



**The Dental Material Commission —
Care and Consideration**

Mercury in dental-filling materials — an updated risk analysis in environmental medical terms

Maths Berlin

An overview of scientific literature published in
1997–2002 and current knowledge

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The Dental Material Commission — Care and Consideration

'The Dental Material Commission — Care and Consideration' assigned Maths Berlin, in autumn 2002, to report on the past five years' research literature on amalgam and the health hazards, if any, of mercury.

Maths Berlin is a Professor Emeritus with long experience of the effects of mercury on animals and humans. He chaired the WHO Task Group on Environmental Health Criteria for Inorganic Mercury (WHO Environmental Health Criteria 118, 1991) and a similar group with the function of drawing up health criteria for methylmercury.

Professor Berlin compiled the environmental medicine risk analysis of mercury and amalgam issued by the Swedish Council for Planning and Coordination of Research (FRN) in 1998 (FRN, Report 1998:22). This risk analysis was based on literature published between 1993 and November 1997. The present risk analysis builds further on this material, and analyses literature published between November 1997 and November 2002.

Available on order from:

*The Dental Material Commission — Care and Consideration
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www.dentalmaterial.gov.se*

1. Background

In April 2002 the Swedish Government appointed a Special Investigator to propose measures to boost knowledge of health problems relating to amalgam and other dental materials, and to improve care of patients who associate their symptoms with such materials. The directives for the Commission emphasise that the Special Investigator should assess the knowledge situation with respect to such health problems and pinpoint areas on which further studies should focus. The Investigator was also assigned to report on key research in recent years, focusing on the past five-year period.

The author was assigned by the Investigator to summarise and evaluate research findings, regarding the environmental medical aspects of exposure to mercury from amalgam, that were published during the period from November 1997 to November 2002. The summary is to continue and supplement the risk analysis that was carried out for the Swedish Council for Planning and Coordination of Research in 1997.

1.1 Data collection

The task of collecting relevant publications was conducted according to the same principles as in 1997. A Medline search for 'mercury' yielded 3,600 references. From these, 936 references of conceivable relevance were selected. After abstracts and summaries had been studied, just over 700 references remained to be read and assessed, and this activity generated an additional number of secondary references of importance to the assessment.

Jointly with the Swedish Research Council, the Commission held a seminar to which Swedish mercury researchers were invited¹. These were briefed on the key features of the past five years' research findings and my assessment of the same. The results were discussed, and an opportunity for commenting on the presentation and proposing additions was provided. A preliminary report was then drawn up and dispatched, along with a request for written comments. Based on the opinions received, this report was completed and submitted to the Special Investigator.

The present report starts by summarising the results from the 1997 risk analysis (FRN Report 98:22). An account of the new research findings follows. Finally,

¹ The seminar, at Lastberget near Stockholm on 6 February 2003, was attended by Maths Berlin (rapporteur), Gunnar Bergenholtz (moderator), Göran Möller, Per Hultman, Marie Vahter, Lars Friberg, Karin Warfvinge, Jan Marcusson, Staffan Scherfving, Gunnar Nordberg, Mats Hanson, Ulf Lindh, Jan Ekstrand, Sven Langworth and Per Dalén. Certain members of the Dental Material Commission were also present: Helena Starup (chairman), Mariana Blixt, Bo Jordin, Christer Malmström, Lars Sjödin, Bengt Järholm (expert), and Ann-Marie Lidmark and Ann-Kristin Myrman (secretaries).

these are summarised, along with an evaluation of the risks and hazards entailed by amalgam mercury and proposals on how to manage the same.

2. Summary of the 1997 risk analysis

In 1997, the Swedish Council for Planning and Coordination of Research was commissioned by the Swedish Government to review and extend knowledge of the health hazards, if any, of mercury from amalgam. I was then assigned to carry out a review of literature, in the form of published research findings, on the subject. This report was written as a continuation of the 1997 report.

In the 1997 risk analysis, it was found that:

- The WHO estimate of amalgam bearers' daily mercury uptake was 3–7 μg , which was the best estimate available at the time. This uptake gives rise to urinary mercury secretion of around 5 $\mu\text{g/g}$ creatinine. However, WHO found wide variation between individuals.
- In subsequent studies of amalgam bearers, uptake of up to 100 μg daily has been observed in extreme cases. The individuals concerned had urinary secretion of around 50 $\mu\text{g/g}$ creatinine. This secretion rate is as high as, or higher than, the lowest exposure shown to provoke clinically demonstrable symptoms in mercury-exposed workers.
- There are no scientific grounds for assuming that the prevalence of clinically demonstrable effects of mercury exposure from dental amalgam exceeds 10 per cent.
- No known epidemiological population study has demonstrated any adverse health effects in amalgam bearers.
- Mercury is a potent toxin that affects the basic functions of the cell by bonding strongly with sulfhydryl and selenohydryl groups on albumen molecules in cell membranes, receptors and intracellular signal links, and by modifying the tertiary structure.
- The structure of albumen molecules is genetically determined, and this leaves ample scope for genetic polymorphism to manifest itself in varying sensitivity and types of reaction to mercury exposure.
- It is probable that, besides local hypersensitivity reactions, mercury in amalgam fillings exerts side-effects just like most potent pharmaceuticals. Some support for this conclusion is to be found in clinical observations reported to date. At a rate that is probably below 10 per cent, however,

these side-effects cannot be demonstrated by means of population-based epidemiological studies.

Mercury is thus a multipotent cytotoxin that intervenes in the primary processes of the cell. This creates scope for a broad spectrum of possible side-effects. The analysis performed in 1997 identified the following health risks from mercury in dental fillings:

- Risk of impairment in the functions of the central nervous system.
- Risk of impairment in kidney function.
- Risk of impairment in the immune system.
- Risk of impairment in foetal development, especially development of the nervous system.

The presentation below is an account of the past five years' research publications, in so far as these may prompt us to supplement or modify the assessments and conclusions contained in the 1997 risk analysis.

3. New research findings

3.1 Studies in molecular biology

In the past five years, several studies of the effects of mercury at cell level have been conducted and published. These studies were performed on cell lines in cultures or suspensions of various origins. Intracellular measurement of mercury concentration has not, however, been feasible. The dose has therefore been represented by the estimated concentration in the medium concerned. Media usually contain proteins and other molecules that can bind mercury. It is therefore impossible to gauge any cellular concentration.

