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Mercury Release from Dental Amalgams: An *in vitro* Study Under Controlled Chewing and Brushing in an Artificial Mouth

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Abstract. The release of mercury vapor from class I amalgam restorations prepared in human molar teeth was studied during chewing simulations in an artificial mouth of a bi-axial servo-hydraulic mechanical test system. So that the total mercury released from the restoration over a fixed time could be determined, a closed chamber surrounded the envelope of chewing motion. In addition, the influence of sampling frequency on mercury release was corrected by the use of different sampling frequencies over a fixed time interval of mercury release measurement and extrapolation to zero sampling time. Thus, a combination of a closed environment and an extrapolation method to determine the mercury release under continuous sampling was used to determine the mercury released under normal breathing conditions. The measured mercury release rate data were used to calculate the potential daily mercury dose in a patient due to a single amalgam restoration, following the method previously outlined by Berglund. The mercury release from both a conventional and a high-copper amalgam was evaluated at different age intervals after the restoration was placed in the teeth. The results show that while the age of the amalgam and the amalgam type influence the extent of mercury release during the initial non-steady-state conditions, the steady-state value of mercury daily dose due to a single amalgam filling is 0.03 μg/day, which is well below the calculated threshold-limiting value (TLV) of 82.29 μg/day considered dangerous for occupational exposure in the United States.

Key words: dental restoration, dental amalgam, mercury, chewing, toothbrushing.

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Introduction

Since the early 1800's, mercury has been an important component of dental amalgams, used in various forms and compositions. It has been estimated that 75% of all single-tooth restorations are amalgam restorations and that this percentage has remained stable for many years (Rupp, 1973). The ease of manipulation and placement, the relatively low cost, and the well-known history of performance in the oral cavity have made dental amalgams an extremely popular restorative material. Yet, the safety of dental amalgams for both the dental patient and dental personnel has been questioned and debated intermittently since the inception of amalgam use (*e.g.*, Burgh, 1863; Lawrence, 1866; Stock, 1926a,b; Fuhner, 1927; Souder and Sweeney, 1931; Grossman and Dannenberg, 1949; Phillips and Schwartz, 1949; Eames *et al.*, 1976; Merfield *et al.*, 1976; Pinto and Huggins, 1976; Gay *et al.*, 1979; Huggins, 1982; Bauer and First, 1982; Bauer, 1985; Paterson *et al.*, 1985 Vimy and Lorscheider, 1985a,b; Craig, 1986; Vimy *et al*, 1986, 1990; Hahn *et al.*, 1989, 1990). Notwithstanding these reports, there are only about 50 documented cases of known allergic reaction to amalgam, while billions of amalgam fillings have been placed in patients during the last 150 years (American Dental Association, 1990). It has been aptly pointed out that the long history of amalgam, successfully used for more than 165 years, and the number of amalgam restorations placed with no documented toxicity clearly confirm the safety of amalgam for restorative applications (Stanford, 1984).

Unfortunately, however, controversial reports from scientists have created apprehension among patients about the nature and extent of mercury release from amalgam fillings. The apprehension among patients is understandable, because mercury is a well-known toxic element in vapor and in some combined forms (Stokinger, 1981). Clearly, there is considerable evidence linking mercury to potential health hazards. One of the primary hazards from dental amalgams is the possible mercury

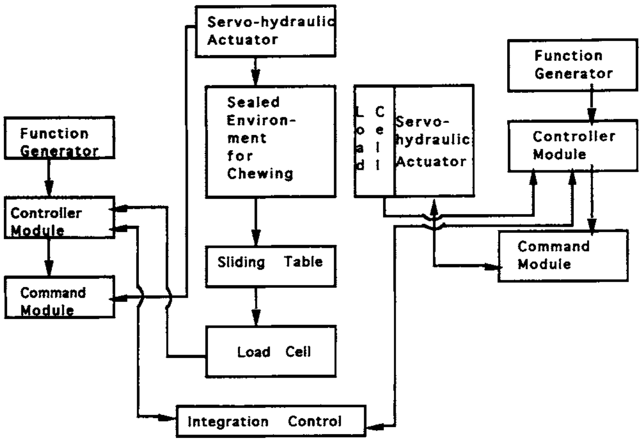

Figure 1. Block diagram of the operational components of the MTS Bi-axial Oral Environmental Testing System.

