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Mercury exposure from amalgam fillings

Analysis of mercury in different biological matrixes and speciation in the feces

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Abbreviations:

AAS	Atomic Absorption Spectrometry
AES	Atomic Emission Spectrometry
AFS	Atomic Fluorescence Spectrometry
AP	Amalgam Particles
F-Hg	Mercury in Feces
CSF	Cerebrospinal Fluid
CVAAS	Cold Vapor Atomic Absorption Spectrometry
GC	Gas Chromatography
GC/CVAFS	Gas Chromatography in combination with Atomic Fluorescence Spectrometry
GC/MPD	Gas Chromatography in combination with Microwave-induced Plasma Atomic Emission Spectrometry
ICP	Inductively Coupled Plasma
N	Number of amalgam fillings
NAA	Neutron Activation Analysis
O-Hg	Mercury in Oral-air
Red-Hg	Reducible Mercury
RNAA	Radiochemical separation with Neutron Activation Analysis
TLV	Threshold Limit Value
Tot-Hg	Total Mercury
U-Hg	Mercury in Urine
XRF	X-ray Fluorescence Spectroscopy

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Aims of the thesis

The aims of this thesis were to:

a) develope and evaluate methods to measure exposure to mercury
b) determine different forms of mercury present during exposure
c) establish the background levels of mercury for non-occupationally exposed individuals

Introduction

Mercury

Mercury is a liquid metal at ordinary temperature with a vapor pressure of 0.16 Pascal at 20°C. The vapor pressure is approximately five times higher at normal body temperature. Mercury is also a very toxic metal, which occurs naturally in the environment in different chemical and physical forms. Inorganic mercury can exist in three different oxidation states, Hg°, Hg^{1+} and Hg^{2+}. Elemental mercury is slightly soluble in water, 0.28 μmol/l at 20°C, but the solubility in biological solutions (e.g. blood) is higher. Elemental mercury can easily form alloys with other metals. Inorganic mercury has also a high affinity for sulfhydryl and selenide compounds (49, 84).

Organic mercury i.e. mercury covalently bound to a carbon atom can also exist in different forms. Methyl mercury is the most frequent of the organic compounds with regard to human exposure and is also a very toxic compound. The covalent binding is strong but methyl mercury can readily bind to a sulfhydryl group such as are present in proteins (49, 82, 83, 84).

History of amalgam

Amalgam also called silver amalgam, is a mixture of silver and elemental mercury which has been used as a tooth filling material as early as the 7th century by the Chinese but with a different composition than that used today. In the beginning of 19th century, silver amalgam was introduced as a tooth filling material in Europe and in north America (4). There was disagreement about using amalgam for tooth fillings. Some dentist claimed that amalgam could be poisonous as they were aware of the poisonous properties of mercury. The American Dental Association, founded in 1859, declared that amalgam was harmless and that it could be used as a tooth filling material.

It was not until the beginning of 20th century that the possibility of human poisoning was focused on again when the famous German chemist Alfred Stock (73) showed that mercury was released from amalgam. He demanded that the use of amalgam be ceased but when World War II broke out this debate lost importance and was forgotten. In Sweden, in 1950 (34), some studies were conducted on the release of mercury from amalgam. However, it was not until the end of 1970 that the debate on the safety of mercury resumed when the subject received much public attention due to publicity through newspapers and television (70, 84). New investigations proved (1, 7) that mercury was released from amalgam fillings not just from newly inserted fillings but that there was a continuos leak of mercury

from amalgam fillings. Hence, it was realized that amalgam was not as stable material as had been presupposed.

Dental amalgam

Now a days dental amalgam consist of approximately 50% by weight of metallic mercury and 50% of a mixture of silver, tin and copper powder (84). Elemental mercury is mixed with this metallic powder (alloy) to form a plastic amalgam. Amalgam is mixed just before insertion in the cavity of the tooth. This can be carried out in a mercury automata or more commonly by the use of amalgam capsules. Amalgam capsules are produced with mercury and alloy separated by a thin plastic wall in the capsules. During preparation of amalgam the capsule is vigorously shaken breaking the plastic separator which results in the mixing of the components. The advantages of using amalgam is that it is a ”handy” material for tooth fillings and it is cheap.

Dental care consumed approximately 1.7 tonnes of mercury in 1991 in Sweden (71). However, the amount used per year is decreasing.

The number of new fillings used with amalgam in adults in Sweden in 1991 were approximately 2 million. On average a middle aged person has 30 amalgam fillings (71).

Tissue uptake and retention of mercury

The main transport of elemental mercury in the human body is probably by the blood. Elemental mercury diffuses from the blood into the brain and other tissues followed by oxidation to Hg^{2+} in cells. Mercury is then bound to sulfhydryl groups of protein-molecules (30). Inorganic mercury, e.g. mercuric mercury, absorbed by the gastrointestinal system is transported by red blood cells, bound to sulphydryl-groups in hemoglobin or gluthathione, or by plasma cells bound to albumin or other macro molecules (30). Divalent mercury is accumulated in the kidneys where almost 90% of the body burden is found at steady state. This explains why the kidneys are especially susceptible to mercury-induced toxicity. Radioactive mercury release from amalgam inserted in the teeth of sheep and uptake has been followed by whole-body image scan (35). The results from this study indicate three possible absorption routes namely, the lungs, the gastrointestinal system and the jaw or gum mucosa. High concentrations of mercury were found to be localized in the liver and kidneys, which confirms the human studies.

The elimination of inorganic mercury from the human body is complicated by biological half-times that differ for different tissues, and time and concentration of exposure to mercury. The half-life for mercury is long and the concentration in blood and urine gradually reaches a steady state which reflects the equilibrium between intake and excretion. The excretion half-time of mercury in blood probably follows a two compartment model. Initially excretion is fast with a half-time of 3-8

days (75) followed by a slower excretion half-time of approximately 45 days. Human studies from intake of radioactive labeled mercury, either protein bound or free ionic inorganic mercury, indicate a biological half-time for the whole-body of approximately 42 days (56). The half-time of mercury excretion in urine after cessation of exposure is approximately 40-60 days (75, 65).

A high level of mercury has been found in the brain of a deceased dentist who have not recently been occupationally exposed to mercury (52). This indicates that the biological half-time of mercury in brain tissue is high. Similar results are reported from studies of the presence of mercury in brain tissue of primates (43). In two other studies with human volunteers (39, 25) the half-time in brain was approximately 20 days. The release of mercury from brain tissue may follow a multi-compartment model: an attempt to create such a model in order to calculate accumulation in the brain has been conducted (84).

Toxicity

The toxic health effects from extensive exposure to inorganic mercury have been known for some considerable time. The classical symptoms of mercurialism are tremor, behavioral and psychological changes, such as irritability, memory disturbances, insomnia, depressions, and other effects including gingivitis and protein uria (84).

Studies of low and prolonged dose exposure to mercury have been conducted for occupational exposed individuals (33, 54, 59, 67). However, very little is published on the toxicity of mercury due to exposure from amalgam fillings and for those few studies conclusions are often conflicting.

In a study by Hansson (36) the same symptoms for non-occupationally exposed individuals with amalgam fillings was reported which are described for individuals with high occupational mercury exposure. In another study, no significant correlation was obtained, at least not at the population level, for symptoms and complaints in relationship to the number of amalgam fillings (3, 12).

Teratogenic effects of mercury released from amalgam fillings were not observed neither in epidemiological data nor in animal experiments (41).

A slight effect on the kidneys has been described for individuals exposed to inorganic mercury from amalgam fillings (31) but Herrström (37) could not confirm that mercury derived from amalgam fillings was responsible for the observed kidney dysfunction.