Nevertheless, the estimated concentration in the medium is, in many studies, very high. These concentrations are both non-physiological and, in the amalgam context, unrealistic. Publications referring to medium concentrations of mercury exceeding 1 μM have therefore, as a rule, been regarded as irrelevant and excluded from this summary.

Modified redox potential

One hypothesis often propounded in the literature is that mercury is toxic because it induces production of free oxygen radicals and modifies the redox

potential of the cell. Several mechanisms for this effect have been proposed (Ercal et al. 2001) and are reviewed in brief below.

Olivieri et al. (2000) reported that mercuric chloride (HgCl_2) in a concentration of 50 $\mu\text{g}/\text{l}$ reduces the cellular content of glutathione by 30 per cent in neuroblastoma cells, thereby decreasing their reductive capacity. Another observation was an increased release of β -amyloid ($\text{A}\beta$) peptide and elevated phosphorylation of tau protein.

Mahboob et al. (2001) found that mice exposed to HgCl_2 (0.8 μg in two peroral doses per week, for two weeks), which showed no influence on weight increase or food intake, had increased lipidoxidation in the kidneys, testicles and epididymides, and an elevated concentration of glutathione (GSH) and superoxide dismutase in the testicles. Administering a dose 10 times as large resulted in a significant reduction in weight increase, in GSH concentration in the epididymides, and also in the activity of glutathione disulphide reductase (GR) and glutathione reductase (GPx) in the kidneys and epididymides.

Goering et al. (2002) exposed rats to 1.2 and 4 mg/m^3 of mercury vapour for two hours daily during 11 days. The rats showed no clinical or histopathological signs of toxic influence. A dose-related increase in the mercury concentration in the brain and kidneys and a 30% increase in free oxygen radicals in the frontal cortex at a dose of 1 mg/m^3 were observed. A statistically significant decrease in GSH concentration and GPx activity was seen in the kidneys at a dose of 2 mg/m^3 . No such change in the brain was detectable at any dose. The authors' conclusion is that neither oxidative stress nor changes in GSH concentration and activity of antioxidant enzymes play any significant part in the toxic effect of mercury vapour on the brain and kidneys.

Wolfreys and Oliviera (1997) found that the increase in sensitivity to IgE stimulation in the peritoneal mast cells of mercury-sensitive rats is due to intracellular increase of free oxygen radicals produced by mercury. Mice exposed to mercury vapour, at 0.5 mg/m^3 for two hours, showed an elevated mercury concentration in motor neurons in the spine and signs of oxidative damage to DNA (Pamphlett et al. 1998).

The difference in results may be explained by the fact that Goering et al. determined the degree of oxidative stress in whole tissues, while the other authors determined oxidation in individual cell types.

In determining mercury concentrations in amalgam bearers' saliva, Pizzichini et al. (2001, 2002) found a significant correlation between mercury in saliva and the number of amalgam fillings in both men and women. Determination of total

antioxidant activity (TAA) in saliva and plasma showed a significant inverse correlation between mercury concentration in plasma and TAA in both genders. In addition, antioxidant activity showed a significant negative correlation with mercury concentrations in women's saliva. In men, no such correlation was found.

The question of the importance of oxidative stress in causing an early toxic effect of mercury exposure is still uncertain. Nevertheless, it is difficult to believe that this effect alone could explain the differences in toxicity for various organs and species to which the mercury gives rise.

Phosphorylation and intercellular signalling

It has been suggested that mercury in low concentrations may affect phosphorylation and thereby intercellular signalling. Huang and Narahashi (1997) used voltage-clamp technology to study the effect of $0.5\mu\text{M}$ HgCl_2 on GABA-induced currents from dorsal root ganglia in rat neurons. They found that mercury increases GABA-induced currents, and attributed this effect to an inhibition of protein kinase A (PKA).

Rosenspire et al. (1998) found that $0.13\ \mu\text{M}$ HgCl_2 boosted phosphorylation of tyrosine in proteins from B-cell lymphoma cells from mouse. The same research group (Mattingly et al. 2001) reported that $0.6\ \mu\text{M}$ HgCl_2 inhibits T cell-receptor-mediated activation of RAS in Jurkat cells, which are a human T cell line. Königsberg et al. (2001) studied the effect of $0.5\mu\text{M}$ on mitochondrion function in a foetal liver-cell line. They found ultrastructural modification of the mitochondria. The respiratory functions of the cell remained intact, but they found that the modification had involved uncoupling from signal links in the cell.

Cytoskeleton of the nerve cells

Mercury inhibits the development of, and breaks down, cytoskeleton structures in nerve cells. This was shown by Pendergrass et al. (1997) when they made rats inhale mercury vapour for 14 days. At approximately $0.35\ \mu\text{g/g}$ mercury in brain tissue, bonding of GTP to tubulin was inhibited. This process is necessary for polymerisation of tubulin, which in turn is a key component of the cytoskeleton.

The same group of researchers, Leong et al. (2001), added HgCl_2 to cultures of neurons from a snail with growing nerve germs. They were able to show that concentrations of HgCl_2 below and close to $0.1\ \mu\text{M}$ inhibit the growth of nerve germs and also cause retrograde degradation of the cytoskeleton in nerve cells.

Apoptosis in nerve tissue

Monnet-Tschudi (1998) studied the incidence of apoptosis (programmed natural cell death) in cultures of foetal rat brain. She found that a concentration of 1 nM of HgCl₂ speeds up spontaneous apoptosis in immature cultures. A concentration of methyl mercury a thousand times higher was required for the same effect. In more differentiated cultures without spontaneous apoptosis, no effect was observed. A high proportion of the apoptotic cells were astrocytes.

Retinal pigment epithelial cells

Toimela and Tähti (2001) studied the effect of HgCl₂ on cultured retinal pigment epithelial cells from pig and from a human cell line. They observed that 0.1 µM mercury reduced glutamate uptake by some 25 per cent. They interpreted this effect as due to inhibition of protein kinase C (PKC).

3.2 The nervous system

Knowledge of the mechanisms of neurotoxic effects exerted by mercury vapour is highly deficient. Perhaps as a result, we lack specific indices of nervous-system impairment caused by mercury vapour.