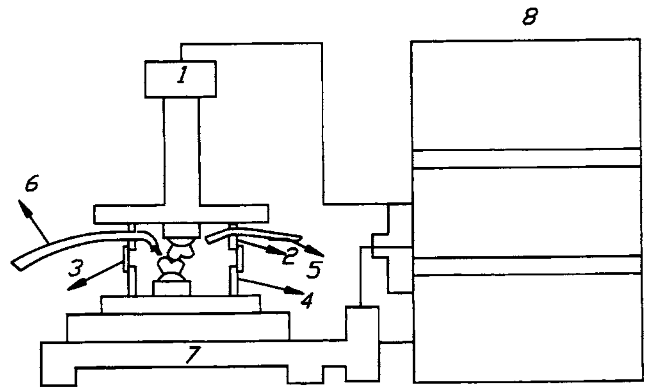

Figure 2. The arrangement of the closed environmental system used for chewing. The identified parts are: (1) vertical actuator, (2) top cylinder containing maxillary tooth, (3) clamped wide rubber band, (4) bottom cylinder containing mandibular tooth, (5) tube connecting mercury analyzer to the closed environment for mercury vapor measurement, (6) water spray tube, (7) horizontal actuator, and (8) electronic control console.

vapor release from amalgams over their lifetime of service in the oral cavity. Although there is considerable literature in this area (Abraham *et al.*, 1984; Vimy and Lorscheider, 1985a,b, 1990; Hahn *et al.*, 1989; Mackert, 1987; Berglund *et al.*, 1988; Berglund, 1990, 1993; Ferracane *et al.*, 1992; Svare *et al.*, 1981; Marek, 1990, 1994), most of the controversy is also focused on the extent of mercury vapor release from amalgam fillings. The public concern on the safety of amalgam has been heightened by the reports of dangerous levels of mercury release in recent controversial studies, where ewes (Hahn *et al.*, 1989) and sheep (Vimy *et al.*, 1990) were used as animal models. These animal models tend to overestimate the release of mercury because: (a) the ewes regurgitate, and consequently they chew much more, and the food that they re-chew contains gastric fluids from the stomach, which may also accelerate the mercury release; and (b) there are differences in the exposed surface areas and chewing conditions between sheep and humans. Moreover, Mackert (1987) and Berglund *et al.* (1988) have demonstrated that, due to incorrect calculations and assumptions, Vimy and Lorscheider had overestimated the extent of mercury release by as much as 16 times. Unfortunately, however, there is considerable anxiety among the public because of the concerns often expressed in the media, where the results from controversial studies are often given more exposure than well-accepted scientific reports. As a result, there is a critical need for additional research on the release of mercury from amalgam fillings in well-controlled experiments, where mercury release can be sampled to simulate a clinical situation, and we can determine the highest possible mercury release during chewing and brushing and calculate the resulting daily dose of mercury to the patients. A unique approach to measuring mercury is through the use of an artificial mouth, where masticatory motions can be controlled in a predictable, repetitive manner and the effects of independent variables can be studied carefully. Although previous investigations on mercury release are extensive, most reports are based on measurements of mercury release acquired *in vivo*. Since a closed environment is difficult to duplicate clinically, measurements of mercury release in a sealed environment of an artificial mouth may provide valuable information not only on the mercury release but also on the release kinetics.

The objectives of this investigation were:

(1) to develop a closed environment for mercury release measurement during chewing experiments involving teeth restored with dental amalgams in a commercial bi-axial mechanical testing system;
(2) to test the hypothesis that amalgam age and composition will have significant effects on the mercury release during mechanical chewing and/or brushing experiments; and
(3) to compare the *in vitro* mercury release and calculated daily mercury dose resulting from this release from an amalgam restoration with the mercury release and dose levels reported in the past.

Materials and methods

An artificial mouth used in this study was very similar to the oral environmental system reported by DeLong and Douglas (1983). The system is based on a bi-axial servo-hydraulic mechanical test system (MTS Inc., Minneapolis, MN). The arrangement of the component parts is shown in the schematic diagram (Fig. 1). The test system is capable of approximating the three-dimensional motion of the mandible with a two-dimensional movement involving proper rotation of the planes of motion. The system has two actuators with separate electronic control systems and an integration control system for inter-actuator synchronization. One actuator operates vertically, one horizontally, and the motions of the two are synchronized and integrated to create chewing simulation. By rotating the planes of motion so that straight-line motion of the horizontal plane lies parallel to the long axis of the horizontal actuator, we can reproduce the three-dimensional motion of mastication by two-dimensional control. The testing system also has the capability of spraying the specimens with a salivary fluid or water at the desired temperature under controlled continuous or pulse modes.

