

Dental Amalgam Mercury Daily Dose Estimated From Intra-Oral Vapor Measurements: A Predictor of Mercury Accumulation in Human Tissues

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Recent misconceptions regarding Hg exposure from dental amalgams have been based on several questionable assumptions. The present paper reexamines earlier estimations of Hg daily dose from dental amalgam in order to elaborate and refine the mechanical and volumetric parameters of open-mouth Hg vapor sampling. This facilitates a comparison with the physiological parameters of human respiration. Corrections for the sampling factors of flow rate and sampling dilution, and the respiratory factor of Hg accumulation in the closed mouth between oral inhalations, reduce our original daily dose estimates by approximately 50%. Application of a general pharmacokinetic model with our revised Hg daily dose estimates results in predictions for brain, kidney, blood, and urine which approximate tissue Hg measurements reported in subjects with dental amalgams. When tissue Hg predictions are made based upon alternate Hg daily dose estimates proposed by other investigators, the resultant error was as much as 11-fold lower than were actual tissue measurements in humans. It is concluded that intra-oral air Hg vapor measurements can be useful for estimating Hg daily dose and tissue Hg levels.

Key words: tooth fillings

INTRODUCTION

Investigations have demonstrated the release of mercury (Hg) from in situ dental amalgams in both exhaled air [1,2] and intra-oral air [3-6]. Chewing stimulation in subjects with amalgams increases post-chewing Hg concentration six-fold in intra-oral air [4] and 15-fold in exhaled air [1] over the pre-chewing concentration. Control subjects with no dental amalgams had small basal Hg levels which did not change as a function of mastication.

Both the pre- and post-chewing Hg concentrations for intra-oral air [4] and exhaled air [1] have been shown to correlate with the number and type of occlusally involved dental amalgam restorations. Furthermore, correlations have been reported between the number or surface area of dental amalgams and Hg levels in blood [3], urine [7], and brain tissue [8].

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Although the evidence for Hg vapor release from amalgams has been generally accepted, the absolute measures and the extrapolation of these data to estimate Hg daily dose and compartmentalized body burden have met with recent criticism [9–11]. The critics suggest as much as a 16-fold error in the actual measurement and subsequent daily dose estimations because volume, time, and flow rate factors differ significantly between machine sampling and human respiration. The principal issue of contention is their opinion that the sampling time of the Hg vapor analyzer (20 sec), when compared to the respiratory time for one inhalation (2.5 sec), will bias the estimates of Hg exposure eight-fold. The secondary issue is that the volumetric difference between the machine sample (0.25 L) and the lung tidal volume (0.50 L) will bias the results an additional two-fold. Thus, they assume the combined effect of these two apparent issues will result in a 16-fold (8×2) overestimation of Hg dose.

In contrast, it is our view that one can equate the mechanical and volumetric parameters of the open-mouth sampling technique with the physiological parameters of human respiration, if one accounts for the factors of serial dilution during sampling, Hg vapor dose accumulation in the closed mouth during nasal breathing, and flow-rate differences between the Hg vapor analyzer and the lung.

Mackert [10] and Olsson and Bergman [9] base their critiques on two questionable premises:

First, they assume that mercury vapor escaping from the amalgam restorations is being quantitatively (i.e., totally) collected during the fixed time of Hg vapor measurement because the sample volume exceeds the volume of the oral cavity. In other words, they contend that in an open-mouth system the collection rate of the Hg vapor analyzer equals the vaporization rate of the amalgam Hg ([10], equation no. 1; [9], equation no. 1).

However, we contend that in order for their assumption to be true, the oral cavity would have to be a closed system at equilibrium, such that Hg can neither escape nor be diluted. Furthermore, the sampling process of this equilibrium volume would have to remove all the vapor contents of the mouth. These conditions are not likely to be met during open-mouth sampling, since it may be assumed that some Hg readily diffuses across the oral tissues into the systemic circulation, Hg may also be absorbed via swallowed saliva, and the sampling technique from the wide open mouth can permit a substantial dilution of the sample with room air as intra-oral air is evacuated by the Hg vapor analyzer.

Second, they assume that only that portion of Hg vaporized during oral inhalation (2.5 sec) would be available for absorption ([10], equation no. 7; [9], equation no. 1).

