Blood and urine mercury levels in adult amalgam patients of a randomized controlled trial: Interaction of Hg species in erythrocytes

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Abstract

Parts of the population are permanently exposed to low levels of Hg\textsuperscript{+} and Hg(II) from dental amalgam. It was the aim (1) to investigate the internal exposure to amalgam-related mercury from the kinetics of inorganic Hg in plasma and erythrocytes after amalgam removal, and (2) to estimate the amalgam-related absorbed dose. Dietary coexposure was monitored by determination of blood organic-Hg. Postremoval steady-state Hg concentrations were measured for 18 months. Eighty-two patients had been randomized into three groups: (A) removal of the fillings; (B) removal and non-specific detoxification, and (C) a health promotion program without removal. After amalgam removal, inorganic Hg dropped rapidly in plasma and red cells, stabilizing at 27% of preremoval levels after 60 days. Concentrations of organic Hg in plasma remained unchanged, indicating no change in dietary uptake of organic Hg. The concentration of organic Hg in red cells of group A was in the early postremoval phase lower and in the late postremoval phase higher than the preremoval control ($p<0.01$ for low-high difference). A protracted increase in organic Hg was also found in red cells of group B after 60 days. Thus, the effect of removal on organic Hg levels in the combined group A + B was compared with the values of group C in a linear mixed effects (LME) model which showed a significant increase with time in group A + B ($p = 0.028$). In all groups, time profiles of urinary concentration and excretion of total-Hg were very similar to those of inorganic-Hg levels in plasma. From extrapolations of blood and urine data it was estimated that the amalgam-related inhalation and ingestion of Hg species were within the limits proposed by WHO, ATSDR and EPA. The integrated daily Hg dose absorbed from amalgam was estimated up to 3\micro g for an average number of fillings and at 7.4 for a high amalgam load.

Conclusions: This is the first study on adult amalgam patients which continuously monitored the postremoval decline of inorganic Hg and the coexposure from dietary organic Hg in a randomized-controlled-trial design. The integrated daily dose of 7.4\micro g absorbed from a high amalgam load is well below the tolerable dose of 30\micro g (WHO, 1990). The unexpected postremoval increase in erythrocyte organic Hg, which is associated with the depletion of cellular inorganic Hg, might result from binding of organic Hg to cellular sites previously occupied by inorganic Hg.

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1. Introduction

Dental amalgam fillings are the main source of permanent low-level exposure of the general population to mercury vapor (Hg\textsuperscript{+}) and inorganic Hg (Hg(II)). The internal Hg burden is best reflected by Hg concentrations in blood and urine. Yet measurements of total Hg in blood cannot unambiguously be related to the origin of exposure with Hg because of the presence of dietary organic Hg, in particular if similar amounts of both forms of mercury are taken up. In spite of the confounding effect of dietary
methylmercury in the mixed exposure, many studies on amalgam patients had been based solely on the determination of total Hg and had assessed fish consumption mostly by subjective information from questionnaires.

Analytical speciation of total and inorganic Hg, providing direct observation of the uptake of amalgam-relevant as well as of dietary Hg species, has been used rarely in studies on amalgam patients. If it was applied, then only for whole blood (Kingman et al., 1998; Evens et al., 2001), although Hg species show marked differences in the distribution to plasma and blood cells. In some instances, total Hg has been determined separately in whole blood and plasma (Sandborgh-Englund et al., 1993) or in erythrocytes and plasma (Molin et al., 1990; Berglund and Molin, 1996). The latter reports provided evidence that the correlation with amalgam-related parameters was stronger for total Hg in plasma than for total Hg in red cells. Thus, the simultaneous exposure to inorganic and organic Hg and the peculiarities of their partition to the fractions of whole blood make it necessary to determine both species in plasma and red blood cells for a realistic assessment of the contribution of amalgam to the internal Hg burden. In an inland population, a low interference from dietary organic Hg can be an advantage for an amalgam study.