Allergic and immunological effects of mercury from amalgam fillings have been reported in three studies (28, 72, 74) but in one study by Langworth (40) a relationship between the presence of amalgam fillings and immunologic disturbance was not demonstrated.

There are very few data about the carcinogenic effects of mercury and mercury compounds. A study of dentists and nurses have shown a possible increased risk of cancer in the brain and other epidemiological studies indicate a risk for lung and kidney tumors (2, 18).

Mercury exposure from amalgam.

Mercury is believed to be released from amalgam in different forms e.g. as mercury vapor, oxidized mercury and amalgam particles. Mercury vapor can be inhaled and is readily absorbed by the lungs, approximately 80%. The target organs for chronic low level exposure are the nervous system and the kidney (13, 29, 30, 82, 84).

Mercury vapor can be absorbed through the mucous membrane (19), for example, in the mouth. Mercury vapor can also be dissolved in saliva, swallowed and absorbed in the gastrointestinal system. Very little, however, is known about the magnitude of mercury absorbed.

Oxidized mercury may emigrate as corrosion products from amalgam, or released mercury vapor may be oxidized in saliva and then swallowed. Inorganic mercury salts are only absorbed by about 5 to 10% in the gastrointestinal system. Exposure to oxidized mercury mainly produces effects on the kidneys, and high level exposure to mercury can lead to damage of the kidneys (30, 82, 84).

Mercury particles or dust can be inhaled or swallowed. The uptake from the lungs and the intestine are probably low.

Occupational exposure.

The staff working at the dental clinics, dentists, nurses and hygienists are exposed both to mercury vapor and mercury dust. The dentist and nurses are exposed when preparing amalgam. The mercury levels in air can momentarily exceed the threshold limit value (TLV) several times during the working day even when capsules are used (16). The exposure can also be very high when inserting new fillings, during polishing and when drilling out old amalgam fillings. The staff are also exposed when they clean dental instruments (20, 50, 55, 57, 58, 64, 86).

Employees in the dental industry which manufacture dental material can be exposed as well as dental technicians (71).

Employees at crematories can also be exposed to mercury vapor during the cremation of corpses having amalgam fillings (71).

Non-occupational exposure

Individuals having amalgam fillings are exposed to mercury from their fillings: the fillings continuously release mercury (1, 7, 15, 66). Just as for the dentist the exposure can be momentarily very high when amalgam fillings are inserted or drilled out. The exposure is also increased when individuals with amalgam restorations are chewing, biting, eating hot meals or grinding teeth during sleep or when stressed (7, 77, 78).

Individuals are also indirectly exposed to mercury originating from amalgam present in the environment via breathing air, drinking water and eating food contaminated with this source of mercury. Distribution of mercury from amalgam into the environment occurs at different life-cycle stages. For instance, emission of mercury occurs in flue gas released from crematories (71). Mercury from amalgam

is also released into the sewage system (60) from dental surgeries despite the use of amalgam separators. In addition, mercury from amalgam is excreted in human feces and enters into the sewage system and thereafter, it may reach the sea where mercury is deposited and can be methylated by microorganisms and stored in fish.

However, indirect exposure from the environment is insignificant compared with the exposure from personal amalgam fillings.

Methods

Cold vapor technique and AAS

To analyze total mercury in biological samples the cold vapor technique together with Atomic Absorption Spectrometry (CVAAS) is often used. Biological samples are often digested before analysis for example with an acid permanganate solution or with a strong acid to a homogenous solution as in study I and II. This treatment releases bound mercury as Hg^{2+} from protein-sulfur complexes.

After addition of a reductant for example, stannous solution, which is the most commonly used agent, Hg^{2+} is reduced to elemental mercury. The released mercury vapor is purged or sucked into the analyzing instrument. In study I and II, an acid stannous-solution was used to reduce mercury in the digested samples and the vapor was purged into the atomic absorption spectrophotometer and the amount of mercury determined by absorption spectroscopy at 253.7 nm. Mercury can also be analyzed in biological samples without previous digestion. To release mercury from undigested samples a reductant with a stronger reducing capacity is required such as Sn^{2+} in alkaline solution: this reduces all mercury compounds with the exception of organo mercury compounds (62). Estimation of total mercury, organo-mercury compounds included, from undigested samples have been conducted by using a alkaline stannous solution in combination with cadmium as reductant (47). In study II, mercury was released from the dried feces samples without previous digestion by using a strong alkaline stannous solution with heating. A similar method was used to analyze mercury in whole blood without previous digestion (32, 48).

The mercury concentration in the sample was determined against a standard curve of different concentrations of mercury added to a matrix or to the sample.

The cold vapor technique together with AAS is very sensitive and the detection limit is sufficient for most biological applications. If necessary, the detection limit can be decreased considerably if the released mercury vapor is concentrated on an gold film previous to analysis (10).

Different kinds of atomic absorption instruments are available for mercury analysis. There are instruments specialized only for mercury analysis and there are ordinary atomic absorption instruments for multi-element analysis.

Mercury was analyzed in study I and II with a Mercury Monitor from Tillquist. The instrument has a cuvette of 0.3 m which was heated to 40°C to prevent the formation of condensation. The instrument was equipped with a gas wash-bottle with a glass filter as reaction tower. All connections to the instrument were made of Teflon.

A single beam UV-instrument (a rebuilt Zeiss spectrophotometer) with a 1 m pre-heated cuvette and a mercury lamp was used for the analyses of mercury in the oral-air as determined in study I. The mercury vapor was sucked into the cuvette by a vacuum pump. Calibration of this UV-instrument was made with a head space technique. An exact volume of saturated mercury vapor was sampled with a syringe and diluted with air into an air sampling bag. The temperature and the volume of the air was measured and the concentration present in the bag was determined by calculation of the gas vapor pressure of mercury. The concentration in the samples were evaluated against this standard curve.

Other techniques than AAS to analyze mercury

Mercury can also be analyzed in biological samples after digestion and dilution by the Inductively Coupled Plasma (ICP) technique. This technique can detect several elements simultaneously which can be useful. The detection limit for this technique is in about the same order of magnitude (ng Hg/g biological sample) as for AAS. Another method used for mercury analysis is neutron activation analysis (NAA), which is sometimes used in combination with radiochemical separation (RNAA) (27). This is a very sensitive method with a high degree of accuracy and is often used as a reference method for AAS. However, as the instrumentation is very specific and not so common it is not used for routine analysis. Atomic Emission Spectrometry (AES), and Atomic Fluorescence Spectrometry (AFS) (61) are also used with the cold vapor technique, the detection limits are in about the same order of magnitude as for AAS but these techniques are not as commonly used as AAS. A pre-concentration step by gold amalgamation increases the sensitivity of these methods: the detection limit can be increased by ten times or more and low amounts of mercury and very small quantities of samples can be analyzed (81).

To analyze organic mercury, for example methyl mercury, a cold vapor technique using AAS has been described by Magos (48). More sensitive methods utilize different gas chromatographic instrumentation (38), sometimes in combination with other techniques (69).

A method for speciation of mercury in biological materials and determination of picogram levels of methyl mercury after ethylation and separation by gas chromatography with cold vapor atomic fluorescence detection (GC/CVAFS) is used by Liang (42) and Bloom (17). Speciation studies and analysis of methyl-mercury in whole blood after extraction, butylation, separation by capillary gas chromatography and detection with microwave-induced plasma atomic emission spectrometry (GC/MPD) is presented in a study by Bulska (24).