However, we contend that amalgam Hg vaporizes continuously after chewing stimulation [5], whether the mouth is open or closed, whether one breathes orally or nasally, and whether one is inhaling or exhaling. The average resting respiratory rate is 12 breaths/min (5 sec/breath or 2.5 sec/inhalation). Thus, in addition to the Hg which vaporizes during the time of oral inhalation (2.5 sec), Hg which accumulates in the closed mouth during nasal inhalation (2.5 sec) and nasal exhalation (2.5 sec) ([11], fig. 4) would also be available for lung uptake when the next oral inhalation occurs. Only during the oral exhalation phase is Hg blown off and not available for inhalation uptake. This uptake occurs because it is generally assumed that the first phase of an oral respiratory cycle begins with an inhalation

[12]. During all other respiratory phases, the Hg vaporizing from the amalgams must be considered as part of the potential dose.

If the oral/nasal breathing ratio is 0.5, then 25% of the time one is exhaling orally and 75% of the total Hg vaporizing over time is available for uptake. Similarly, if an oral/nasal breathing ratio of 0.35 is used, only 17.5% of the time is one exhaling orally. The oral/nasal correction factor now becomes 0.825 (to include Hg vaporizing during oral inhalation, nasal inhalation, and nasal exhalation). The less frequently one exhales orally, the greater should be the total dose exposure to Hg because of the higher Hg concentration in the closed mouth.

The misconceptions raised in these commentaries [9,10] have prompted us to reexamine some facets of our earlier estimations of Hg daily dose from dental amalgam [5] and projected Hg body burden [13] with a view to elaborating and refining the concepts of Hg measurement and daily dose estimation from dental amalgam. The present paper estimates Hg daily dose attributable to dental amalgam from human autopsy data [8,14] and compares these estimates to Hg daily dose estimates published by others. Second, this paper reevaluates our original lung uptake approach for Hg daily dose estimation [5]. New daily dose estimations are made with a proposed generic equation and are employed with various metabolic models for prediction and comparison to actual Hg concentrations in blood and urine and Hg accumulation measured by others in autopsied brain and kidney tissues of subjects with dental amalgams. A similar analysis is also undertaken with the Hg daily dose estimates proposed by Mackert [10] and Olsson and Bergman [9], allowing evaluation of the various dose estimates.

METHODS

Total brain inorganic Hg content was calculated (Table I) for subjects with and without dental amalgams from published autopsy data correlating brain cortex Hg with the presence of dental amalgam restorations, the presumption being that both groups of subjects were from environmentally similar backgrounds [8,14]. The Hg attributable exclusively to dental amalgam could be estimated by subtracting the baseline data from amalgam-free subjects from the data of subjects with amalgam. The average daily doses necessary to produce this brain Hg content were estimated (Table II), employing a single compartmental distribution model [15,16] and assuming that brain uptake is either 2% [17,18] or 7.1% [19] of the total daily dose. The biological half-time for Hg in the brain is assumed to be 21 days [19].

TABLE I. Mean Brain Cortex Inorganic Hg Levels (ng/g wet wt.) From Human Autopsy Data

	Hg in amalgam-free Ss	Hg in amalgam Ss	Hg attributed exclusively to amalgam
Eggelston et al. [14]	$(6.7 + 3.8)/2 = 5.3$	$(15.2 + 11.2)/2 = 13.2$	7.9
Nylander et al. [8]	6.7	12.3	5.6
$\bar{X}$	6.0	12.8	6.8
Total brain (1,200 g)	7,200 (7.2 μ g)	15,360 (15.4 μ g)	8,160 (8.2 μ g)

TABLE II. Mean Hg Daily Dose Necessary to Produce Known Brain Hg Content* as Predicted by a Distribution Model†

	Amalgam-free Ss (μg/day)		Amalgam Ss (μg/day)		Attributed exclusively to amalgam (μg/day)	
	2%	7.1%	2%	7.1%	2%	7.1%
Brain uptake ^a	2%	7.1%	2%	7.1%	2%	7.1%
Daily dose	11.9	3.4	25.4	7.2	13.5	3.8

*From Table I (7.2, 15.4, 8.2 μg).

†Uses a single compartment distribution model for inorganic mercury ([15]; [16], equation no. 2): $B = d(f)(T_{1/2}/\ln 2)$, with B = compartment burden, d = daily dose, f = fraction of daily dose going to compartment, $T_{1/2}$ = biological half-life.

