. Hence, for this sub-group, it was concluded that no lasting neuropsychological effects were shown.

A clinical neurological examination was done supported by neurophysiology and neurography measurements. Some slight lasting neurological and neurophysiological abnormalities were found. These were observed, on average, 12.3 years after cessation of exposure (*Ellingsen et al 1993 and Andersen et al 1993*).

In the study of *Bast-Pettersen et al (2005),* the average yearly exposure was 16.6 µg/g 4.8 years after cessation of exposure. This was a follow-up to the *Ellingsen et al (2001)* study. It showed that the modest effect of the then current exposure, as measured by blood mercury (B-Hg), had disappeared completely.

4.3. Neurotoxicity studies in dental health workers

A number of studies have been carried out on dental professionals (Table 3).

The study of *Echeverria et al (1995)* was a small pilot study in dentists. 19 were currently exposed and 20 currently unexposed. The "unexposed" group was, however, not without potential exposure in their practices. Furthermore, age distribution, drinking habits and use of NO_2-anaesthesia were not equally distributed in the two groups. The exposed group had a current mean U-Hg of 36 µg/l (≈27.7 µg/g) and the "unexposed" had 0 µg/l. The basis for inclusion was self-selection. The Digit span and Simple reaction time were associated with Hg exposure. Some of the confounding factors were accounted for by statistical treatments.

This study was followed up by *Bittner et al (1998)* in a larger study which was also recruited by self-selection. The study of 230 dentists was run, without any reference group, as a study of co-variability between the outcome variables (test-scores) and measures of exposure and potential confounders as determinants. The study group could easily have been divided into high and low exposed sub-groups as distinguished by the exposure distribution shown in the article. However, that possibility was not utilised in any statistics shown in the article.

Of the five neuro-behavioural tests done, only one, the Intentional Hand Steadiness Test, showed a statistically significant co-variation with the Hg^0 exposure. Even though information about smoking habits was collected, it was obviously not used in order to control this confounding factor in the study. Finger tapping, One-hole test, Simple reaction time or Hand tremor did not show any statistically significant co-variation with the current U-Hg exposures.

This study was inconclusive because of the self-selection into the study, the use of current U-Hg as the only estimator of occupational exposure and the lack of a reference group. In this case the comparison was between a "high" exposure sub-group (≈25-30 µg/l) and a low one (≈2-2.5 µg/l).

Echeverria et al (2005) made a study of 193 male dentists randomly drawn from a self-selected group consisting of 40% of the target population. The study population was supported by 233 dental assistants from the practices of the study-group. The study utilised statistical treatment of test scores and results (for example to separate the cognitive elements in a test from the motor ones). The validity of these statistical treatments may be open to question.

The average current exposure for dentists, measured as U-Hg, was 3.32 µg/l and 1.98 µg/l for the dental assistants. The current U-Hg could have been expressed in terms of µg Hg/g creatinine in urine since effort and costs were spent for that additional analysis. However, in the statistical analysis reported, the uncorrected U-Hg values were used.

This study also lacked a reference population or sub-group. Again, the comparison was merely between the high and low exposure groups - see *Bittner et al (1998)* above). For this reason it was not possible to analyse the reliability of the regression coefficients.

The male dentists were generally significantly older and drank more alcohol than the female dental assistants. They were also exposed to approximately twice the level of Hg. Even so, the crude scores look rather similar for the two study groups. The coefficients from the multivariate regression analysis related to the uncorrected U-Hg, fall into the same range for the two groups. Out of the 23 tests/test derivatives, 8 are statistically significant in the dentists and 7 in the dental assistants. The two study groups have five statistically significant β–coefficients in common. These were Digit span (Forward), Symbol digit (Rate), Finger tap (Dominant/non dominant hand), Finger tap (Alternate partial) and Hand steadiness (Factor 1). No significant associations were found between the outcome variables and the indicators for either chronic or peak Hg exposure. Multiple testing was accounted for in the statistical analysis.

The article does not describe the co-variations reported upon in any other ways than giving the β–coefficients and their p-values. Hence, with the questions mentioned above, the interpretation of this study is difficult. With no reference group, this study is not included in Table 3.

The study of *Gonzalez-Ramirez et al (1995)* was so small and the differences in gender distribution and age so great between exposed and reference groups, that adequate control of confounding factors was probably not possible. Furthermore, multiple tests were done but the statistical methods used were not reported.

The dentists in the study of *Ngim et al (1992)* were divided into 4 sub-groups by median B-Hg and median length of exposure. In the sub-group analysis, those with the highest cumulative exposure (18.6 µg/l and 13.4 years) showed a statistically significant poorer performance in the digit symbol, digit span, trail-making, logical memory delayed recall, and visual reproduction tests compared with both the reference group and the lowest exposed sub-group (7.2 µg/l and 3.5 years). The study assumed that exposure had been constant over the years. The selection process of the study group (32% of all Singaporean dentists) was not reported. (There may have been a percentage of self-selection.)

The *Ritchie et al (1995)* study utilised computerised tests where visuo-motor speed and memory were the main elements. Word recall, Simple reaction time, Number vigilance, Choice reaction time, Spatial memory, Memory scanning, Delayed word recall and Word recognition were tested. The results of those tests with a component of immediate memory did not differ between the groups, nor did recognition tasks. Older dentists performed significantly worse than the older reference group on both immediate Word recall and Delayed word recall, but better on Simple reaction time. Confounding factors cannot be excluded.

In the study by *Uzzell (1988)* of neuropsychological function in male dentists, the exposed group had an average of 23.1 years in practice. Their hair concentration ranged from 20 to 129 µg Hg/g hair. The average ambient Hg air concentration during 8 hours had exceeded 50 $\mu g/m^3$ in 15-20% of the surgeries. The tests used were WAIS, visuo-graphic skills with Bender Gestalt Test (BGT), response latencies with disjunctive reaction time, Visuo-motor Skills with Purdue Pegboard, Grooved Pegboard, Finger tapping, and steadiness with vertical and horizontal groove. 18 parameters were examined and the statistics were corrected for multiple testing. Only the BGT showed a statistically significant negative difference from the controls.

The study of *Uzzell and Oler (1986)* investigated dental assistants. They were divided into high and low exposed sub-groups. The selection process of the study groups seems unbiased. The average age was 41 years and the period of exposure was 14.8 years for both groups. They were subjected to a battery of tests covering attention, motoric/fine-motoric tempo and memory. Only for short time memory did the highly exposed group perform significantly worse.

The study of dentists by *Ritchie (2002)* is the largest one in dental health. 72% of the dentists were recruited by random choice and 28% by self-selection. 60% were men. The reference group was recruited from university staff by self-selection. The composition by age and gender did not correspond with the exposed group. It was attempted to correct for these differences by statistical procedures. No effects were found that were related to Hg exposure.

Table 3. Neurotoxicity studies of mercury in dental health workers

Reference	N (Cases/control)	Mean current exposure	Effect
Echeverria et al 1995	19/20	36.4 µg/l	Yes?
Bittner et al 1998	230 : participation from 169 to 86 according to test type	Not given Median ≈ 10 µg/l	Yes for Hand steadiness test. No for 4 tests
Echeverria et al 2005	194 males 233 females	3.32 µg/l 1.98 µg/l	Yes? current Hg No peak exp. No cum. exp.
Gonzales-Ramirez et al 1995	10/13	3.3 µg/g	Yes?
Ngim et al 1992;	98/54	18 [a] µg/g (B-Hg: 13.1 µg/l)	Yes
Ritchie et al 1995	Younger: 19/20 Older: 20/20	Younger: 3 (1-29) µg/g Older: 6 (4-31) µg/g	Some
Uzzell 1988	26/14	36 µg/g [b]	Yes
Uzzell and Oler 1986	13/13	30 µg/g [b]	Yes
Ritchie 2002	162/167	4.6 µg/g	No

Exposures are given as current spot U-Hg at the time of the investigation unless otherwise indicated. Concentrations are given either corrected for by U-creatinine or uncorrected (µg/g means µg Hg/g creatinine). If uncorrected values are given, an indication of what a corrected value might have been is given in brackets: (≈ µg/g).

[a] U-Hg calculated from B-Hg by Meyer-Baron et al 2002

[b] Estimated by Meyer-Baron et al 2002 from information about mean exposure for dentists the same year

4.4. Visual impairment

The visual system is one of the targets of chronic metal toxicity (*Grant and Schuman 1993*) manifesting itself as chronic toxic neuropathy. One of the first clinical signs of this disorder can be impaired colour vision (*Rosen 1965*). In animal exposure studies it has been shown that Hg also accumulates in the retina (*Khayat and Dencker 1984*). Interference of Hg with Zn in the sensory function of the retinal cone cells might be one possible explanation for this disturbed function or toxicity.