Worried by anticipated toxic effects from amalgam Hg and by the symptoms of an undefined clinical condition called amalgam disease, 1500 amalgam patients filed a law suit at the State Court in Frankfurt/Main in 1995 against a major amalgam manufacturer in Germany. The ensuing court ruling commissioned a comparative investigation on the outcome of various therapeutic interventions in amalgam patients. Hence a three-armed randomized clinical trial was selected as the most appropriate approach and was applied for the first time to adult amalgam patients (German Amalgam Trial, GAT). This report is part of the GAT and is focused on the toxicological-analytical results, while those of the psychometric evaluation will be reported elsewhere (Melchart et al., 2006).

It was the aim to investigate the internal exposure to amalgam-derived Hg by following the kinetics of total and inorganic Hg in blood as well as the levels of total Hg in urine after intervention, while the exposure to dietary methylmercury was monitored from the blood levels of organic Hg. The treatment consisted in amalgam removal in 1/3 of the cohort and in a health promotion program without removal in the remaining 2/3. In particular, the postremoval follow-up period of 18 months for the psychometric protocol facilitated the observation of lowered steady state levels which allowed the estimation of the daily Hg dose absorbed from amalgam.

2. Methods

2.1. Design and patients

The study was a randomized, controlled, three-armed trial comparing three treatment strategies. (A) removal of dental amalgam, (B) removal of dental amalgam combined with a so called biological detoxification therapy, and (C) participation in a health promotion program without removal of amalgam.

2.2. Inclusion criteria

Patients with dental amalgam fillings who suspected that their health complaints (at least 10 symptoms) were caused by dental amalgam; age 20–50 years. Besides this limitation, the influence of age on the number of amalgam fillings was further reduced by stratified randomization of the number of amalgam surfaces into three subgroups (1–12, 13–18, 19–25 surfaces) within each group. Exclusion criteria were: patients with bridges, crowns, gold inlays or dentures, and patients with unsuccessful endodontic treatment, and those with relevant organic or mental disorders.

2.3. Treatments

In groups A and B, amalgam was replaced by other filling materials at the University Clinic of Restorative Dentistry. Fillings were removed by one quadrant at a time with at least one week between visits; the cavities were temporarily sealed with calcium hydroxide before being filled with ceramic or gold inlays, or composite filling. In patients with many fillings, the quadrant-oriented procedure extended the treatment period up to six weeks between removal of the first and the last amalgam. Beginning four weeks before amalgam removal, patients of group B were additionally treated with high doses of vitamins and trace elements for 12 weeks; these patients, however, did not receive therapeutically proven Hg antidotes such as chelating agents. Patients in group C kept their amalgams and participated in a health promotion program in the form of group therapy, aiming at developing a health-related lifestyle management suitable for patients’ everyday life (Wunderlich and Melchart, 2002).

2.4. Blood and urine sampling

Concentrations of total, inorganic and organic Hg in red blood cells and plasma had been measured at the time of prescreening (pre-1) and after randomization into groups (pre-2), i.e. when all participants had their fillings still in place. At the same time, samples of morning urine had been taken for measurement of concentration and excretion of total Hg. Posttreatment followup started at day zero, when the last amalgam had been removed (groups A and B) or the first therapeutic session had been attended (group C). Thereafter, blood and urine samples were taken at days 60, 360 and 540, and additionally at days 1, 3, 9 and 30 in group A. Additional urine samples were collected at day 180 in groups A and B.

Venous blood was collected in 10 ml syringes pretreated with EDTA (Primavette blood-collecting system, Kabe, Nümbrecht, Germany). Shortly after collection, plasma and red cells were separated by centrifugation and stored at 4 °C for no longer than three days before Hg determination. Morning urine was collected between 10 pm and 6 am into 1 L polyethylene bottles that contained 1 mL of 10% nitric acid to avoid volatilization of Hg.

2.5. Mercury speciation

Mercury was determined in a cold vapor atomic absorption spectrometer equipped with a gold trap (Hg-Mess-2-87, Leunawerke, Leuna, Germany) by reduction of samples of 0.4 mL red cells, 0.5 mL plasma or 1 mL urine with alkaline SnCl2 (Magos and Clarkson, 1972). This method was modified by replacing CdCl2 with CuSO4 to break the C–Hg bond for the determination of total Hg, or by protecting the C–Hg bond with hydroxylamine hydrochloride (without CuSO4) for the determination of inorganic Hg (Manthey and Berge, 1980; Roschig and Wünscher, 1988). All analyses were carried out in duplicate, and the concentration of organic Hg was obtained as the difference between the duplicates’ means of total and inorganic Hg, as had been reported previously (Halbach et al., 2003). The concentration of Hg in whole blood was calculated from the
concentrations in red blood cells and plasma, assuming a hematocrit of 45% (average for men and women of this age group (Diem and Lentner, 1974).