Analysis

Test subjects

Individuals participating in these studies were well informed of the projects design and intent to ensure that they were aware of the importance of following instructions to avoid uncontrolled exposure. It was also crucial to have a number of individuals as reference subjects in order to follow the natural variations among individuals.

The individuals which participated in study I and II were all healthy and had no known occupational exposure to mercury. They were all non-smokers and did not excessively eat fish. Prior to sampling they were requested not to eat fish. Their amalgam fillings had not recently been inserted and none of the individuals had undergone any other dental treatment two months prior to sampling.

Two individuals which had voluntarily ingested amalgam particles, inorganic-mercury and mercury vapor were also included in order to follow the excretion of different mercury compounds into feces and to use these samples with known mercury content in the speciation study II.

Two individuals, one who never had had any amalgam fillings and one who had removed all fillings served as references.

Amalgam fillings

Amalgam fillings can differ greatly in their size. In order to determine if there was a relationship between amalgam fillings and excretion of mercury in biological samples it was necessary to estimate a measure of the amount of mercury in the amalgam fillings.

Determination of the size and shape of each amalgam area, or the number of fillings counted after a rough correction for sizes has been reported (11, 86). To obtain the correct measure of the ”active” size of a filling the actual area that is used for chewing has to be estimated.

For the individuals in study I and II the number of amalgam surfaces were counted by a dentist using a scale of 0 to 6 where 6 areas represented an amalgam crown. The total number of surfaces for all the individuals in the study were in the range 0-84. This represented a rough estimate as the amalgam surfaces differ greatly in size, as the microscopic area, the composition and aging of the amalgam are unknown, however, this was a useful approach. The relationship between the number of amalgam surfaces and mercury released in oral-air, urine and feces are shown in Figure 1, for ten individuals in study I.

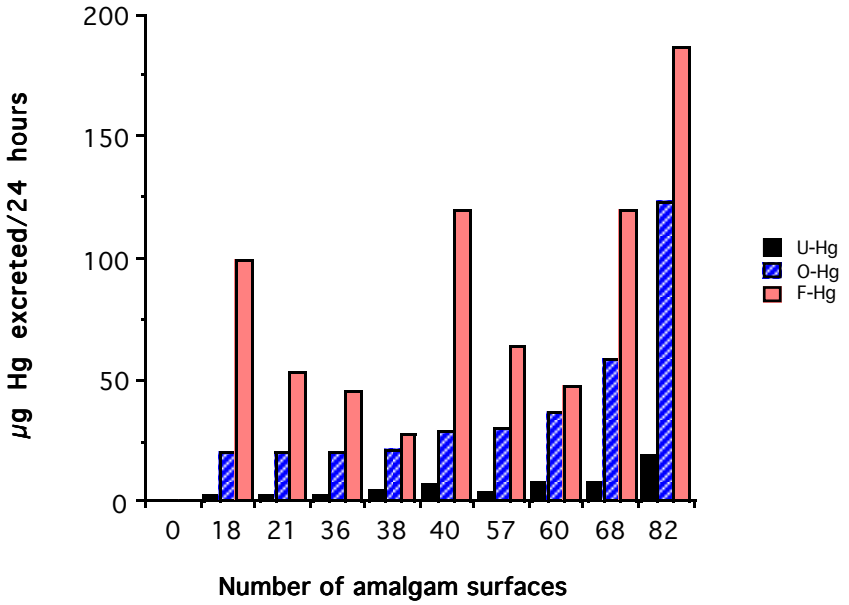

Figure 1. The relationship between the number of amalgam surfaces and mercury excreted during 24 hours in urine (U-Hg), oral air (O-Hg) and feces (F-Hg) for 10 individuals with 0-82 amalgam surfaces in study I.

A high correlation coefficient was observed for the number of amalgam surfaces versus mercury excreted in urine. Almost the same value was calculated between number of amalgam surfaces and mercury released into oral-air. The correlation with feces was somewhat lower, probably dependent on that the larger variance obtained with feces samples due to the higher uncertainty in sampling, Table 1.

Table 1. Correlation coefficients for the 24 hours excretion of mercury in oral air, urine, feces and number of amalgam surfaces for ten individuals in study I with 0-82 amalgam surfaces are presented in a correlation matrix. All correlations are significant at 95% level.

24-hours samples	N*	O-Hg§	U-Hg#
O-Hg	0.83		
U-Hg	0.84	0.97	
F-Hg##	0.66	0.84	0.80

* N number of amalgam surfaces.
§ O-Hg emission of Hg into oral cavity.
# U-Hg excretion of Hg in urine.
F-Hg excretion of Hg in feces.

Mercury in oral-air

Investigations of mercury release from different amalgam materials have been made in vitro and from old cut out amalgam fillings. Studies of mercury release from amalgam in artificial saliva solutions and from dental amalgam in an artificial mouth has also been conducted under controlled conditions. The release of mercury was strongly influenced by changes in temperature, pressure, type of solutions and mixtures used and age of the amalgam (9, 21, 22, 23). Therefore, it was necessary to use a method for measuring in situ mercury release (15) from amalgam fillings. In paper I, a method to measure mercury in the oral air was developed. A mouthpiece of polyethene which looked like a bulb was constructed. The mouthpiece was placed in the mouth and the oral air was sucked through the mouthpiece with a vacuum pump to an UV-instrument in which released mercury vapor was directly measured. The test subjects were very carefully instructed not to eat or drink just before the sampling as the condition in the mouth readily changes and consequently influences the release of mercury. Mercury vapor could not be detected in the oral air of individuals without amalgam fillings or from individuals with their amalgam fillings covered with paraffin. With the lowest quantifiable concentration of 1ng Hg°/L it was possible to measure the mercury emission from all individuals with at least three amalgam surfaces.

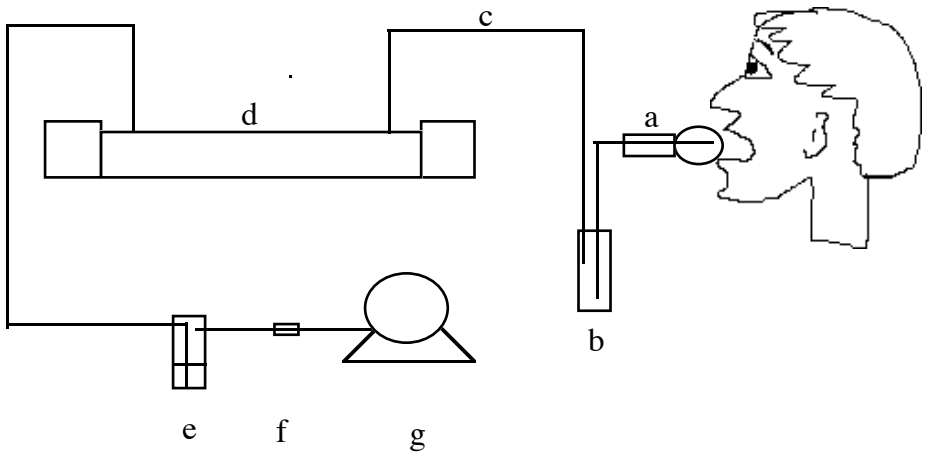

Figure 2. The release of mercury from amalgam fillings measured in the oral-air. a) Mouthpiece of polyethene big enough to prevent contact between amalgam surfaces in the mouth. b) An ice-cooled impinges to prevent condensation in the UV-instrument. c) Connection tubes in Teflon. d) UV-spectrophotometer with mercury lamp and 1 m cuvette. e) Gas wash-bottle filled with $KMnO_4$ to sample Hg-vapor. f) Capillary orifice for regulation of the flow. g) Vacuum pump.