Table 4. Studies of visual impairment caused by exposure to mercury

Reference	N (Cases/control)	Mean current exposure	Effect
Roels et al 1985	170/158	♂: 52 µg/g ♀: 37 µg/g	No
Canto-Pereira 2005	15/13	1.97 µg/g	Yes, in some but not all tests
Urban et al 2003	24/24	20.5 µg/g 14.7 years exposure	(Yes) but confounded
Cavalleri et al 1995	33/33	99.5 µg/g	Yes
Cavalleri and Gobba 1998	21 before and after hygienic measures	10 µg/g	No

Exposures are given as current spot U-Hg at the time of the investigation unless otherwise indicated. Concentrations are given corrected for by U-creatinine (µg/g means µg Hg/g creatinine).

Roels et al (1985) studied colour vision in a mixed gender cohort exposed to metallic Hg vapour. Colour vision was tested with the 28-Hue test of Roth. This test was used because it permits a quantitative evaluation of the colour discrimination in the examinees. The test was applied to each eye separately under daylight conditions with standard illumination.

The cumulative frequency distributions of the total error score for colour discrimination calculated in either gender sub-group did not differ between the exposed and the reference groups. Results were similar for both eyes. Also, the prevalence of entirely correct performance of the colour discrimination task was not different between controls and Hg exposed subjects, either for the left or right eye.

In a study of visual impairment of dentists related to occupational mercury exposure, the dominant eye was tested for colour vision with the Lanthony de-saturated test and Cambridge colour test. Contrast sensitivity was measured with Luminance contrast, Red-green chromatic contrast and Blue yellow chromatic contrast tests. Inclusion criteria for the dentists were not reported (*Canto-Pereira 2005*).

The Lanthony de-saturated test did not show any statistically significant difference between the exposed and the reference groups. However, for the dentists the Cambridge colour vision test showed a significant loss of colour vision of a diffuse nature. Contrast sensitivity function tests as well as chromatic contrasts (red-green and blue-yellow) also showed significant adverse effects of exposure to mercury. The authors proposed two possible explanations for their findings; either an influence at the level of the outer retina, or of the entire visual system in a diffuse way. They favoured the latter explanation.

However, linear regression analysis did not reveal any associations between U-Hg and the visual functions. This was probably because of the “narrow range” in U-Hg concentrations according to the authors. (NB - the Range was between 0.7 and 5.8 µg/g. The top concentration was eight times the lowest.) Hence the relationship of their observed findings to occupational Hg exposure might be dubious.

In the *Urban et al (2003)* study of currently Hg-exposed male workers in the chlor-alkali industry in the Czech Republic, none of the subjects had any awareness of any colour vision impairment. The only index of cumulative exposure used, was the product of duration of exposure and U-Hg concentrations.

Alcohol consumption, cigarette smoking, and history of diseases or drug use which had the potential to influence colour vision were registered among the exposed and reference

populations. The groups differed significantly with respect to alcohol consumption and cigarette smoking; both self-exposures were higher in the exposed group. Alcohol consumption is a known confounder in the tests performed, but it did not contribute significantly in a multiple regression analysis of the data.

Colour vision was tested with the Lanthony 15-Hue de-saturated panel. In the exposed group the CCI (colour confusion index) was statistically significantly higher than in the reference group. Further, the frequency of subjects with errorless performance among the exposed group was significantly lower. However, no measure of the magnitude of exposure used (cumulative or current) showed any significant dose-effect or dose-response association in the regression analysis.

Industrial workers (N=33) engaged in production of precision instruments were compared with unexposed controls (*Cavalleri et al 1995, Cavalleri and Gobba 1998, Gobba and Cavalleri 2003*). The authors successfully matched age, gender, alcohol consumption and cigarette smoking in the two groups. All but one of the exposed workers exceeded 35 µg Hg/g creatinine. Colour vision was assessed by the Lanthony 15-Hue de-saturated panel. The results showed a loss of colour vision and a higher colour confusion index (CCI) in the exposed population.

After hygienic measures were taken to reduce exposure to mercury in the workplace, a sub-set of the 1995 cohort (21 due to the drop-out of one factory from the study) was re-examined. No-one exceeded a level of 35 µg/g, and the colour perception had returned to normal.

4.5. Very low exposure in children

A recent randomised, prospective study in which 534 children aged 6-10 years without any dental amalgam fillings were followed up for 5 years. They were randomised into amalgam or composite treatment for dental caries when fillings were required.

They were tested for general IQ, memory and visuo-motor skills at inclusion and after 5 years. The average restored tooth surfaces were 15 per individual (median 14) at the end of the study.

The exposure measured as U-Hg was 0.9 µg/g in the amalgam group and 0.6 µg/g in the reference group. This was a statistically significant difference.

No statistically significant differences were found in the 5-year changes of the full scale IQ score between the exposed and the control groups. Neither was there any difference in the 4-year change in the general memory index, nor in the 4-year change in the visuo-motor composite. According to the authors, these findings suggest that the health effects of amalgam restorations in children need not be the basis of decisions regarding dental treatment (*Bellinger et al 2006*).

4.6. Summary and discussion

Several studies on this topic appear to suffer from selection bias, either in the exposed group by self-selection (which might have been triggered as a result of symptoms experienced) or in the reference groups by, amongst other things, the selection from an inappropriate pool. This potential bias is often impossible to evaluate due to poor description in the literature, both of the selection process and of the resulting study groups (i.e. exposed and reference populations).

In this review no emphasis has been put on studies based upon self-reporting of symptoms due to the high probability of experiencing information/knowledge bias.

A difference in the relationship between effect and exposure level is observed in studies of dental health professionals as compared with studies of industrial workers. In several studies of dental health professionals effects are observed at exposure levels where none are found in equivalent industrial groups. Most often in the “dental” studies the effects are related to current exposure, which might not be representative of the full exposure histories. This may well have resulted in an overestimate of the effects of a present low exposure. Also, self-recruitment into studies might give an over-representation of persons with health problems expected to occur as a result of Hg toxicity. This might result in both a selection bias and an information bias in these

studies. Hence, great care must always be taken with such studies, especially with regard to self-reported symptoms.

Children are often considered to be more vulnerable to toxic exposures than adults. The recent randomised, prospective study of very low exposure by dental amalgam (Hg^0) in young children has not shown any harmful effects on the central nervous system by neuropsychological testing (*Bellinger et al 2006*).

From a meta-analysis of studies on neurobehavioral function, the motor nervous system seems to be the most sensitive indicator of the influence of Hg on the central nervous system. The lowest group threshold was an average U-Hg of 18 µg/g based upon data of current exposure. The lowest measures of effect were seen in dental health personnel. Tests with memory components indicated a reduced performance by the Hg-exposed groups whereas the speed tests did not. Tests measuring verbal memory showed, unambiguously, no effects of Hg exposure; whereas visual memory tests showed an effect if an independent reproduction of the figural material was required.

Attention deficit tests were more ambiguous, showing diverging results. The latest additional analyses have confirmed that the influence of Hg on separate psychological functions differs. Results on motor performance compared with memory revealed the greatest impairment in Hg-exposed workers. Attentional performance was hardly affected. The over-all correlation coefficient of combined test effects and Hg exposure for this meta-analysis was 0.5 (*Meyer-Baron et al 2002, 2004*).

Influence on colour vision seems to be the most sensitive indicator of Hg effects. It appears that this effect is also dependent on dose, having a threshold at a group level somewhere below 50 µg/g. An effect shown in industrial workers with high exposure was totally reversed when the group level of exposure was reduced to 10 µg/g (*Cavalleri et al 1995, Cavallieri and Gobba 1998, Gobba and Cavalleri 2003*).

Five articles, summarised above, cover effects of historical exposure (*Ellingsen et al 1993 a and b, Mathiesen et al 1999, Bast-Pettersen et al 2005 and Frumkin et al 2001*). They all show signs of partial or a full reversibility. In group average exposure levels corresponding to 25 µg/g or less, no lasting effects have been shown. For high historical Hg exposures, the lasting effects are comparable to those seen at moderate current exposure (*Meyer-Baron et al 2004*).

In tremor studies, smoking (nicotine exposure) is an important confounding factor which, in some cases, has not been accounted for at all or omitted from the analysis (e.g. *Fawer et al 1983, Roels et al 1985*) (see *Ellingsen 2006*).

5. Renal effects of moderate and low exposure

5.1. General nephro-toxicology (Valko et al 2005)

Metallic mercury vapour is converted into ionic mercury, which is recovered predominantly (approximately 98%) in the kidney. The species of mercury most likely to be taken up into the proximal tubular epithelial cells are mercuric conjugates of low-molecular-weight ligands. The mechanism for this uptake is by the organic anion transporter. The majority of the mercury present in plasma is, however, bound to albumin and other large proteins. The organic anion transport system cannot transport these mercuric conjugates of proteins.

Two conjugates (glutathione and cysteine) have been implicated in the transport of Hg conjugates due to their size. Glutathione has a net negative charge at physiological pH. Because of this charge, glutathione has been postulated to be the preferred substrate at the site of the organic anion transporter.

