FINAL REPORT

TO: INTERNATIONAL ACADEMY OF ORAL MEDICINE AND TOXICOLOGY

ON BEHALF OF FUNDERS, INCLUDING THE PARKER HANNIFIN FOUNDATION

MERCURY EXPOSURE AND RISKS FROM DENTAL AMALGAM, PART 1: UPDATING EXPOSURE, RE-EXAMINING REFERENCE EXPOSURE LEVELS, AND CRITICALLY EVALUATING RECENT STUDIES

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EXECUTIVE SUMMARY

Background

This report was prepared in order to estimate current levels of mercury (Hg) exposure from dental amalgam in the US general population. The report also reviews and discusses a variety of issues regarding that exposure, Hg vapour (Hg^0) toxicology, risk assessment, reference exposure levels, and uncertainties, limitations and data gaps that continue to surround the issue of Hg exposure from dental amalgam.

Currently in the United States, 181.1 million Americans of all ages carry a grand total of 1.46 billion restored teeth. Based on past dental practice, and recently available data on the relative use of different restorative materials, the majority of these restorations, if not the vast majority, are composed of dental amalgam. However, the exact proportion of those fillings that are composed of amalgam versus alternate materials cannot be precisely quantified with currently available information.

Hg^0 continuously evolves from dental amalgam fillings. That Hg^0 is inhaled, predominantly during mouth breathing, and is absorbed from the lungs into systemic circulation where it is

distributed and deposited to tissues throughout the body, including the brain. For the fetus and infant, amalgam-associated Hg exposure arises from maternal amalgam load, via cord blood (fetus) and breast milk (infant).

Dental amalgam is the primary source of exposure to Hg^0 in the general, non-occupationally exposed population. Amalgam-related Hg exposure exceeds that from fish or other sources for the majority of the population. Amalgam-associated Hg is detected not only in urine but also in: feces; exhaled breath; saliva; blood; various organs and tissues including the kidney, pituitary gland, liver, and brain; in amniotic fluid, placenta, cord blood, meconium and various fetal tissues including liver, kidney and brain, due to maternal amalgam load; and in colostrum and breast milk in association with maternal amalgam load.

Amalgam fillings are sufficiently significant to personal Hg exposure that the influence of amalgam load on blood and urine Hg concentration can be detected despite moderate occupational Hg exposure.

Exposure to Mercury from Amalgam in the US Population

Data on the occurrence of dentally restored tooth surfaces in the US general population were drawn from the 2001 to 2004 National Health and Nutrition Examination Surveys (NHANES) conducted by the US National Center on Health Statistics (NCHS).

Employing the latest research (predominantly US-based) on the incremental increase in urinary Hg concentration per amalgam-filled tooth surface, and other information such as body weight recorded in the NHANES, estimates of Hg exposure from amalgam fillings were determined for 5 age groups of the US population: toddlers (aged 2 to < 5 years), children (aged 5 to <13 years), adolescents (aged 13 to <21 years), adults (aged 21 to <60 years) and seniors (aged ≥ 60 years). Children as young as 26 months were recorded as having restored teeth.

Four specific exposure scenarios were considered. These were:

- All reported restored tooth surfaces were assumed to be composed of amalgam;
- All reported restored tooth surfaces, but excluding 5 surface fillings (which were assumed to be non-amalgam crowns) were assumed to be composed of amalgam;

- Only 50% of all reported restored tooth surfaces, but excluding 5 surface fillings, were assumed to be composed of amalgam;
- 30% of persons with filled teeth were assumed to have no amalgam, and of the remainder only 50% of all reported restored tooth surfaces, but excluding 5 surface fillings, were assumed to be composed of amalgam.

Derived exposures for these 4 scenarios are summarized in Table ES-1. Average exposure levels across all age groups, on a µg/day per filling basis (approximately 2 filled surfaces per filled tooth, on average based on NHANES data), are consistent with previous estimates presented by Health Canada in 1995. Table ES-2 presents the estimated proportion and total number or US citizens possessing amalgam that exceed the dose associated with each of the various reference exposures levels for Hg^0 published by US regulatory agencies and other agencies/authors.

With reference to scenario 4, above, which is the least conservative of the scenarios evaluated (predicts the lowest levels of exposure for any of the scenarios), it was determined that some 67.2 million Americans would exceed the Hg dose associated with the REL of 0.3 ug/m^3 established by the US Environmental Protection Agency in 1995, whereas 122.3 million Americans would exceed the dose associated with the REL of 0.03 ug/m^3 established by the California Environmental Protection Agency in 2008. Other published RELs, and the populations exceeding them, fall between these two extremes.

Presented in Table ES-3 are the estimated numbers of amalgam-filled tooth surfaces that will not result, on average, in exceeding the doses associated with the various RELs.

Fetal and Infant Exposure to Hg from Amalgam

The Fetus

The fetus and young infant are vulnerable or 'sensitive' receptors with respect to exposure and risks to neurotoxic substances such as Hg^0. An immature blood-brain barrier, and the continuing development and maturation of the brain in utero and well beyond birth are the primary reasons for this vulnerability.

The fetus is exposed to Hg as a result of amalgam fillings present in the teeth of pregnant women. Although the placenta and fetal liver provide some protection of the brain and other organs and tissues from this Hg, that protection is not complete. Hg concentrations increase with increasing maternal amalgam load in amniotic fluid, cord blood, placenta, meconium, and various fetal and neonatal tissues including liver, kidney and brain.

Concentrations of amalgam-related Hg in fetal cord blood have been reported to range between 1.2 and 2 times the concentration of Hg in maternal blood, with incremental increases in cord blood per maternal amalgam fillings of between 0.76 and 1.4 ug Hg/L per amalgam filled tooth. At baseline (amalgams = 0), cord blood Hg concentrations are already elevated relative to maternal blood Hg levels, further demonstrating the cumulative or bioconcentrating nature of Hg exposure from mother to fetus.

It was estimated that for every maternal amalgam filling, the Hg concentration in cord blood increases by an average of 0.11 ug Hg/L. This is essentially the same incremental increase per filling as observed in maternal blood, indicating that, on a blood concentration basis, the dose received by the fetus is equal to that in the mother. This would equate to approximately 0.05 ug Hg/L of blood for every amalgam filled tooth surface, assuming approximately 2 surfaces per filling on average (determined from NHANES data).

Using these relationships derived from the published literature, fetal cord blood concentrations were estimated for various numbers of amalgam fillings in the mother. Those estimates are presented in Table ES-4.

The breast feeding infant

The concentration of Hg in breast milk increases with increasing maternal amalgam load. However, overall risks posed to breast-feeding infants cannot be determined with any degree of certainty until data on the further speciation of inorganic Hg (as Hg^{2+} and Hg^{0}) in breast milk are available, and the gastro-intestinal absorption rate of the Hg^{0} from ingested breast milk is better understood. Based on currently available information, this pathway is not considered to be problematic relative to fetal exposure, and there are no data or information to suggest that the continued promotion of breast feeding, for its significant health and developmental benefits, should be altered for mothers possessing amalgam fillings.

Potential developmental effects associated with Hg exposure from dental amalgams

There is virtually no data on the neurotoxicological or neurodevelopmental effects posed by Hg^0 exposure in the fetus or young infants. One study, related to amalgam, reported no adverse outcomes in infants born to women bearing relatively low numbers of amalgam fillings. Another study, again related to amalgam, reported a 4-fold increase or greater in the incidence of cleft palate of children born to women who received dental treatments with amalgam during the first trimester of pregnancy. This latter study is currently being repeated.

Given this paucity of neurotoxicological and neurodevelopmental data, the California EPA applied additional precaution by increasing the uncertainty factors within their derivation of their chronic reference exposure level for Hg^0, establishing their regulatory REL at 0.03 ug Hg/m^3. Until further data are available on developmental and neurological outcomes associated with Hg^0 exposure in humans, it is essential that precaution be applied in the determination of updated and revised reference exposure levels for the protection of public health.

Toxicology of Hg^0

Toxicological reviews of Hg^0 were recently prepared in 2008 by Health Canada and the California Environmental Protection Agency. Additional, but older, reviews have been prepared by the World Health Organization, the US Agency for Toxic Substances and Disease Registry, and the US Environmental Protection Agency. As a result of the availability of these previous reviews, a detailed review is not presented herein. Instead, this report presents an examination of existing reference exposure levels for Hg^0 for protection of public health, including a new REL just proposed in 2010, as well as a critical discussion of the Casa Pia and New England Children's Amalgam Trials (CATs), the latter with reference to a very recent (currently in press) dose-response analysis of porphyrin excretion in participants of the Casa Pia Children's Amalgam Trial.

Reference exposure levels (RELs) for Hg^0

At present, six agencies and authors have prescribed reference exposure levels (REL) for Hg^0, for risk assessment of general (non-occupational) population exposures:

- the California Environmental Protection Agency in 2008: 0.03 $\mu g/m^3$;
- the Canadian Federal Department of Health (Health Canada) in 2008: 0.06 $\mu g/m^3$;
- Lettmeier et al. (2010): 0.07 $\mu g/m^3$;

- the US Agency for Toxic Substances and Disease Registry in 1999: 0.2 µg/m^3;
- the US Environmental Protection Agency in 1995: 0.3 µg/m^3; and
- the European office of the World Health Organization in 2000: 1 µg Hg^0/m^3.

On the basis of the key toxicological studies employed for REL derivation, it is apparent that RELs established by the USEPA, the USATSDR and the WHO can no longer be considered valid. The USEPA acknowledges within their entry for 'mercury, elemental' on their Integrated Risk Information System website that significant new toxicological literature was identified as early as 2002 that could significantly influence the determination of their REL. Also, these three agencies all relied on occupational studies of chloralkali workers whose Hg exposure and effects would have been reduced by concomitant chlorine gas (Cl_2) exposure. The concomitant exposure to Cl_2 that occurs in chloralkali plants reduces Hg respiratory absorption, reduces deposition of Hg to the brain, and reduces the resulting toxicity of Hg exposure. This makes chloralkali studies unsuitable for establishing a REL for public health protection from Hg^0 exposure alone. The REL from California EPA is also based on those same chloralkali studies.