The artificial mouth was modified to an air-and-water-sealed environment. The sealed enclosure was provided by two transparent resin cylinders, representing the maxillary and mandibular halves of the mouth, respectively. The maxillary tooth was attached to the vertical actuator and the mandibular tooth to the horizontal actuator. The two cylinders were connected with each other by a wide flexible rubber band which was tightened to the cylinders with metal ring clamps. All the interfaces between the cylinders and the artificial mouth were sealed by high-vacuum silicon grease (Dow Corning Co., Midland, MI). Fig. 2 shows the arrangement of the sealed environment in the overall experimental set-up. The design permitted the two cylinders to change their relative positions during the chewing or brushing cycles and created a compartment enclosing the chewing envelope of motion. On the wall of the cylinder attached to the vertical actuator, four holes were drilled to accommodate appropriate tubes. One hole accommodated the tube to the mercury vapor analyzer (Fig. 2), one to the zero filter, one for the delivery of the distilled water spray (Fig. 2), and the fourth for the tube used to remove the water from the sealed environment. The zero air filter traps mercury. During the sampling of the chamber, the vapor mercury analyzer draws air from outside the chamber, and the incoming air goes through the zero air filter, which traps any mercury that might be present in the room air. The total approximate volume of the cylinder, without the teeth present, was 120 mL.

Before each use, all the attachments to the artificial mouth were dipped in 20% warm solution of potassium hydroxide (KOH) for 10 min, rinsed with distilled water, and dipped again in 20% chromium tri-oxide (CrO_3). After another 10 min in the chromic acid, they were rinsed with distilled water and dried. This process removes all the traces of mercury from the high-vacuum silicon grease. The attachments were then soaked in 10% nitric acid solution (HNO_3) for 15 min to remove all the mercury traces left on their surfaces.

The mercury released into the air of the chamber was measured with the use of a mercury vapor analyzer (Jerome 431-X Mercury Analysis System, Arizona Instrument Co., Tempe, AZ). The instrument utilizes the change in the resistivity of gold when in contact with air containing mercury vapor. The instrument has a 12-second sampling time at a flow rate of 750 mL/min. During this time, the instrument acquires 150 mL of air. According to the manufacturer, the instrument's detection range is from 0.000 to 0.999 mg Hg/m^3, the sensitivity is 0.003 mg Hg/m^3, and the resolution is 0.001 mg Hg/m^3. It also has ± 5% accuracy at 0.100 mg Hg/m^3, and 5% relative standard deviation at 0.100 mg Hg/m^3. We also calibrated the instrument by transferring known quantities of mercury vapor to a sampling trap, thermally desorbing the trap, and measuring the mercury vapor by the instrument. The least-squares fit (by linear regression) of the measured and actual mercury values yielded a coefficient of determination value (R^2) of 0.993, indicating a very close fit.

The mercury vapor analyzer was interfaced to a IBM PS/2, Model 70/386 (International Business Machines Corporation, Armonk, NY), computer, equipped with Asyst software (Asyst Software Technologies, Inc. Rochester, NY), for data collection and storage. The acquired data were stored in files and then retrieved and analyzed.

Human molar teeth, extracted and stored in distilled water containing thymol, were used in the study. A Class I cavity was prepared in each tooth by means of a high-speed handpiece with a #330 carbide bur under water spray. We placed the amalgam using hand instruments and removed the superficial layers rich in mercury. The material was carved to follow the anatomy of the tooth. The prepared specimens were stored in plastic bottles filled with distilled water at 37°C for the appropriate storage time.

Two amalgam alloy types were used: a conventional alloy, SDI (Southern Dental Industries LTD., Victoria, Australia); and a high-copper alloy, Tytin (Kerr USA, Michigan). The SDI is a micro-grain alloy containing 70% Ag, 26% Sn, 3.5% Cu, and 0.5% Zn. The SDI amalgam used in the study contained 400 mg alloy and 440 mg mercury in capsules. Tytin is a spherical alloy and contains 60% Ag, 28% Sn, and 12% Cu, also supplied in capsules containing 600 mg alloy and 443 mg mercury. Energy-dispersive elemental analysis of the alloys studied gave a composition range which was in close agreement with the manufacturer's specified composition. The specimens were in two categories, depending upon the type of amalgam with which they were restored. One category received the conventional type (SDI) and the other the high-copper type (TY). Each specimen was tested at six different time points. The first test was performed exactly 2 hrs after the preparation, and the rest were performed 1, 7, 15, 30, and 31 days later. All the tests involved chewing cycles except those on the 31-day-old amalgams, which involved brushing cycles. In each group, there were 7 replicates. A total of 70 samples (2 amalgams x 5 aging times x 7 replicates) was evaluated under the chewing protocol and 14 samples (2 amalgams x 1 aging time x 7 replicates) under brushing. The teeth were randomly assigned to each group. A controlled spray of distilled water at 37°C was injected onto the teeth (at the rate of approximately 5 mL/min in a pulsed mode) during both chewing and brushing experiments.