Another method for determining the emission from amalgam fillings is also presented in study I. It was intended that this procedure would be used as a screening method for example by dentists. In brief, the subjects rinsed their mouth with a portion of water at 37°C for an exact time. The remaining water was then carefully collected. The content of mercury in the water was analyzed after digestion with acid permanganate solution. There was good agreement between this method and the method previously described. The result from these methods indicates that most of the mercury released from unstimulated amalgam surfaces was in the vapor state. The correlation between the number of amalgam surfaces and mercury vapor in oral-air was high but even a higher correlation was achieved between mercury vapor in oral-air and mercury excretion in urine (see Table 1). This strongly indicates that there is an uptake of mercury from amalgam fillings via released mercury vapor (this is further discussed below).

Release of mercury from stimulated amalgam fillings, for example after chewing or drinking hot water, was momentarily 10 to 100 times higher than from unstimulated amalgam surfaces.

Released mercury vapor can either be inhaled, or absorbed through mucous membranes or the vapor can be dissolved in saliva and swallowed. Because the uptake from the lungs, mucous membranes and the intestinal system are different as are the eating and drinking habits for different individuals, the oral-air mercury values could not be used solely to estimate the body uptake of mercury from amalgam fillings.

Mercury in urine

Biological monitoring of mercury in urine samples collected from individuals occupational exposed has been conducted for many years. However, the correlation between mercury in occupational air and mercury recovered in urine correlate poorly (46, 75) because mercury accumulates in the body with a long half-time of excretion in urine of approximately 40 days (65). By contrast, there is a significant correlation between the excretion of mercury in urine and mercury released from amalgam fillings as exposure is continuous. Consequently, an equilibrium between exposure and excretion is established as long as fillings are not inserted or removed.

The method used for analysis of mercury in urine in paper I was a modification of an earlier used method (45). The sensitivity was increased by about ten times making it possible to detect mercury in urine samples from individuals without amalgam fillings. This improvement together with a different sampling technique where the individuals collected all voided urine samples during 24 hours made it possible to determine a correlation between the excretion rate of mercury in urine and number of amalgam fillings. The relative standard deviation for analysis of mercury in the urine sample excreted within 24 hours was improved in our studies

by 10% compared to 25% in spot samples after correction for dilution (unpublished).

The correlation between the number of amalgam surfaces and mercury excretion in urine was significant for the ten individuals studied in study I. The correlation coefficients are presented in Table 1. Urine samples are therefore suitable to follow mercury exposure from amalgam fillings. It was proved by oral air measurements that mercury release from amalgam fillings was increased upon chewing. This can also be seen from the mercury excretion in urine in a group of dentists who chewed chewing gum frequently. They all had a statistical significant increase of mercury excretion in urine (64).

Mercury excretion in urine also correlated significantly with the excretion of mercury in feces, although this correlation is weaker (Table 1).

Mercury in feces

Very little is known about the excretion of mercury in feces. In 1938, Stock (73) showed that there might be a connection between the presence of mercury in amalgam fillings and mercury excreted in feces. It was therefore considered of great interest to determine if there was a correlation between the number of amalgam fillings and mercury excreted in feces. Hence, there was a need for a reliable sampling technique and a sensitive analytical method to analyze total mercury in feces. In order to determine how large the total flow of mercury through the intestinal system may be, feces samples were collected from individuals in study I and II with and without amalgam fillings. As mercury in feces originates both from intake of food contaminated with mercury and amalgam fillings it was necessary to sample all feces for a period of several days to obtain representative samples.

The feces samples were freeze-dried and then mixed to a homogeneous sample. The feces samples were digested with nitric acid in order to estimate the total mercury content. Subsequently the samples were analyzed by the modified method described above (see also Ref. 45).

The correlation between the number of amalgam surfaces and total mercury excretion in feces during 24 hours for the individuals in study I was significant. An even stronger correlation was obtained for total-mercury excretion in feces with mercury release in oral-air, and with mercury excretion in urine during 24 hours (Table 1).

The difference between Table 1 and 2 show the importance of sampling feces during a period of several days and subsequently calculating the excretion for 24 hours as the correlation coefficients were much higher for the 24-hours excretion values of mercury in urine compared with the number of amalgam surfaces than the corresponding concentration values for mercury in random samples of feces.

Table 2. Correlation coefficients show the advantage of using the value of reducible mercury (in which mercury emanating from particles is not included) instead of using the total mercury feces value in the correlation of the number of amalgam surfaces or the 24-hours mercury excretion in urine. An even stronger correlation is obtained when mercury excreted in the feces sample within 24-hours is used instead of the concentration obtained from random samples.

24-hours feces sampling	Random feces sampling	N*	U-Hg§
	Total-Hg#	0.54	0.61
	Red-Hg##	0.58	0.65
Total-Hg		0.66	0.80

* N number of amalgam surfaces.
§ U-Hg 24 hours excretion of Hg in urine.
# Total-Hg the total amount of Hg in feces.
Reducible-Hg the amount of Hg in feces which not consist of particles.

In study I, the average mercury excretion was about twenty times higher in feces than in urine. Some very high values of mercury excretion in feces are seen for individuals with a large number of fillings (7, 66). They are at the same level as is recommended for the highest intake of total mercury from food (85). The concentration of total-mercury in feces should not be considered as an indirect indicator of mercury uptake but rather only to indicate the level of mercury flow through the body. Because the sampling of feces is more complicated and given the larger variations between samples than for urine samples, such samples are not suitable for routine analysis.

Mercury in other biological samples

Other samples which are used for biological monitoring of mercury uptake are blood, blood-plasma or blood-serum (1, 5, 7, 68, 75). Routine sampling of blood and urine are often used for individuals who are occupational exposed. This procedure is also recommended by ”Socialstyrelsen” in Sweden to follow the uptake of mercury from amalgam fillings. Blood samples show the present total systemic uptake of mercury in contrast to mercury in urine samples which indicates the past internal exposure as the half-life of mercury excretion in urine is about 40 days (65). To distinguish methyl mercury exposure from inorganic mercury exposure in a blood samples, the blood cells have to be separated from plasma, as methyl mercury is mainly accumulated in the red blood cells (30). Analytical methods to determine inorganic mercury and organic mercury in biological samples are also available (6, 38, 42, 48, 61).
Hair samples have been used especially to detect methyl mercury exposure (10, 53). There is a correlation between methyl mercury intake and the mercury concentration in hair. There is very little known about inorganic mercury exposure

and the mercury concentration in hair. Hair samples are easy to collect but there can be a problem with contamination from the surroundings and to correlate the sample concentration of mercury with the time of exposure.
Studies of mercury excretion in breast milk have been conducted by Oskarson and co-workers (53). According to these authors, there was a correlation between the number of amalgam fillings and mercury excretion in breast milk.
Analysis of mercury in the brain from deceased persons have shown that there is a correlation between the number of amalgam fillings and mercury concentration in the brain (13, 29). In a study with deceased dentists, increased mercury concentrations were found in the brain compared to non-exposed individuals.
Another biological sample which has recently been used is cerebrospinal fluid (CSF) (76). The concentration of mercury in CSF is proposed to reflect mercury concentration in the brain. This is the only method currently used to determine the in vivo amount of mercury in the brain. The sampling is complicated and places the patient at risk and hence, cannot be used routinely. The mercury concentration in CSF is approximately 50 times lower than the corresponding urine sample which presents a problem during analysis as this is close to the detection limit.
High concentrations of mercury have been detected in liver and kidney in individuals occupational exposed (52). Correlation studies have also shown higher concentrations of mercury in liver and kidney in individuals with amalgam fillings compared with those without fillings (51, 79). Attempts to take muscle biopsies from living individuals to determine the body burden of mercury has also been conducted. Moreover, this sampling technique is not suitable for routine analysis.
In vivo X-ray fluorescence spectroscopy (XRF) analysis of specific organs has been successfully performed on occupationally exposed individuals. However the method is not sensitive enough for reliable estimations of individuals nor for determining levels in individuals exposed to low doses (Börjesson J, unpublished).
Attempts to measure mercury in exhaled air and the concentration of mercury in saliva has been undertaken in order to calculate the uptake of mercury. The concentrations of mercury in these samples are low and contamination from mercury release from amalgam fillings can be a great problem. Furthermore, the sampling technique must be carefully standardized in order to correlate these concentrations with the plasma values (1, 11, 30).