The degree of protection offered to the developing central nervous system (CNS) of the fetus and young infant, including the brain, by the RELs from the USEPA, USATSDR and the WHO are not defensible. With respect to the appropriate uncertainty factors to be applied in the derivation of a valid REL for Hg^0, the USEPA REL does not comply with their own current guidance with respect to protection of the CNS of the fetus and infants from exposure to neurotoxic chemicals. As reviewed and discussed by the California EPA, the database of studies concerning the exposure and risks posed to the fetus, infants and children is minimal to non-existent, requiring additional adjustments to (lowering of) the REL to account for these data deficiencies.

The RELs developed by Health Canada and by Lettmeier et al (2010) employ studies of Hg exposures that were free of concomitant Cl_2 exposure, making them more reliable for public health protection. The REL of Lettmeier et al (2010) is particularly interesting as it was based on a very recent study of Hg^0 exposure, rather than on studies published decades ago.

The study of Lettmeier et al (2010) is particularly important to the assessment of risks from Hg^0 exposure, and ultimately to the determination of a valid and up-to-date REL for Hg^0, because of the following:

- The mercury vapour exposures can be safely assumed to have been free of concomitant exposure to Cl_2, so that confounding is avoided with respect to the chemical form of Hg, the absorption and the toxic effects of Hg^0.

- The toxicological data relate to what are clinical signs and symptoms rather than sub-clinical measures of neurotoxicity, the latter often the cause for debate regarding significance for human health risk assessment;

- A dose-response analysis was conducted in which 'cut-off' exposure values or points of departure from the dose-response relationship were determined, rather than relying on simple group average exposure levels.

The Children's Amalgam Trials

The Casa Pia and New England Children's Amalgam Trials (CATs) were an attempt to resolve debate regarding health risks posed by the Hg exposure that arises from amalgam fillings in children and adolescents. These clinical trials assigned participants randomly to 2 groups – those having carious lesions restored with dental amalgam, and those having caries restored with composite resin. Health effects investigated included neurobehavioral and neuropsychological functions (including IQ), renal effects, and immune function.

The New England CAT has reported on the follow up of these cohorts for a period of up to 5 years to date, whereas the Casa Pia CAT cohorts have been followed for up to 7 or 8 years (depending on toxicological endpoint) so far. Both of these studies have identified no significant differences in the average incidence or types of health effects or neuobehavioural deficits between cohorts receiving dental amalgam fillings versus those receiving composite resin fillings, with the exception of increased or altered porphyrin excretion in amalgam recipients in the Casa Pia CAT, excretion of which is reported to diminish with time since amalgam placement.

The Casa Pia and New England Children's Amalgam Trials (CATs) do nothing to resolve the debate surrounding potential health effects associated with the Hg exposure arising from dental amalgam fillings. The presence or absence of amalgam fillings aside, it is the difference in Hg exposure which is at issue. Whereas occupational studies ensure that Hg exposure in the control or referent group is significantly less than that in the exposed cohort (control groups generally having Hg exposure 3 to 10 times less than the exposed group), the referent groups (children receiving composite resin fillings) in both these CATs had the same (Casa Pia CAT) or perhaps greater (New England CAT) Hg exposure, as measured by urinary Hg concentration, when compared to the cohorts receiving amalgam fillings. Given that Hg exposure was the same or possibly greater in the referent groups, it would have been impossible to detect any significant differences in the types and average incidence of effects between exposed and referent cohorts. The effects and effect levels would be the same, irrespective of the source (amalgam or other) of the Hg^0 exposure.

Another major issue regarding interpretation of the CATs will be their relatively short duration thus far. The USEPA considers the minimum study duration to be 7 years for consideration as a chronic study. The New England trial has been reported for a total of only 5 years and, therefore, cannot be considered to represent chronic exposure. The Casa Pia Trial did report on follow ups for a total of 7 or 8 years post-recruitment (depending on endpoint considered). However, given the known cumulative nature of Hg in the body, and particularly the brain, it would not seem reasonable to accept just 7 or 8 years of exposure to Hg^0 as sufficiently representative of chronic exposure applicable to the average 80 year lifespan currently realized in the US population.

The only way that these CATs may benefit the debate regarding potential health effects of Hg exposure from amalgam will be to continue study follow up in future years, and to conduct a thorough quantitative analysis of their dose-response relationships. In other words, to plot health effects on an individual participant basis as a function of a Hg exposure metric that is appropriately designed to control for confounding factors and that incorporates both exposure level and exposure duration. To date, no such dose-response analysis has been published from these CATs.

Another approach may be to re-analyze the data in the same manner as reported to date by study authors, but to *post-hoc* exclude all members of the referent cohorts that had measured urine Hg concentrations exceeding 0.5 µg/g creatinine, the background level in the US population associated with the absence of amalgam fillings. Owing to time constraints, and the inability to access the data of these CATs within the timelines for this project, neither the former nor latter analysis could be undertaken.

These CATs, or at least the publications related to them, have a variety of other limitations that further undermine their ability to contribute meaningfully to the debate surrounding potential health effects of Hg exposure from amalgam. These include:

- The maximum total numbers of tooth surfaces restored with amalgam (24 for the New England CAT; unknown for the Casa Pia CAT) was less than that observed in the general US population.
- The vast majority of members of the New England CAT amalgam cohort received a total of less than 15 amalgam surfaces, which is only the average observed in the US population for children; this information is unknown for the Casa Pia study.
- Statistical power is low for dose-response analysis. Although efforts were made to ensure a good study design, the uneven distribution of study participants across all dose groups, and in particular the far greater abundance of participants at the low end of the exposure range, greatly undermines statistical power. That power determines the ability to detect significant differences between different dose groups, as well as the ability of only a relatively few high exposed individuals to significantly influence average effect levels for the group as a whole.
- The analytical treatment has not, to date, effectively controlled for confounders, and in particular, the need to apply an exposure metric that incorporates both dose and duration of exposure. The analysis of mercury in urine data from the New England CAT clearly demonstrated that an exposure metric that integrated both exposure level (number of amalgam surfaces) and exposure duration (years) explained greater variability in the urine Hg data than either of dose or duration alone. This same result will quite likely also be evident within the toxicological data.

Porphyrin Profiles as a Toxicological Endpoint for Hg^0 Toxicity

Porphyrins are formed in the production of heme, with redundant excess production being excreted via the urine. Disruption of the heme synthesis pathway results in alteration of the concentrations and ratios (profiles) of the various porphyrins in urine. The inhibition of enzymes within this essential synthesis pathway can be viewed, in and of itself, as a toxic effect. Porphyrin concentrations and profiles in urine are a direct measure of inhibition of heme pathway enzymes.

Data from the Casa Pia CAT indicate that amalgams are associated with elevated urinary concentrations of certain porphyrins. A recent re-analysis of the Casa Pia porphoryn data demonstrated a persistent, strong and significant dose-response relationship with alteration of normal porphyrin profile increasing with increasing Hg exposure.

The USEPA has previously employed enzyme inhibition as a toxic endpoint, specifically with regard to the establishment of their regulatory reference dose (RfD) for zinc. Enzyme inhibition by zinc was employed by USEPA, with addition of appropriate uncertainty factors, to establish their RfD for zinc of 0.3 mg/kg-day. It stands to reason, therefore, that the inhibition of heme synthesis enzymes by Hg^0 exposure, as measured by porphyrin concentrations and profiles, can be employed as another toxic endpoint for determination of an exposure level for Hg^0 that should be free of anticipated impacts in the general population. Due to time constraints, this analysis was not completed in this report, owing to insufficient time to review all relevant data and information pertaining to porphyrin production, heme pathway enzyme inhibition, and the defensible basis for appropriate uncertainty factors needed for the final determination of an appropriate REL based on this endpoint. The Casa Pia CAT may provide a suitable basis for this determination.

Recommendations for Further Research

- As part of a future NHANES survey, compile data on the specific restorative materials used to fill tooth surfaces within the US population. At the very least, recording whether the material used was amalgam versus some other material should be relatively simple. This distinction is relatively easy as it can be based solely on restoration color (silver versus other).

- The USEPA and USATSDR should immediately initiate the review of Hg^0 toxicology, including all studies conducted in the past 2 decades, towards updating and revising their RELs for Hg^0. This review and update should include consideration of heme synthesis enzyme inhibition as one of the toxic endpoints.
- A post-hoc analysis should be undertaken of the statistical power offered by the Casa Pia and New England children's amalgam trials to quantify precisely the degree of difference in incidence of neurological impairments that can be statistically differentiated between higher exposure subgroups and lower exposure subgroups within the amalgam cohorts of each study.
- Quantitatively determine the impact of urinary Hg concentrations in the CAT referent groups (those that received composite resin fillings) relative to the amalgam groups to determine if non-amalgam sources and levels of Hg^0 exposure in the referent groups negate any ability to rely on these studies as a means of demonstrating the absence of health effects due to Hg exposure from amalgam. This could include a *post-hoc* re-screening of referent group members to select only those with a urine Hg concentration ≤ 0.5 µg Hg/g creatinine.
- Combine the New England and Casa Pia studies in a meta-analysis, thereby providing increased statistical power for detecting differences in incidence of neurological effects between higher dose and lower dose members of these combined cohorts.
- Enhance the dose-response analysis of both (and combined) amalgam trials data on neurological and other outcomes by better controlling for confounders and ensuring a dose metric that reflects both exposure level and exposure duration. Data must be presented and analyzed with respect to individual CAT participants, and not simply as overall averages for exposed and referent cohorts.
- Consider future follow up of both cohorts to increase the data available on duration of exposure, thereby extending the exposures to more effectively represent true chronic exposure, particularly given Hg's accumulation in the brain and other tissues over time.
- Clarify the average numbers of amalgam filled tooth surfaces possessed by the different cohort groups that should be considered as in-place for the full duration of the CAT studies. It is apparent that members of these cohorts had varying numbers of amalgam fillings throughout the duration of these studies. The more detailed dose response analysis of these data recommended above could make this unnecessary, however.

- Explicit publication of the urine Hg concentrations from the Casa Pia study, with an analysis of the association of urine Hg concentration with amalgam load.
- Efforts should be expended to define a more appropriate reference group for future CAT studies, the members of which are free of mercury exposure (to the limits possible), not just free of amalgam.