It is, however, clear that thiols, especially homologues of cysteine, such as homocysteine and N-acetylcysteine, can significantly influence the manner in which inorganic mercury is handled in the kidney.

Inorganic or organic mercuric cysteine conjugates have also been proposed to be highly relevant as species transportable by the organic anion transporter, even though cysteine has a net neutral charge at physiological pH. This hypothesis is derived from studies in which organic S-conjugates of cysteine have been shown to be taken up by the proximal tubular cells by a mechanism consistent with the activity of the organic anion transporter.

Once inorganic mercuric ions have entered into the proximal tubular cells, it appears that they distribute in all intracellular pools. The cytosolic fraction has been found to contain the greatest content of mercury.

When rats were treated chronically with mercuric chloride, the relative specific content of mercury was shown to increase to the greatest extent in the lysosomal fraction. Increases in the lysosomal content of mercury may reflect the fusion of primary lysosomes with cytosolic vesicles containing complexes of inorganic mercury bound to proteins.

The high affinity of mercuric ions for binding to thiols naturally suggests a consequent depletion of intracellular thiols (especially glutathione). This may cause (either directly or indirectly) or predispose proximal tubular cells to, oxidative stress. *Lund et al (1993)* demonstrated that the administration of mercury as Hg^{++} in rats resulted in glutathione depletion, increased formation of H_2O_2, and lipid peroxidation in the kidney mitochondria.

A high affinity for inorganic Hg in the kidney has been shown after exposure to elemental mercury. After exposure to metallic Hg vapour, around 98% is recovered in the kidneys. A suggested reason for this is an induction of metallothionein in the kidneys which is mediated by inorganic mercury. The administration of a single, daily, non-toxic dose of mercuric chloride over several days caused a near doubling in the concentration of metallothionein in the renal cortex of rats (*Zalups 1993*). Mercury vapour exposure in rats over the course of several days has also given an enhanced synthesis of metallothionein in the kidney (*Cherian and Clarkson 1976*).

A positive correlation exists between accumulation of some stress proteins and acute renal cell injury. The role of accumulation of glucose-regulated proteins and heat-shock proteins *in vivo*, prior to or concurrent with nephro-toxicity, remains to be completely understood. These stress proteins may be a part of a cellular defence in response to nephro-toxicants. It has been proposed that renal tubular cells that do not, or are unable to, express stress proteins are more vulnerable to toxic exposure.

5.2. Clinical nephro-toxicity

There is not much evidence that exposure to metallic mercury vapour causes gross kidney damage. Case histories of nephrotic syndrome, characterised by gross albuminuria and oedema following exposure to inorganic Hg and metallic Hg vapour are rare (*Kazantzis et al 1962, Gaultier et al 1968, Agner and Jans 1978*).

5.2.1. Mortality and cancer related to the urinary system

Studies have been done on mortality and cancer incidence in Swedish and Norwegian chlor-alkali workers exposed to inorganic Hg. The incidence and mortality rates were compared with the national averages for the general male population (Barregård *et al 1990, Ellingsen et al 1993*). The Swedish cohort came from 8 Swedish plants, and the exposed group consisted of 1,190 subjects. The Norwegian one came from 2 plants and consisted of 674 exposed men covering 16,170 person years. The average yearly Hg exposure levels as U-Hg were around 100 µg/l (≈77 µg/g) for the whole group in Sweden and 93 µg/l (≈71 µg/g) in Norway. No excess of specific cancers of the bladder or kidneys was found, neither incidence nor mortality. Furthermore, no excess mortality from non-malignant diseases in the kidneys or bladder was found.

5.2.2. Toxicity studies during current exposure in industrial situations

There have been several toxicity studies done. Some of the most important ones are summarised in Table 5.

Table 5. Summary of renal toxicity studies done in industrial situations

Reference	**Exposure U-Hg**		**U-β2-micro-globulin**	**U-Protein/ albumin**	**U-NAG[a]**	**U-NAG comments**	**Other kidney markers**
	µg/g	**years**					
Roels et al 1982	22	5	Neg.	(Pos.)			
Stonard et al 1983	67	8	Neg.	Neg.	Neg.		
Piikivi and Ruokonen 1989	18	13.7		Neg.	Neg.		
Barregård et al 1988	30	10		Neg.	(Neg.[b])	Pos. > 35 µg/g	
Langworth et al 1992	25.4	13.5	Neg.	Neg.	(Neg.[b])	Pos. > 45 µg/g	
Cardenas 1993	22		(Neg.? [c]) Inconclusive	Neg.	(Neg.[b])	Pos. > 50 µg/g	Positive for some other markers.
Ellingsen et al 2000	11	13.3	Neg.	Neg.	Pos.		

Exposures are given as current spot U-Hg at the time of the investigation unless otherwise indicated. Concentrations are given corrected for by U-creatinine or uncorrected µg/g means µg Hg/g creatinine.

[a] *U-NAG: Urinary N-acetyl-beta-D-glucosaminidase.*

[b] *No effect at low exposures.*

[c] *Negative association to Hg exposure, but probably a urinary pH phenomenon.*

In the studies referred to in the table the current average cohort exposure levels were in the range 11-67 µg/g. There was little evidence for gross kidney pathology as manifested by proteinuria or elevated serum creatinine. Antibodies to the glomerular basement membrane were generally not present. U-NAG seems to be a relatively sensitive indicator of exposure. The pathophysiological meaning of this indicator is, however, uncertain.

In one of the studies (*Cardenas et al 1993*) there was a significant increase of several other possible indicators of renal toxicity. However, most of these effects have not been reproduced in other studies with corresponding exposure levels.

5.2.3. Kidney toxicity studies of historical exposure in industrial groups

Few studies have been done on industrial workers with historical moderate to low occupational Hg exposure. The most important markers, including N-acetyl-beta-D-glucosaminidase (NAG), have been covered in three recent studies (*Ellingsen et al 1993, Frumkin et al 2001, Efskind et al 2006*).

Table 6. Chronic renal effects of historical Hg exposure

Reference	N (Cases /controls)	Mean current U-Hg	U-Hg yearly mean	Years under exposure	Effect
Ellingsen et al 1993	77/53	1.8 µg/g	107 µg/l (≈ 75 µg/g)	7.9; range 1.1-36.2	No
Frumkin et al 2001	147/132	2.8 µg/g	72 µg/l (≈ 55 µg/g)	Not given	No
Efskind et al 2006	49/49	3.1 µg/g	16.7 µg/g	13.1; range 2.8-34.5	No

Exposures are given as current spot U-Hg at the time of the investigation unless otherwise indicated. Concentrations are given either corrected for by U-creatinine or uncorrected. µg/g means µg Hg/g creatinine. If uncorrected values are given, an indication of what an corrected value might have been is given in brackets: (≈ µg/g).

The mean annual exposure levels during exposure, as measured by U-Hg, were 107 µg/l (≈ 75 µg/g), 72.1 µg/l (≈ 55.5 µg/g) and 16.7 µg/g. The average times since cessation of exposure were 12.3, 5.7 and 4.8 years respectively. No differences between the exposed and control groups were found on the markers studied.

The most recent of these studies of historical moderate to low exposure (*Efskind et al 2006*) included a sub-cohort of 41 formerly exposed and 40 reference individuals from the cohorts studied by *Ellingsen et al (2000).* While the levels of U-NAG were statistically significantly higher in the exposed group during exposure, they ended up 5 years after cessation of exposure, with a nearly identical mean level in U-NAG as compared with the corresponding controls. Both the previously exposed and the unexposed in this sub-cohort showed a statistically significant increase in U-NAG by ageing, which is expected, but hence, a significantly less increase by ageing in the formerly exposed sub-group. Thus, this study shows a full normalisation of the NAG excretion in urine after cessation of exposure, and no indication of a chronic co-effect between aging and Hg exposure.

5.3. Summary and discussion of renal toxicity

The review of the relevant literature shows that most markers of renal toxicity caused by metallic mercury vapour at low exposure levels (< 35 µg/g) are within the normal range, and not different from occupationally unexposed controls.

Some dose-effect and dose-response relationships have, however, been found. Especially those related to urinary N-acetyl-beta-D-glucosaminidase (U-NAG), where effects have been found even at very low exposure levels. Refined analytical methods and good epidemiological design have made such observations possible.

U-NAG shows considerable day to day variation within individuals. Hence, it has been proposed to sample and analyse urine over a period of several days and use the mean results (*Barregård et al 1988*). It has also been noted that storage time below – 20^{o} C also influences the measured concentration (*Ellingsen et al 1993*). These two sources of unaccounted-for variation could have affected the power of studies of U-NAG creating a "bias" towards the null hypothesis. The difference in the results summarised above could be explained by this. For example, no effect of Hg exposure was found by *Piikivi and Ruokonen (1989)* where an increased U-NAG in the exposed population was detected by *Ellingsen et al (2000)* even though the Hg exposure levels in those studies were similar.