Reports in the literature regarding the wear simulation in the artificial mouth (DeLong *et al.*, 1985; Coffey *et al.*, 1985) have indicated that one month's wear can be simulated by use of a 13.4-N (Newton) force every 0.25 s for 30,000 cycles. When these data are extrapolated, 1000 chewing cycles in the artificial mouth can be considered to be clinically equivalent to one day of intra-oral chewing. In this experiment, we used 200 chewing cycles during a $^1/_2$-hour period in each experiment. Two hundred strokes were also used in the brushing experiments, in which the lateral movement was also adjusted to ensure that brushing covered the complete surface area of the restoration. A section of a Colgate Plus toothbrush was cut and mounted in the top half of the artificial mouth for the brushing experiments, in place of the mandibular tooth used in the chewing experiments. No toothpaste was actually used in the brushing experiments.

Before the chewing experiments, the specimen and the opposing tooth were placed inside the sealed environment, and the hydraulic system was adjusted to deliver a 13.4-N force with 0.84 mm of lateral excursion at 4 Hz. Then the chamber was evacuated with the pump of the vapor analyzer until the mercury reading on the instrument indicated zero and the zero filter was closed. When the artificial mouth was prepared for brushing cycles, a 4.5-N load was used, because it is marginally higher than the loads used in other toothbrush wear studies (Aker, 1982; Svinnseth *et al.*, 1987; Efraimsen *et al.*, 1990; Dean, 1991; Goldstein and Lerner, 1991). The loads applied in these studies ranged between 1.5 and 4.5 N.

Pilot experiments showed that the mercury release rate depends upon the frequency of the sampling. The more frequently the chamber was evacuated, the higher the release rate observed. Another finding was that the concentration of accumulated mercury in the chamber did not significantly increase with time intervals higher than 45 min, indicating attainment of equilibrium conditions. Therefore, analysis of the data suggested that the maximum release rate could be obtained if the mercury was continuously withdrawn from the chamber. If it were possible to estimate the mercury release rate during continuous sampling of the chamber, it would approximate the oral environment during normal breathing. Continuous sampling of the chamber was not possible, because too-frequent sampling resulted in a mercury build-up level below the detection limit of the instrument. To overcome this problem, we used a different approach. A time period (30 min) was chosen shorter than that required for the system to reach equilibrium. We sampled the mercury using different intervals over the 30-minute period. Thus, samples were taken at intervals of 3 min (10 samples), 5 min (6 samples), 10 min (3 samples), 15 min (2 samples), and 30 min (1 sample), and the release rate was determined. During the 30-minute period, 200 chewing cycles were divided equally among the sampling intervals. The overall period of sampling varied slightly with the sampling frequency, because multiple sampling increased the duration of mercury release as a result of the finite time it took for each sampling to be carried out. However, this variation was corrected by recalculation of the mercury release for 30 min for each sampling frequency. The amount of mercury released over the 30-minute interval was plotted against the sampling interval (Fig. 3), and the curve with the best-fit regression was determined from among linear, polynomial, logarithmic, and exponential regression models by *Grafit* software (Graficus Inc., Kirkland, WA, USA). The best-fit curve was invariably described by an exponential equation of the form $y = \mathbf{a}\exp(\mathbf{b}x)$. The exponential regression fit finds a curve with the least-squares fit of the (x,y) measured data points and calculates the constants **a**,**b** of the linearized equation $\ln y = \ln \mathbf{a} + \mathbf{b}x$ and the value of the coefficient of determination (R^2). The extrapolation of the curve to the Y axis gives the release rate under conditions of constant evacuation of the chamber. This rate is the maximum release rate and can be used to calculate the maximum possible daily dose under normal breathing conditions of patients with a single amalgam restoration. An additional advantage of multiple sampling and the extrapolation to zero sampling time is that this provides a reasonable correction for the time-dependent absorption of mercury, if any, by the plastic materials used in the artificial mouth.