^aAssumes 2% [17,18] or 7.1% [19] of total daily dose taken up by brain and a $T_{1/2}$ for brain of 21 days [19].

Our earlier approach emphasized lung uptake and used a lung flow rate of 6 L/min. This has incorrectly given the impression that we believe that Hg vapor release is proportional to lung flow rate. Rather, this flow rate was meant to be employed as a constant in an attempt to account for factors of dilution during sampling, accumulation of Hg in the closed mouth, and factors of respiratory efficiency due to the much larger cross-sectional area of the trachea than that of the machine sampling tube [20]. The analyzer flow rate of 0.75 L/min could have been a more appropriate term, if these confounding factors were able to be individually assessed and incorporated into the equation. From our original integrated data [5], one can estimate the amount of Hg collected by the analyzer during each of the integrated time periods by simply multiplying the integration (μg min/1,000 L) by the minute flow rate of the analyzer (L/min). This approach is similar to that employed by Mackert [10] and Clarkson et al. [7]. However, this estimate merely establishes the average collection of Hg by the analyzer per integrated time, not the release rate of Hg from the amalgams, and must be corrected for the dilution effect during open-mouth sampling in order to obtain a more realistic approximation of the actual amount of Hg released intra-orally. Other factors which must be considered in estimating lung uptake include closed-mouth accumulation of Hg (oral/nasal breathing ratio) and Hg retention across the lung alveoli.

Determination of the approximate volume of a wide-open adult mouth was performed by a water displacement technique in five subjects. Subjects sat upright in a dental chair with their heads tilted posteriorly while water was poured into their wide-open mouths until the entire intra-oral space was filled. This water volume was expectorated into a beaker and measured in a graduated cylinder to the nearest milliliter. Water volumes ranged from 114 to 133 ml with a mean of 125.4 ml.

A generic equation which would represent the foregoing conditions including dilution and oral/nasal breathing is as follows:

$$\left[\frac{\mu\text{g min}}{1,000 \text{ L}} \right] \times \left[\frac{\text{analyzer flow rate}}{\text{rate}} \right] \times \left[\frac{\text{dilution factor}}{\text{factor}} \right] \times \left[\frac{\text{oral/nasal uptake ratio}}{\text{uptake ratio}} \right] \times \left[\frac{\text{lung retention of Hg}}{\text{of Hg}} \right] \\ = \text{Hg dose for integration time,}$$

where $\mu\text{g min}/1,000\text{ L}$ = integration of Hg released from amalgams ([5], table 1); flow rate = 0.75 L/min fixed for Hg analyzer; dilution factor = V_1/V_2 , where V_1 = sample volume (0.25 L) and V_2 = open mouth volume (0.125 L); oral/nasal uptake ratio = $1 - (\text{oral/nasal breathing ratio})/2$, e.g., for O/N ratio of 0.5 = 0.75 and for O/N ratio of 0.35 = 0.825; and lung retention of Hg = 0.80 [5], e.g., $(323\ \mu\text{g min})/1,000\text{ L} \times 0.75\text{ L/min} \times 2 \times 0.75 \times 0.80 = 290.7\text{ ng Hg}$.

Our previous estimate of the Hg daily dose for subjects with various numbers of dental amalgams [5], the alternate daily dose estimate suggested by Mackert [10] and Olsson and Bergman [9], and our present revised estimate based upon the generic equation (Table III) are used to predict brain levels of Hg by employing the metabolic model of Bernard and Purdue [21] as we have previously described [13]. These results are compared with predictions obtained with an alternative metabolic model ([15,16], equation no. 2), using various reported brain uptake percentages of the Hg daily dose and a brain half-time for Hg retention (Table IV). The predicted brain levels of Hg derived from the metabolic models were in turn compared with the actual concentration of Hg (6.8 ng/g) reported in brain tissue autopsy samples from subjects with dental amalgams (Table I). The same revised estimates of daily dose were also used to predict kidney, blood, and urine levels for comparison to actual measures reported in humans and correlated with the presence of dental amalgams.

RESULTS

Table I shows the average total brain content of Hg reported in autopsied specimens from two studies. Amalgam-free subjects had total brain Hg of 7.2 μg , while subjects with varying numbers of dental amalgams had 15.4 μg Hg. Brain Hg content attributed exclusively to amalgam, when background levels from amalgam-free subjects are subtracted, is 8.2 μg .