Speciation of mercury excreted in feces

Analysis of the total mercury in feces only reflects the flow of mercury through the gastrointestinal system which can be large. Mercury present in the feces consists of inorganic and organic mercury derived from intake of food and inorganic mercury released from amalgam fillings which is swallowed. Inorganic mercury from amalgam fillings may be released in different ways, for example, as vapor or as oxidized mercury through corrosion of amalgam surfaces, or as particles formed when chewing. It is of great interest to determine in which chemical form and in what proportions mercury is excreted in the feces as the uptake and the toxicity vary for different mercury compounds. To be able to calculate the uptake of mercury released from amalgam fillings it is also necessary to know the source from where mercury originated.

Methyl mercury

In vitro studies with bacteria have shown that inorganic mercury can be methylated by intestinal bacteria in small amounts. As the total mercury flow through the digestive canal is high for some individuals with amalgam fillings there is a possibility that methylation may occur in vivo. In study I, a method to analyze methyl mercury in fish was modified to analyze methyl mercury in feces. Methyl mercury could not be detected in feces from individuals in study II without amalgam fillings. Probably methyl mercury from food is demethylated before excretion. Methyl mercury could neither be found in the feces from individuals in study II with amalgam fillings indicating that methylation does not occur to any great extent in the gastrointestinal system.

Elemental mercury

Elemental mercury release from amalgam fillings has been measured in oral air. Calculations have also been made on the uptake of mercury in human lungs, but little is known about the flow of mercury vapor dissolved in saliva that is swallowed. Quantitative information of that flow and if mercury vapor is absorbed through the mucous membrane or just excreted without any uptake is missing. There is a possibility that mercury vapor can be formed through oxidation-reduction processes or by bacterial metabolism of mercury compounds in the intestinal system. In order to reproduce the conditions in the mouth one individual in study II swallowed known quantities of mercury vapor dissolved in water. The excretion of total mercury in feces was thereafter followed (Figure 3). The total excretion of mercury in the feces was less than 50%, which indicates that the uptake of mercury

vapor in the intestinal system may be considerable. In study II, elemental mercury could not be released from any of the feces samples from the individuals with and without amalgam fillings indicating that mercury vapor either is absorbed or oxidized before excretion.

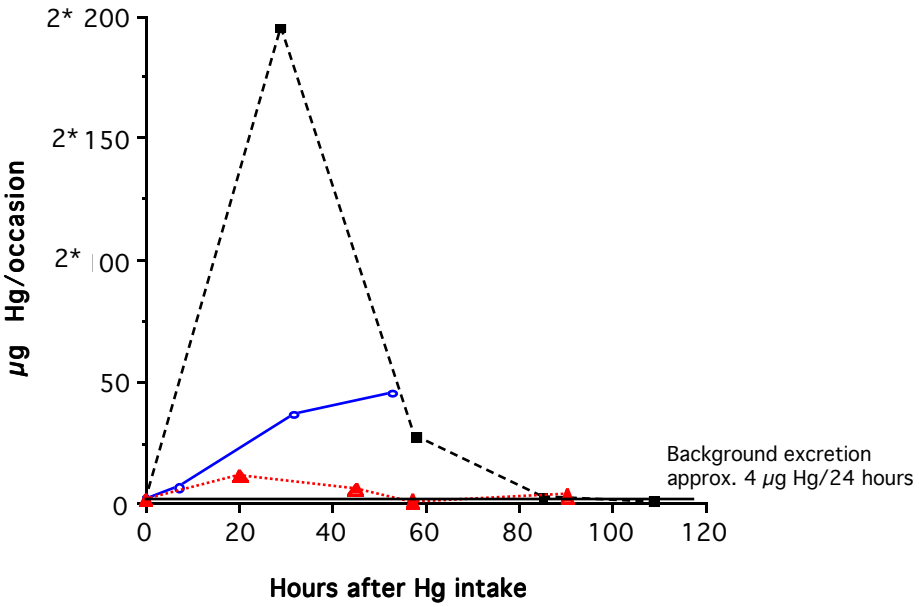

Figure 3. The excretion of mercury in feces after intake of: ■, 560 μg mercury as amalgam particles; ❍, 100 μg mercury bound to cysteine, and; ▲, 30 μg mercury vapor dissolved in water of one individual without amalgam fillings. The background excretion originate from mercury contained in food.

Amalgam particles and reducible mercury

Amalgam particles are formed in large quantities when amalgam is polished or carved with a drilling machine. The particles have been studied using the electron microscope and the contents of the particles have been analyzed. A photograph of the amalgam particles and the results of the analysis of one of the particles is shown in Figures 4 and 5, respectively.

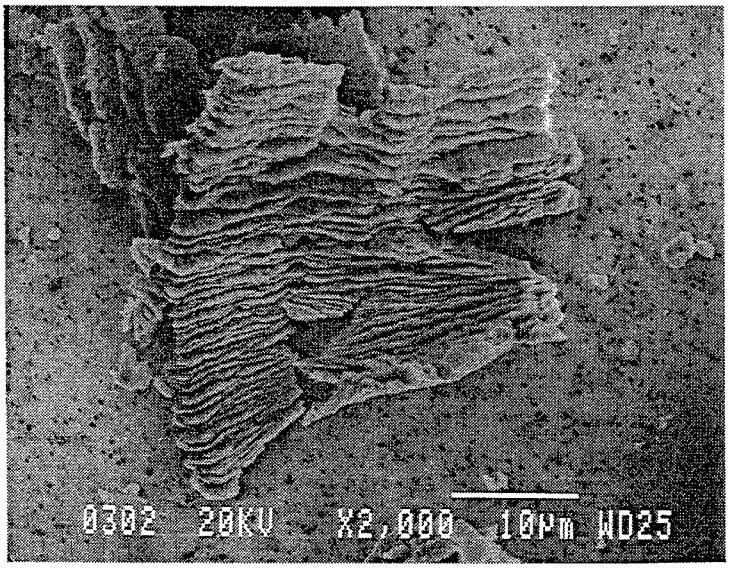

Figure 4. Amalgam particles formed from drilling a piece of amalgam

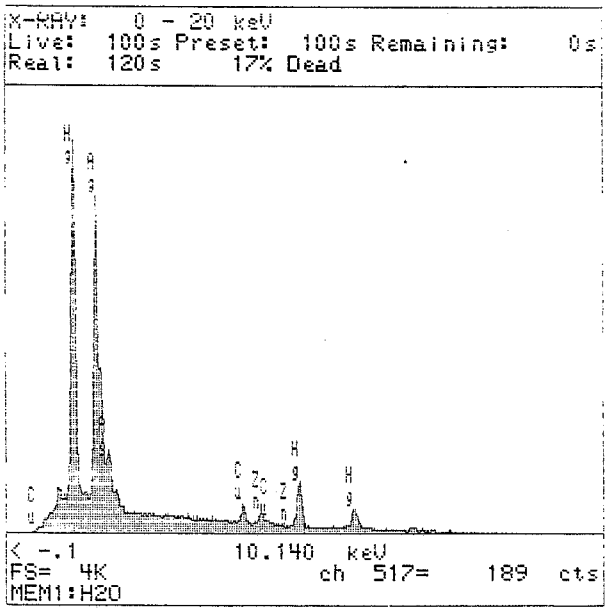

Figure 5. X-ray analysis of one of the particles formed during the drilling of a piece of amalgam.