The daily dose of mercury vapor from dental amalgam was estimated based on mercury release rate data gathered by the method proposed by Berglund (1990). The method differentiates mouth breathing from nasal breathing and assumes that no mercury is absorbed during nasal breathing. According to this method, data on the mouth-to-nose-breathing ratio were based on studies from Uddstromer (1940), Camner and Bakke (1980), and Gleeson *et al.* (1986). The factors 0.8 and 0.5 refer to retention of inspired mercury and the inspiration/expiration rate, respectively. According to Uddstromer (1940), a normal individual at rest has 0.4% oral respiration; according to Camner and Bakke (1980), male non-smokers with no acute respiratory symptoms have 58% oral respiration during conversation; according to Gleeson *et al.* (1986), healthy persons without respiratory disease, hypersomnolence, or sleep complaints have 17% oral respiration while sleeping. The results of these studies indicate that conversation, unlike work, increased mouth breathing considerably. So, the oral/nasal breathing ratio corresponding to conversation was used for the calculations of mercury daily intake during an active period of a patient's day. For calculation purposes, it was assumed that, during a single day, the oral/nasal ratio for 8 hrs follows the sleeping pattern, that for 8 hrs follows the conversation pattern, and that for 8 hrs follows the resting pattern. Therefore, if α is the rate of mercury release *per* minute, the amount of mercury absorbed would be: (a) (rest) $\alpha \times 60 \times 8 \times 0.004 \times 0.8 \times 0.5$; (b) (conversation) $\alpha \times 60 \times 8 \times 0.58 \times 0.8 \times 0.5$; and (c) (sleep) $\alpha \times 60 \times 8 \times 0.17 \times 0.8 \times 0.5$. The overall mercury dose is the sum of the mercury intake calculated for the rest, conversation, and sleep periods in a day. The calculated mercury release data were statistically tested by two-way ANOVA and Tukey's multiple-range test for the comparison of means at the 95% confidence level.

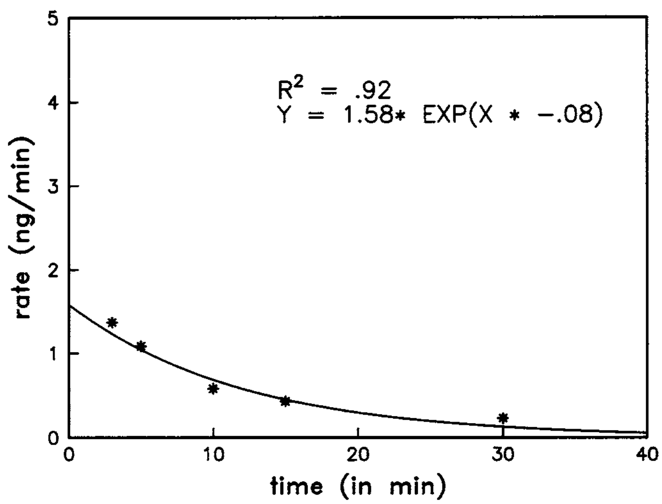

Figure 3. Typical exponential regression fit of the mercury release values for 30 min with different sampling intervals used during the period. Note the determination of the maximum mercury release *via* extrapolation to zero sampling time.

Results

The data were grouped by the different independent variables (amalgam type, age, chewing *vs.* brushing) used in the study and then analyzed for investigation of the effects of these variables on the mercury release rate and the estimated daily mercury intake from amalgam restorations.

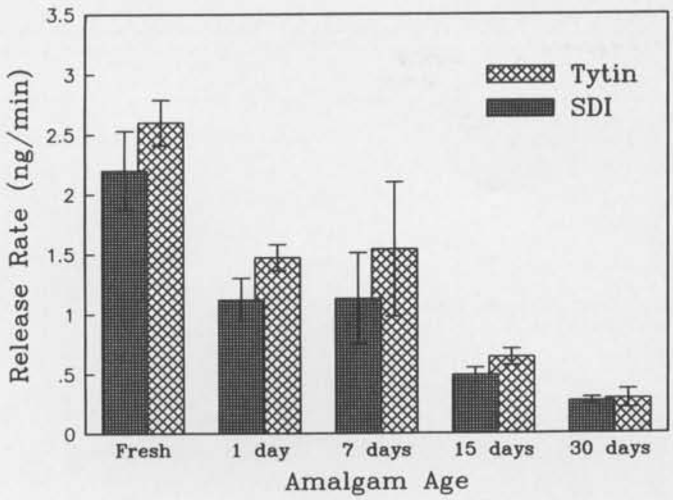

Figure 4. Maximum mercury release rate over a 30-minute period as a function of amalgam age and brand. Note the effect of age in reducing the mercury release rate. Also note the higher release rates associated with the high-copper amalgam Tytin for amalgams aged 15 or fewer days.