In the Norwegian studies of present and past low exposure (*Ellingsen et al 2000, Efskind et al 2006*), taking the average of U-NAG from two consecutive days and doing the analysis of the urine samples at day no. 60 of each individual last sample, significantly improved the detection rate in those two studies.

In the latest study of historical exposure to metallic mercury vapour (*Efskind et al 2006*), U-NAG and also U-Alb showed an inverse co-variation with the selenium status. This was independent of any Hg exposure. Hence, the selenium status might confound this type of study. This is especially a risk in meta-studies since the selenium status might vary significantly between populations in different regions.

6. Immunotoxicity

6.1. General aspects

Mercury is responsible for hypersensitivity reactions. A toxic agent may induce immunological effects because:

- it binds to self-constituents (e.g. proteins) and triggers an anti-hapten (hapten is an incomplete antigen) immune response;
- hapten-modified auto-antigens may lead to a true auto-immune response directed against the auto-antigens alone;
- it disturbs the regulation of the immune system resulting in the appearance of more generalised auto-immune manifestations.

Mercury seems to involve all three mechanisms. Additionally, both cellular and humoral mechanisms are involved. All isotypes are affected, but the increase in total serum Immunoglobulin (IgE) is particularly prominent. Often B-cell (lymphocyte) hyperactivity is induced, at least in susceptible species or strains. Auto-reactive T-cells seem to play a crucial role in the induction of this B-cell hyperactivity (*Druet et al 1989*).

The immunological effects of inorganic mercury are mainly related to their interactions with sulfhydryl-containing residues. By breaking R⁻S⁻S⁻R bonds in proteins, the tertiary structure of proteins might be changed to such a degree that antigenicity is changed, making autologous proteins “foreign” and, hence, vulnerable to the attack by lymphocytes. Metallic Hg may induce cell-mediated immunity as in contact hypersensitivity.

The finding of Hg-specific lymphocytes in the blood of patients with symptoms of Hg intoxication indicates that Hg behaves as an independent antigenic determinant.

Furthermore, Hg might interfere with cellular functions as an immunotoxin, causing a dysfunction of the immune system. This would most often be expressed as immunosuppression. However, unspecific immunostimulation might also ensue (*Moszczynski 1999*).

6.2. Human epidemiology

Table 7. Summary of human epidemiology studies on immunotoxicity

Reference	Mean current exposure	Effect studied	Result
Roels et al 1982	95.5 µg/g	RF, MAG-agglutinator	No
Lauwerys et al 1983	56 µg/g	Anti-laminin	Yes
Bernard et al 1987	72 µg/g	Anti-laminin	No
Langworth et al 1992	25.4 µg/g	IgA, IgG, IgM, Anti-laminin, anti-GBM	No
Queiroz et al 1994	24.7 µg/g	IgA, IgG IgM	Yes?
Ellingsen et al 2000	16.6 µg/g (yearly mean)	Anti-GBM, ANA TNF, anti-MPO, anti-R3	No Yes
Ellingsen 2004 *(unpublished report)*	3.1 µg/g 4.8 years past exposure	TNF anti-MPO, anti-PR3	No Yes?/No?

Exposures are given as current spot U-Hg at the time of the investigation unless otherwise indicated. Concentrations are given corrected for by U-creatinine, (µg/g means µg Hg/g creatinine). Ig is an abbreviation for immunoglobulin. RF: Rheumatoid factor, MAG: myelin-associated glycoprotein, GBM: glomerular basement membrane, ANA: anti nuclear antibodies, TNF: tumor necrosis factor, MPO: myeloperoxidase; PR3: proteinase 3.

The results of the human epidemiological studies are equivocal for low or moderately low exposures to inorganic Hg. Studies have shown both elevated and reduced levels of immunoglobulins (elevated*: e.g. Queiroz et al 1994, Bencko et al 1990;* reduced*: Moszczynski et al 1990*).

For the auto-antibodies towards the glomeruli a possible threshold effect might be present. For other auto-antibodies (e.g. anti-MPO, anti-PR3) a reversible effect might be present. People still working in a chlor-alkali plant showed elevated levels of those two auto-antibodies. For those having left this plant a full recovery to normal levels has been seen. For anti-nuclear antibodies (ANA) no effect is shown at low exposure. For immune regulators like TNF a reversibility is shown after cessation of long term low exposure (*Ellingsen et al 2000, Ellingsen 2004 unpublished report*).

In a Polish cohort from the chlor-alkali industry no increased incidences of infections in the skin or of the respiratory and urinary tracts were found (*Moszczynski 1999*).

6.3. Summary and discussion

Animal experimental studies have shown that inorganic Hg has a marked capacity to influence the immune system. A problem in several epidemiological studies is confounding by other immuno-active exposures. Effects shown in some studies of workers in chlor-alkali plants, might be due to, or modified by, other chemical or physical confounders (with the capacity of modifying the immune or inflammatory response) which were also present in the working environment. Moderate or low exposures to inorganic Hg, however, do not seem to create adverse effects at a clinical level. The epidemiological studies performed to date, have failed to find conclusive evidence of increased mortality from infections or malignancies (where the immune system also serves as a guard).

7. Cardio-vascular and cerebro-vascular toxicity of Hg

7.1. General studies

An increased risk of cardio-vascular disease and death in Finland has been related to a high intake of organic Hg from freshwater fish (*Salonen et al 1995, Salonen et al 2000, Virtanen et al 2005*). This is the main source of Hg exposure in the general population. It has been postulated that this effect was due to increased lipid peroxidation counteracting the beneficial effects of n-3 fatty acids.

In a follow-up study, the coronary risk associated with organic Hg exposure was investigated further. The effect of Hg exposure, measured as the concentration in hair, and the potential cardio-protective effect of docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) were investigated. Men in the highest quintile of DHA and DPA concentrations and who had a low content of Hg in hair (<2.0 µg/g), had a 67% reduced risk of an acute coronary event compared with those in the lowest quintile with high (>2.0 µg/g) Hg in hair.

This finding was supported by another international epidemiological case control study done jointly in nine countries (*Guallar et al 2002*). Cumulative Hg exposure was measured as the toe-nail concentration, and the potential cardio-protective effect was measured as the concentration of DHA. The conclusion here was also that the risk of myocardial infarction was related to the body burden of mercury, and inversely related to the adipose tissue concentration of DHA.

This linking of coronary heart disease to organic Hg exposure has raised the question of similar risk attached to inorganic Hg. Hence, a mechanistic *in vitro* study was done investigating the effect of Hg on peroxidation of Low-Density Lipoprotein (LDL) (*Seppänen et al 2004*).

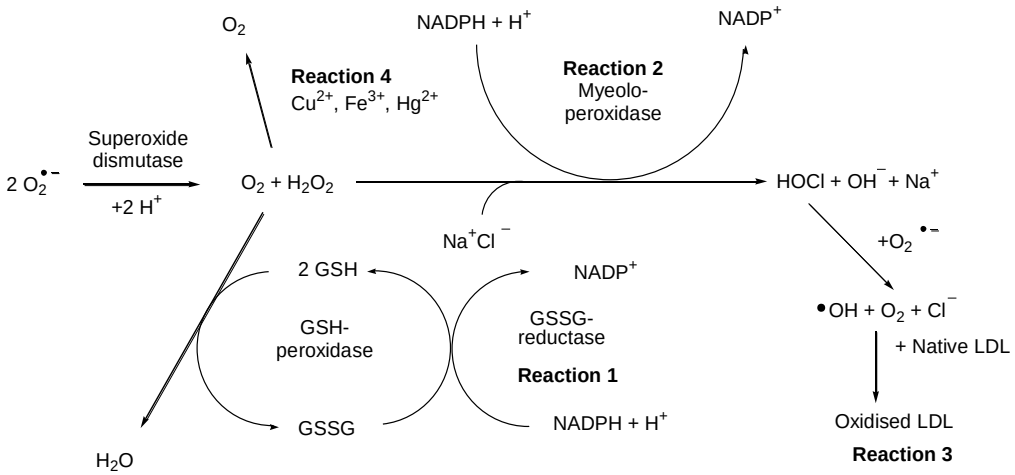

Figure 4. Generation and quenching of radicals in peroxidation (After Seppänen et al 2004). GSH: glutathione, GSSG: glutathione disulphide, NADP+: nicotinamide adenine dinucleotide phosphate, NADPH: reduced nicotinamide adenine dinucleotide phosphate.

Nuclear magnetic resonance (NMR) experiments showed that inorganic Hg does not promote a direct non-enzymatic peroxidation of LDL. (Oxidised LDL is the lipoprotein carrying most of the atherogenic potential of the lipoproteins.) It does, however, in contrast to methyl mercury (MeHg), effectively inhibit the glutathione peroxidase (GSH-Px). It thus reduces the antioxidant protection against oxidative stress. However, the combined effect of additional simultaneous high exposure to iron and copper combined with a low selenium can lead to a condition in which inorganic Hg can promote lipid peroxidation.