Table II shows the average estimations of the Hg daily dose necessary to produce the brain Hg content found in Table I assuming 2% or 7.1% of the total daily dose goes to the brain. For subjects with no dental amalgam, the Hg daily doses were 11.9 and 3.4 $\mu\text{g/day}$, and for subjects with dental amalgams, the daily

TABLE III. Comparison of Daily Hg Doses (μg) From Amalgams

No. of amalgams	Vimy and Lorscheider ([5], table 2) original estimates	Mackert [10] calculations	Vimy and Lorscheider revised estimates
1-16	19.85	1.24	9.98 ^a
≥ 12	29.24	1.83	14.68
≤ 4	8.10	0.51	4.01

^a0-15 min snack: $\frac{323\ \mu\text{g min}}{1,000\text{ L}} \times 0.75\text{ L/min} \times 2 \times 0.75 \times 0.80 = 0.2907\ \mu\text{g}$;

0-30 min meal: $\frac{791\ \mu\text{g min}}{1,000\text{ L}} \times 0.75\text{ L/min} \times 2 \times 0.75 \times 0.80 = 0.7119\ \mu\text{g}$;

30-120 min after chewing: $\frac{1,174\ \mu\text{g min}}{1,000\text{ L}} \times 0.75\text{ L/min} \times 2 \times 0.825 \times 0.80 = 1.1623\ \mu\text{g}$;

daily dose = Hg vapor released during and after three meals and three snacks, e.g., meal = 0-30 min + 30-120 min, snack = 0-15 min + 30-120 min ([5], table 1).

TABLE IV. Model Predictions of Hg Levels in Human Brain* of Subjects With 1–16 Dental Amalgams Based Upon Three Different Estimates of Hg Daily Dose

Source	Body Daily Hg dose (μg)	Brain			
		WHO [15]; Gerstner and Huff [16] model ^a (ng/g)		Bernard and Purdue [21] model ^b	
		2% ^c	7.1% ^d	ng/g ^e	ng/g ^f
Vimy and Lorscheider [5]	19.85	10.0	37.8	2,600	45.7
Mackert [10]	1.24	0.6	2.4	170	2.9
Vimy and Lorscheider revised (present study)	9.98	5.1	19.0	1,340	23.0

*All predictions based upon 1,200 g brain.

^aTwenty-one day half-life for brain [19].

^bTen year projection with compartment R4 half-life of 10,000 days [13].

^cBrain uptake of 2% of Hg dose [17,18].

^dBrain uptake of 7.1% of Hg dose [7].

^eAssumes R4 is only brain.

^fAssumes R4 is uniformly distributed in 70 kg body.

doses were 25.4 and 7.2 $\mu\text{g}/\text{day}$. Thus, the Hg daily dose attributed exclusively to amalgam was 13.5 and 3.8 $\mu\text{g}/\text{day}$.

Table III compares the Hg daily doses from dental amalgams as estimated by several approaches. Our present revised Hg daily dose estimate of 9.98 μg for all subjects with 1–16 occlusal amalgams (14.68 μg with 12 or more amalgams and 4.01 μg with 4 or less amalgams) as calculated using the new generic equation is approximately 50% lower than our original estimates [5]. This is in contrast to the recent Mackert [10] calculations which remain eight-fold lower than our present revised estimates of the Hg daily dose.

Table IV shows the predicted brain levels of Hg for all subjects with 1–16 dental amalgams when Hg daily doses of 19.85 μg [5], 1.24 μg [10], and 9.98 μg (present study) are used in two different metabolic models. For the WHO [15] and Gerstner and Huff [16] model, we have used a 21-day half-life for Hg in brain [19] and have assumed either a 2% brain uptake of the daily Hg dose [17,18] or a 7.1% brain uptake [7]. For the Bernard and Purdue [21] model as we employed previously [13], we have estimated a 10 year projection after amalgam placement with our assumption that compartment R4 (which has a half-life of 10,000 days) is either confined only to brain or is uniformly distributed throughout an adult body.