Table ES-01. Summary of Hg doses estimated for the US population with amalgam fillings

		Number with fillings		Number of filled surfaces			Dose as ug Hg/kg-day			Dose as ug Hg/day			Hg concentration (ug Hg/g creatinine) [3]	
		NHANES 2001-04	US population [1]	Mean [2]	Min	Max	Mean [2]	Min	Max	Mean [2]	Min	Max	Min	Max
Scenario 1	Toddlers	94	740,404	14.6	1	72	0.15	0.02	0.54	2.53	0.18	9.87	0.58	6.76
All restored	Children	1181	12,806,364	9	1	72	0.11	0.01	0.45	3.72	0.27	22.9	0.58	6.48
tooth	Adolescents	2059	17,671,696	7.1	1	84	0.09	0.01	0.37	5.79	0.49	33.53	0.56	6.13
surfaces	Adults	4454	120,199,880	20.2	1	128	0.16	0.01	0.49	12.98	0.44	58.79	0.56	8.82
assumed to														
be amalgam	Seniors	2031	29,711,241	32.9	1	109	0.22	0.01	0.5	16.87	0.46	55.39	0.57	5.81
Scenario 2	Toddlers	87	667,166	7.8	1	36	0.1	0.01	0.37	1.63	0.18	6.51	0.58	3.38
Same as	Children	1109	11,987,269	5.4	1	32	0.08	0.01	0.31	2.71	0.23	22.9	0.58	3.15
Scenario 1,	Adolescents	2038	17,561,152	6.7	1	47	0.08	0.01	0.37	5.53	0.49	32.17	0.56	4.15
but 5 surface	Adults	4402	120,298,407	13.2	1	72	0.12	0.01	0.39	10.11	0.44	45.6	0.56	4.82
fillings														
excluded	Seniors	1972	28,902,381	14.9	1	67	0.13	0.01	0.39	10.43	0.46	39.55	0.56	4.62
Scenario 3	Toddlers	84	625,582	4	1	18	0.05	0.01	0.19	0.83	0.18	3.25	0.58	1.94
Same as	Children	1025	11,064,670	2.8	1	16	0.04	0.01	0.15	1.37	0.23	11.45	0.58	1.83
Scenario 2,	Adolescents	1898	16,362,871	3.4	1	23	0.04	0.01	0.18	2.8	0.44	15.22	0.56	2.33
but only 50%	Adults	4315	118,460,911	6.5	1	36	0.06	0.01	0.19	4.94	0.43	22.31	0.56	2.66
of surfaces														
as amalgam	Seniors	1940	28,583,321	7.3	1	33	0.07	0.01	0.19	5.11	0.39	19.77	0.56	2.78
Scenario 4	Toddlers	57	379,004	4.4	1	16	0.06	0.01	0.19	0.95	0.2	3.25	0.58	1.89
Same as	Children	714	7,714,637	2.7	1	16	0.04	0.01	0.15	1.37	0.24	8.22	0.58	1.83
Scenario 3,	Adolescents	1341	11,289,979	3.3	1	23	0.04	0.01	0.18	2.77	0.44	15.14	0.56	2.33
but 30% with	Adults	3003	82,524,655	6.6	1	31	0.06	0.01	0.19	5.05	0.43	22.31	0.56	2.54
no amalgam	Seniors	1387	20,403,213	7.3	1	33	0.07	0.01	0.19	5.11	0.39	19.77	0.56	2.78

1. Determined from the statistical weighting provided by NCHS for NHANES.

2. Derived as the weighted US population mean, not the mean of NHANES participants.

3. Urine Hg concentration = Background urine Hg concentration + (number of amalgam surfaces X incremental increase in urine Hg concentration per amalgam surface) (see Methods). Background urine Hg concentration set equal to 0.5 ug Hg/g creatinine, consistent with Dye et al (2005) for women of child-bearing age with no amalgam fillings in the US population.

Table ES-2. Proportion and numbers of US citizens with amalgam fillings that exceed doses associated with published reference exposure levels for Hg^0 (1)

		TODDLERS	CHILDREN	TEENS	ADULTS	SENIORS	Total population N > REL
Scenario 1	Total population with fillings	740,404	12,806,364	17,671,696	120,199,880	29,711,241	181,129,584
All restored surfaces assumed to be amalgam	% > CalEPA REL	100	100	100	100	100	181,129,584
	% > Richardson et al REL	100	100	99.4	99.5	99.7	180,400,644
	% > Lettmeier et al REL	100	100	99.0	99.0	99.5	179,613,884
	% > US ATSDR REL	84.3	81.5	74.3	92.0	95.4	163,078,979
	% > US EPA REL	74.6	68.8	62.5	87.1	92.3	152,539,776
Scenario 2	Total population with fillings	667,166	11,987,269	17,561,152	120,298,407	28,902,381	179,416,376
Same as Scenario 1, but 5 surface fillings excluded	% > CalEPA REL	100	100	100	100	100	179,416,376
	% > Richardson et al REL	100	100	99.3	99.5	99.4	178,500,660
	% > Lettmeier et al REL	100	100	98.9	98.9	98.9	177,551,107
	% > US ATSDR REL	72.2	77.4	73.0	90.1	91.4	157,330,552
	% > US EPA REL	60.7	61.6	60.3	84.1	84.8	144,115,315
Scenario 3	Total population with fillings	625,582	11,064,670	16,362,871	118,460,911	28,583,321	175,097,356
Same as Scenario 2, but only 50% of filled surfaces as amalgam	% > CalEPA REL	100	100	100	100	100	175,097,356
	% > Richardson et al REL	100	100	97.6	97.7	97.7	171,253,842
	% > Lettmeier et al REL	100	99.8	95.7	95.6	96.0	168,068,173
	% > US ATSDR REL	48.6	50.0	50.5	74.7	77.6	124,708,512
	% > US EPA REL	37.1	29.6	31.7	58.0	62.1	95,120,044
Scenario 4	Total population with fillings	379,004	7,714,637	11,289,979	82,524,655	20,403,213	122,311,488
Same as Scenario 3, but 30% with no amalgam	% > CalEPA REL	100	100	100	100	100	122,311,488
	% > Richardson et al REL	100	100	97.2	98.0	97.9	119,908,745
	% > Lettmeier et al REL	100	99.7	95.3	96.2	96.0	117,784,675
	% > US ATSDR REL	60.0	48.8	49.4	75.7	77.3	87,852,641
	% > US EPA REL	45.2	29.2	30.8	59.0	61.9	67,220,662

1. REL-equivalent doses derived as: Dose (µg/kg-day) = REL (µg Hg^0/m^3) X 15.85 m^3 inhaled/day X 80% Hg^0 absorbed ÷ 80 kg average adult body weight.

Table ES-3. Numbers of amalgam-filled surfaces that will not exceed doses associated with published reference exposure levels (RELs) for Hg^0.

Age group	REL source	REL (µg Hg/m^3)	REL-associated dose (ug/kg-d) [1]	No. of surfaces not exceeding REL dose [2]
Toddlers, children & young teens	California EPA (2008)	0.03	0.005	0.6
	Richardson et al (2009)	0.06	0.01	1.3
	Lettmeier et al (2010)	0.07	0.011	1.4
	US ATSDR (1999)	0.2	0.032	4
	USEPA (1995)	0.3	0.048	6
Older teens, adults & seniors	California EPA (2008)	0.03	0.005	0.8
	Richardson et al (2009)	0.06	0.01	1.7
	Lettmeier et al (2010)	0.07	0.011	1.8
	USATSDR (1999)	0.2	0.032	5.3
	USEPA (1995)	0.3	0.048	8

1. REL-associated doses derived as per footnote to Table ES-2.
2. Calculations employed non-conservative assumptions; alternate possible values would predict fewer numbers of fillings.

Table ES-4: Predicted cord blood Hg concentrations versus number of maternal amalgam-filled teeth.

Number of maternal amalgam filled teeth	Estimated maternal blood Hg concentration (based on Oskarsson et al. (1996)	Estimated cord blood concentration
1	0.99	1.01
2	1.14	1.20
5	1.59	1.46
10	2.34	2.03
20	3.84	3.16
23 [1]	4.29	3.50
6.3 [1]	1.79	1.61

1. for US female population aged 16-49 yrs from NHANES 2001-2004; omits 5-surface fillings.

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1 INTRODUCTION

The Environment Division of SNC-Lavalin Inc. (SNC-Lavalin Environment, or SLE) was funded to undertake an updated assessment of exposures and risks posed by mercury (Hg) exposure from dental amalgam, specific to the US general population. Funding for this project was provided by corporate foundations, including the Parker Hannifin Foundation, Cleveland, OH.

Contract management on behalf of funding foundations was provided by the International Academy of Oral Medicine and Toxicology (IAOMT), ChampionsGate, FL.

This project was divided into two parts. Part 1 quantifies US population exposure from dental amalgam, examines and discusses fetal exposure due to in utero exposure, discusses infant exposure through consumption of mercury-contaminated breast milk, critically reviews currently published reference exposure levels for mercury vapour which are designed to protect public health, and critically reviews recent clinical trials that compared health effects in children and adolescents receiving amalgam fillings versus those receiving composite resin.

Part 2 presents a critical review of concomitant exposure to mercury from amalgam, methyl mercury from fish consumption and exposure to environmental lead. An analysis is presented of the need to consider these exposures as additive, less than additive (antagonistic) or more than additive (synergistic) during the conduct of population risk assessments from simultaneous exposure to these three common contaminants.

1.1 Background

For the general population, on average, dental amalgam is the most significant single source of mercury (Hg) exposure, compared to food (including fish), indoor and outdoor air, drinking water and soil (Health Canada 1996; WHO 1991).