(This *in vitro* experiment does not seem to provide any explanation of the epidemiological findings from organic mercury exposure arising from eating fresh-water fish. The authors

suggest, however, that transformation of MeHg to an inorganic form may provide the explanation.)

7.2. Human epidemiology (mortality studies) – Metallic mercury

The results of the epidemiological studies mentioned above have come from studies of exposure to organic Hg. The epidemiological studies on cardiovascular effects of exposure to metallic Hg are equivocal.

A study of the causes of mortality in 1,190 chlor-alkali workers in Sweden has been conducted. A borderline significant excess was found with a standardised mortality ratio (SMR) of 1.3 both for ischemic heart disease and cerebro-vascular disease when allowing for a latency time of 10 years. The average U-Hg levels, according to the authors, would probably be around 100 µg/l (≈75 µg/g). This was based on approximate levels of 200 µg/l in the 1950s, 150 µg/l in the 1960s and less than 50 µg/l in the 1980s (*Barregård et al 1990*).

The smoking habits of individuals within this cohort were not known. It was assumed that they were similar to those of the general Swedish population at the time. A sample of 81 men, alive in 1984 and born between 1900 and 1939, showed an equal prevalence of current smoking (30%) as the then general population. Estimations by the authors showed that a 5% excess in the prevalence of smokers would not distort the statistical significance.

A parallel study of 674 chlor-alkali workers, employed before 1980, was done in Norway (*Ellingsen et al 1993*). This group had an average exposure level of U-Hg of 100 µg/l (≈75 µg/g). No excess of mortality, (from vascular, cardiac or CNS (central nervous system) causes) was seen in the study group compared to the general population.

The standardised mortality ratios (SMRs) were around unity (generally slightly below 1.0), either when accounting for time under exposure or different latency times. These results do not indicate an increased cardio-toxic or vascular risk. The prevalence of current smoking in that cohort was 56%, some 15-20% higher than in the general population. This was accounted for in the analysis.

A study of causes of mortality in an American cohort of 2,133 subjects exposed to metallic mercury showed that there was no effect on cerebro-vascular deaths even in the highest exposed sub-cohort of 858 workers. This latter group had an exposure measured as U-Hg exceeding 300 µg/l (≈230 µg/g) (*Cragle et al 1984*). Neither was there any excess for cardio-vascular diseases in this cohort (*personal communication from D. Cragle to Barregård 1990*).

7.3. Summary and discussion

A relationship appears to have been established between cardio-vascular mortality and exposure to organic Hg (MeHg) (as measured by the Hg content in hair or nails).

There is a possible increased risk of acute coronary events, coronary heart disease (CHD) and cardio-vascular disease. However, the results of some of the studies are equivocal.

It is of interest that one study (*Yoshizawa 2002*) showed no such effect on CHD in the total cohort. However, in the sub-group excluding occupational exposure to metallic Hg vapour (dentists), a weak relationship could not be excluded.

The discrepancy between two of the mortality studies referred to above (*Barregård et al 1990, Ellingsen et al 1993*), might be explained by the co-effects of Hg with other trace metals. Selenium is known to protect the blood lipids, such as LDL, from being oxidised.

The Norwegian population is expected to have a higher level of body selenium compared to the Swedish. This is because of different levels of selenium in corn. Swedish people consume mainly European corn which is considered low in selenium. Norway imports a substantial amount of corn from overseas which is richer in selenium.

It should also be noted that, if the prevalence of the smoking habits in the Norwegian cohort in relation to the general population is valid also for the Swedish chlor-alkali workers, then the likelihood of smoking confounding the Swedish study might not be negligible.

8. Mutagenicity and carcinogenicity of exposure to inorganic Hg

8.1. General toxicology

The different stages in the carcinogenic process are initiation, promotion, progression and metastasis. The carcinogenic potential of metals can be expressed in one of two possible ways on this process; either directly or indirectly. The former involves the direct formation of a DNA adduct by the metal. The indirect mechanisms include modifications in DNA repair (not relevant for Hg) and DNA methylation status. Other indirect mechanisms may affect the DNA-replication and expression processes. Reactive intermediates from lipid peroxidation or redox reactions can oxidise DNA or form DNA adducts.

Chronic low-level exposure to Hg^{++} that is not cytotoxic in mammalian cell studies in intact human KB cells and in Chinese hamster ovary cells, might give an indication of possible carcinogenic risk. The studies showed a dose-dependent binding of Hg to DNA. In cultured human whole blood, inorganic Hg induced micronuclei and sister chromatid exchanges in lymphocytes. Evidence of Hg carcinogenicity in humans is, however, inadequate (*Madden 2003*).

Toxic and carcinogenic metals are capable of interacting with nuclear proteins and DNA, causing site-specific damage. The “direct” damage may involve conformational changes to bio-molecules by the metal. On the other hand, “indirect” damage is a consequence of metal-driven formation of reactive oxygen species. This can involve the superoxide and hydroxyl radicals, nitric oxide, hydrogen peroxide and/or other endogenous oxidants. Certain carcinogenic metals, including cadmium (Cd), arsenic (As) and nickel (Ni), are known to inhibit DNA repair mechanisms. The major oxidative effects in DNA include base modification (e.g.chromium (Cr), Ni), cross-linking (e.g. iron (Fe) in combination with an oxidant, copper (Cu) in combination with an oxidant and Ni), strand scission (e.g. Cr in combination with an oxidant, Cd and Ni), and de-purination (e.g. Cr, Cu, Ni) (*Valko et al 2005*).

Studies with Hg, Cd and Ni, revealed that the primary route for their toxicity is depletion of glutathione and bonding to the sulfhydryl groups of proteins. Various antioxidants, both enzymatic and non-enzymatic, provide protection against free radical attacks mediated by metals. Some of the most effective antioxidants are thiol compounds, especially glutathione (GSH). These provide significant protection by trapping radicals, reducing peroxides and maintaining the redox state of the cells (*Valko et al 2005*). The availability of the selenium-dependent GSH-Px is an important factor for maintaining the net antioxidant capacity of the GSH redox cycle. The amount of biologically available Se is an important factor in counteracting oxidative stress and, hence, this aspect of Hg toxicity (*Gordana 1998*).

The non-enzymatic antioxidant vitamin E can prevent the majority of metal-mediated damage, both *in vitro* and *in vivo*, by preventing lipid peroxidation. This is also the case *in vivo* for ascorbic acid (vitamin C).

Zinc (Zn) counteracts oxidative stress by protecting sulfhydryl groups of proteins and enzymes against oxidation. It also prevents hydroxyl radical formation from H_2O_2 through the prevention of free radical formation (antagonism of redox active metals). Zn also interacts with components of the immune system and is an essential trace element for immune function (*Rink 2000*).

Oxidative stress as a consequence of Hg exposure was studied in a “highly exposed” cohort (*Chen 2005*). In this study DNA-damage was measured by determining urinary 8-hydroxy-2’-deoxyguanosine (8-OHdG). The antioxidant status was evaluated by measuring the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), the total concentration of reduced glutathione (GSH) and protein-bound thiols in serum.

The exposed group consisted of 13 environmentally exposed residents and 35 occupationally exposed workers of both sexes. Some of them showed signs and symptoms of Hg toxicity. The reference group consisted of 35 age- and gender-matched healthy residents from a non-Hg-contaminated area of Beijing.

The mean U-Hg in the whole exposed group was 76.5 µg/g with a range from 8.2 to 293 µg/g. The mean, for the occupationally exposed sub-group, was104 µg/g. The mean serum concentration (S-Hg) for the whole group was 38.5 µg/l (60.8 µg/l in the occupationally exposed sub-group). The non-exposed had a mean U-Hg of 0.95 µg/g and an S-Hg of 0.91 µg/l.

The urinary 8-OHdG was 243 µg/g compared with 2.08 in the controls. Also the antioxidants (SOD, GSH-Px, GSH and thiols) were significantly increased in the exposed group when compared to the reference population. The relative increase was especially marked for SOD. The concentrations of these determinands were significantly correlated with the U-Hg level. The authors concluded that oxidative stress could be involved in Hg-induced cytotoxicity in humans *in vivo*. They also considered that antioxidative functions could be activated to protect the functions of the human body.

The pre-exposure levels of antioxidants within the body should protect against such oxidative DNA damage, at least to a certain degree. Only when such mechanisms are saturated or overwhelmed, is damage likely to occur. Thus, the relevance of this study for a low exposure situation can be disputed.

8.2. Clinical mutagenicity and carcinogenicity

In cancer incidence and mortality studies of chlor-alkali workers exposed to inorganic Hg in Sweden and Norway (*Cragle et al 1984, Barregård et al 1990, Ellingsen et al 1993*) no over-all significant excess of malignancies was found. This was especially the case for the brain and the kidneys. However, in both studies there was an excess of lung cancer. This was statistically significant in the Swedish cohort with a standardised incidence rate of 2.0. It was borderline in the Norwegian cohort with an incidence rate of 1.66.