When our revised Hg daily dose of 9.98 μg (Table IV) is used, the 21-day Hg half-life in the WHO [15] and Gerstner and Huff [16] model predicts a brain Hg concentration of 5.1 ng/g (for 2% of dose uptake) and 19.0 ng/g (for 7.1% of dose uptake). The actual measured brain Hg concentration of 6.8 ng/g (Table I), attributable exclusively to amalgams, falls within the range (5.1–19.0 ng/g) predicted by the model ([15,16], equation no. 2) as presented in Table IV. With the longer half-life of 10,000 days, the Bernard and Purdue [21] model predicts a brain Hg level of 23.0 ng/g when Hg is uniformly distributed (Table IV). An assumption in Table IV that compartment R4 is exclusively brain for the Bernard and Purdue [21] model would probably be erroneous, since such an assumption

results in a Hg prediction of 1,340 ng/g, which is considerably above the actual concentration measured in brain of 6.8 ng/g (Table I). It should be noted in Table IV that the Mackert [10] Hg daily dose calculation (1.24 μg), when applied to the metabolic model ([15,16] equation no. 2) for a 2% brain uptake, results in tissue level estimations (0.6 ng/g) that are as much as 11-fold lower than levels actually found in brain of 6.8 ng/g (Table I).

Table V compares the Hg daily dose estimations from dental amalgam for nine reports including the present study. These daily dose estimates range from 1.24–27.0 $\mu\text{g}/\text{day}$.

DISCUSSION

The present report calculates that, for a brain content of 8.2 μg Hg (Table I), the mean Hg daily dose attributable exclusively to dental amalgam is between 3.8–13.5 $\mu\text{g}/\text{day}$ depending upon which percent of total daily dose (2% or 7.1%) one assumes goes to brain (Table II). Furthermore, when our previous integrated data [5] is modified by a generic equation accounting for analyzer flow rate, dilution during sampling, and closed-mouth accumulation of Hg vapor, the revised Hg daily dose estimations from dental amalgam (Table III) will validly predict (Table IV) the concentration of Hg actually found in human brain exclusively attributable to dental amalgam (Table I), especially for a 2% brain

TABLE V. Various Hg Daily Dose Estimations From Dental Amalgam

Study	Daily dose (μg Hg/day)	Method
Patterson et al. [2]	27.00	Experimental study estimating the Hg daily dose from dental amalgam from exhaled air before and after tooth brushing
Vimy and Lorscheider [5]	19.85	Experimental study estimating the Hg daily dose from dental amalgam from serial measurements of intra-oral air after chewing
Vimy and Lorscheider (present study)	9.98	Mathematical study reevaluating authors previous data
Aronsson et al. [6]	9.80	Experimental study estimating the Hg daily dose from dental amalgam from measurements of intra-oral air after chewing
Clarkson et al. [7]	7.70	Review paper mathematically reestimating the Hg daily dose from a variety of experimental studies of others
Langworth et al. [28]	3.00	Experimental study comparing the concentration of Hg in intra-oral and tracheal air
Snapp et al. [24]	1.30	Experimental study measuring the clearance of Hg from blood
Mackert [10]	1.24	Mathematical reappraisal of Vimy and Lorscheider [5]
Olsson and Bergman [9]	1.24	Mathematical reappraisal of Vimy and Lorscheider [5]
Average of all studies	9.00	

uptake. Similar predictions can be made for kidney, blood, and urine Hg concentration. Our present predictions are clearly supported by the published tissue and fluid results of others.

Variation of some parameters in the generic equation could alter the Hg dose. For example, the oral/nasal breathing ratio is a significant factor because during nasal breathing Hg accumulates in the closed mouth, tending to increase the Hg dose at the next oral inhalation cycle. Similarly, lung retention of Hg could be reduced in instances where respiratory volume or lung absorption were impaired, thereby reducing the Hg dose. Other parameters of the equation remain relatively constant under measurement conditions employed in this study.

The metabolic compartmental model of Bernard and Purdue [21] for Hg accumulation and distribution was first employed by us [13] because it was current and we initially felt that the relatively small amounts of Hg escaping from amalgams would only accumulate in tissues in substantial amounts over a very long time. The Bernard and Purdue model [21] comprises four compartments, one of which has a 10,000 day half-life. We agree with Mackert [10] that there is a paucity of evidence for the existence of such a long-term compartment. It is evident from the data in the present study (Table IV) that application of the Bernard and Purdue model [21] will substantially overestimate the amount of dental amalgam Hg projected to accumulate in brain (Table I), particularly if the long-term compartment is presumed to be exclusively brain. Utilization of the WHO [15] and Gerstner and Huff [16] model may be more appropriate. However, the Bernard and Purdue [21] model is useful as a worst-case estimation for amalgam exposure [22].