External quality control was ascertained by regular participation in interlaboratory trials organized by the German Scientific Society for Occupational and Environmental Medicine. Hg concentrations of blood and urine test samples were below 5 ng/mL (environmental range) and above 10 ng/mL (occupational range); our laboratory had correctly quantitated 95% of the test samples in each range. Internal quality was surveyed by analyzing control samples of urine (reference 8.2–9.8 ng/mL) and whole-blood (reference 2.9–3.4 ng/mL; Clincheck, Recipe, Munich, Germany). Inserted in the daily series, these samples had to be within ±3 SD of the above reference values as determined by independent reference laboratories. Day-to-day coefficients of variation were between 3.5% (standard solution, 50 ng/mL) and 12.6% (certified blood, 3.4 ng/mL). Detection limit was 0.1 ng Hg/ml.

2.6. Statistics

Results were expressed as median concentrations. Differences were considered significant at \( p \leq 0.05 \). Linear and nonlinear regressions were calculated by the least-squares method.

3. Results

3.1. Patient disposition

Patients were included in the study between April 1998 and July 2002. Most participants were recruited through reports in local newspapers. Approximately 1200 patients expressed interest in the study, of which 164 entered the baseline, and 91 were randomized. Due to dropouts, blood and urine data of 26 patients in each of groups A and B, and of 22 in group C were evaluated up to day 540. Mean numbers of amalgam surfaces were 23.6 ± 10.3 (A), 26.4 ± 10.6 (B) and 23.1 ± 9.8 (C; \( n = 25–28 \)).

3.2. Hg concentrations in blood before intervention

Concentration data were not distributed normally, hence medians were used for the descriptive statistics. With regard to the pre-1 and pre-2 data of each Hg species, the three groups did not differ significantly (Kruskal–Wallis test). The values at pre-2 served as baseline because of the proximity to the treatment phase. As shown in the notched box plot of Fig. 1, the overlap of the baseline data indicated the absence of significant differences between the groups. For all participants (\( n = 75–77 \)), the median concentration of organic Hg was fivefold higher in cells than in plasma and represented the highest accumulation (37%) of either Hg species in either blood fraction (Table 1). This indicated a strong affinity of erythrocytes for organic Hg in contrast to inorganic Hg which was almost equally distributed between cells and plasma. It can also be seen that organic Hg in both blood fractions makes up 47% of total Hg in whole blood, and that it still contributes 25% to total Hg in plasma (Table 1).

3.3. Hg concentrations in blood after intervention

In each of the groups, the time course of total-Hg levels in erythrocytes showed an initial decline followed by an early (group A), intermediate (group B) or late increase (group C). The corresponding concentrations in plasma showed an initial decline to nearly steady-state levels in groups A and B and a protracted moderate decline in group C (Fig. 2). Inorganic Hg levels displayed very similar time courses in red cells and plasma within each group (Figs. 3 and 4). There was an initial drop after amalgam removal in groups A and B which was followed by steady state after day 60, while there was little change in group C during the entire
observation period. The median decline of inorganic Hg in whole blood (pre-d-540) was 0.36 ng/mL for the combined group A+B (*n* = 49; data not shown). A fit of single values and medians of the postremoval elimination of inorganic Hg from plasma of group A showed halftimes of 17 and 21 days leading to a new steady state (97% of maximal decline) of 0.17 and 0.15 ng/mL after 85 and 105 days, respectively (Fig. 5). The depletion kinetic of inorganic Hg from red cells of group A was very similar with halftimes of 8 days (single data) and 10 days (medians; data not shown).