Data from animal experiments

Exposure to mercury vapour in rat (Warfvinge et al. 1992), mouse (Warfvinge 1995) and monkey (Warfvinge et al. 1994; Warfvinge 2000) causes accumulation of mercury in the brain and spinal cord. Mercury was often concentrated in neurons, especially motor neurons and astroglia cells. With toxic exposure, loss of Purkinje cells and granulocytes in the cerebellar cortex arises in rat (Sørensen et al. 2000). Whether similar changes arise in other parts of the brain has not yet been investigated by means of modern methods. Myelin sheaths of dorsal nerve roots also manifest changes (Schionning et al. 1998).

Accumulation in the retina

The retina of the eye accumulates mercury when there is exposure to mercury vapour. Mercury remains in the retina for a very long time — often for years. Accumulation of mercury is seen, in monkeys, in the inner portion of the retina, in pigment epithelial cells and capillary walls (Warfvinge and Bruun 2000).

Brain development and toxicokinetics in the foetus and mother

During the past five-year period, there have been few publications elucidating the effect of mercury vapour on foetal development. Studies clarifying its effect on the growing brain and foetal development in general are entirely lacking. According to information received, however, several major epidemiological studies are under way in the USA.

A German prospective study of 3,946 pregnant women was carried out. The women were interviewed regarding mercury exposure at the workplace. The mothers-to-be exposed to mercury or mercury compounds showed a significantly elevated risk of giving birth to babies who were small for their gestational age (Seidler et al. 1999). Nevertheless, the exposure criteria were dubious: they mean that other exposure to chemical substances also took place. Nor can chance significance be excluded.

Studies of the toxicokinetics of mercury in humans, including pregnant and lactating women, have been conducted by Swedish researchers. These studies confirm the picture previously obtained from animal experiments, and have provided quantitative information. The mother's amalgam fillings are reflected in the quantities of inorganic mercury in the placenta (Ask et al. 2002), in umbilical-cord blood, in breast milk (Vahter et al. 2000) and in amniotic fluid (Luglie et al. 2000).

The conclusion from the information available is that the mercury contained in breast milk is not a substantial source of infants' mercury exposure (Oskarsson et al. 1996; Drexler & Schaller 1998).

Amalgam removal involves a rise of some 30 per cent in plasma levels of inorganic mercury. After a phase of rapid decline, the plasma level decreases with a half-life of around 46 days (Sandborgh-Englund G 1998).

Neuropsychological tests

In occupationally exposed workers, it has been clinically feasible to demonstrate changes in brain potentials induced by visual stimulation and changes in conduction velocity in peripheral sensory nerve fibres. This result suggests that both the central nervous system (CNS) and the peripheral nervous system (PNS) are affected. These effects arise at relatively high exposure levels (Urban et al. 1999). At lower exposure levels, impairment of cognitive, sensory and motor functions occurs. Mood may also be modified. These changes have been quantified using batteries of neuropsychological tests.

At a level of mercury exposure caused by one of their duties, 13 men (mean age: 45 years) were exposed to mercury vapour for two to four weeks. After the exposure ceased, the men's blood mercury concentration averaged 48 µg/l of blood (corresponding to approx. 150 µg/g creatinine), with a range of 21–84 µg/l. One year after exposure had ceased, all the men were subjected to a battery of neuropsychological tests, and compared with a control group of 13 non-exposed workers.

Compared with the control group, the exposed group displayed cognitive deficits in terms of motor coordination, rapid reception of information with and without motor elements, verbal capacity, verbal memory, visual problem-solving and comprehension. The men exposed also had more emotional problems, such as an increased focus on bodily functions, depression, anxiety and being more socially withdrawn (Haut et al. 1999).

With batteries of neuropsychological tests, several studies of populations that are occupationally exposed to mercury vapour have been conducted. These studies have had two main purposes: to identify the lowest exposure level that gives rise to demonstrable health effects, and to investigate how far the health effects that have arisen are reversible if exposure ceases.

Early 2002 saw the publication of a meta-analysis of 44 epidemiological studies of populations that are occupationally exposed to mercury vapour. Twelve of these studies were included in the analysis, which comprised 686 exposed persons and 579 controls. In nine neuropsychological performance parameters, statistically significant differences between exposed persons and controls were found, with a dose-response association for exposure corresponding to 18–34 µg Hg per litre of urine (Meyer-Baron et al., 2002).

In an Italian multicentre study of 122 workers exposed to mercury vapour and 196 controls, a statistically significant decline in motor performance and a significant decrease in blood prolactin concentrations were found, with a dose-response association. Mean secretion of mercury in urine was 10.4 ± 6.9 µg/l for the exposed subjects and 1.9 ± 2.8 µg/l for the controls (Lucchini et al. 2002).

Persistent effects of mercury exposure

In one American survey, the reversibility of symptoms induced by exposure to mercury vapour was studied. The survey covered 205 workers whose mean age was 71 years. Of these workers, 104 had been heavily exposed more than 19 years previously, with mercury secretion in excess of 600 µg/l urine. The other 101 workers had not been exposed. Conduction velocity in peripheral nerves was significantly correlated with cumulative mercury exposure, which suggests residual peripheral neuropathy. Motor co-ordination was also reduced to a statistically significant degree, with a dose-response association (Letz et al. 2000).

In a Norwegian survey of 75 chloralkali workers compared with 52 controls, a dose-related effect on attention capacity and visual-motor capacity was found 12 years after termination of exposure. This group's exposure to mercury was considerably lower than that of the above-mentioned American cohort. For the

Norwegian workers, mean mercury secretion was roughly 100 µg/l urine during their work period (Mathiesen et al. 1999).

Alzheimer's disease

The question of whether mercury exposure from amalgam can cause Alzheimer's disease (AD) has been raised. This is because some *in vitro* studies have found effects of inorganic mercury on nerve tissue that resemble those seen in Alzheimer's.

In a study of 68 Alzheimer's patients and 33 controls, no significant difference was detected between the patients and controls in terms of mercury concentrations in the various parts of the brain. Nor was there any difference with respect to the presence of amalgam fillings (Saxe et al. 1999).

Another study involved a comparison of mercury concentrations in blood between 33 Alzheimer's patients on the one hand and, first, a group of 45 patients suffering from depression and, secondly, a group of 65 patients with a variety of non-psychiatric illnesses, on the other. The mercury concentrations were more than twice as high in the Alzheimer's patients as in both the control groups. Nevertheless, no association was found between elevated mercury concentrations and the presence of amalgam fillings (Hock et al. 1998).