In the present paper we have taken Mackert's [10] suggestion to use a half-life of 21 days for Hg in brain [19]. If our present revised daily dose estimate is employed in the basic pharmacologic equation of WHO [15] and Gerstner and Huff ([16], equation no. 2), predictions for Hg accumulation closely agree with actual brain measurements, when combined with this shorter half-life and fractions of Hg daily dose for brain uptake (2% and 7.1%, see Table IV). Although Mackert [10] suggested this approach, he failed to take this important step with his Hg daily dose calculation (1.24 μg). If he had, he would have found that his results were as much as 11-fold lower than previously published autopsy data correlating brain Hg levels with the number of dental amalgams [8,14,23].

The percentage of daily Hg dose taken up by the rat brain was 2% [17,18] and may be too low for extrapolation to humans who have a larger brain/body mass ratio. On the other hand, the 7.1% brain uptake [19] may be too high, since it is based upon total human head content of Hg and not exclusively brain Hg. A more realistic percentage for brain uptake of Hg may lie somewhere between these two values. Similarly, the biological half-time of 21 days for Hg in human brain suggested by Hursh et al. [19] might be too low, since it is based upon total head count, which does not separate the Hg half-time in oral and nasal epithelial tissues from that in brain. This factor becomes important because the exposure in their experiment was Hg vapor via the inhalation route.

If our present revised daily Hg dose estimate (9.98 μg) is employed in the WHO [15] and Gerstner and Huff ([16], equation no. 2) model to predict blood, urine, and kidney concentrations of Hg, one obtains values of 1.0 ng/ml, 3.0 ng/ml, and 894 ng/g, respectively, when using the distribution and organ weight

criteria of Clarkson et al. ([7], table 2). Such predictions compare favorably with actual measures for blood of 0.7 ng/ml [3] and 1.13 ng/ml [24], for urine of 4.2 ng/ml [25], and for kidney of 433 ng/g [8] in subjects with aged dental amalgams. Therefore, our present revised method for estimating the Hg daily dose from dental amalgam will reliably predict Hg distribution in a variety of tissues.

With regard to volume differences between sampling and respiration, we originally [5] saw no need to account for dilution, since the dilution of sampling (250 ml sample/125 ml open mouth) appeared to be counteracted by the dilution of respiration (250 ml sample/500 ml tidal volume). This factor has now been clarified in our generic equation.

Mackert [10] and Olsson and Bergman [9] assume that the collection rate of the Hg vapor analyzer equals the vaporization rate of Hg from the amalgam, but we contend that their assumption is questionable. If one accounts for a dilution effect (room air) and some Hg loss in the open-mouth system (saliva, mucosa), then the average release rate will be: $r_{av} = [d(C_f)/t] - k$, where r_{av} = average Hg release rate from the amalgam fillings, d = dilution factor (two-fold), C_f = final concentration reading of the vapor analyzer, t = sampling time (20 sec), and k = unknown amount of Hg lost within the system. The difference between a lung uptake model as described by Clarkson et al. ([7]; appendix I) and the machine uptake for the Hg vapor analyzer [4] is that in the latter case a serial dilution of intra-oral air with room air is occurring in a non-equilibrium system while the Hg being sampled continues to vaporize off the amalgam surfaces during the sampling time.

With regard to time differences between sampling and respiration, the critics have identified a potential discrepancy in our original estimates if one merely takes a mechanistic approach, since there is an eight-fold time differential between machine and lung uptake of a sample (20 sec/2.5 sec). However, by employing this mechanistic approach, Mackert [10] and Olsson and Bergman [9] inappropriately limit exposure to that Hg vaporizing only during the oral inhalation phase (2.5 sec). We strongly disagree with their restriction, because Hg is continuously vaporizing from amalgams, particularly after chewing stimulation [5]. Machine sampling and normal lung respiration cannot be directly equated until one accounts for potential Hg vapor dose accumulation in the closed mouth, especially during nasal respiration.