To date, at least 13 assessments quantifying Hg exposure from dental amalgam have been published, determining Hg dose rather than simply reporting Hg concentrations in urine or other bodily fluids or tissues. These were summarized by Richardson (2003) and are depicted in

Figure 1. More recently, a series of studies have reported urinary Hg concentrations (variably corrected or uncorrected for urine creatinine content) as a function of amalgam filling load[1] (Barregard et al. 2008; Dunn et al. 2008; Melchart et al. 2008; Woods et al. 2007; Bellinger et al. 2006; Dye et al. 2005; Factor-Litvak et al. 2003; Pesch et al. 2002; Kingman et al. 1998; among others). In these studies, as with earlier studies reviewed by Richardson and Allan (1996; see also Health Canada 1995), the average urine Hg content is consistently greater in groups with amalgam fillings than in those without, and urine Hg content consistently increases as amalgam load increases. Numerous studies have also demonstrated that the Hg exposure or concentration increases with increasing amalgam load in the following tissues and situations:

- Due to chewing, brushing and bruxism (Hansen et al. 2004; Ganss et al. 2000; Isacsson et al. 1997; Sallsten et al. 1996; Berdouses et al. 1995; Bjorkman and Lind, 1992; Forsten 1989; Vimy and Lorscheider 1985a,b; Berglund 1990; Svare et al. 1981; Gay et al. 1979);
- In exhaled or intra-oral air of persons with amalgam fillings (Halbach and Welzl, 2004; Skare and Engqvist 1994; Gay et al. 1979; Svare et al., 1981; Patterson et al. 1985;Vimy and Lorscheider, 1985a,b; Berglund et al. 1988; Jokstad et al. 1992);
- In saliva of persons with amalgam fillings (Fakour et al. 2010; Melchart et al. 2008; Zimmer et al. 2002; Ganss et al. 2000; Pizzichini et al. 2000; Bjorkman et al. 1997; Berglund 1990);
- In blood of persons with amalgam fillings (Gerhardsson and Lundh, 2010; Halbach et al. 2008; Melchart et al. 2008; Lindberg et al. 2004; Pizzichini et al. 2003; Ganss et al. 2000; Vahter et al. 2000; Kingman et al. 1998; Oskarsson et al. 1996; Skare and Engqvist 1994; Akesson et al. 1991; Abraham et al. 1984; Snapp et al. 1989; Molin et al. 1990; Jokstad et al. 1992; Svensson et al. 1992; Herrstrom et al. 1994);
- In various organs and tissues of amalgam bearers, including the kidney, pituitary gland, liver, and brain or parts thereof, (Barregard et al. 2010; Bjorkman et al. 2007; Guzzi et al. 2006; Barregard et al. 1999; Weiner and Nylander 1993; Nylander et al. 1989: Nylander

[1] Amalgam filling load variably reported as numbers of amalgam-filled teeth, total numbers of amalgam-filled surfaces, numbers of amalgam-filled occlusal surfaces, surface area of total or occlusal surfaces filled with amalgam.

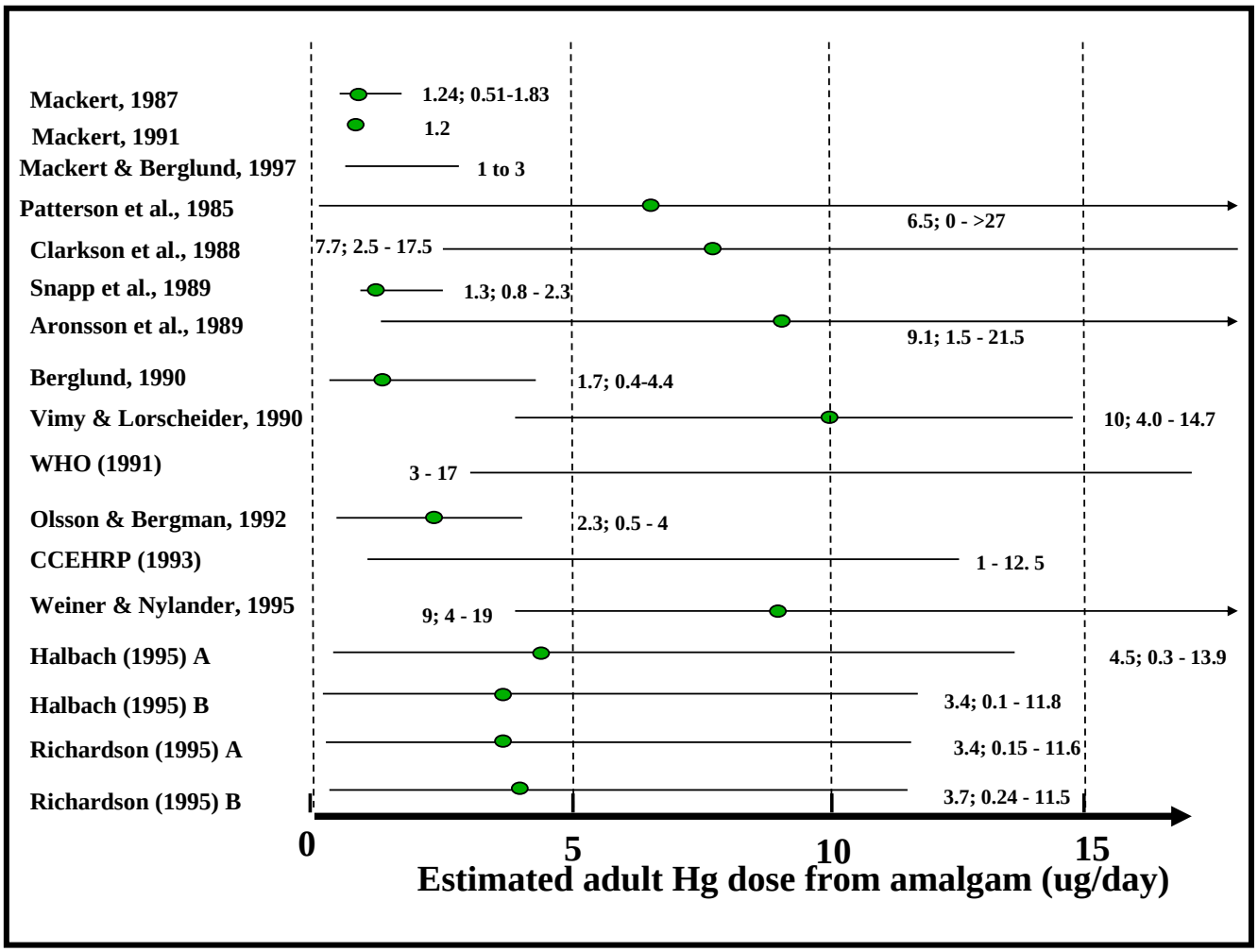
et al. 1987; Eggleston and Nylander 1987);

- In feces of amalgam bearers (Engqvist et al. 1998; Bjorkman et al. 1997; Skare and Engqvist 1994);
- In amniotic fluid, cord blood, placenta, and various fetal tissues including liver, kidney and brain, in association with maternal amalgam load (Palkovicova et al. 2008; Ursinyova et al. 2006; Luglie et al. 2005; Ask-Bjornberg et al. 2003; Lindow et al. 2003; Ask et al. 2002; Vahter et al. 2000; Lutz et al. 1996; Drasch et al. 1994);
- In colostrum and breast milk in association with maternal amalgam load (Ursinyova et al. 2006; Ask-Bjornberg et al. 2005; Da Costa et al. 2005; Drexler and Schaller, 1998; Drasch et al. 1998; Oskarsson et al. 1996).

Amalgam fillings are sufficiently significant to personal Hg exposure that the influence of amalgam load on blood and urine Hg concentration can be detected despite moderate occupational Hg exposure, that results in up to about 10 µg Hg/L (Skare et al. 1990; Martin et al. 1995; Soleo et al. 1998a; Jokstad 1990). Increased Hg exposure in amalgam bearers has even been reported to result from magnetic resonance imaging and cellular telephone use (Mortazavi et al., 2008).

Methyl Hg has also been detected in the oral cavity at higher levels in amalgam bearers than those with no amalgams (Liang and Brooks 1995; Sellars et al. 1996; Leistevuo et al. 2001). The methylation of Hg by oral and intestinal microflora has been demonstrated *in vitro* (Heintze et al. 1983; Rowland et al. 1975; Yannai et al. 1991).

Figure 1. Summary of published exposure assessments of Hg^0 from dental amalgam fillings (after Richardson, 2003).



Perhaps the most quantitative assessment of Hg exposure from dental amalgam was prepared for the Canadian Federal Department of Health (Health Canada, 1995; see also Richardson and Allan, 1996). That investigation was initiated in 1994 at the request of the Medical Devices Bureau of Health Canada. That assessment combined data on the frequency of filled teeth in the Canadian population, and specific Canadian data on body weight and other required information to assess exposure in Canadians as young as 3 years of age who were recorded as possessing fillings, up to the elderly that included individuals >90 years of age. The frequency of filled teeth in the various individuals for whom data were available ranged from 1 filled tooth (those with no filled teeth were not considered part of the exposed subpopulation) up to 25 filled teeth. Based on the analysis presented in the Health Canada Report, it was possible to quantify the proportion of the Canadian population that exceeded the level of exposure (dose) associated with a toxicologically-based reference exposure level (REL). Also, the analysis contained in that report permitted the determination of the numbers of amalgam-filled teeth that could be toxicologically 'acceptable' or 'tolerable'; the number of filled teeth that would not lead to exceeding the specified REL.

To date, no population-based assessment of Hg exposure from dental amalgam specific to the US general population has been undertaken. The quantification of Hg dose associated with dental amalgam is required to complete a proper risk assessment. Determining the amalgam-associated dose can be directly compared to the dose associated with regulatory reference exposure levels (RELs) prescribed for the protection of the health of the general population. Such RELs are published by the USEPA (1995), the USATDSR (1999), the California EPA (2008), and others; these RELs are discussed in greater detail later in this report.

Dye et al (2005) provided a statistical analysis of the association between estimated [2] numbers of amalgam filled tooth surfaces and urinary Hg concentrations for US women aged 16 to 49 years. However, no dose conversions/calculations were provided to permit comparison to regulatory reference exposure levels. Lacking a reference urinary Hg concentration considered '*safe*' for the general population [3], the analysis provided by Dye et al (2005) is of limited use for risk assessment purposes.

The Health Canada (1995) report remains the single most quantitative assessment of Hg exposure from dental amalgam published to date. However, that report presents certain limitations with respect to its application and relevance to the US population. Those limitations include:

- The data employed within the Health Canada report were collected from the Canadian general population. Dental care systems (social versus private dental programs and the relative coverage of the population by dental care insurance, for examples) may be sufficiently different that the Canadian statistics are not directly applicable to the US population.
- The data on frequency of filled teeth within the Canadian population were collected as part of a Canadian population health survey (the Nutrition Canada Survey) conducted from 1970-72. These data are now some 40 years old and may not represent current dental health statistics, in Canada or the US.
- Body weight data, required to standardize exposure estimates in units of dose per unit weight (typically micrograms per kilogram of body weight), were likewise collected between 1970 and 1972. Population trends in body weight over the intervening 40 years

[2] Dye et al (2005) based their analysis on data compiled by the National Health and Nutrition Examination Survey (NHANES) of 1999-2000 (data available at http://www.cdc.gov/nchs/nhanes.htm); NHANES does not report the composition of dental restorations, only the presence/absence of such restorations. Dye et al assumed all dental restorations, except for 5-sided restorations, were composed of amalgam.