Individuals are exposed to these particles during treatment at the dentist and many particles are swallowed even if good suction apparatus is used. Studies of amalgam pieces in vitro indicate that particles even are formed at normal friction, e.g. when chewing. In study II, the electron microscope was used to show that the particles were formed when one test subject was chewing intensively. The photograph of one of these particles and the test result from the particle are presented in Figures 6 and 7, respectively.

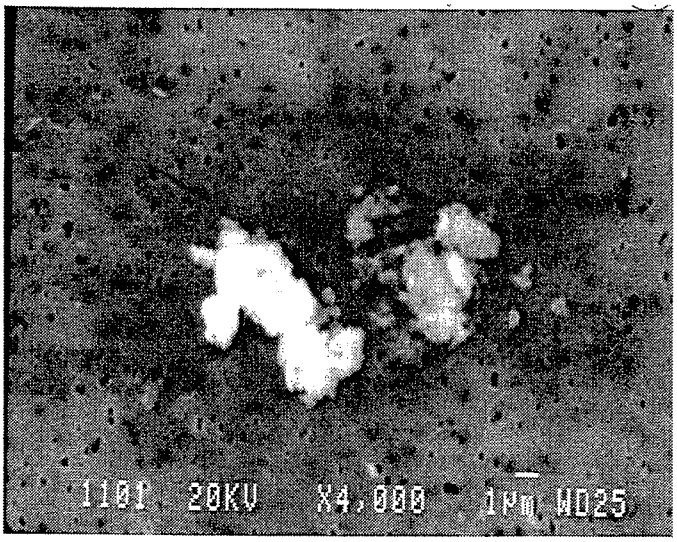

Figure 6. Particles collected on a filter from one test subject with amalgam fillings after intensively chewing.

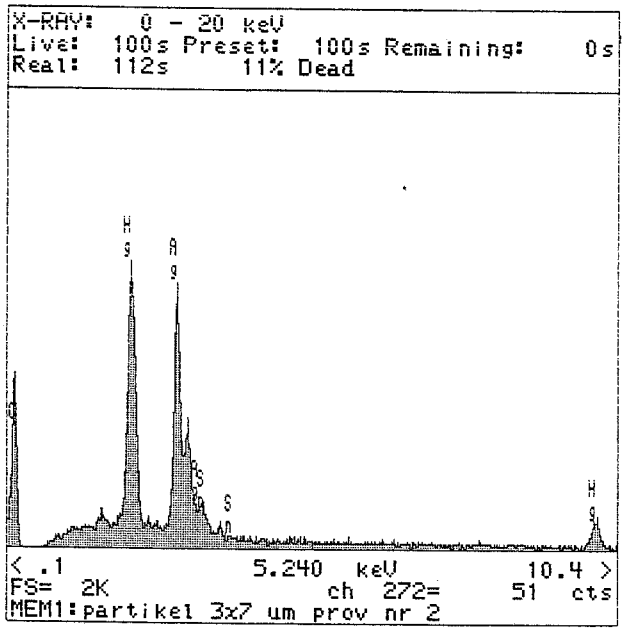

Figure 7. X-ray analysis of one of the particles found after intensively chewing.

There are few studies dealing with the estimation of the proportion of particles formed, as particles are believed to pass through the gastrointestinal system without causing any harm to man. In study II, two individuals consumed amalgam particles by the oral route (Figures 3 and 8). Subsequently, the excretion of mercury was analyzed in feces and followed until the levels decreased to the original values. The particles were excreted almost intact in feces i.e. they apparently reacted in the same way as amalgam particles added to a dried feces sample in vitro. Approximately 80% of the amalgam particles were recovered, measured as total mercury.

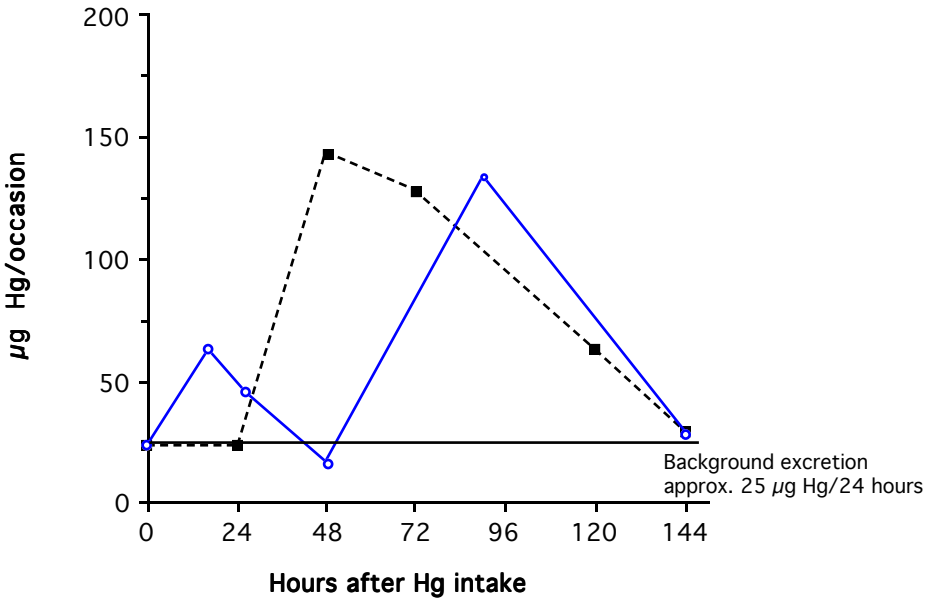

Figure 8. The excretion of mercury in feces after intake of: ■, 310 μg mercury as amalgam particles, and; ❍, 200 μg mercury bound to cysteine of one individual with 30 amalgam surfaces. The background excretion from food and amalgam fillings are presented.

Corrosion of amalgam or oxidation of elemental mercury released from the fillings in the mouth may give rise to Hg^{2+} probably bound to a sulfur containing compound. To determine how these compounds may have reacted after passing through the intestinal system and being excreted, the same individuals in study II were given a cysteine complex of mercury by oral administration. About 80% of the mercury-cysteine complex was recovered in the feces, however, the excretion time seemed to be somewhat longer than for mercury particles. These feces samples, with known content of mercury, were used as standards to separate the contribution of particles from other mercury compounds excreted in feces. A method was established in study II to analyze total mercury and reducible mercury in feces samples. As the feces samples neither contained methyl mercury nor elemental mercury the difference between total mercury and reducible mercury was assumed to be equal to the mercury particles. The analysis that resulted from these standard samples were also used to develop a model to calculate the proportion of particles in feces samples from individuals with amalgam fillings. With this knowledge, speciation of the mercury excreted in feces by the individuals in study II with amalgam fillings showed that only a minor part, less than 26% of the mercury

excreted in feces consisted of amalgam particles. This was less than expected as the general opinion has been that most of the mercury released from amalgam fillings consisted of particles. The main portion of the mercury excreted in feces probably consisted of mercury originating from mercury vapor swallowed, absorbed and oxidized, and then excreted bound to sulfhydryl containing groups. The relative proportions between total mercury, reducible mercury (Hg bound to sulfhydryl groups) and amalgam particles are presented in Figure 9 for the test subjects in study II.