In our measurements of intra-oral Hg vapor [4], the dimensions of the Hg vapor analyzer sampling tube were 30 cm in length with an inside diameter of 3 mm. The internal volume of this tube is always filled with clean room air prior to sampling. Thus, the tube volume would reduce the absolute Hg measurement by $\text{tube vol./sample vol.} \times 100$. We did not consider this factor to be important because it tends to underestimate, rather than overestimate, the Hg measurement. At the onset of sampling, we instructed the subject to expectorate the gum, swallow, and only breathe nasally during the duration of sampling. Immediately, at the onset of vapor sampling, the tube was first moved between the occlusal surfaces of the upper and lower teeth (in a wide open mouth) from the posterior to the anterior region on the right side. The tube was then rotated from this anterior position across the midline of the mouth to the posterior region on the left side, and then moved between the upper and lower occlusal surfaces from the posterior to the anterior region on the left side. Then, as before, the tube crossed

the midline again, completing a "figure 8" pattern. This process was continuously repeated for the 20 sec sampling duration. We estimate that approximately eight to ten complete "figure 8" passes will occur during 20 sec. In subjects with 1–16 occlusally involved amalgams, typical intra-oral Hg vapor values obtained before and after 10 min of chewing stimulation are $4.91 \pm 0.90 \mu\text{g}/\text{m}^3$ and $29.10 \pm 6.07 \mu\text{g}/\text{m}^3$, respectively [4]. A more recent study of intra-oral Hg vapor in similar subjects who chewed for 5 min confirms our findings [6].

At time zero during a machine sampling (20 sec), the intra-oral air Hg concentration approximates zero, because expectorating the gum and the subsequent swallow clears the mouth of fluid and atmospheric contents. Therefore, the Hg collected during machine sampling, in the absence of oral breathing, primarily represents Hg vaporizing during the 20 sec [10,11,20]. On the other hand, during lung uptake (2.5 sec) at time zero, the intra-oral air Hg concentration can be very high. This reflects the Hg which has accumulated in the closed mouth during the nasal respiratory cycles as demonstrated by Berglund et al. ([11], fig. 4) immediately prior to the oral inhalation. This accumulated Hg dose now becomes available for oral inhalation uptake. Where the critics err is that they apply the mechanistic sampling premise (time zero = zero oral Hg concentration) to lung uptake during normal respiration as if prior Hg accumulation in the mouth does not occur. Thus, we have revised our oral/nasal breathing factor (75–82%) to account for this accumulation effect.

Berglund et al. ([11], fig. 7) demonstrate that after 1 min further Hg accumulation in the closed mouth is not evident. This result could be due to Hg vaporization reaching equilibrium, a corrosive layer or saliva inhibiting further vaporization, or that intermittent swallowing is clearing the mouth of all fluid and atmospheric contents. The first two possibilities are highly unlikely, since we have shown that Hg vaporizes for prolonged periods after chewing cessation even in the presence of saliva [5]. Berglund et al. [11] apparently did not control for intermittent swallowing. Had this been done, the intra-oral air Hg level would likely have continued to rise.

It is interesting to note that Berglund et al. ([11], fig. 4) have demonstrated that Hg accumulates in the closed mouth of subjects with dental amalgams even when no chewing stimulation has occurred. If their subjects had stimulated their amalgam restorations with chewing, then the rate and amount of Hg accumulation in the closed mouth would have been much higher as we have mentioned [20] for observations made several years ago.

It is also very important to note that the Hg daily dose calculations proposed by Mackert [10], and supported by Olsson and Bergman [9] and Berglund et al. [11], will not satisfy the autopsy levels of brain Hg found in subjects with varying numbers of aged dental amalgams [8,14,23]. Unlike other laboratories involved in resolving the issue of amalgam safety, neither Mackert [10] or Olsson and Bergman [9] provide any mammalian experimental evidence to support their claims that amalgam Hg release and Hg accumulation in tissues are substantially less than that which has been reported. Berglund et al. [11] conclude their paper with an equation (no. 15) which is essentially the same as Mackert's [10] equation no. 1. Application of either equation would be in error for the foregoing reasons.