[3] The American Conference of Governmental Industrial Hygienists (ACGIH) has published a Biological Exposure Index (BEI) of 35 µg/g creatinine; this is a reference level for occupational exposures and is not relevant to the general population that includes infants and children, pregnant women, etc. ACGIH does not guarantee that BEIs are safe for all workers.

would suggest that more recent body weight data should be employed for dose standardization.

- At the time that the Health Canada report was being prepared (1994-95), it was generally accepted that the vast majority of in-place fillings were composed of amalgam. This was particularly true for fillings present in 1970-72, the years for which Canadian filling frequency data were available. However, since 1994-95 the sales of dental amalgam by dental materials suppliers has reportedly steadily declined (see Van Boom et al. (2003) for trend in Canada), due in part to the continuing controversy surrounding dental amalgam, and to the increasing availability of alternate filling materials, particularly aesthetic (white colored) alternates. Therefore, for 2010, the exposure assessment should be adjusted for the relative proportion of dental fillings that are composed of amalgam versus alternate materials.

1.2 Why was this Report Prepared?

To date, no population-based assessment of Hg exposure from dental amalgam specific to the US general population has been undertaken. The quantification of Hg dose associated with dental amalgam is required to complete a proper risk assessment. Determining the amalgam-associated dose can be directly compared to the dose associated with regulatory reference exposure levels (RELs) prescribed for the protection of the health of the general population. Such RELs are published by the USEPA (1995), the USATDSR (1999), the California EPA (2008), and others; these RELs are discussed in greater detail later in this report.

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1.3 What this Report did not Evaluate

This document does not revisit, repeat nor re-evaluate every aspect of Hg exposure, toxicity, pharmacokinetics, etc. These topics are addressed in detail elsewhere (USATSDR, 1999; WHO, 2000, 2003; Health Canada, 1995; Richardson et al. 2009; etc.) and need not be reproduced herein.

This report does not attempt to quantify exposure to Hg^{2+} associated with amalgam corrosion, wear and subsequent ingestion. Health Canada (1995; see also Richardson and Allan 1996) demonstrated that inclusion or exclusion of this ingestion exposure resulted in essentially the same estimates of exposure, indicating that ingestion of amalgam particles and Hg^{2+} ions is insignificant compared to exposure to Hg^{0} alone.

This report did not attempt to assess or quantify the potential exposure to methyl Hg associated with the methylation of amalgam-related Hg in the oral cavity or gastrointestinal tract.

Although a brief review is provided of infant exposure via consumption of breast milk from mothers with amalgam fillings, the dose associated with this exposure is not quantified.

This report does not evaluate nor assess the association of amalgam fillings or Hg exposure to specific diseases or disorders such as Alzheimer's Disease, Autism, Multiple Sclerosis, Amyotrophic Lateral Sclerosis (ALS), or Parkinson's Disease.

2 PROBLEM FORMULATION

2.1 What is Dental Amalgam?

Dental amalgam is a solid emulsion composed of a mixture of metals comprising approximately 50% metallic Hg by weight. Formulations vary in their Hg content, ranging from 43 to 50.5% Hg by weight, mixed with a powder of other metals typically containing silver (40 to 70%), tin (12 to 30%), copper (12 to 30%), indium (0 to 4 %), palladium (0.5%) and zinc (0 to 1%) (Berry et al. 1994). Typically in North America, dental amalgam is prepared and sold in sealed single use capsules, where the liquid Hg and alloy mixture are separate. Immediately prior to use, the Hg and alloy are mixed together with the aid of an amalgamator. The amalgam sets within about 30 minutes of mixing and placement. Prior to setting, the material is a soft metallic paste which is installed into the prepared tooth surface (Horsted-Bindslev et al. 1991).

2.2 What is the Controversy?

Hg has been listed consistently as a priority pollutant by the US EPA since the 1970s. It was included as substance 45 in the first list of toxic pollutants, as published on January 31, 1978 in the Federal Register (43 FR 4108). Hg was later included as substance 123 in the subsequent list of Priority Pollutants, published by the EPA in Appendix A to 40 CFR Part 423. Regulatory concern for this substance is due primarily to its neurotoxic and fetotoxic effects and its widespread distribution in the environment, including air, water, soil, and foods (particularly fish) (Richardson and Allan, 1996).

Hg is one of the most studied chemical substances; whether it is ***the*** most studied substance is difficult to quantify. This qualifier as “the most studied” is routinely applied to many toxic substances, in both science journal articles and science news articles in the general media. However, a simple search of PubMed® using the key word ‘mercury’ produced >31,700 hits (as of August 21, 2010); a similar search for ‘Hg’ returned >72,000 hits. Specifically categorizing Hg as the substance name in PubMed’s advanced search engine resulted in 16,784 hits. Similar searches for other popular toxic substances produced the following results:

- 'Pb' and 'lead': 25,796 hits as a simple search of Pb; 21,563 hits as lead[substance name];
- dioxin/PCDD/TCDD: 15,545 hits, 2,266 hits and 7,074 hits, respectively, as simple searches only;
- bisphenol-a: 5,797 hits as a simple search; 1,601 as bisphenol-a[substance name];
- formaldehyde: 24,043 hits as a simple search; 15,537 as formaldehyde[substance name].

As previously mentioned, dental amalgam is composed of approximately 50% elemental (liquid) Hg by weight. It has been used in North American dentistry for perhaps 150 years (Clarkson and Magos, 2006) and during that time has been the subject of repeated controversy, often referred to as the *Amalgam Wars* (Clarkson and Magos, 2006). A brief historical account of its introduction, use and controversy is provided by Molin (1992). Scientific articles regarding amalgam's potential toxicity date back at least to 1885 (Talbot, 1885). These *wars* or debates have been due to the recurring concern for the potential health risks posed by exposure to the Hg used in the manufacture of dental amalgam.

The quantity of Hg^0 released from amalgam is often referred to as 'minute' (ADA, 2008; CDA, 2005) or 'very small' (AGD 2007). However, it is not the dose itself that determines safety, it is how that dose compares to levels considered 'safe' or without anticipated harm that determines whether or not the dose is significant (hazardous or free from harm). Irrespective of quantity, a minute dose can be very hazardous if the substance is very toxic and the received dose exceeds the toxic dose. Dental amalgam has been identified as the single largest source of continuous Hg exposure for members of the general population who possess amalgam fillings (WHO, 1991; Heath Canada, 1996). Also, previous assessments have demonstrated that the dose of Hg received from amalgam exceeds what is considered to be a safe or reference dose (see HC, 1995; Richardson and Allan, 1996).

The Academy of General Dentistry (AGD 2007) goes on to say that "mercury *in dental amalgam is not poisonous*". However, the mercury that evolves from amalgam, as for any other source, is toxic; for example, Hg^0 originating from amalgam has been shown to cause neurobehavioral and other toxic effects in dental staff that place amalgam fillings. The Hg^0 from amalgam is no different chemically or toxicologically than the Hg^0 from any other source. The American Dental Association (ADA) recommends storing scrap amalgam and used amalgam capsules in airtight

containers (ADA 2007). The ADA's mercury hygiene recommendations are predicated on protecting the safety of dental professionals (ADA 2003; ADA Council on Scientific Affairs).

In a recent survey of members of the Society of Toxicology (STATS et al 2009) Hg was surpassed as the primary toxic substance of concern only by smoking-related issues (direct use of cigarettes, use of chewing tobacco, second-hand smoke).

Adding further to the controversy surrounding the continued use of amalgam in North America is the fact that Norway has now banned the use of amalgam in dental treatment (with certain minor exemptions) (Norway Ministry of Environment, 2007). Sweden has also banned the further use of amalgam in general dentistry (Sweden Ministry of Environment, 2009). Those bans were based predominantly on the health concerns related to exposure to Hg^0 from this dental material.

2.3 Who is Exposed and How are They Exposed?

Those people who possess one or more amalgam filled tooth surfaces will be directly exposed to Hg from dental amalgam. The fetus is also exposed due to maternal amalgam load, as are breast-fed infants as breast milk Hg content reflects maternal amalgam load. Excluding the fetus and infants, the primary route of exposure to Hg from dental amalgam is via inhalation of Hg^0 emanating from in-place amalgam fillings (WHO, 1991; Richardson and Allan, 1996; USFDA, 2009).

2.4 What is Mercury and What are Its Forms in the Environment?

Hg (quicksilver) is a dense silver-white metal that is liquid at room temperature and is characterized by low electrical resistance, high surface tension, and high thermal conductivity (Andren and Nriagu 1979; Environment Canada 1981). Hg is found in the environment, not as the liquid metal, but mainly in the form of amalgams, inorganic salts and minerals which have lower vapour pressures than elemental Hg (Andren and Nriagu 1979).