3.3 The immune system and blood cells

Data from animal experiments

Substantial research inputs have been made over the past five-year period to survey the mechanisms underlying autoimmune reactions provoked by mercury in sensitive rat and mouse strains. These studies have essentially increased our knowledge; nonetheless, they have not succeeded in elucidating this complex phenomenon.

The effects of mercury on the immune system are governed by genotype, mercury dose and the status of the immune system concerned. Reactions to mercury vary between different bred strains and between species. Reaction intensity increases with the mercury dose, while there appears to be a dose threshold below which no reaction can be produced (Nielsen and Hultman 1999). In mercury-sensitive strains, too, the reactions decrease after a certain period of exposure (Roether et al. 2002).

If mercury-sensitive newborn rats are injected with HgCl₂, resistance to mercury arises. This suggests that the system can offset the stimulation of mercury (Field et al. 2000).

Amalgam fillings in the teeth of mercury-sensitive rats give sufficiently high mercury exposure to provoke an autoimmune syndrome with a rise of immunoglobulins in plasma and immunocomplex deposition in the kidneys (Hultman et al. 1998).

In animal experiments, mercury can modify the functioning of the immune system in various pathological states. Mice treated with injections of subtoxic doses of HgCl₂ are, for example, more susceptible to leishmaniasis infestation than untreated animals (Bagenstose et al. 2001).

Both mercury-sensitive and mercury-resistant mice show reduced immunity against malaria protozoa after injection of subtoxic doses of HgCl₂ (Silbergeld et al. 2000). In mice with a genetically conditioned tendency to develop the autoimmune syndrome systemic lupus erythematosus (SLE), development of the disease is accelerated if mercury is injected in subtoxic doses (Pollard et al, 2001). In mice with a genetic predisposition for diabetes (non-obese diabetic [NOD] mice), the development of diabetes is inhibited if subtoxic doses of HgCl₂ are injected (Brenden et al. 2001).

Lichen

One side-effect of amalgam fillings that is not particularly unusual is oral lichen. Larsson (1998) describes accumulation of mercury in the tissue affected, and accumulation of dendritic cells. Little et al. (2001) showed that a culture of human oral keratocytes, on exposure to subtoxic concentrations of HgCl₂ (10 µM), expresses ICAM-1, which in turn induces T cell binding, release of TNF-α and interleukin-8 and down-regulation of interleukin-1α. This induces activation of the immune system, which is not seen in experiments with cutaneous keratocytes.

Occupational exposure

Effects on the immune system of occupational exposure to mercury vapour have been studied in several surveys of worker populations. The workers were exposed to mercury levels below and at around the threshold value for permitted exposure, which corresponds to a urinary secretion rate of mercury of some 50 µg/g creatinine. These results were summarised by Moszczynski (1999). The studies reported statistically significant deviations in the number of cell elements, cytokine concentrations and immunoglobulin concentrations in the exposed workers. Nevertheless, these findings are contradictory: both stimulating and inhibitory effects were found to exist.

In a later study, 20 workers exposed to mercury vapour had mean urinary secretion of mercury of 45 µg/l. The study reported that the number of CD4+ and CD45RA+ and the total number of CD4+ T-lymphocytes were significantly

lower than in the controls. The numbers of CD57+ and CD16+ NK (Natural Killer) cells were also found to be negatively correlated with the mercury concentration in urine (Park et al. 2000).

Another group of 19 workers exposed to mercury vapour had a mean urinary secretion of mercury of $9.7 \pm 5.5 \mu\text{g/l}$. In this group, Vimercati et al. (2001) found an inverse correlation between mercury in urine and the numbers of CD13+ and CD15+ leucocytes and NK cells. A reduced capacity for chemotaxis in polymorphonuclear leucocytes was also found. Loftenius et al. (1998) studied the effect of amalgam removal on mononuclear lymphocytes from 10 patients. They found no statistically significant change in the number of cell types. However, they found a rise in IL-6 in plasma after 48 hours. The mercury concentration in plasma rose by some 10 per cent.

In 47 chloralkali workers with mercury exposure corresponding to 5.9 nmol/mmol creatinine, an increase in autoantibodies against myeloperoxidase and proteinase 3 was observed. This increase was correlated with the mercury concentration in urine (Ellingsen et al. 2000a).

Reduced enzyme activity in erythrocytes

Zabinski et al. (2000) reported that enzyme activity for several enzymes in erythrocytes — G-6PD, AchE, GR and SOD — was significantly reduced in a group comprising 46 chloralkali workers, with a urinary mercury concentration of $77 \mu\text{g/l}$. Bulat et al. (1998) observed reduced activity for GPx and SOD in erythrocytes for a group of 42 chloralkali workers, with a urinary secretion rate of $23.2 \pm 11.3 \text{ nmol/mmol creatinine}$.

In a group of 16 workers exposed to mercury vapour, reduced levels of glutathione and elevated catalase activity in red blood cells were observed. Mean urinary secretion of mercury in this group was $18.5 \pm 8.8 \mu\text{g/l}$ (Queiroz et al. 1998).

Autoimmune diseases

The tendency of mercury to induce autoimmunity gives rise to suspicion that mercury may boost the risk of autoimmune diseases, such as multiple sclerosis (MS). In a Canadian case-reference study, this hypothesis was tested (Bangsi et al. 1998). The findings of this survey, which covered 143 MS patients and 128 controls, provided no support for the hypothesis. True, persons with more than 15 fillings showed an excess risk of 2.57 times the risk of getting MS among persons without fillings, but this difference was not statistically significant.

Similar results were obtained in an Italian survey comprising 132 MS patients and 423 controls (Casetta et al. 2001). A British survey of 39 female MS

patients and 62 matched controls showed a significant correlation between the prevalence of caries and the risk of MS. However, no significant difference was found between the MS patients and the controls in terms of how many amalgam fillings they had (McGrother et al. 1999).

Mercury-resistant and antibiotic-resistant bacteria

Results from experimental studies have aroused suspicions that release of mercury in the oral cavity could produce mercury-resistant bacterial flora and, by the same token, antibiotic resistance. In several surveys of humans, this suspicion has not found support. In a British survey of 83 children, half of whom had amalgam fillings and the other half of whom lacked them, no differences were found in the prevalence of mercury-resistant or antibiotic-resistant bacteria (Pike et al. 2002).