Concentrations of organic Hg in plasma did hardly deviate from control values over the whole study period (Fig. 4). The concentration of organic Hg in red cells, however, increased in groups A and B above control values in the late postremoval phase (Fig. 3). This was preceded by a transient decrease in concentration of organic Hg, as revealed by frequent sampling in the first month (group A, Fig. 3). The time-coordinated bidirectional change in red-cell organic Hg was reproducible and contrasted the steadiness of organic-Hg levels in plasma, a coincidence that can hardly be explained by spontaneous changes in diet. Interestingly, the initial depletion of organic Hg from group-A red cells was significantly correlated with that of inorganic Hg (regression day 1–9: organic Hg = inorganic Hg*1.82 + 0.33, *r* = 0.38, *p* = 0.0006, *n* = 77; regression day 0–9: organic Hg = inorganic Hg*1.76 + 0.34, *r* = 0.46, *p* < 0.0001, *n* = 101). In erythrocytes of group C, the concentration of organic Hg decreased slowly within one year and regained the initial level thereafter (Fig. 3).

3.4. Effect of amalgam removal on cellular concentrations of organic Hg

The increase in organic-Hg concentration in red cells of groups A and B was examined further as it was not related to changes in the concentration in plasma (Figs. 3 and 4). First, the postremoval data of group A were partitioned in two subgroups composed of the individual medians of the concentrations of days 1–30 and of those of days 60–540. This permitted the comparison of the early low medians with the late elevated medians in a paired *t*-test which revealed a significant increase in red-cell organic Hg in the late postremoval phase (*p* = 0.01; 26 pairs). Second, a linear mixed-effects model was applied (LME; Pinheiro and Bates, 2000). In this approach, the influence of the factor amalgam removal (fixed effect) on cellular organic-Hg levels was characterized by the comparison of the combined data of groups A and B (treated group) with the...
untreated group C. The grouping of the data into serial measurements associated with individual patients required the consideration of a patient effect. This and the random assignment of the patients to the groups was the motivation to choose a LME model which incorporates both effects. Complete data sets for the time points 0 (pre-removal), 60, 360 and 540 days were entered in the analysis ($n = 39$ for A and B; $n = 19$ for C). Because of the skewed distribution of the data and their generation from concentration differences, a double-log transformation was performed for normalization which confirmed the homogeneity of variances and the normal distribution of the transformed data. The LME model was based on the regression equation

$$H_{\text{organic}} = a + \beta \cdot \text{day} + \varepsilon,$$

with $\varepsilon$ representing an error term. Intercept and slope were treated as random effect. The ANOVA of the LME model showed a significant difference between the groups (treated vs. untreated) with respect to the time course of organic-Hg levels ($p = 0.0288$). The group-specific regressions (fixed effects) were for group A+B:

$$\log(\log(H_{\text{organic}} + 1) + 1) = 0.432986 + 0.000261312 \cdot \text{day};$$

untreated group C. The grouping of the data into serial measurements associated with individual patients required the consideration of a patient effect. This and the random assignment of the patients to the groups was the motivation to choose a LME model which incorporates both effects. Complete data sets for the time points 0 (pre-removal), 60, 360 and 540 days were entered in the analysis ($n = 39$ for A and B; $n = 19$ for C). Because of the skewed distribution of the data and their generation from concentration differences, a double-log transformation was performed for normalization which confirmed the homogeneity of variances and the normal distribution of the transformed data. The LME model was based on the regression equation

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with $\varepsilon$ representing an error term. Intercept and slope were treated as random effect. The ANOVA of the LME model showed a significant difference between the groups (treated vs. untreated) with respect to the time course of organic-Hg levels ($p = 0.0288$). The group-specific regressions (fixed effects) were for group A+B:

$$\log(\log(H_{\text{organic}} + 1) + 1) = 0.432986 + 0.000261312 \cdot \text{day};$$
and for group C:

$$\log (\log (H_{\text{organic}} + 1) + 1) = 0.416044 - 0.000005567 \times \text{day}.$$ 

The regression curves after backtransformation to concentration values are shown together with the median concentrations in Fig. 6.

On the whole, the data on Hg concentrations in blood showed that the postremoval changes of inorganic Hg in both blood fractions were very similar, suggesting a rapid equilibration of this species between plasma and cells. However, the data of organic mercury in cells and plasma were at variance with such a postremoval equilibration which would be expected from the high mobility of organic Hg in the organism (WHO, 1990; see Discussion).