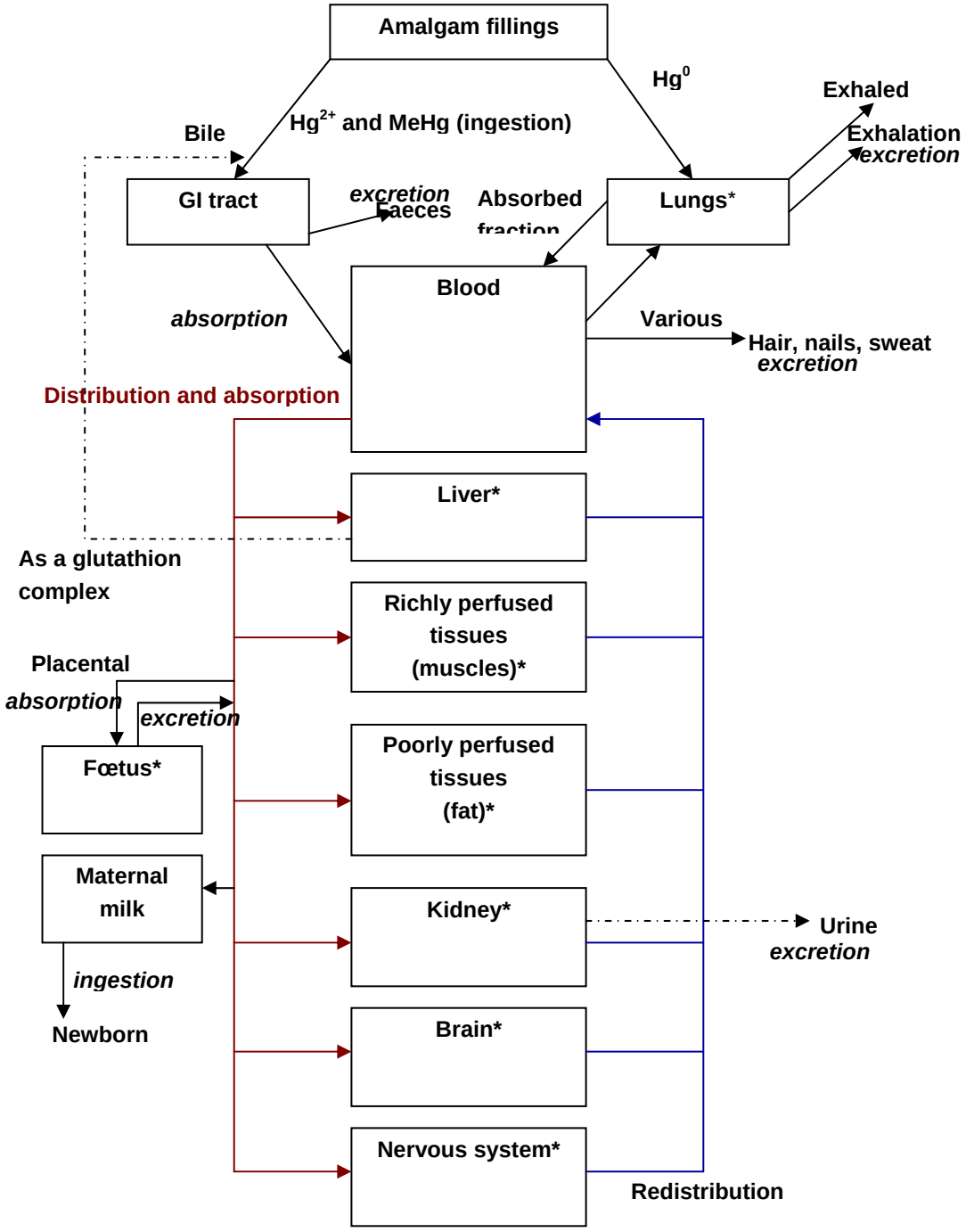
The two properties that largely determine the environmental behaviour of Hg are the high vapour pressure of metallic Hg, and the relative insolubility of ionic and organic forms. The

vapour pressure of metallic Hg is highly dependent on ambient temperature, and the tendency of liquid Hg to form small droplets increases its rate of evaporation (by presenting greater surface area). Hg can exist in three stable oxidation states: elemental Hg (Hg^0), mercurous ion (Hg_2^{2+}/Hg(I)), and mercuric ion (Hg^{2+}/Hg(II)). Hg (II) forms both inorganic and organic salts, such as chlorides and sulphates, and organoHg compounds. Organo-Hg compounds are characterized by covalent bonding of Hg to one or two carbon atoms to form compounds of the type R-Hg-X and R-Hg-R', where R and R' represent the organic moiety, and X represents a halogen. The organic moiety may take the form of alkyl, phenyl and methoxyethyl radicals (WHO 1976). A subclass of short-chained alkylmercurials, which includes monomethyl Hg (CH_3Hg^+) and dimethyl Hg ($(CH_3)_2Hg$), are the predominant organic Hg compounds found in nature. Dimethyl Hg is less stable and more volatile than monomethyl Hg (Environment Canada 1981).

2.5 Exposure Assessment Conceptual Model

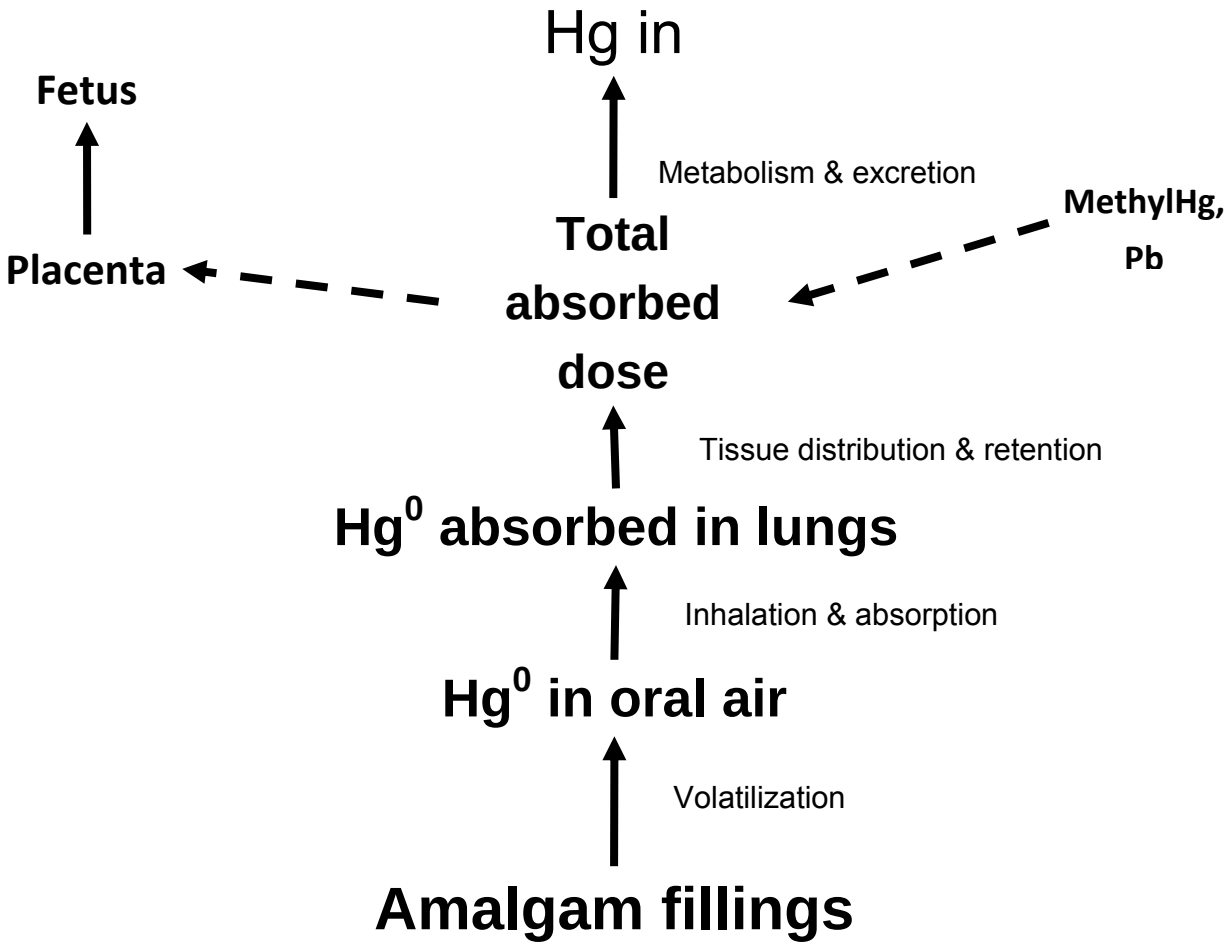
Shown in Figure 2 is the theoretical fate of Hg^0 in the human body. However, full physiologically-based pharmacokinetic (PBPK) modeling was beyond the scope of this report. The working conceptual model developed for this risk assessment is depicted in Figure 3. In essence, as Hg^0 evolves from amalgam fillings, it is taken into the lungs with air that is inhaled predominantly through the mouth. Once in the lungs, the Hg^0 is absorbed at a rate of approximately 80%, thereby entering the systemic circulation. The Hg^0 is transported to the blood brain-barrier and crosses this barrier into the CNS. Once in the brain, the Hg^0 binds with sulfhydryl groups in CNS cellular proteins. Once bound to CNS proteins, the Hg remains in the brain for a prolonged period of time during which it elicits its neurotoxic effects.

Figure 2: Theoretical model of Hg^0 uptake, distribution and excretion in the human body.



*Elemental or metallic mercury is oxidized to divalent mercury in tissues, probably by catalases. Once Hg^0 and MeHg cross the blood-brain barrier, they convert to Hg^{2+} and bind to sulphydryl groups which leads to accumulation in the brain.

Figure 3: Conceptual model for mercury exposure from dental amalgam.



Fetal exposure occurs when the pregnant woman possesses one or more amalgam fillings, the Hg from which crosses the placenta into the fetus. The fetal exposure (dose) is proportional to the number of amalgam fillings in expectant mothers' teeth.

For those persons with amalgam fillings, they are simultaneously exposed to Hg^0 from their amalgam fillings, to methyl Hg from the fish and shellfish they consume, and to lead (Pb) from their general environment. These concomitant exposures may give rise to risks that are additive, less than additive (antagonistic) or more than additive (synergistic) relative to the toxicities of the individual substances.

3 THE TOXICOKINETICS OF MERCURY

3.1 Summary

The toxicokinetics (uptake, tissue distribution and retention, metabolism, excretion) of Hg^0 will not be reviewed in detail. A review of Hg^0 metabolism is provided by Lorscheider *et al.* (1995), while the pharmacokinetics of Hg have been reviewed in detail by the USATSDR (1999) and the World Health Organization (WHO 2000, 2003). Additional valuable information is provided by Clarkson and Magos (2006) and Mutter et al. (2007).

Exposure to Hg^0 is predominantly via the lung, with reported absorption ranging from 61 to 86% of the vapour inhaled (Neilsen-Kudsk 1965; Teisinger and Fiserova-Bergerova 1965; Hursh et al. 1976; Oikawa et al. 1982).

The primary organ of deposition is the kidney, with lesser amounts in the liver, CNS and other tissues (WHO 1991). The ratio of plasma:erythrocyte Hg concentrations is approximately 1 or 2 for Hg^0 (WHO 1991), compared to 0.05 for methyl Hg (WHO 1990). WHO (1991) concluded from *in vitro* studies of Hg oxidation in blood (Hursh et al. 1988) that transport from the lung to the blood-brain barrier is direct and rapid with little oxidation (<10%) of Hg^0 to Hg^{2+} before reaching the blood-brain barrier. A greater relative proportion of Hg^0 absorbed via the lungs is deposited in the brain than for any other route of exposure or form of Hg (WHO 1991). Hg^0 crosses the blood-brain barrier (WHO, 2003) and once in the brain, it is oxidized to Hg^{2+}

(Lorscheider et al., 1995) which binds to sulphydryl groups of proteins. Hg^{2+} can not readily cross the blood-brain barrier (WHO, 2003) and is thereby 'trapped' in the brain or CNS (Lorscheider et al. 1995). Whereas the whole-body half-life of Hg^0 is approximately 60 days (Clarkson and Magos, 2006), the half-life of Hg from the brain extends for decades (reviewed by Mutter et al. 2007). Modeling of Hg accumulation and elimination in the brain suggests that a small elimination phase may exist with a half life approaching 30 years (Bernard and Purdue, 1984).