Very recently Snapp et al. [24] have demonstrated that blood Hg levels fall when amalgam fillings are removed. They estimate that the average blood level of

Hg attributable to dental amalgam is 1.13 ng/ml in subjects averaging 14 amalgam surfaces, seven of which were occlusally involved. They employ a 30 day half-time for Hg elimination via blood to estimate a daily Hg dose of 1.30 μg from dental amalgam. Their 30 day blood half-time for Hg is in contrast to a value of 3.3 days established by Cherian et al. [26]. The Hg elimination from the whole body varies from 1.2 to 13 days in rat [18] to 4–5 days for 85% of a single Hg dose in human subjects [27]. Hg released from dental amalgam should be considered as a series of doses, each acting independently with regard to tissue uptake and elimination. Even if Hg is considered to have a biphasic elimination pattern over any period of time, the daily dose calculated by Snapp et al. [24] would in any event not satisfy autopsy levels of amalgam Hg found in human brain and kidney. If instead Snapp et al. [24] had used the generally accepted blood half-time of 3.3 days for Hg as employed by Clarkson et al. [7], then a daily Hg dose of 11.9 μg would be obtained, which is in close agreement with the midpoint (10 μg) of the Hg daily dose range (2.5–17.5 μg) listed by Clarkson et al. [7], the mean of 9 μg (Table V), and which is also in close agreement with our revised daily dose of 9.98 μg in the present report (Table III). It is clear that one cannot totally discount a blood phase having a biological half-time of 30 days; however, the Environmental Protection Agency document employed by Snapp et al. [24] clearly points out that greater than 50% of the blood Hg has a half-time of 3.3 days and that the 30 day compartment is very small. What Snapp et al. [24] were likely measuring was not the clearance of Hg originally found in blood, but rather the release of Hg from tissue compartments into blood.

Clarkson et al. ([7], table 2), in reinterpreting our original data [5] while using their own criteria for time of exposure, have helped to clarify the issue of flow rate. However, they did not account for any effect of sampling dilution or dose accumulation in the closed mouth. If this were done, it would increase their daily dose estimate of 2.9 μg employing our data [5] by approximately three-fold (8.7 μg). Their subsequent calculated predictions of blood, urine, brain, and kidney Hg concentrations would likewise be elevated, placing the tissue concentration estimates in closer agreement with actual blood levels reported by Abraham et al. [3] and Snapp et al. [24], urine levels reported by Langworth et al. [25], and with kidney and brain levels reported by Nylander et al. [8].

To date, a variety of attempts have been made to estimate the Hg daily dose from dental amalgam (Table V). These studies range from a high of 27.0 $\mu\text{g}/\text{day}$ to a low of 1.24 $\mu\text{g}/\text{day}$. The overall average of all studies is 9.0 $\mu\text{g}/\text{day}$, which is a reasonably good approximation of the daily dose which would be necessary to produce the tissue levels established in human autopsy studies (Table I). This assumes that Hg which is continuously released from dental amalgam fillings conforms to distribution patterns of tissue uptake and release which can be described by standard first-order pharmacokinetic equations. It should be noted that the autopsy data employed in the calculations herein (Table I) is based on studies of people who had their dental amalgams for many years. It is possible that newly placed dental amalgams would vaporize a substantially larger quantity of Hg on a daily basis than would a similar number of aged amalgams. Thus, one might expect tissue Hg levels also to be initially high and then to gradually decrease over time commensurate with a progressive decrease of the Hg daily dose as amalgams age. In individual subjects, factors which would enhance Hg

vapor release, such as prolonged chewing, grinding of teeth and thermal or acidic mouth conditions, can markedly elevate individual daily doses as much as 10-fold above the average of 9.0 μg Hg/day.

Recent retrospective human postmortem tissue analysis of Hg, while making an important contribution to this debate, is of limited predictive value. However, preliminary evidence in guinea pigs [29] and monkeys [30] suggests, prospectively, that Hg accumulates in brain tissue from in situ amalgams. Results of studies recently completed in our laboratory using sheep clearly indicate that both maternal and fetal tissues begin to accumulate Hg soon after amalgam placement, even though blood and urine Hg levels remain relatively low [31]. Radioactive Hg from dental amalgam deposits in adult tissues to such an extent that the Hg can be readily visualized by whole-body image scan within 29 days after amalgam placement [32].

Since the cyclical release of Hg vapor from dental amalgams is now an established fact, it is appropriate that Clarkson et al. [7] have concluded that the Hg released from in situ dental amalgams is the major source of inorganic Hg exposure (including Hg vapor) in the general population. We conclude that the sampling of Hg released from dental amalgams in situ is a valid and useful approach for estimating Hg daily dose and tissue Hg levels resulting exclusively from dental amalgams when data is corrected to reflect principles of sampling and respiratory physiology.

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