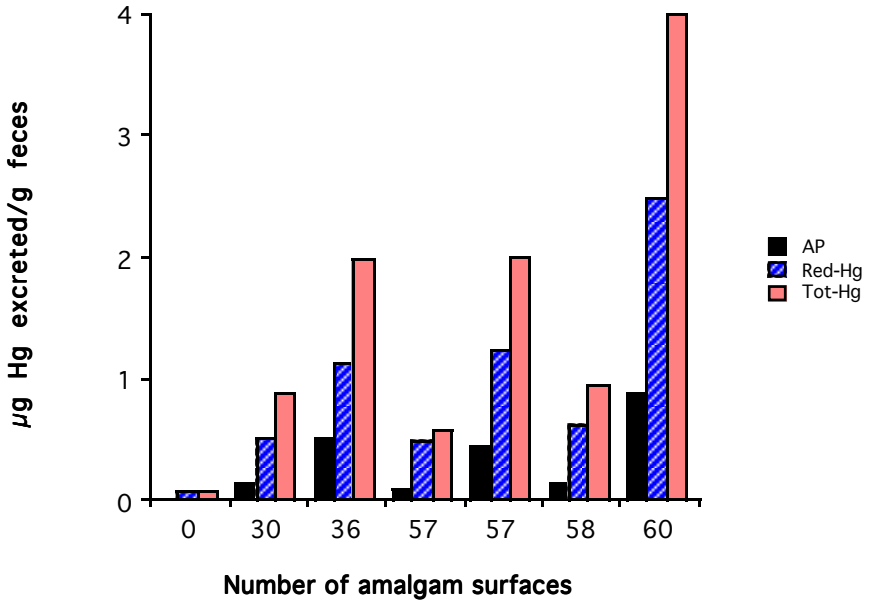

Figure 9. The relationship between total mercury (tot-Hg), reducible mercury (Red-Hg) and amalgam particles (AP) in feces samples from seven individuals with 0-60 amalgam surfaces in study II.

According to study I there is a significant correlation between total mercury excreted in feces and mercury excreted in urine, but an even better correlation was obtained if reducible mercury instead of total mercury was correlated with mercury in the urine (Table 2). This was expected as particles pass almost intact through the intestinal system and is not available for uptake. However, as the amount of particles in feces was low for the individuals in study II with amalgam fillings, the uptake of mercury released from amalgam fillings may be greater than has previously been believed. It has to be pointed out that the results presented here are only based on a very small material and may not be representative for all individuals with amalgam fillings.

Quality control

To be able to compare data between external studies (44) quality assurance of data is very important. It is also necessary to control the quality of data from internal analysis in order to assure that correct conclusions are derived from data, such as for the for correlation studies. To assure the accuracy of determination of mercury in biological samples has been carried out at all stages. It is very important to have a well established standard operating procedure with clear and well defined instructions to how sampling should be performed. This is aided by informing the subjects to how important it is to follow the instructions for the quality of the end results. In study I and II, a written instruction was given to all participating individuals together with personnel verbal clarification. Once collected, the samples also have to be handled correctly as soon as possible so to prevent the material from changing before analysis. In addition, in study I and II, all material were rinsed before use to avoid contamination from glass and plastic materials during the analysis.

The analysis results of mercury in urine using the modified method in study I was compared with the results from an external laboratory which used an almost identical method. In study II, feces samples were sent to an external laboratory for analysis of total mercury with Neutron Activation Analysis. Furthermore, reference standards were used and analyzed at all possible stages. Sometimes there were no reference samples available, as in study II concerning the speciation of mercury. Reference material was in these cases prepared from feces samples with known contents of mercury after ingestion of amalgam particles (AP), mercury in complex with cysteine (Hg-S) and mercury vapor dissolved in water (Hg-vapor) .

The method used to measure mercury in oral-air by direct-UV, in study I, was compared with quite a different method to analyze mercury from oral-air after wet digestion. This latter method used two different techniques to analyze the same sample which additionally enhances the accuracy of the estimation.

Uptake of mercury from amalgam fillings

Mercury release from amalgam fillings is influenced by the composition of the amalgam, temperature and pressure changes in the mouth which normally occur when individuals are eating a hot meal, chewing gum, brushing teeth etc. Hence, there is large variations for mercury released from amalgam from day to day and even more so between individuals. The uptake of mercury from fillings is influenced by the chemical form of mercury released and the site in the body where uptake occurs. Several studies deal with the estimation of the uptake of mercury from amalgam fillings. Mercury release into oral-air has been measured in some individuals during the whole day and estimations of uptake has been based on breathing habits, as breathing through the mouth or the nose can markedly influence the amount of mercury absorbed. In different studies it has been estimated that the range of uptake is 2-25 μg/24 hours (11, 26). However, in none of the studies has the proportion of amalgam particles been estimated, nor has the uptake from mercury vapor dissolved in saliva and swallowed been established. With a better knowledge of the relative proportions of the different forms of mercury, calculations of the mass flow of mercury through the body can been determined in a more reliable way and a prediction of the uptake, for example, for an individual with a certain number of amalgam surfaces can be established, as in study I.

Background values

Based on the correlation equations between the number of amalgam surfaces and 24 hours mercury excretion in urine, feces and oral-air, background levels of mercury can be calculated for individuals with a different number of fillings. For an individual with no fillings, the value for mercury obtained in study I corresponds very well with urine values for amalgam-free individuals in other studies. Most individuals who never have had any fillings or have had their fillings removed had urine values of less than 1 μg Hg/24 hours (11, 64, 66, 86).

A background value of 15 μg Hg/24 hours in feces for individuals without fillings derived with the correlation equation in study I seemed high compared with other studies, however, this value is markedly influenced by the presence of mercury in the diet. Becker et al., (8) found a daily intake of mercury from ordinary food to be approximately 2 μg/24 hours. This was also in accordance with the mercury values determined in feces sampled during ten days from an amalgam-free individual in study I (28, 66).

Oral-air mercury for an individual without fillings gave a mercury value which was under the detection limit with the method described in study I. Mercury accumulated in the body, from uptake of mercury in food, are in balance with all body fluids and also with exhaled air hence, mercury can be found in oral-air although the individual has no amalgam fillings (30).

With help of these correlation equations the non-occupational excretion of mercury in urine, feces and oral-air can be calculated for a particular individual with a known number of amalgam surfaces.

Table 3. Background values of 24 hours mercury levels excreted from food and amalgam fillings in urine, feces and oral air are presented for individuals with a different number of fillings. These values are estimated according to correlation equations and compared with values found in the open-literature.

Mercury excreted in μg/24 hours according to the correlation equations.				Background values according to literature in μg/24 hours
Number of amalgam surfaces	0	30*	120**	0
U-Hg	0.4	2.8	10	< 1
F-Hg	15	60	189	1-4
O-Hg	0.1	22	88	<0.5

* an average load for a Swedish middle aged population.
** if all premolars and molars are counted as amalgam crowns.

It is valuable to estimate such values to separate occupational exposure from non-occupational exposure (80). For individuals who are occupational exposed to low concentrations of mercury, for example, dentists and nurses it is especially important to determine the background excretion of mercury from their own amalgam fillings.