Excretion of Hg following exposure to Hg^0 is predominantly via urine and feces, although a small proportion of excretion may also occur via expired air, saliva, sweat and breast milk (WHO 2003). Urinary excretion is considered the primary excretion route (58%) following long term occupational inhalation exposure (WHO, 2003). However, the proportion of Hg excreted by the urinary route is dose dependent at lower exposure levels (Reviewed by Richardson 1999). This phenomenon is most readily apparent in the curvilinear relationship between measures of amalgam load (primary non-occupational source of exposure to Hg^0) and Hg concentration in urine (see data reported by Maserejian et al. 2008; Halbach et al. 2008; Factor Litvak et al. 2003; Herrmann and Schweinsberg, 1993; Skerfving 1991; Akesson *et al.* 1991; Langworth *et al.* 1988, 1991). As exposure level (number of amalgam fillings) increases, the proportion of Hg excreted in urine also increases, producing the observed curve. Based on published evidence, Richardson (1999) determined that the proportion of daily Hg excretion by the urinary route increases progressively from about 10% for a dose of 0.2-0.45 µg/day, to 40% for persons receiving a daily dose of 9-12 µg/day.

3.2 Gender Differences in Hg Pharmacokinetics

Available evidence indicates gender differences in uptake, distribution, and excretion of Hg^0. However, the evidence is too limited for quantitative evaluation. The available information on this issue was recently reviewed by Richardson et al. (2009). Most studies indicate that males metabolize and eliminate Hg more quickly than do females and that, after exposure, Hg tends to be distributed differently in males and females, with a greater proportion of dose going to the brain and CNS of females. While Hg appears to be distributed more quickly to the kidney and urine in males, it appears to be retained for a longer time in females.

3.3 Interaction of Chlorine Gas and Hg^0

When simultaneous exposure occurs to Hg^0 and chlorine gas, the interaction alters the chemical form of the Hg and reduces uptake and alters tissue distribution (Richardson et al., 2009). The occupational studies underlying most current reference exposure levels (RELs) for Hg^0 were conducted on chloralkali workers. Although Air-Hg^0 concentrations are generally elevated among such workers, concomitant exposure to chlorine gas (Cl_2) also occurs. Data on airborne Cl_2 levels in chloralkali plants were recently summarized by the European Union (EU, 2007). Cl_2 levels in the air of chloralkali plants averages about 1 ppm (0.3 mg/m^3) and ranges between 0 and 6.5 ppm (0-19.5 mg/m^3) depending on the specific work environment where sampling was conducted.

The concomitant exposure to Cl_2 and Hg^0 effectively reduces worker exposure to Hg^0 by decreasing the amount of airborne Hg^0 available for inhalation and absorption. Hg^0 converts to $Hg^{2+}Cl^{-1}{}_2$ in the presence of Cl_2 at room temperature (Menke and Wallis, 1980; Viola and Cassano, 1968). Also, the inhalation absorption of $HgCl_2$ is only half or less that of Hg^0 (USATSDR, 1999; Viola and Cassano, 1968). Hg deposition to the brain is also altered. Hg^{2+} (associated with $HgCl_2$) does not effectively cross the blood-brain barrier as does Hg^0 (WHO 2003; Lorscheider et al., 1995; Viola and Cassano, 1968). Following Hg^0 exposure, the red blood cell (RBC) to plasma Hg concentration ratio typically ranges between 1:1 to 2:1 (WHO, 1991). However, much less Hg is associated with RBCs in the blood of choralkali workers (with Cl_2 present). Suzuki et al. (1976), investigating Hg^0-exposed chloralkali workers versus workers from 2 other industrial sectors (who were all exposed to Hg^0 at similar airborne concentrations (0.01 to 0.03 mg/m^3)), observed that the RBC to plasma Hg concentration ratio in the chloralkali workers was only 0.02:1 whereas workers of the two other industries (with no concomitant exposure to Cl_2), had RBC to plasma Hg concentration ratios between 1.5:1 and 2:1. A study by Viola and Cassano (1968) of rodents (rats, mice) exposed to Hg^0 alone or in the presence of Cl_2, demonstrated reduced Hg absorption in the presence of Cl_2, and the deposition of Hg to the brain of rodents exposed concomitantly to Hg^0 and Cl_2 was only 1/5th of that when exposure was to Hg^0 alone.

Based on the information above, reference exposure levels prescribed for the protection of the general non-occupational population should not be based on the toxicological results of studies of chloralkali workers (Richardson et al. 2009).

4 EXPOSURE ASSESSMENT METHODS

Exposure to Hg in the US population resulting from the presence of amalgam fillings was undertaken following the general methods of Weiner and Nylander (1995), Richardson (1999) and Richardson and Allan (1996; see also Health Canada 1995). This was the general method employed by Health Canada in their assessment of mercury exposure and risks from dental amalgam (HC 1995). Details to apply this methodology are described below. In general terms: 1) the incremental Hg concentration in urine (µg Hg/g creatinine) was determined as a function of number of amalgam-filled tooth surfaces; 2) the total Hg excreted via the urine in 24 hours was determined by multiplying the Hg concentration in urine (as µg Hg/g creatinine) by the amount (grams) of creatinine excreted in urine over 24 hours; 3) the total daily absorbed dose of Hg from amalgam was then determined by dividing the total Hg from amalgam excreted in urine over 24 hours by the proportion of total daily Hg excretion that occurs via the urine pathway alone, thus accounting for excretion via both urine and feces.

In general:

$$UHg_{Incremental} = N * B \qquad \text{(Equation 1)}$$

where,

$UHg_{Incremental}$ = incremental urinary Hg concentration (µg Hg/g creatinine), above background, that is due to the presence of amalgam-filled tooth surfaces;

N = number of amalgam-filled tooth surfaces

B = increase in UHg per amalgam-filled surface ([µg Hg/g creatinine]/N)

$$UHg_{Excreted} = UHg_{Incremental} * Cr * BW \qquad \text{(Equation 2)}$$

where,

$UHg_{Excreted}$ = Hg excreted via urine in 24 hours (µg Hg/day)

$UHg_{Incremental}$ = incremental urinary Hg concentration (µg Hg/g creatinine), as

calculated with Equation 1;

Cr = creatinine excreted per kg body weight in 24 hours (g creatinine/kg-day)

BW = body weight (kg)

$Hg_{Absorbed} = UHg_{Excreted} / (P*BW)$ (Equation 3)

where,

$Hg_{Absorbed}$ = Total Hg absorbed in 24 hours (µg/kg-day)

$UHg_{Excreted}$ = Hg excreted via urine in 24 hours (µg Hg/day), as calculated in Equation 2;

P = proportion of total Hg excretion via urine (unitless)

BW = body weight (kg)

Finally, combining equations 1 to 3:

$Hg_{Absorbed}$ (µg/kg-day) = [N * B * Cr]/ P (Equation 4)

4.1 Frequency of Restored Tooth Surfaces, Body Weight and Age Data Representative of the US General Population

The US National Center for Heath Statistics (NCHS) is a division of the Centers for Disease Control and Prevention (CDC), under the US Department of Health and Human Services. The NCHS conducts the National Health and Nutrition Examination Survey (NHANES) on a continuous basis. NHANES samples a statistically representative subset of the US population, generally involving approximately 12,000 participants per cycle. NHANES is designed to assess the health and nutritional status of the US population, and to track changes over time. The surveys combine both interviews and physical examinations. The interview collects demographic, socioeconomic and dietary information, as well as answers to specific health-related questions. The examination component involves medical, dental and physiological measurements, as well as laboratory tests (such as measurement of substances in blood and urine) collected or administered by medical personnel. Details on NHANES surveys are available at: http://www.cdc.gov/nchs/nhanes.htm.

The oral health exam of the NHANES collects information on, among other variables, the presence/absence of teeth, and the condition of those teeth including the presence of restored tooth surfaces. The NHANES data for 2009-10 are not yet released. NHANES surveys conducted in 2007-08 and 2005-06 only recorded the presence/absence of at least one tooth with at least one dental restoration to at least one tooth surface. No information regarding the total numbers of restored teeth per individual, or the location of those restorations on individual tooth surfaces (lingual, facial, mesial, distal, occlusal) was recorded.

In 2003-04, NHANES conducted a detailed oral health survey of a representative subset of the US population aged 24 months and older in which data were recorded on the presence/absence of dental restorations on individual tooth surfaces (lingual, facial, mesial, distal, occlusal) of each individual tooth of each survey participant. These 2003-04 US population data represent the most recent data upon which to base the assessment of exposure to Hg from dental restorations for the US population. Of 8,847 initial participants in this oral health survey, complete records on the status of dental restorations were recorded in a total of 8,257 participants, aged 24 months to >85 years. Data from the oral health exam were subsequently merged with NHANES data from the same survey year on demographics (age, gender) and body dimensions (body weight) using the sequence number (SEQN) associated with each record. The sequence number is an identifier that is unique to each participant in these surveys. Upon merging of relevant oral health data, demographics data and body weight data, a data file containing the combined records for total of 8,257 participants was created. For 76 of the oral health survey participants, body weight was not recorded. Rather than lose these data, an estimate of body weight was imputed as the arithmetic average weight for all individuals of the same gender, and same age in months ± 6 months. Summary information on the final data set for 8,257 participants in the 2003-04 NHANES survey is presented in Table 1.