3.4 Kidneys

Understanding of the mechanisms whereby the kidneys absorb and secrete mercury has improved considerably, largely thanks to new methods in molecular biology. The current state of knowledge has been summarised in an article in *Pharmacological Reviews* (Zalups 2000).

In a cross-section study in Scotland, 180 dentists were compared with 180 academics at Scottish universities. Kidney disease was found to be ten times more common among the dentists (6.5%) than in the controls. The dentists' mean urinary secretion was 2.58 nmol/mmol creatinine (Ritchie et al. 2002).

Among 47 chloralkali workers with a mean urinary mercury concentration of 5.9 nmol/mmol creatinine, secretion of N-acetyl- β -D-glucosaminidase (NAG) was measured. The results showed that in those with mercury secretion that exceeded the mean for the group, NAG secretion was also elevated (Ellingsen et al. 2000 a).

3.5 Thyroid and muscular atrophy

Ellingsen et al. (2000b) reported finding impaired thyroid function in a group of 47 chloralkali workers, whom they compared with 47 controls. The exposed workers showed a statistically significant rise in reverse T₃ (rT₃) — a rise that was dose-related. The mean urinary concentration of mercury was 5.9 nmol/mmol creatinine, with a range of 1.1–16.8.

Atrophy and capillary damage in thigh muscle were observed in five out of six workers in dental care who had a urinary mercury-secretion rate of 13–67 μ g/l at the time of the biopsy. These changes may, according to the authors, have been

induced by the effect of the mercury on the nervous system or on capillaries. There might also be a direct effect on muscle fibres (Nadorfy-Lopez et al. 2000).

3.6 Testicles

Exposure to mercury vapour causes mercury to accumulate in the testicles, where it is eliminated very slowly. Daily administration of HgCl₂ to mice in a dose that did not affect body weight caused a reduced sperm count, modified sperm morphology and lower fertility. It proved possible to offset this effect by administering vitamin E (Rao and Sharma 2001).

Monsees et al. (2000) studied the *in vitro* effect of HgCl₂ on Sertoli cells from rat. They observed that concentrations below 1 µM of HgCl₂ sharply reduced inhibin production. Clinical observations have prompted suspicions of associations between acrodynia (Pink Disease) and epididymis obstruction (de Kretser et al. 1998).

3.7 Polymorphism

During the five-year period under review, several case descriptions involving acute mercury exposure, with concentrations usually well above what may be expected from amalgam, have been published. These case descriptions have been published because the symptoms are unexpected. Mercury concentrations are documented with urine and blood figures, and the symptoms have subsided when the exposure ceased. Accordingly, there is no doubt that the high mercury concentrations genuinely caused the symptoms.

Besides oral lichen — which is sometimes combined with facial exanthema — the symptoms present have been a range of dermal syndromes, such as systemic contact dermatitis (baboon syndrome) (Alegre et al. 2000; Bartolome et al. 2000). Three cases of nummular dermatitis, which were cured by amalgam removal, are described by Adachi et al. (2000) and Pigatto et al. (2002). In a review article, Britschgi and Pichler (2000) assert that mercury can induce acute generalised exanthematous pustulosis. In another review article, Boyd et al. (2000) summarise experience of skin diseases caused by mercury.

One article describes a five-year-old boy who, after massive mercury exposure, developed tics, extensive blinking, head-twisting and shoulder-jerking as his sole symptoms (Li et al. 2000).

There have also been descriptions of several cases where, in children with hypertension and elevated catecholamine secretion induced by mercury exposure, the symptomatology has resembled phaeochromocytoma (Laurans et

al. 2001; Torres et al. 2000; Wössmann et al. 1999; Kosan et al. 2001). A 48-year-old man developed aspects of severe, acute polyarthritis (Karatas et al. 2002) as a result of massive mercury exposure. Dalén (2000) describes a historical case with symptoms suggesting gastroenteral influence.

The cases referred to above evince pronounced polymorphism in ways of reacting to mercury exposure. The conclusion is that the clinical picture of exposure to mercury vapour may vary greatly.

3.8 Gender differences

Knowledge of the dose-response association for exposure to mercury vapour and inorganic mercury compounds is derived mainly from epidemiological studies of occupationally exposed populations. The great majority of subjects studied have been men.

To permit conclusions to be generalised to the whole population, one must assume that sensitivity to mercury is equally distributed. There is well-founded reason to question support for such an assumption. Data from animal experiments do not show a consistent picture; but neither do they provide support for the thesis that men and women are equally sensitive to mercury.

In one study, 30 Sprague-Dawley rats received a daily dose of HgCl_2 by gastric tube, in doses from 0 to 10 mg/kg. The rats were killed after 14 days, and distribution and uptake of mercury were studied. No significant gender difference emerged with respect to signs of toxicity or concentration of mercury in various organs.

Previous studies of rats and mice have shown gender differences in the kidneys' uptake of mercury, but in divergent directions (Khan et al. 2001). In mice that had received intraperitoneal injections of HgCl_2 corresponding to 0.5mg/kg or been exposed to mercury vapour in low doses, gender differences were demonstrated. With autometallography, uptake of mercury in motor neurons was shown to occur to a larger extent among females than among males. Males were also found to accumulate more mercury in the kidneys than females (Pamphlett et al. 1997; Pamphlett and Coote 1998).

Hultman and Nielsen (2001) studied the importance of dose, gender and genetic composition in two mouse strains. They found that the same dose produced quantitative differences in mercury uptake both between the two strains and between the genders. This suggests differences in toxicokinetics between the genders and different strains. They also found that the concentration of mercury in tissue that is required for an autoimmune reaction to be induced varies

between strains and the genders. This suggests variation in sensitivity to mercury between strains and between genders.

Data from humans are notably scant. One study was carried out in which diurnal variation in the kidneys' mercury secretion was investigated. No demonstrable diurnal variation in men, but significant diurnal variation in women, was found (Woods et al. 1998).

Barregård et al. (1999) determined mercury concentration in test biopsies from 36 kidneys donated for transplantation — half from men and half from women. Mercury concentration in the kidneys was statistically significantly higher in women than in men. As discussed above (3.1), TAA in saliva was found to be significantly inversely correlated with mercury concentration in saliva in women, but not in men (Pizzichini et al. 2001, 2002).