### 3.5. Urine

In presence of amalgam fillings, the concentration of total Hg in the urine of all participants was related to the number of amalgam surfaces by the regression: ng/mL = nr. surfaces *0.091–0.181 (r = 0.47; p < 0.0001; n = 78). After amalgam removal, time-dependent changes in total-Hg concentration in urine of groups A and B were very similar to those in inorganic-Hg levels in plasma (data not shown). This parallelism was also observed for the 8-h excretion rate. In group A, the postremoval 8-h excretion rate of total Hg in urine declined with halftimes of 43 and 52 d to low steady states of 0.17 and 0.23 mg/8 h after 9 and 12 months when calculated from the medians or from individual values, respectively (Fig. 7). Thus, the excretion rate has decreased in group A by 0.39 to 0.45 mg/8 h from its preremoval steady-state level of 0.62.

Postremoval steady-state levels of excretion rate were also assessed from the disappearance of the significant relation between excretion and number of amalgam surfaces. The combined data of groups A and B showed that the slopes of the pertaining regressions decreased with progressing postremoval time (Fig. 8) until they were no more different from zero after 180 days. The y-intercepts of the not-significant regressions of d-360 and d-540 data crossed at 0.21 mg/8 which agreed with the postremoval background excretion in Fig. 7. Under a high amalgam load (45 surfaces), the difference of 1.24 mg/8 h between background and the preremoval regression represented the upper range of the amalgam-related excretion; likewise, the
difference in excretion was 0.69 µg/8 h under a medium amalgam load (median 25 surfaces). Assuming for long-term exposure to Hg that urinary excretion equals about half the total excretion, and that the latter equals uptake (Clarkson et al., 1988), the daily mercury dose absorbed from amalgam was estimated from 2.3 to 2.7 µg at an average amalgam load (e.g. 0.45 µg*24 h/(8 h*0.5) = 2.7) and up to 7.4 µg for individuals with many fillings.

In order to investigate a possible amalgam-related gender difference in urinary Hg levels, the data of groups A and B were combined. It can be seen in Table 2 that the preremoval concentrations were higher in female than in male samples (not significant), whereas they were equally low at day 540. The larger pre-post difference in women than in men, however, was not quite significant (p = 0.058; one-tailed Mann–Whitney test).

4. Discussion

This is the first study on mercury kinetics after amalgam removal which applied the design of a randomized controlled trial to adult amalgam patients, and which surveyed the concomitant exposure to dietary mercury by continuous determination of organic Hg in blood (Clarkson et al., 1988; WHO, 1990). In order to reduce pain from dental procedures and to avoid gnathological complica-

tions, in particular in patients with many fillings, the amalgams were exchanged for each quadrant in separate sessions which led to varying exposures over several weeks. Therefore, day zero of the protocol was set at the removal of the last amalgam. This protracted partial exposure blurred the expected transient increase in Hg levels in blood.
and urine occurring immediately after a one-session removal of all fillings (Molin et al., 1990; Begerow et al., 1994; Kremers et al., 1999).

4.1. Amalgam-specific and background Hg concentrations in blood

The between-group comparison of time profiles of inorganic Hg levels in plasma and erythrocytes clearly demonstrated that this Hg species is the specific indicator for the absorption of amalgam-derived Hg in plasma and cells. Nearly as representative was the time course of total-Hg concentration in plasma (Fig. 2) because of the constantly low background level of organic Hg (Fig. 4). In contrast, the measurement of total Hg in whole blood included 47% organic Hg even in a population with low fish intake (Table 1). This percentage can be expected to increase in subjects with elevated fish consumption or few amalgam fillings. Hence the specificity for amalgam-derived mercury becomes the more important once the post-removal concentrations of the relevant species decline.