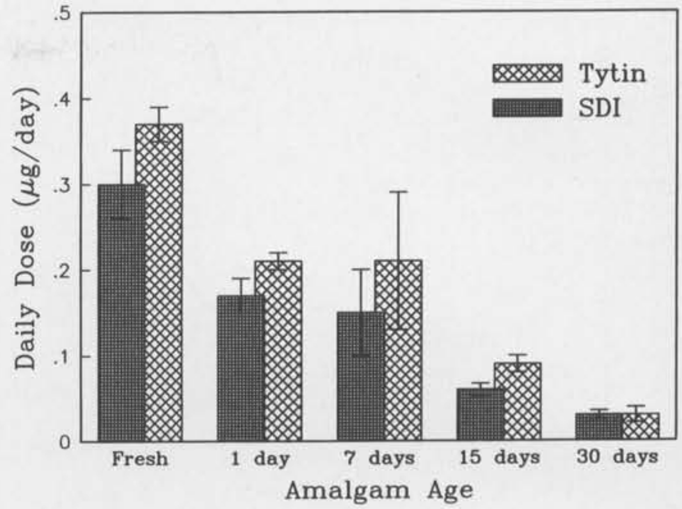

Figure 5. Daily dose as function of amalgam age and amalgam brands. Note the decreasing daily dose with increasing amalgam age. Also note the higher dose associated with the high-copper amalgam Tytin when the age is 15 or fewer days.

Effects of chewing and age on mercury release rates and mercury daily intake from amalgam restorations

Fig. 3, shown previously, illustrates a typical exponential regression and determination of the maximum rate of mercury release by extrapolation to zero sampling time. In this Fig., the rate of mercury released is shown as a function of sampling time during a fixed period (30 min) of mercury release measurement. Note the increasing trend of the rate of mercury release with increasing sampling frequency (or decreasing sampling time). The best-fit exponential regression equation and the corresponding value of the coefficient of determination (R^2) are also indicated in the Fig. The maximum rate of mercury release corresponds to the Y-axis intercept of the curve (corresponding to zero sampling time), and this is given by the constant 1.58 in the regression equation. Fig. 4 shows the mean maximum mercury release rates and corresponding standard deviations determined in this way for each of the different amalgam types and ages. Fig. 5 shows the amount of mercury absorbed daily (mercury daily dose) due to a single amalgam restoration, as estimated by Berglund's method. The results indicate that the SDI amalgam released mercury at a lower rate for each amalgam age, indicating a lower estimated mercury intake. In addition, the amount of mercury released shows a decreasing trend with increasing amalgam age. Tytin amalgam releases from 20 to 35% more mercury vapor than SDI, depending on the age of the specimen. There is also nearly a 90% decrease in mercury release during the first 30 days of amalgam age in both amalgam types. ANOVA results, presented in Table 1, showed that there is a significant difference in mercury release rates between the two amalgam types as well as among the different ages ($p < 0.0005$). The type * age interaction effect was non-significant ($p > 0.05$). Tukey's multiple-range test ($\alpha = 0.05$) results are presented in Table 2. Note the significant differences between the means for pairs of groups except for the means between the ages of one- and seven-day-old amalgam restorations. Similarly, Tukey pairwise comparison of mean mercury release rates between amalgam types in Table 4 indicates that the mercury release rate of SDI is significantly higher than that of Tytin. Because the daily intake values are obtained only when multiplied with a constant number, the statistical differences found for the mercury release rates will also be true for the daily mercury intake estimations.

Effects of chewing and brushing on the mercury release rate

To compare the effects of chewing and brushing on the mercury release rate, we compared the group of samples treated with brushing cycles with the 30-day-old specimens that underwent chewing cycles. The ages of the two groups were selected to be close (30 and 31 days) to minimize any effect on the mercury release rate due to age differences. The same amalgams were used in the two groups so that sample differences would not influence the data analysis. The data, presented graphically in Fig. 6, show that brushing causes higher release rates from conventional amalgam than high-copper amalgam.

ANOVA and Tukey pairwise comparisons were utilized to evaluate the data for statistical differences. The summary of ANOVA results and Tukey contrast is presented in Tables 3 and 4. The analysis showed that, while the difference in the release rate between the two amalgam types is not significant ($p > 0.05$), statistically significant difference of means was found between the two treatments ($p < 0.005$).