3.9 Side-effects and their incidence

'Side-effect' is a clinical pharmacological term relating to unintended repercussions over and above the therapeutic effect. In toxicology, reference is made to especially sensitive populations, who have a dose-response association and/or a way of reacting that significantly deviates from the majority of the population. These deviant populations may be conditioned by genetic differences, age and gender differences or pathological states.

The fact that a person feels ill as a result of amalgam fillings may be due to various factors. It may be because the person perceives a connection between the symptoms and the oral cavity, or that the symptoms are connected with a dentist's manipulations. Alternatively, amalgam may be perceived as an explanation for malaise of a different origin, if a credible explanation is sought. Research has been carried out to find methods of distinguishing between these alternative explanations.

Clinical surveys

In a summary of just over 400 patients referred to Huddinge Hospital with suspicion of amalgam-related conditions, the authors consider that some 30 per cent of cases were attributable to diagnoses other than amalgam influence. These diagnoses included, for example, heart disease, chronic collagenosis, neurological disease and cancer; in the authors' opinion, these could explain the patients' condition. In other cases, there was speculation about the causes and it was found that the summary did not support the hypothesis that amalgam had contributed to the patients' pathological condition. The argument for this was that no connection between their symptoms and elevated mercury concentrations in their blood or urine were demonstrable (Langworth et al. 2002).

This survey supports the hypothesis that, among those who believe themselves to be suffering as a result of amalgam, the true cause is not always amalgam. However, it does not rule out the possibility that amalgam influence can be found in some of these persons. The diagnoses mentioned in this study include impaired thyroid function, oral lichen, kidney disease, fatigue, vertigo, somatisation tendency, depression and anxiety — all of which are symptoms that may be associated with mercury exposure.

A Swiss dentist followed up 75 of the 90 patients he had treated with amalgam removal according to the patients' own wishes. All the patients had psychoneurological symptoms or muscular and joint pains of various kinds. Sixty-eight per cent of the patients felt that they were much better at the time of their annual check-ups following the removal. Another 12 per cent felt better, 9 per cent were slightly better, 7 per cent were unchanged and one of the patients felt worse after the removal (Engel 1998).

In a similar Swedish questionnaire survey comprising 445 patients of one dentist, the patients' amalgam fillings were removed because of prolonged, unexplained ailments. Here, the health of 80 per cent of the patients whose fillings had been removed was found to be good or better, while that of 11 per cent was unchanged and 9 per cent felt that it had deteriorated or were doubtful. More than half the patients stated that they had experienced symptoms in connection with having their fillings removed. These symptoms often began after a few days and commonly lasted about a week (Strömberg and Langworth 1998).

Provocation tests

One study was carried out in the form of provocation tests. Initially, an advertisement was placed in the daily press inviting people suffering from amalgam-related disease to apply. Of those who registered their interest, 39 were tested by being given gas to inhale through a mouthpiece for five or 10 minutes. The gas was blindly switched from each occasion to the next between pure air and air containing mercury. The mercury concentrations varied between 25 and 200 $\mu\text{g}/\text{m}^3$. Exposure occurred at intervals of two to three weeks. Each patient's symptoms were registered after every exposure occasion. In two persons, the results showed unequivocal mercury sensitivity, while suspected sensitivity was found in another two, although not with statistically significant results (Strömberg et al. 1999). The survey appears to be highly illuminating. The provocation dose corresponded, at its highest level, to the daily exposure dose for an amalgam bearer, or roughly one-hundredth of the permitted daily dose for an industrial worker. It is possible that optimal discrimination would have been increased a slightly higher exposure dose.

Allergy diagnostics with epicutaneous tests (patch testing) can sometimes, besides skin reactions, provoke systemic effects with such symptoms as headache, vertigo, fatigue and general malaise (Kunkeler et al. 2000; Inerot and Möller 2000). A group of 65 patients who had all reacted with intensified subjective symptoms in conjunction with amalgam removal, were subjected to provocation experiments by means of patch testing.

The tests were carried out blind, with a concentration of roughly 10 µg of metallic mercury, 4 µg phenylmercuric acetate and mercury-free substances. For a week after the skin application, the patients had to keep a log according to a questionnaire on their symptoms. Some reacted with increased symptoms of substances containing mercury, and were described as 'mercury-intolerant'. The patients who did not react were described as 'mercury-tolerant' (Marcusson 1996).

Neutrophils from 14 intolerant and 14 tolerant patients and 14 controls were tested. The cells were exposed to HgCl₂ and compared in terms of the release of superoxide. A statistically significant difference between tolerant and intolerant patients was observed. There was a correlation between the activity of superoxide dismutase (SOD) in lymphocytes and the symptom score, and also between superoxide formation and the symptom score for the mercury-exposed patients (Marcusson et al. 2000).

4. Risk analysis — definition of three new hazards

Not infrequently, progress in research raises more questions than it answers. Since 1997, three new health risks have emerged that, with reasonable suspicion, may conceivably be attributed to mercury from amalgam. These hazards involve influence on the retina of the eye, testicle function and thyroid function.

Suspicion of effects on the retina is founded mainly on the fact that mercury accumulates in the retina, with lasting retention especially in the pigment epithelium. Whether this mercury accumulation can contribute to the incidence of degenerative changes, such as retinal detachment or macular degeneration, cannot be assessed without further research.

In the testicles, too, accumulation of mercury takes place with lasting retention as a result of exposure to inorganic mercury. Clinical observations and experimental studies confirm that functional impairment may arise from exposure to mercury. Information on dose-response association is, however, lacking and amalgam risk therefore cannot be assessed at present.

Mercury accumulates in the thyroid as a result of exposure to mercury vapour. This may be associated with observed impairment of T₄ deiodisation. In this case, too, the information available is insufficient to permit assessment of whether there is a risk of amalgam causing thyroid disease.

Scientific support for influence at low concentrations

The 1997 risk analysis assumed that the minimum exposure level that gives rise to demonstrable impairment of the nervous system is represented by urinary secretion of mercury at roughly 50 µg/l. Subsequent research findings have shown that influence arises at considerably lower exposure levels. There is scientific evidence for influence from mercury concentrations in urine of some 25 µg/l, and from even lower levels.