The Swedish cohort came from 8 plants and consisted of 1,190 subjects. Of these, 457 had a possible, mostly low, exposure to asbestos. Asbestos might have been a causal factor in the Norwegian cohort as well. Smoking might also have been a further potential confounder in both studies. In the Norwegian study where smoking habits were, to a large extent, known from the medical records (in contrast to the Swedish study), smoking was more prevalent in the cohort (64%) than in the general Norwegian population (48% in 1975 and 39% in 1986).

The exposure levels of the Swedish workers had declined with time. This was reflected by the fall of U-Hg from about 200 µg/l (≈ 140 µg/g) in the 1950s to about 50 µg/l (≈ 35 µg/g) at the time the study was completed, three decades later. The parallel study in Norway, also of mortality and cancer incidence, was done in a cohort of 674 workers who were exposed for an average of 9.6 years at 2 chlor-alkali plants. Their mean U-Hg during the years of exposure based upon more than 20,000 urine samples throughout their employment under exposure, was 465 nmol/l (= 93 µg/l or ≈ 65 µg/g).

Finally, a Polish study of workers exposed to metallic mercury vapour for nearly 20 years has shown no increased incidence of malignant neoplasms (*Moszczynski 1999*).

8.3. Summary and discussion

In contrast to other metals investigated with respect to mutagenic or carcinogenic potential, the influence of Hg seems to rest upon metal-induced oxidative stress (*Valko et al 2005*). This is an indirect effect through depletion of available reduced glutathione (GSH) which is an important antioxidant. The level of other available antioxidants might also be of importance in this respect.

If a mutagenic or carcinogenic potential of Hg in humans is present, a threshold effect is hypothetically possible. This is due to the fact that Hg binds easily to available thiols, for example those in macromolecules outside the cell.

The evidence for a mutagenic or carcinogenic potential of Hg in both animal and epidemiology studies, is equivocal. It is so far totally lacking in humans at low exposure concentrations (e.g. <50 µg/g).

9. Reproduction toxicity

9.1. General Hg reproduction toxicity

Heavy metals are not normally transferred to the foetus. This has been demonstrated experimentally in pregnant mice. After injection of high doses of inorganic Hg, significant accumulation occurred in the placenta. However, the accumulation was much less in the foetus. Such experiments show that the placental membrane constitutes a barrier against the penetration of mercuric Hg into the foetus (*Berlin and Ullberg 1963, Yang et al 1996*).

It has, however, also been shown that mercury does penetrate the placental barrier and accumulates in the foetus when the mother is exposed to metallic Hg vapour. In animal studies, Hg levels in the foetus were 10-40 times higher than in animals exposed to the equivalent amount of mercuric Hg. The uptake increases with gestational age and the pattern of distribution changes as well. The most pronounced uptake was in the foetal liver and heart. In pregnant female guinea pigs exposed to 200-300 µg/m^3 Hg vapour per day in late gestation until birth, the Hg concentration in foetal liver was twice as high as in the maternal liver. For all other organs the foetal Hg concentrations were lower than the maternal ones (*Yoshida 2002*).

Even though there are inter-species differences, limited epidemiological studies in humans show that there is a transfer from mother to foetus during Hg vapour exposure (*WHO 1991, Drasch 1994, Yang et al 1996*).

Hg vapour passing through the placental barrier is oxidised in the foetal liver and accumulates in that organ. In foetal liver, the content of metallothionein (MT) is high and associated with zinc and copper. MT is a low molecular weight protein with a high cysteine content. It has an important role in the transport and storage of essential metals (zinc, copper).

No Hg was detected in the CNS of pups after exposure to Hg vapour at 50 µg/m^3, but after an exposure of 500 µg/m^3, Hg was detected in the blood vessels and sensory ganglia. The CNS Hg concentration increased during the postpartum period, in parallel to the reduction of the Hg bound to metallothionein (MT) in the liver. This increase might have an adverse effect on developing neonates (*Yoshida 2002*).

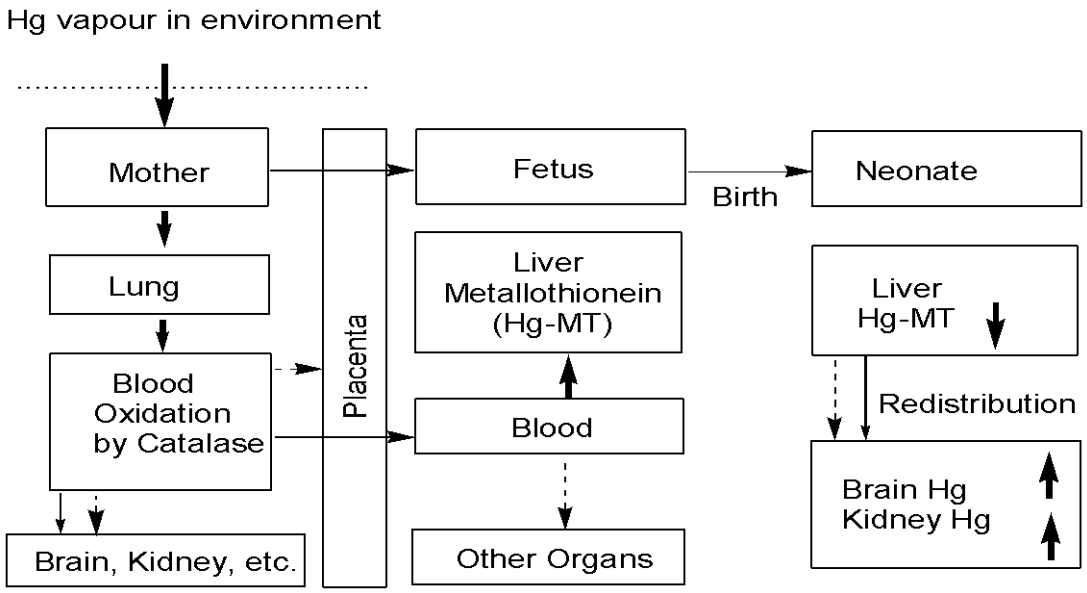

Figure 5. Schematic representation of the transport, distribution and oxidation of inhaled mercury vapour in pregnant animals. Dotted arrows represent inorganic Hg. Arrows otherwise represent metallic Hg vapour. (After Yoshida 2002.)

Foetal liver MT concentration, which is much higher than in the maternal liver, decreases during postnatal development. The bulk of the Hg in the maternal liver is, however, bound to high molecular weight proteins. More than 50% of the foetal burden of Hg is bound to the low molecular weight MT. There is a postpartum redistribution of the Hg in the foetus from the liver to, among others, the brain due to a progressively diminished content of hepatic MT. This is an indication that MT plays a role in preventing further distribution of Hg within the foetus after *in utero* exposure to Hg vapour.

Any agent preventing the oxidation of Hg vapour in the mother will increase the foetal uptake. Thus, maternal alcohol consumption during Hg vapour exposure will increase the foetal Hg burden.

Metallothionein (MT) is also reported to be present in the human and rodent placenta where it is localised in the trophoblasts. Studies in mice have shown that the foetal Hg levels were significantly higher in placental MT-deficient strain mothers where Hg was associated with high molecular weight placental proteins, than those in foetuses from wild strain mothers. Hence, placental MT seems to play a role in preventing maternal-to-foetal transfer of Hg (Yoshida 2002).

In studies of foetal development, no increased incidence of gross malformations was seen in rats after maternal exposure to mercury vapour at 100 µg/m^3. However, acute exposure at 500 µg/m^3 gave an increased number of resorptions. Chronic exposure at the same level also gave cranial defects. In a teratological study in rats, embryo toxicity and teratogenic effects were not observed at 50 µg/m^3, but were evident at 1000 µg/m^3 when this was maintained during the entire gestation or organogenesis period (*Steffek et al 1987*).

9.2. Clinical toxicity in humans

9.2.1. Fertility

Male fertility was investigated in a mercury-exposed industrial cohort from a zinc-mercury amalgam factory, a chlor-alkali plant, and plants manufacturing electrical equipment (N=51) (*Lauwerys et al 1985*). There were no statistically significant differences in any age group in the numbers of children between the exposed and reference groups. Potential confounding factors appear to have been properly accounted for in this study.

The average U-Hg was 52.4 µg/g in the exposed group, the median was 36.9 µg/g and the 95th percentile was 147 µg/g. The controls had an average U-Hg of 0.9 µg/g and all measurements were below 5 µg/g.

9.2.2. Spontaneous abortions in industrial workers exposed to mercury

The incidence of spontaneous abortions has been studied both in female industrial workers (*de Rosis et al 1985*) and in the wives of male industrial workers exposed to inorganic Hg (*Cordier et al 1991*).