Due to its high mobility in the organism, methylmercury is uniformly distributed to the tissues a few days after absorption (WHO, 1990). Thus, in blood, a small portion is bound to plasma proteins while 90% or more is accumulated by red cells (Berlin, 1986; Clarkson et al., 1988). In our study population, about 80% of the organic Hg in blood was found in erythrocytes (Table 1). After long-term exposure, it is reasonable, therefore, to assume a steady-state distribution between plasma and erythrocytes for all Hg species. This means that an additional fish intake by some participants during the study should have resulted in an increase in erythrocyte organic-Hg as well as in a simultaneous relative increase in plasma organic-Hg at some undefined time point but this was not observed (Fig. 4). The increase in red-cell organic Hg was consistently preceded by a transient drop below the preremoval level as shown by frequent sampling in the early postremoval period of group A, in which time plasma organic-Hg values did also not change (Figs. 3, 4). This sequence of early postremoval decrease and late increase in erythrocyte organic Hg was common to all subjects of group A although zero-time of the individual protocols varied over a period of one year because of the enrolment pattern. Such a time-coordinated decrease and increase in red-cell organic Hg is unlikely to have resulted from unscheduled changes in the diet of randomized subjects; it rather is evidence for an association with the termination of Hg emissions from amalgam.

4.2. Interaction of inorganic and organic Hg in blood

The kinetics of mercury binding had initially been investigated in isolated human red blood cells in which about 25% of sulfhydryl groups show high-affinity reactions with Hg(II) or reduced-affinity binding to organic mercury compounds (bifunctional sites; Weed et al., 1962; Van Steveninck et al., 1965). Later, GSH and SH groups of hemoglobin have been identified as intracellular ligands for methylmercury (Naganuma et al., 1980; Doi and Tagawa, 1983). Detailed examinations of the isolated reaction partners revealed a high exchange rate of each Hg species at thiol-containing ligands which promotes the high mobility of mercury in biological systems as well as a fast concentration-dependent Hg extrusion from the cells (Rabenstein, 1989). This seems also consistent with the assumption of a transmembrane GSH–GSSG shuttle which can efficiently remove thiolate-S conjugates of xenobiotics from the cells (Beutler and Dale, 1989).

In view of the rapid intracellular redistribution and/or extrusion of mercury, the significant correlation between inorganic and organic Hg in group-A erythrocytes in the early postremoval phase suggests a possible sequence of interactions between these Hg species. While the cessation of Hg\textsuperscript{2+}\ emissions had reduced the preremoval uptake of Hg(II) from plasma into red cells, the efficient extrusion of GS-Hg(II) conjugates still continued which resulted in a rapid decrease in red-cell inorganic Hg of group A and B patients. (Fig. 3). With ongoing depletion of high-affinity bound Hg(II), some of the GSH-mediated mercury-efflux capacity became available for organic Hg, which was then eliminated together with inorganic Hg (group A, Fig. 3). In fact, regression coefficients between 1.76 and 1.82 for the organic/inorganic-Hg concentration ratio in the initial depletion phase (see Results) suggested the stoichiometric replacement of inorganic Hg (reacting with one or two SH groups) by organic Hg (binding to one thiol only; Rabenstein, 1989). Similar to the mercury efflux, organic Hg may also gradually have substituted the inorganic Hg removed from other intracellular binding sites in the late postremoval phase. In addition, with progressing post-removal time, young erythrocytes appeared in the peripheral blood which had been exposed mostly to organic Hg during erythropoiesis, as is the case with chronic lead exposure (Woods, 1996). These cells had then a priori a higher capacity for organic Hg than mature preremoval cells. The stability of organic-Hg levels in plasma of all groups might be explained from their regulation by intestinal absorption and excretion, the capacity of which is large in comparison to the total release and uptake by red cells.

The notion that the elevated postremoval concentration of red-cell organic Hg was a genuine effect of Hg(II) decrease on the cellular balance of organic Hg was supported by several observations. (1) the initial decrease in inorganic and organic Hg in group-A erythrocytes was well correlated, (2) the biphasic changes in the organic-Hg concentration of group-A erythrocytes were significant (Fig. 3), and (3) the longterm increase in organic Hg of the combined-group A + B red cells was also significant (LME model, Fig. 6). The last aspect, being characterized by the random effect in the LME model, is representative for the general population and is compatible with the findings of a Swedish study (Molin et al., 1990). In this patient...
group, the 12-months decrease in plasma total Hg of 2.2 nmol/L (comparable to that of 1.9 nmol/L for group A in Fig. 2) was paralleled by unchanged levels in erythrocytes although a nearly equal postremoval depletion of inorganic Hg in cells and plasma (Figs. 3 and 4) could have been expected. These findings also include the possibility of organic Hg compensating the loss of cellular inorganic Hg.