In a cross-section study of 49 dentists and dental nurses, mercury secretion in their urine was measured before and six hours after administration of sodium-2,3-dimercaptopropane-1-sulfonate (DMPS), a mercury-chelating substance (Echeverria et al. 1998). Before chelation, the mercury concentration in urine averaged 0.95 µg/l; after six hours it was 9 µg/l. The statistical analysis showed, throughout the dose range, a significant correlation between dose in terms of secretion after chelating and aggregate subjective symptoms. Conversely, there was a correlation between secretion after chelation and the results of tests of motor function.

The dose-response curve for this group of dental-care personnel covers roughly the same dose range as that incurred by amalgam bearers. Nevertheless, it is unclear how far the mercury concentration in urine before chelation is representative of exposure further back in time. It cannot be excluded that the dental-care staff's exposure may have been higher further back in time.

In the Scottish study referred to above (Ritchie et al, 2002), 180 dentists were compared with an equal number of controls of university employees. Mean urinary mercury secretion was four times as large among the dentists as among the controls and five times as large as that in the dental-care personnel above before chelation. Statistically significantly more often than the controls, the dentists showed memory impairment and deterioration in psychomotor function. These changes were not, however, correlated with the mercury secretion in their urine.

A Swedish prospective cross-section study of 1,462 women aged 38–60 was conducted, with a follow-up after five years. In this study, no correlation was found between symptoms and exposure to mercury from amalgam (Ahlqwist et

al. 1999). The yardstick of exposure used was the mercury content of serum, and effects were gauged by responses to a questionnaire concerning symptoms.

The statistical sensitivity of this Swedish study is much greater, but the effect measure is relatively insensitive and the dose measure less specific than in the chelation study. Nevertheless, it should be emphasised that the effects referred to here are subclinical effects, i.e. observed functional impairment, and that the symptoms fall within the normal variation in the population. Accordingly, these effects can be demonstrated only at group level.

At present it may be considered unproven, but not excluded, that subclinical psychomotor functional impairment caused by mercury is demonstrable in groups at the mean exposure level for amalgam bearers.

Influence on foetal development

The risk of influence on foetal development was pointed out in the 1997 risk analysis. This is not contradicted by more recent results that may suggest an elevated risk, among women exposed to mercury in the course of their work, of giving birth to babies who are small for their gestational age. In addition, there are experiments on animals indicating that one expected effect of exposure to low doses of mercury vapour is inhibition of brain development. In these experiments, this inhibition resulted in reduced cognitive and motor capacity. Such inhibition of brain development falls within the normal range in the population.

These effects in animal experiments resemble those observed after exposure to methyl mercury. However, the dose of mercury that yields the effect has been only about one-tenth of the dose of mercury that exerts an effect following exposure to methyl mercury. Only through epidemiological studies using batteries of neuropsychological tests and possibly neurophysiological survey methods can these effects be demonstrated.

The risk of inhibition of brain development during the foetal stage and early childhood is obvious. This hazard is a contraindication for amalgam fillings in children and women of fertile age, until a quantification of the risk prompts a different assessment.

Influence on the immune system

The clinical studies of how mercury vapour influences the immune system show clearly that effects can be demonstrated down to dose levels corresponding to exposure to amalgam. The clinical significance of these effects, on the other hand, is unclear. The observations based on animal experiments provide

evidence that genetic make-up and gender have a bearing on the nature and intensity of reactions.

Published surveys of the association between amalgam and multiple sclerosis are of limited sensitivity, but appear to rule out amalgam as a major aetiological factor in the development of MS. Available clinical information provides no guidance as to whether mercury from amalgam can affect the course of the disease of MS.

Experimental data prompt the question of whether removing amalgam in the event of autoimmune diseases is justified. No general reply to this question can be given; instead, in the current situation the circumstances must be weighed up in each individual case. Nevertheless, it would seem imperative for clinicians to bear this option in mind. The same applies to parasitic diseases, such as malaria.

Risk of kidney disease

Over the past five-year period, another survey has emerged that shows an elevated risk of developing kidney disease among those who are occupationally exposed to mercury. This observation was made on a group of dentists whose exposure was fairly low. The survey confirms the findings of earlier surveys.

The question is whether this is an effect induced solely by mercury exposure or whether it is the result of a combination of factors. It would appear vital for nephrologists to devote attention to this issue.

Varying sensitivity between individuals

There are strong indications of a gender difference in terms of mercury metabolism in data from animal experiments and in clinical observations. Information on what this may entail regarding differences in sensitivity to mercury exposure is entirely lacking. This is a fundamental shortcoming that invalidates every risk analysis.

The cases of acute or subacute mercury intoxication referred to above illustrate a pronounced polymorphism in the range of symptoms. This suggests that the toxic effect of mercury has several targets, and this probably contributes to the variation in sensitivity between individuals. This is not surprising, in view of the omnipotence of the mercury atom in the biochemical dynamics of the cell. For genetic reasons, particularly sensitive groups in the population may be expected to show equally marked polymorphism in their mode of reaction to amalgam.

In purely theoretical terms, it is highly probable — verging on certainty — that individuals with genetically conditioned deviant sensitivity to mercury exist. The clinical observations referred to above support this conclusion. Diagnosis is

a problem that requires further research. At present, the golden diagnostic standard appears to be blind provocation with realistic concentrations of mercury vapour. However, this method is too laborious, time-consuming and costly to be incorporated into clinical routine.

The most probable side-effect of amalgam seems to be a reaction mediated by the immune system. This does not exclude the possibility of genetically conditioned high sensitivity to mercury in the nervous system. Mercury is not the only environmental factor that provokes an immune-system-mediated reaction. Other metals and organic molecules can also induce such reactions in sensitive individuals.

There are no facts indicating that all those who believe that they are affected by amalgam are in fact so affected. It is therefore more probable that, for many people, the symptoms have other causes. But it is also likely that many people with side-effects from amalgam fillings are unaware of a causal connection.

There is no evidence that the frequency of pathological side-effects of amalgam due to genetically conditioned high sensitivity exceeds 1%. It is therefore impossible to demonstrate these states by means of epidemiological studies of representative population samples. It is unclear whether subclinical influence on mood and motor function can be caused by the mercury concentrations to which amalgam bearers are exposed. These effects have been observed in occupationally exposed persons within the same dose range.