The exposed women did not have a higher rate of abortions compared to the control group, while the wives of the exposed male workers did. However, the excess in the latter case was not clearly statistically significant. The average Hg air concentration to which the group of female workers was exposed was approximately 10 µg/m^3.

In the study where the men were exposed, three levels of exposure were identified on the basis of average U-Hg concentrations. These were 1-19 µg/l (“low”), 20-49 µg/l (“medium”), and 50+ µg/l (“high”) respectively.

The overall crude ratio of abortions per 100 pregnancies amongst the wives of exposed men was 7.6 compared to 9.6 in the control group. The ratios for the three separate sub-groups based on exposure levels (low, medium and high) were 4.5, 13.2 and 18.4 abortions per 100 pregnancies respectively. The national average ratio was 9. The authors considered that the excess observed in the “high” sub-group was statistically significant (borderline) based on a

stratified statistical analysis. (The exposure giving U-Hg above 50 µg/l was approximately equivalent to 40 µg Hg/g creatinine).

9.2.3. Spontaneous abortions in dental health personnel

Spontaneous abortion rates of women referred to hospital have also been studied using records from public registers for the years 1980-1981 (*Ericson and Källén 1989*). Spontaneous abortions were identified from a Hospital Discharge Registry. No excess risk was attached to women working in the exposed occupations (dental health). In such a study there is no possibility for a knowledge and/or recall bias.

For the years 1964 -1965 an historic prospective study was performed in the third largest city in Sweden covering 78 people in dental occupations (13 dentists and 65 dental assistants). In this study group, 7 spontaneous abortions were observed while 6.0 were expected calculated from all women with a gainful occupation, standardised for maternal age and parity. The observed rate was not statistically different from the expected. The study is small but is free of any recall and knowledge biases. The authors conclude that these dental health workers who were exposed to metallic Hg vapour showed no indication of any reproduction hazard (*Ericson and Källén 1989*).

9.2.4. Perinatal harmful events of mercury

Pregnancy outcome in dental health professionals has been studied using data from public registers (*Ericson and Källén 1989*). Its main source was the Swedish birth registry, and 8,157 infants born to dentists, dental assistants and dental technicians were identified. The outcome variables studied were perinatal death, low birth weight and malformations. Comparisons were made against all births registered in Sweden during the same period (1982-1986). Except for a significantly low perinatal death rate in the exposed population, no statistical deviations were observed. The strength of this study is that it is unbiased with respect to recollection, knowledge and self-selection.

There is one case report of an accidental long-term exposure of a woman during the first trimester which gave her a U-Hg (24 hour sample) of 230 µg/l (≈177 µg/g) when exposure was discovered and stopped. Subsequently U-Hg was reduced to 7.5 µg/l at the end of the second trimester, and then to 2.7 µg/l in the third. Ultrasound examinations during the pregnancy showed normal growth and development. At birth the weight was 3,515 g and the Apgar scores were 8 and 9 at 1 and 5 minutes. At birth the baby had a Hg content of 16.2 ng/g in hair; i.e. 3 orders of magnitude greater than normal (0.008 ng/g). The follow-up at 2 years of age showed a normal development with the child meeting all standard milestones. However, no formal psycho-developmental testing had been done (*Thorp et al 1992*).

9.3. Summary and discussion

In a summary article on reproduction toxicity of Hg exposure (*Gardella and Hill 2000*), the authors stated that only a few epidemiological studies have been performed and that these were mostly in the field of dentistry. The most reliable source in epidemiological research is good public register data. The information is free of recollection and selection bias.

As a whole, the limited data presently available provide no conclusive evidence for occupational exposure to mercury vapour being harmful to reproduction. There is no link to an increase in teratogenic or other adverse pregnancy outcomes. It seems there is little risk for dental health personnel working under the hygiene conditions practised in North Europe. Unfortunately good exposure data are not available.

For the industrial cohorts investigated, exposure data are available, but these are often not complete throughout the exposure periods. The studies referred to in this report, show no negative effects attributable to inorganic (metallic) Hg exposure giving a U-Hg less than 50 µg/l (≈ 40 µg/g).

10. Endocrine toxicity

10.1. Hg-related basal endocrine toxicology

It is known that mercury accumulates to a large extent in the thyroid gland (*Kosta et al 1975*). Hence, it might be a target for Hg toxicity. Furthermore, type 1-iodothyronine deiodinase (ID-I) is a seleno-enzyme. This enzyme converts thyroxine (T_4) into 3-iodothyronine (T_3) (the active form of the thyroid hormone) or to reversed 3-iodothyronine (rT_3) (an inactive isomer of T_3). It also inactivates these hormones by further de-iodinisation.

It is further known that Hg binds selenium (Se) in a 1 to1 relationship forming complexes, and thereby reduces the bio-availability of Se. Furthermore, the de-iodination of the thyroid hormones takes place in the liver and kidneys. The tissues of these organs accumulate a substantial amount of Hg. In the kidneys the proximal tubuli are the principal target for Hg toxicity. Also, it is in the S3 segment of the proximal tubuli that ID-I is expressed.

The rT_3 is produced when the organism has a sufficient level of T_3 in the body to maintain a normal level of metabolism. It also plays a significant role in the regulation of body temperature. More T_4 is converted to rT_3 when the body temperature is (endogenously) down-regulated.

Hg also accumulates in the pituitary gland. It is found intracellularly in the lisosomes and granules of the somatotrophs, thyrotrophs and corticotrophs. Except for vacuolation of lysosomes, no structural damage was seen in the cells containing Hg. (*Møller-Madsen. et al 1986*)

10.2. Human epidemiology

10.2.1. Thyroid

In a study on the influence of Hg exposure on thyroid function at a chlor-alkali plant, 47 exposed workers were compared with 47 individually matched unexposed controls from the same industrial complex. The exposed group had a current U-Hg of 5.9 nmol/mmol (≈10.5 µg/g) and a yearly average over the time of exposure of 9.0 nmol/mmol (≈16.2 µg/g) (*Ellingsen et al 2000*).

The exposed workers had a significantly higher concentration of reversed 3-iodothyronine (rT_3), the inactive form of the thyroid hormone, in serum than the reference population (268 pmol/l compared to 232 pmol/l). There was a significant association of rT_3 with the “cumulative intensity of Hg-exposure” (Cum. U-Hg/total years of exposure = Cum. Hg $year^{1}$). There was also a significant negative association between the active hormone (T_3) and the cumulative exposure. However, there was no significant difference when comparing the exposed group with the reference group.

The ratio of free T_4: free T_3 was higher in the exposed group than in the controls (with borderline statistical significance). In the regression analysis, this ratio was also almost significantly associated with both Cum. U-Hg and Cum. Hg $year^{-1}$. The sub-group (33% of the exposed cohort) with the highest Cum. Hg $year^{-1}$ of 12.6 nmol/mmol (=22.7 µg/g) had, however, a significantly higher ratio than their individually age-matched controls. These increases were most pronounced in those exposed subjects with a low iodine content in urine (U-I). U-Se levels did not influence any of the effect measures in the statistical analysis.

A follow up study at the same plant was conducted five years later when the Hg exposure had ceased. At that time, the mean concentrations of the most clinically relevant thyroid parameters TSH, free T_3, and free T_4 were of the same magnitude in the two groups under study. The mean ratio free T_4: free T_3 was identical in the two groups. The annual mean individual exposure (expressed in terms of the urinary mercury concentration, U-Hg) was calculated to be 9.3 nmol/mmol creatinine/year (≈ 6.7 µg/g/year). The range of individual results was from 4.0 to

25.4 nmol/mmol creatinine. The average duration of exposure had been 13.1 years (range 2.8 - 34.5 years).

The geometric mean concentration of rT_3, which is of no clinical significance because it is biologically inactive remained significantly higher in the previously exposed workers (*Ellingsen 2008 in press*).

10.2.2. Pituitary gland

The effects of Hg exposure on the pituitary gland have also been investigated.

Erfurth et al 1990 investigated the basal serum concentrations of the pituitary hormones thyrotropin (TSH), prolactin (PRL), follicle stimulating hormone (FSH) and luteinising hormone (LH), and also of cortisol in 11 male workers and nine male dentists. They also studied the release of these hormones after administration of thyrotropin and gonadotropin releasing hormones. These effects were compared with age matched controls. The mean exposures as U-Hg were 26 nmol/mmol (49 µg/g) for the workers and 1.3 nmol/mmol (2.3 µg/g) for the dentists.