Discussion

The data from this study show that the mercury released decreases as the age of the amalgam increases up to 30 days. The same finding has been previously reported by Brune (1981) when he studied the corrosion of amalgam, and by Dérand (1989) in an *in vitro* study of mercury release from amalgams. Since the release of mercury in both corrosion and air depends on mercury transport *via* bulk diffusion within the filling (Olsson *et al.*, 1989), it is not surprising that

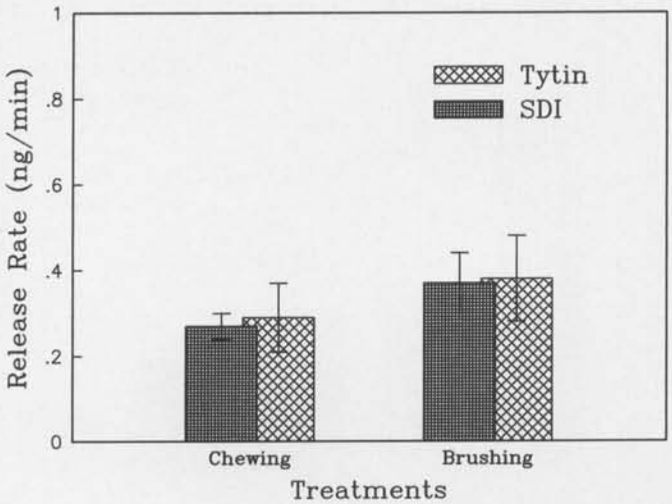

Figure 6. Effect of brushing *vs.* chewing on maximum mercury release rate. Note the increase in mercury release during brushing in both brands.

the kinetics of mercury release follows the same pattern in these cases. However, our findings suggest a rather continuous decreasing trend of mercury release rate during the first 30 days of an amalgam restoration, while the results of Dérand suggest a steady-state release rate after 3 days. These differences may arise from the differences in the experimental procedures. Dérand measured the mercury release at room temperature of < 25°C, while we measured mercury release under a continuous exposure of water spray at 37°C and under simulated chewing cycles. Moreover, we used an extrapolation method to determine the mercury release rate corresponding to a zero sampling interval, and this procedure was not followed by Dérand. In spite of these differences, the dependence of mercury release with the amalgam age is borne out in both studies. This dependence of mercury release rate on amalgam age can be attributed to: (a) the oxidation of the amalgam surface, leading to a protective film (Dérand, 1989; Ferracane *et al.*, 1992;

Table 2. Tukey subsets based on variables of amalgam type and age in chewing experiments[a]

Variable	N	Mean Mercury Release Rate (ng/min)
Amalgam Type		
Tytin	35	1.312
SDI	35	1.075
Amalgam Age		
Fresh	14	2.404
1 day old	14	1.367
7 days old	14	1.341
15 days old	14	0.573
30 days old	14	0.284

[a] Mean values separated by discontinuous vertical lines indicate significant differences between them. Homogenous subsets are indicated by connecting vertical lines.

Table 1. Summary of ANOVA results from chewing experiments

Source	DF	SS	MS	F[a]	P
Amalgam type	1	0.98	0.98	14.4	< 0.0005
Amalgam age	4	38.23	9.56	140.24	< 0.0001
Type * age	4	0.39	0.098	1.45	> 0.05
Error	60	4.09	0.068		
Total	69	43.69			

[a] Critical F values for comparison are: $F_{0.0005}$ (1.60) = 13.5, $F_{0.0001}$ (4.60) = 7.05, and $F_{0.05}$ (4.60) = 2.53.

Berglund, 1993); and (b) time-dependent microstructural changes in the amalgam (Marshall *et al.*, 1979). Since chewing and brushing are expected to remove any oxide film present, the decrease in mercury release with amalgam age may result from the microstructural changes associated with the solid-state transformations known to occur during amalgam aging.

The comparison of the mercury release between the two amalgam types showed a significantly higher mercury release for the high-copper amalgam than for the conventional one. This finding has also been reported in the literature (Dérand, 1989; Brune, 1981; Herø *et al.*, 1983; Kozono *et al.*, 1982). These are *in vitro* studies investigating the mercury release and corrosion in the amalgam. The differences may arise as a result of the compositional differences in the amalgams. Ferracane *et al.* (1994) recently reported the release of mercury vapor from a series of experimental amalgam compositions and showed an excellent negative correlation ($R^2 = 0.956$) with the Sn content in the γ_1 phase. Their results also showed that the mercury vapor emission from a high-copper amalgam composition (55% Zn, 30% Sn, 15% Cu) was significantly higher than that from a conventional amalgam composition (70% Ag, 27% Cu, 3% Cu, 0.5% Zn), and the Sn content in the γ_1 phase of the high-copper system was lower than that of the conventional amalgam. Our results are in agreement with these observations.