Future studies

Present day knowledge about the uptake of mercury from amalgam fillings is still insufficient. It would be of interest to determine if the proportion of amalgam particles vary greatly in a large number of individuals, or if they are of the same order as found in this investigation with six individuals with amalgam fillings. There is also a need to further characterize the toxicity and dose-response relationship with low doses of mercury for long-term exposure (14). These latter studies would be of interest as individuals are exposed to mercury from amalgam fillings 24 hours a day for the majority of their life in contrast to occupational exposed individuals. The problem of mercury exposure from amalgam fillings will continue for many years as the middle and old aged populations still have their amalgam fillings intact. In Europe, amalgam is also used as a tooth filling material even for children.

Environmental aspects

Mercury release to the environment is a great problem and a proposition to restrict the use of mercury has been proposed in a parliamentary resolution, in Sweden. There are many restrictions that dental clinics must follow when handling amalgam waste. Amalgam separators with at least 95% efficiency have to be used in the clinics. Waste has to be specifically sent for destruction or recycling. Despite these measures, waste-water from dental clinics in Sweden contains 150-200 kg mercury per year.
The fecal and urinary excretions of mercury from the Swedish population into the sewage system is approximately 150 kg Hg a year.
There is also a release of mercury vapor to the environment from crematories of approximately 255 kg per year (71, 63, 84).
Inorganic mercury released into in the sea can be methylated by micro-organisms and accumulated in the sediment and subsequently through bioaccumulation there will occur a high concentration of methyl mercury in fish (82, 83). An investigation of how to handle mercury-waste in the future is currently ongoing by the Swedish National Chemicals Inspectorate and Swedish Environmental Protection Agency. One possible solution which has been discussed is to store mercury-waste in specially built rock shelters.

Conclusion

It has been established that mercury is released from amalgam fillings and that the amount of mercury which passes through the human body is sometimes very high. For a person with all occlusal surfaces filled with amalgam the excretion was 100 times the mean intake of total mercury from the normal Swedish diet. Today there are sophisticated and sensitive analysis methods for mercury and, therefore, mercury can be detected in almost all parts of the human body. Although there are hundreds of articles published dealing with mercury in amalgam further studies are required on the uptake or on the toxic effects of mercury from amalgam in the human biological system. The Swedish Socialstyrelsen has decided that amalgam shall not be used as a tooth filling material for young individuals. However, the middle aged and elderly people still have old amalgam fillings continously leaking mercury which means that the problem has not yet ended.
It is also established that mercury from amalgam is spread to the environment, for example, into the sewage system. Restrictions on the use of mercury have been introduced and discussions are on-going concerning how to handle existing mercury in a safe way, for example, by storing mercury in specially prepared rock shelters. Compared with this, the opinion that the only currently safe place to stoore mercury is in our teeth as amalgam fillings is rather bewildering.

With this in mind, it is not difficult to understand why people are confused and even afraid of having mercury in their teeth. This in itself may be reason enough to ban amalgam from use in dental care.

Summary

Engqvist A. Mercury exposure from amalgam fillings: Analysis of mercury in different biological matrixes and speciation in the feces. *Arbete och Hälsa* 1998:2.

Techniques for sampling and methods for analysis of mercury in oral-air, urine and feces were developed for individuals exposed to mercury from their amalgam fillings. Mercury can be released from fillings as elemental mercury (Hg^0) which can either be inhaled or dissolved in saliva and swallowed, or as oxidized mercury (Hg^{2+}) from corrosion of the amalgam or oxidation of elemental mercury, or as particles from intensive chewing. In order to follow the exposure to mercury and the excretion of these compounds two individuals swallowed amalgam particles, oxidized mercury as a cysteine complex and mercury vapor. The excretion of amalgam particles and oxidized mercury was about 80%. Less than 50% of mercury from the swallowed mercury vapor was excreted indicating that a large uptake of elemental mercury had occurred.

A method to separate mercury particles from other mercury compounds in feces was also established. These methods were used in a study with ten individuals with and without amalgam fillings in order to follow the excretion of mercury in oral-air, urine and feces. The number of amalgam surfaces were counted (0-82) and the 24 hours mercury excretion in oral-air 0-124 μg/day and in urine 0.4-19 μg/day and in feces 1-200 μg/day were measured. A significant correlation was obtained between the number of amalgam surfaces and the levels of mercury at 24 hours excreted in the different samples. From this correlation the backgrounds levels for non-occupational individuals was calculated. In another group of individuals with 0-60 amalgam surfaces, the amount of amalgam particles in feces were established to be about 25% of the total excreted mercury. The total flow of mercury was sometimes very high (200 μg/day) and as the proportion of particles was low the uptake of mercury may be greater than presently believed. However, it is envisaged with this knowledge about the proportion of particles the calculations of the uptake of mercury will be more reliable in the future.

Sammanfattning

Engqvist A. Mercury exposure from amalgam fillings: Analysis of mercury in different biological matrixes and speciation in the feces. *Arbete och Hälsa* 1998:2.

Avsikten var att utarbeta analysmetoder för att kunna mäta kvicksilver exponeringen från amalgamfyllningar.
En provtagnings och analys teknik togs fram för att bestämma kvicksilveravgången från amalgam fyllningar in situ till luften i munhålan, samt vad som påverkade densamma.

För att kunna följa utsöndringen av kvicksilver härrörande från amalgam fyllningarna, utarbetades känsliga provtagnings- och analysmetoder för bestämning av kvicksilver i feces och urin.

Utsöndringen av kvicksilver i feces följdes från två individer efter intag av kända mängder kvicksilver, dels bundet till cystein dels i form av amalgampartiklar. I båda fallen återfanns ca 80% av intaget kvicksilver i feces, men utsöndringen av partiklarna var något snabbare. Amalgam partiklarna passerade mag-tarm-kanlen opåverkade. Av nedsvald kvicksilverånga, löst i vatten, återfanns endast ca hälften i feces, vilket tyder på ett betydande upptag av elementärt kvicksilver i magtarm-kanalen. Detta tyder på att även upptaget av nedsvald kvicksilverånga löst i saliv härrörande från amalgam fyllningar kan vara betydande.

Storleken på upptaget och toxiciteten för kvicksilver varierar beroende på i vilken form kvicksilver föreligger och var i kroppen upptaget sker. En metod utvecklades för att speciera det kvicksilver som utsöndrades i feces. Med denna teknik kunde mängden amalgam partiklar uppskattas i feces och mängden lättrörligt kvicksilver bestämmas.

Studier har sedan genomförts på individer med och utan amalgam fyllningar. Antalet amalgamytor hos försöks personerna var 0-82 st. Kvicksilveravgången till munhålan var 0-124 μg/dygn och utsöndringen av kvicksilver i feces har uppmätts i området 1-200 μg/dygn och motsvarande urin värden 0,4-19 μg/dygn. Korrelationsstudier visade att signifikanta samband erhölls mellan antal fyllningar och dygns utsöndring av kvicksilver i munhålan resp urin och feces. Från dessa samband kan även uppskattningar av bakgrundsvärden för icke yrkes exponerade individer göras. Hos en grupp av sex individer med amalgam fyllningar bestod endast en fjärdedel av det kvicksilver som passerade kroppen av amalgam partiklar. Flödet av lättrörligt kvicksilver genom kroppen är alltså högt. Det högsta uppmätta fecesvärdet på 200 μg Hg/dygn överskred det genomsnittliga dygnsintaget av kvicksilver via födan med ca 100 gånger. Med kännedom om hur stor del av kvicksilver i feces som består av amalgam partiklar kan säkrare beräkningar göras av upptaget från amalgamfyllningarna.

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