4.3. Dose absorbed from amalgam Hg and relation to reference limits

It is assumed that inorganic Hg is excreted via intestine and kidney at roughly equal rates, while organic Hg is removed predominantly via the intestinal route (Clarkson et al., 1988). This explains the strong correlation between Hg concentration in urine and the amalgam surface area. In fact, we found the same relationship of 0.09 ng/mL in urine per one amalgam surface as did Kingman et al. (1998) and Schuurs et al. (2000). Absorption of inhaled Hg$^+$ and ingested Hg(II) are integrated into one internal mercury dose originating from amalgam, i.e. the increases in whole-blood inorganic Hg and in the renal excretion of total Hg can no more be differentiated as to the relative contribution of these Hg species. Therefore, the estimated absorbed doses of amalgam-Hg were compared with the proposed limits of exposure and intake levels under two separate assumptions, i.e. that this absorption consisted entirely of Hg$^+$ or of Hg(II). (1) As reported in Results (Figs. 7 and 8), the amalgam-related absorbed dose ranged from 2.7 μg/d/person for a medium amalgam load to 7.4 μg/d/person for persons with many fillings. If this dose originated entirely from Hg(II) exposure it can be compared to the tolerable intake of 2 μg inorganic Hg per day and kg bodyweight as proposed by WHO (WHO, 2003) which translates into an absorption of ca. 13 μg per day in a 65-kg person (assuming 10% intestinal absorption). (2) The median pre-/postremoval difference of 0.36 ng/mL (group A + B) in the whole-blood concentration of inorganic Hg facilitates the use of the slope observed in the relation between Hg concentrations in air and blood in Hg$^+$ exposed workers (Roels et al., 1987):

\[
\text{whole-blood Hg (ng/mL)} = \frac{\text{air work (μg/m}^3\text{)}}{0.48}.
\]

In case of permanent exposure to the same amount of Hg$^+$, the concentration at the workplace is diluted in proportion of worktime per week (40 h/168 h) as follows:

\[
\text{whole-blood Hg (ng/mL)} = \frac{\text{air permanent (μg/m}^3\text{)}}{2}.
\]

Inserting the above decrease by 0.36 ng/mL yields an amalgam–associated concentration of 0.18 μg/m$^3$ for permanent Hg$^+$ exposure which can be compared to the EPA reference concentration of 0.3 μg Hg/m$^3$ (EPA, 1997) or to the ATSDR minimal risk level of 0.2 μg Hg/m$^3$ (ATSDR, 1999). However, the assumption of single-species absorption overestimates the amalgam-related Hg$^+$ exposure or Hg(II) intake as the changes in blood inorganic Hg and in Hg excretion are in reality partitioned into both ingestion and inhalation. Nevertheless, a reason for this dual approach is the uncertainty about the relative contribution of each species to the internal Hg burden which may result from the oxidation of inhaled Hg$^+$ to Hg(II) by the high activity of human erythrocyte catalase (Halbach and Clarkson, 1978).

The increase in preremoval urine-Hg concentrations of women exceeding that of men (Table 2) appears to be consistent with a significantly higher Hg concentration in renal tissue of amalgam-bearing women in comparison to that of men (Barregard et al., 1999). Of note is the disappearance of the gender difference in urine Hg at the lowered postremoval steady-state levels which suggests that the amalgam-related increase in tissue-Hg levels of amalgam-bearing women is reversible.

4.4. Conclusions

The integrated daily dose of ca. 7.4 μg absorbed from a high amalgam load is below the tolerable dose of 13 μg. The exposure to Hg vapor, as extrapolated from the amalgam-related increase in inorganic Hg in whole blood, does not exceed the levels proposed for permanent inhalation. The unexpected postremoval increase in erythrocyte organic Hg might result from binding of organic Hg to cellular sites previously occupied by inorganic Hg. The mutual exchange of toxic metal species at background exposure levels has thus far not been reported for a tissue in the living human organism.

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The protocol was approved by the university ethics committees of the Technische Universität München and the Ludwig-Maximilians Universität München.

All study participants provided written informed consent.

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