It was also found that brushing strokes caused higher mercury release than chewing. This finding is also in accordance with those of previous reports (Paterson *et al.*, 1985; Langworth *et al.*, 1988; Berglund, 1990). The absence of significant differences as a function of the types of amalgams

Table 3. Summary of ANOVA results of chewing/brushing experiments of 30-/31-day-old amalgams

Source	DF	SS	MS	F[a]	P
Chewing *vs.* brushing	1	0.142	0.142	0.22	> 0.05
Amalgam type	1	6.41	6.41	10.06	< 0.005
Interaction	1	0.001	0.001	0.00	> 0.05
Error	24	15.3	0.64		
Corrected total	27	21.853			

[a] Critical F values for comparison are $F_{0.05}$ (1.24) = 4.26 and $F_{0.005}$ (1.24) = 9.55.

Table 4. Tukey contrast of mercury release rates as a function of chewing/brushing and amalgam type variations (30-/31-day-old amalgams)[a]

Variable	N	Mercury release rate ng/min
Amalgam Type		
Tytin	14	0.33587
SDI	14	0.32429
Treatment		
Chewing	14	0.37929
Brushing	14	0.28537

[a] Mean values separated by discontinuous vertical lines indicate significant differences between them. Homogenous subsets are indicated by connecting vertical lines.

Table 5. Daily mercury intake as it was published and re-calculated according to Berglund (1990)

Author/Year	Published daily dose (μg/day)	Recalculated daily dose (μg/day)
Svare *et al.*[a,c]/1981	17.57	4.87
Abraham *et al.*[a,c]/1984	8.00	2.20
Patterson *et al.*[a,c]/1985	2.50	0.80
Vimy and Lorscheider[a,c]/1985	19.85	0.90
Mackert[b,c]/1987	1.24	0.90
Vimy and Lorscheider[b,c]/1990	9.98	0.90
Berglund[c]/1990	1.70	1.70
This study[d]/1993	0.03	0.03

[a] Author's initial data recalculated by Clarkson *et al.* (1988).
[b] Daily mercury estimations based on the original data presented by Vimy and Lorscheider (1985b).
[c] *In vivo* studies presenting data from subjects with multiple amalgam restorations.
[d] *In vitro* study presenting data from a single amalgam restoration.

in this analysis indicates that the mercury release in SDI and Tytin amalgams stabilizes to a common release rate when they are 30 to 31 days old, and the only significant differences are due to the treatment choice of chewing *vs.* brushing.

When the mercury daily intake due to inhalation of mercury vapor from amalgam restorations is estimated, different factors need to be considered. Berglund *et al.* (1988) and Olsson *et al.* (1989) reported that the rate of mercury release from amalgam restorations is independent of the air-flow rate through the oral cavity; therefore, this factor was not included in the variables.

The steady-state value of daily mercury intake due to a single amalgam restoration (0.03 μg/day) is far below the maximum daily dose (82.29 μg/day) calculated as the Threshold Level Value (TLV) for safety in occupational hazard situations (Berdouses, 1992). The release of mercury may become higher if multiple restorations are present, but past reports (Abraham *et al.*, 1984; Berglund, 1990) indicate that there is little correlation between mercury release and the number of restorations or surfaces. There is also a need to exercise caution in using the mean daily dose as the sole predictor of public health risk, because the upper range of values of daily exposure may also have important health effects on segments of the patient population of interest. However, even the highest steady-state daily dose due to a single restoration calculated in our study (0.039 μg/day) was also far below the daily dose corresponding to the TLV indicated above. Even the highest non-steady-state daily dose calculated for fresh amalgams (0.39 μg/day), which is of only transient effect of a few hours in the several years of the clinical life of an amalgam restoration, is well below the TLV daily dose.

While there are some limitations with respect to a clinical simulation, the data from this study suggest that the mercury absorbed by the body due to mercury release from amalgam restorations is well below the proposed threshold limit values, as a function of both amalgam type and age. The conventional amalgam released less mercury than the high-copper amalgam during the first 15 days. However, the relatively low levels of mercury released from the two types and the limited difference in the amount of mercury released between the two types do not support the possibility that there is any significant clinical difference between the two types.

Table 5 is based on data that Olsson and Bergman (1992) described recently. In this Table, the daily intake values are presented as originally published, and then the same base data are re-calculated according to the method that Berglund (1990) proposed. All the studies included in the Table are based on *in vivo* studies done on subjects with multiple restorations. Moreover, the format in which the data were presented was not complete and uniform at all times, and that may influence the estimates. Nevertheless, these values give an indication of the range of mercury daily intake due to amalgam restorations.

Comparing the values from this *in vitro* investigation with the *in vivo* reported values, we need to consider the fact that our results are based on a single amalgam restoration, whereas in all the *in vivo* studies, the subjects had multiple restorations. When this factor is considered, the value obtained from this study seems to be comparable with the *in vivo* results. This suggests that the experimental design presented here may be valuable for further investigation of the different factors influencing the mercury release from amalgam restorations.

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