5 Summary and conclusions

The past five years' research has yielded further evidence that amalgam can give rise to side-effects in a sensitive portion of the population. Thus:

- Research in molecular biology has elucidated mechanisms that may underlie the toxic effects of mercury.
- Studies of the effects of mercury on the immune system in rodents have enhanced knowledge of the mechanisms whereby mercury affects the immune system. Clinical studies of occupationally exposed employees have objectively confirmed subclinical influence of mercury on the immune system at low levels of mercury exposure.
- The thyroid has been identified as the target organ for the toxic effect of mercury in occupational exposure to mercury vapour in low doses.

- Experimental studies of primates and rodents have revealed that mercury is accumulated and persists for years in the retina as a result of exposure to mercury vapour. The consequences of this accumulation are, however, unclear.
- Clinical studies of the effects of mercury on occupationally exposed workers, using modern diagnostic methods, have elucidated the connection between dose and effect. They have also identified and quantified neuropsychological symptoms at low exposure levels.
- The lowest exposure, in terms of urinary mercury secretion, that has been found to give rise to a demonstrable toxic effect has fallen from 30–50 µg/l till 10–25 µg/l. Accordingly, the safety margin that it was thought existed with respect to mercury exposure from amalgam has been erased.
- Studies of workers previously exposed to mercury have shown that prolonged exposure to mercury vapour, with mercury concentrations in urine of some 100 µg/l, may result in symptoms emanating from the nervous system that persist decades after exposure has ceased. This suggests that exposure causes lasting damage to the central nervous system, which complicates the interpretation of results of low-dose studies of occupationally exposed populations.
- Clinical reports of acute or subacute cases of mercury intoxication where modern diagnostic methods have been applied have revealed a remarkably high degree of polymorphism in human reactions to toxic mercury exposure.
- Both animal experiments and clinical observations have demonstrated gender differences in the toxicokinetics of mercury.
- Additional facts have come to light that may indicate that mercury vapour can affect human foetal development.
- Clinical provocation studies, with exposure to small quantities of mercury through skin exposure or inhalation, have confirmed that individuals with deviant high sensitivity exist.

With reference to the fact that mercury is a multipotent toxin with effects on several levels of the biochemical dynamics of the cell, amalgam must be considered to be an unsuitable material for dental restoration. This is especially true since fully adequate and less toxic alternatives are available.

With reference to the risk of inhibiting influence on the growing brain, it is not compatible with science and well-trying experience to use amalgam fillings in children and fertile women. Every doctor and dentist should, where patients are suffering from unclear pathological states and autoimmune diseases, consider whether side-effects from mercury released from amalgam may be one contributory cause of the symptoms.

Removal of existing amalgam fillings should not be undertaken unless there are medical reasons for doing so. The reason is that the risk of complications from the removal may exceed the risk of side-effects from the amalgam. The risk of removal is due mainly to the fact that dental substance is drilled away, which may itself result in problems with existing teeth.

6 Environmental medical views of risk management

For medical reasons, amalgam should be eliminated in dental care as soon as possible. This will confer gains in three respects. The prevalence of side-effects from patients' mercury exposure will decline; occupational exposure to mercury can cease in dental care; and one of our largest sources of mercury in the environment can be eliminated.

Dental materials left in patients' mouths should be treated as drugs for administrative purposes. Accordingly, toxicological and clinical testing should be required. Reporting of side-effects should also take place according to the same norms that apply to drugs.

It is imperative for doctors and dentists to be made aware of the fact that all dental restoration materials can give rise to side-effects, and that this eventuality should always be considered when the patient's pathological state is unclear. Side-effects may conceivably both cause, and be contributory factors in, various pathological states.

7 Clinical management

Special clinical units should be created with the function of investigating unclear pathological states when there is any suspicion of an environmentally related cause. These units should have access to all medical specialities and the research skills that are required for assessment and treatment of this category of patients. Mercury exposure from amalgam is only one of many conceivable agents that may conceivably induce syndromes that are difficult to diagnose. Units of this

kind may possibly be linked to environmental-medicine units at regional hospitals.

It is imperative for cost-effective routines to be created for diagnosis of the side-effects of amalgam. At present, the golden standard for specific diagnosis should be blind provocation with mercury vapour. However, this method is not suitable for routine clinical use.

It is essential to develop alternative clinical tests that are simple and cost-effective to use. This requires suspected cases to be assembled in a few locations and systematically studied with all available and relevant methods in a scientific manner.

8 Need for research

In most studies of the effects of mercury, the subjects have been men. It is imperative to elucidate the differences, if any, between men and women in metabolism and the toxicokinetics of mercury after exposure to mercury vapour.

Epidemiological surveys of the *in utero* effects of mercury exposure on foetal brain development should be carried out to further clarify the hazards, if any.

Epidemiological studies designed to investigate associations, if any, between amalgam load and degenerative retinal diseases are urgently required.

Likewise, epidemiological studies designed to find any associations that may exist between thyroid disease and amalgam fillings are advisable.

Co-ordinated clinical studies of people who undergo amalgam removal on suspicion of side-effects from mercury should be carried out. Thorough investigations before, during and after removal, using all clinically available methods and focusing on the immune system, thyroid and nervous system, should be carried out. Muscle biopsy should be performed in cases where there is pronounced muscle pain.

Initiation of clinical and experimental basic research to clarify the mechanisms whereby mercury vapour affects the central nervous system is highly essential. Today, knowledge of these mechanisms is poor.

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Abbreviations

AB	β-amyloid
AchE	acetylcholinesterase
AD	Alzheimer's disease
CNS	central nervous system
DMPS	sodium 2,3-dimercaptopropane-1-sulfonate
GABA	γ-aminobutyric acid (gamma-amino butyric acid)
GPx	glutathione reductase
GR	glutathione disulphide reductase
G-6PD	glucose-6-phosphate dehydrogenase
GSH	reduced glutathione
GTP	guanosine triphosphate
Hg	mercury
ICAM-1	intercellular adhesion molecule 1
MS	multiple sclerosis
NAG	N-acetyl-β-D-glucosaminidase
PKA	protein kinase A
PKC	protein kinase C
PNS	peripheral nervous system
rT ₃	reverse T3
SOD	superoxide dismutase
TAA	total antioxidant activity
U-Hg	urinary mercury

1 nmol Hg/mmol creatinine = 1.79 μg Hg/g creatinine

1 μg Hg/g creatinine = 0.56 nmol Hg/mmol creatinine