No differences were found between exposed and reference populations at a group level. Neither were there any associations between the Hg exposure and the free thyroid hormones T_4 and T_3. Considering the individual U-Hg values for the whole cohort, an association was found for the serum PRL concentration in a regression analysis of borderline ($p = 0.06$) statistical significance when age and plasma Se were taken into account. Looking at the graphical representation of their data, this effect seem to rest upon three observations > 25 nmol/mmol (45 µg/g)

McGregor and Mason (*1991*) investigated the pituitary-testicular axis as well as TSH and PRL in a rather highly Hg-exposed group of 29 workers. A potentially Hg exposed sub-group (U-Hg <3 nmol/mmol) was also included. They were compared with an unexposed referent group of 63 males of similar socio-economic status. The sub-groups differed significantly in age distribution and were accounted for in the statistical analysis. The authors concluded that there were no subclinical effects on thyroid, testicular, pituitary function. There was no relation between the measures of exposure (B-Hg, U-Hg or duration of exposure) and the free testosterone index (Ftel%), sex-hormone binding globulin (SBHG) (except for years of exposure which was statistically significantly associated in regression analysis), FSH and LH, nor for PRL and TSH.

10.3. Summary and discussion

The few studies of thyroid function have shown some different results. *Barregård et al* (1994) found an increased free T_4: free T_3 ratio and increased free T_4. The increase in free T_4: free T_3 ratio is compatible with the results of *Ellingsen et al* (2000). Two other smaller occupational studies did not find any effects on the pituitary-thyroid axis (*Erfurth et al 1990; McGregor and Mason 1991*).

People with a low iodine status (as measured by U-I) who are exposed to inorganic Hg, even at relatively low average exposure over the years, may be at risk of disturbances in their thyroid function. The results from the two studies discussed above, show that the small (but statistically significant) changes in the active thyroid hormones are reversible after cessation of exposure.

However, increased concentrations (group means) of the biologically inactive rT_3 (a biomarker that has not been studied by other investigators) as compared with the controls seem to be more persistent. The post-exposure (4.8 years) concentration of rT_3 was, however, not associated with any of the measures of exposure (e.g. Cum. U-Hg, Cum. Hg. $year^{-1}$ or Current U-Hg). They had U-Hg levels above 40 nmol/mmol (≈ 72 µg/g). Two sub-groups were identified: the long-term exposed (> 5 years, n = 11) and the short-term exposed (< 5 years, n = 18).

There is also no clear influence on the pituitary and male sex-hormone functions by a moderately low Hg exposure.

11. Conclusion

In studying health aspects of occupational exposure to chemicals, there are principally three methods applicable: *in vitro* cellular studies, animal toxicity experiments and human epidemiology. The *in vitro* studies elucidate cellular mechanisms and effects. The animal toxicity studies investigate mechanisms and effects in whole organisms under controlled conditions of exposure/dosage in a model which, hopefully, is relevant for humans.

Human epidemiology can provide information on the association of exposure to a specific substance and its effects in a realistic exposure situation. It may also improve the relevance for humans of the results from animal toxicology. The weakness of the non-human studies on their own relates to the question of their relevance to humans. The weaknesses of stand alone human epidemiology relate to issues about causation and contra co-variation. They also often suffer from weak documentation and understanding of the relevant magnitude of exposure, whether it be acute or chronic. Thus, there is a potential lack of understanding of the accurate dose-effect/response relationships.

A prerequisite for valid epidemiology studies is the careful selection of a reference population. Ideally these controls should not differ from the study population. If they do, it should only be by chance (random difference). Hence, the reference population should not be recruited from a systematically different background pool. Wherever possible it should come from the same "statistical universe" as the study population. An accurate description of the study and the reference populations should be presented together with a description of the selection methods and processes for both groups. Only by understanding these descriptions, can bias and confounding factors be evaluated and understood. While confounding factors may be eliminated or sufficiently controlled for by statistical techniques, bias in a study can never be fully accounted for this way. At best it can only be qualitatively evaluated.

Mercury is a toxic metal. It is bio-persistent with a half life in the kidney of around 60-70 days and, hence, has an elimination time of around one year in that organ. The half life in the brain is considerably longer, probably by at least one order of magnitude. It has a corresponding elimination time of several years (or even decades) in the brain.

With the exception of the urinary excretion of N-acetyl-beta-D-glucosaminidase (NAG) from the proximal tubular cells of the kidney, it seems from this review of the scientific literature that effects on the central nervous system are the most sensitive indicator of Hg toxicity. Some tests appeared to show signs of neurotoxicity at group average levels below 35 µg Hg/g creatinine. However, effects at group-average yearly exposures at the level of 35 µg/g, seems to be fully reversible (some years) after cessation of exposure.

It should be noted that many of the studies use current exposure, as measured by a spot sample (or current group average exposure) at the time of the study as the measure of exposure. With a wide range of actual exposures, many of the effects seen might be attributable to the people with the highest individual exposures. Historical exposure might also have been substantially higher than the current levels at the time of the studies. In such situations the dose-effect or dose-response relationships are (falsely) shifted towards a lower exposure range.

The most sensitive tests of the central nervous system function were the ones measuring the colour vision accuracy. These tests also indicated that a full recovery could occur, even under reduced but ongoing exposure. This was at group-average levels of 10 µg Hg/g creatinine, and occurred relatively soon (within 12 months) after occupational hygiene intervention. Such a threshold level of effect and its full reversibility will hopefully be tested and confirmed in future studies.

The effect of mercury exposure on the proximal tubular cells, as measured by U-NAG, is fully reversible some years after cessation of exposure. Whether the increased excretion of NAG under exposure should be considered a toxic effect or just a concomitant to the excretion of Hg

from these cells at the exposure levels discussed remains an open question. Further studies of this kinetic relationship would be beneficial.

Gaps in current knowledge still remain even though many studies have been done on the three main avenues to obtain toxicological knowledge (*in vitro* studies, animal studies and human epidemiology). Which of these data gaps are addressed will depend on the perspectives of the various research groups, with their different priorities. From a health perspective and with a view to setting exposure limits, not all changes in any particular indicator will be of equal importance. Hence, both qualitative and quantitative considerations have to be taken into account.

It is considered that the toxic effects from high level exposure are sufficiently well known. Sufficient knowledge for defining reasonably tenable occupational exposure limits also seems to be in place. This is based on recent epidemiological studies on cohorts under current or historical low level exposures.

The main gap in current toxicological knowledge is the lack of good epidemiological data on reversibility of long-term exposure (> 10 years) at levels in the range 20 - 55 µg Hg/g creatinine (average ≈ 25 µg/g). Furthermore the kinetics of NAG related to those of Hg in the kidney need to be better understood. This is particularly important as (some) regulators have placed considerable importance on changes in this parameter of the kidney. Sub-clinical disturbances of colour vision have not been accorded similar significance. One study shows full reversibility during ongoing exposure when the average urine level was reduced from 50 µg/g to 10 µg/g. Confirmation of this reversibility is needed.

The conclusion of the author of this review, putting a particular emphasis on the latest *Ellingsen et al* studies (2000, 2001 and 2006) encompassing the magnitude of reversibility after cessation or reduction of exposure, there are reasons to support a NOAEL (no adverse effect level) of 30 µg Hg/g creatinine.

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Other Euro chlor Science publications

Focus on Chlorine Science

Electromagnetic fields in chlor-alkali production - Health effects and regulation – January 2009
Life Cycle Assessment – November 2006
Risk assessment and cycling of natural organochlorines – May 2006
Environmental fate and impact of chlorinated solvents – December 2005
Chlorination by-products – November 2005

Key Science Information Sheets (KSIS)

Bioaccumulation – January 2005
Effluent testing: a review of current status – November 2004
Abbreviations used in (eco)toxicology – September 2004
Effluent testing with cell-based in vitro bioassays: use and limitations – July 2004
Marine Risk Assessment – October 2003
POPs & PBTs – February 2003
Naturally-occurring organochlorines – January 2003
Dioxins – April 2002
Water chlorination – February 2002
Children and exposure to highly chlorinated chemicals – August 2000

Science literature reviews

Bio-dehalogenation: Between 2001 and 2005, Professor James Field (University of Arizona, USA) provided Euro Chlor with quarterly updates of published scientific literature reporting on microbial (de)halogenations of key chlorinated compounds. There are 15 issues.

Natural organohalogens: From 1995 to 2002 Professor Gordon Gribble (Dartmouth College, Hanover, New Hampshire, USA) provided Euro Chlor with a series of periodic updates to the natural halogen literature, focusing on organochlorine compounds. There are 18 issues.

Marine Risk Assessments

1,2-Dichloroethane
1,1,2-Trichloroethane
Tetrachloroethylene
Trichloroethylene
Chloroform
Carbon tetrachloride
Dichloromethane
Hexachlorobenzene
Hexachlorobutadiene
1,2,4-Trichlorobenzene
1,1,1-Trichloroethane
Vinyl chloride
Monochloromethane
1,2-Dichlorobenzene
1,4-Dichlorobenzene
Mercury
Monochlorophenols (2-, 3- and 4-)
PCBs, DDT and dioxin
Pentachlorophenol
1,1-Dichloroethylene
Monochlorobenzene

All the above and more are available on Chlorine Online at

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