Similar to 2003-04, NHANES conducted a detailed oral health survey on 2001-02 of a representative subset of the US population aged 24 months and older in which data were recorded on the presence/absence of dental restorations on individual tooth surfaces (lingual, facial, mesial, distal, occlusal) of each individual tooth of each survey participant. Of 11,039 initial participants, complete records on the status of dental restorations were recorded in a total

of 9,010 participants, aged 24 months to >85 years. Data from the 2001-02 oral health exam were subsequently merged with NHANES data from the same survey year on demographics (age, gender) and body dimensions (body weight) using the sequence number (SEQN) associated with each record. For 313 of these 2001-02 survey participants, body weight was not recorded. Rather than lose these data, an estimate of body weight was imputed as the arithmetic average weight for all individuals of the same gender and same age in months ± 6 months, within the same survey year. Summary information on the final data set for these 9,010 participants in the 2001-02 NHANES survey is presented in Table 1.

Due to relatively small sample sizes of age group subsets of the NHANES surveys, the 2001-02 data were merged with the 2003-04 data to create a single data set that would ensure adequate sample size and statistical representativeness within each of the smaller subsamples represented by the different age groups being considered herein. Such merging of survey data across years is recommended by NHANES when subsample groups are small (NCHS 2005). Upon merging of the 2001-02 and 2003-04 NHANES data, a final data set comprising 17,267 survey participants was created. These combined data are also summarized in Table 1.

It should be noted that the data compiled from NHANES on filled tooth surfaces specifically omitted consideration of the presence of pit and fissure sealants. Although the 2001-02 and 2003-04 NHANES surveys collected information on the presence of pit and fissure sealants, these data were recorded separately and were not included or double counted within the data on the presence of restored tooth surfaces.

Table 1. Summary of NHANES data of 2001-02 and 2003-04.

Age Group	Age range (months)	Total Survey Sample Size (N)			Number of Participants with Restored Teeth			Average number of restored tooth surfaces (all participants) [1]			Average number of restored surfaces (amalgam bearers only) [1]			Maximum number of restored surfaces		
		2001-02	2003-04	2001-04	2001-02	2003-04	2001-04	2001-02	2003-04	2001-04	2001-02	2003-04	2001-04	2001-02	2003-04	2001-04
Toddlers	24-59	697	588	1285	54	40	94	0.9	1.2	1.1	12.2	18.2	14.8	72	60	72
Children	60-155	1653	1419	3072	634	547	1181	3	3.4	3.2	7.9	8.8	8.3	56	72	72
Adolescents	156-251	2096	1990	4086	1055	1004	2059	3.6	3.6	3.6	7.1	7.1	7.1	52	84	84
Adults	252-719	3043	2630	5673	2391	2063	4454	14.6	13.5	14.1	18.6	17.2	17.9	114	128	128
Seniors	≥ 720	1521	1630	3151	990	1041	2031	19.5	17.8	18.6	30	27.9	28.9	100	109	109
Total NHANES Population with dental data		9010	8257	17267												
Youngest age (in months) reported with at least one filled tooth		26	32	26												

1. Averages for NHANES survey participants only; not weighted (adjusted) to US population

4.2 NHANES Data and Statistical Weighting

When NHANES surveys are carried out, specific subsets of the US general population are targeted for over-sampling. This is done to ensure the collection of statistically valid and representative data on specific issues of interest that relate to those specific, over-sampled population subgroups. As a result, the distribution of data across all NHANES participants does not precisely match the distribution across the general population. To correct for this intentional sampling bias, NHANES creates variables for each survey participant that quantifies how many individuals within the general population that each NHANES participant represents. Therefore, the Hg exposure estimates derived herein for participants of the 2001-02 and 2003-04 NHANES surveys were multiplied by their respective weighting factor to accurately adjust distributions of exposure to mirror the actual US population. Since data from two consecutive NHANES surveys were combined for this analysis, spanning a total of 4 years, appropriate 4 year statistical weights, rather than 2 year statistical weights, were applied as recommended by NHANES (NCHS 2005).

4.3 Urine Hg Concentration as a Function of Amalgam Filling Load

There is an incremental increase in urine Hg concentration with each incremental increase in number of amalgam-filled tooth surfaces. Reported values for this incremental increase in urine Hg per amalgam-filled tooth surface are presented in Table 2. Specifically for urine Hg reported in µg Hg/g creatinine, these values range between 0.06 µg/g creatinine per amalgam-filled surface and 0.09 µg/g creatinine per amalgam-filled surface. Based on the compiled data, it appears that children receive greater doses per filled tooth surface than do adults. This is logical given that the dose of Hg delivered by any given filled surface will be diluted in a greater biomass, greater daily urinary output and greater daily creatinine output, in adults as compared to children. Suzuki et al. (1993) demonstrated that creatinine excretion reaches adult levels after approximately 18 years of age. For studies investigating children, the incremental urine Hg concentration per filled surface ranges between 0.08 and 0.09 µg Hg/g creatinine/filled surface, whereas in adults, the incremental increase ranges between 0.06 and 0.07 µg Hg/g creatinine/filled surface.

Based on the foregoing, persons 24 months to 216 months of age (2 yrs to 18 yrs) were assumed to have an incremental urine Hg concentration ranging between 0.08 and 0.09 µg Hg/g creatinine/filled surface, while persons aged >216 months were assumed to have an incremental urine Hg concentration ranging between 0.06 and 0.07 µg Hg/g creatinine/filled surface.

The data were insufficient to define differences on the basis of gender and, therefore, these assumptions were applied equally to males and females within the same age group.

4.4 Daily Creatinine Excretion

Creatinine is a waste product of muscle contraction and daily creatinine clearance is proportional to body mass (Welle *et al.*, 1996; Wang *et al.*, 1996). Twenty-four hour creatinine clearance ranges between 0.015 and 0.025 g/kg body weight in healthy individuals (Thomas, 1993). For the present study a uniform distribution between these two limits was assumed, and applied to all cases irrespective of age or gender.

Table 2. Summary of studies reporting incremental increase in urine Hg concentration as a function of dental amalgam load.

Authors	Year	Age group studied	Slope [1]	Comments
Studies reporting UHg as µg Hg/g creatinine, and quantifying amalgam load as numbers of filled surfaces				
Maserejian et al	2008	267 children 6-10 yrs of age at time of recruitment; gender division not reported	0.082 (± 0.022) (µg/g creatinine/surface)	Data transcribed from Figure 3A; UHg reported as µg Hg/g creatinine
Dunn et al	2007	534 children 6-10 yrs of age at time of recruitment; 54% female; 46% male	0.09 (± 0.01) (µg /g creatinine/surface)	UHg reported as ug/g creatinine
Levy et al	2004	34 children 4-8 yrs; 57% male; 43% female	0.08 (ug/g creatinine/surface)	Slope determined from data presented in Tables 2 & 3; UHg reported as ug Hg/g creatinine
Dye et al	2005	1626 adult females aged 16-49 yrs; results extrapolated to full US female population of same age	0.07 (± 0.004) (ug/g creatinine/surface)	UHg reported as ug Hg/g creatinine; standard error as per Dye (pers. com. 23-09-2010)
Factor-Litvak et al	2003	550 adults 30-49 yrs of age; 38% male; 62% female	0.07 (ug/g creatinine/surface)	Estimated from Figure 1A; standard error could not be determined; UHg reported as ug Hg/g creatinine
Kingman et al	1998	1127 adult males aged 40-79 yrs	0.059 (± 006) (ug/g creatinine/surface)	UHg as ug Hg/g creatinine; standard error of slope not reported for simple model, but apparent from other data reported as ± 10% of slope; also reported the incremental increase as 0.1 µg Hg/L urine.
Other similar studies				
Suzuki et al	1993	Children 3 – 14 yrs; 66% female; 34% male	0.119 (age 3-8 yrs) 0.116 (9-14 yrs) (ug/g creatinine/filled tooth)	Metric for amalgam load was numbers of filled teeth, rather than filled surfaces; standard errors not reported
Soleo et al	1998b	Adult workers; gender division unknown	0.08 (ug/L/surface)	Average reported only
Jokstad et al	1992	3-87 yrs; 64% female; 36% male	0.07 (± 0.02) (ug/L/surface)	Data transcribed from Figure 2B; UHg converted to ug Hg/L from nmol Hg/L
Langworth et al	1988; 1991	Adult industrial workers; precise ages not reported; assumed majority are male	0.085 (± 0.020) (ug/L/surface)	Data transcribed from Figure 1; UHg converted to ug Hg/L from nmol Hg/L. Same data presented in both publications

Table 2 Continued

Authors	Year	Age group studied	Slope [1]	Comments
Skare et al	1990	Dentists and dental nurses, and reference group	0.07 (ug Hg/24 hr/surface)	Standard errors of slopes not reported; same slope but different intercept for dentists & nurses versus referants
Skerfving	1991	80 adult subjects from Sweden	0.173 (ug/g creatinine/filled tooth)	Metric for amalgam load was numbers of filled teeth, rather than filled surfaces; data transcribed from Figure 4; standard error of slope not computed
Halbach et al	2008	54 adult subjects	0.03 (ug Hg/8 hrs/surface)	Data for 'groups A & B' prior to removal of amalgam fillings; UHg data reported as ug Hg excreted in urine over 8 hr sampling period

4.5 Proportion of Total Hg Excreted via Urine and Feces

Hg is excreted in both urine and feces. Chronic exposure to Hg^0, as from dental amalgam, results in a steady state where daily uptake and total daily excretion (urine + faeces) of Hg are in equilibrium (Weiner and Nylander 1995; Rothstein and Hayes, 1964). Knowing the proportion of excretion via urine thereby provides the basis for determining total excretion via urine + feces. In other words:

total Hg excretion = [urinary excretion] / [proportion of total excretion via urine]

At low doses, such as that equivalent to 1 or a few amalgam surfaces, urinary excretion of Hg following Hg^0 exposure represents only about 10% of total excreted Hg (Rothstein and Hayes, 1964), the remainder being excreted via feces. However, at exposure levels sufficient to produce the same urinary Hg concentrations associated with up to 128 amalgam-filled tooth surfaces (the reported maximum number of filled tooth surfaces in the US population; see Table 1), urinary excretion represents 40% of total daily excretion of Hg. This latter value can be determined from the data presented by Roels *et al.* (1987), assuming that adult working males inhale an average of 6.6 m^3 of air in an 8 hour shift (U.S. EPA, 1989a), and that 80% of inhaled Hg is absorbed. From the data of Roels *et al.* (1987), the proportion of total Hg excretion which occurred via the urine was 39.8 ± 12.5 %.

Based on the foregoing, it was assumed that daily Hg excretion by the urinary route ranged progressively from 10% for persons with 1 filled tooth surface, to 40% for persons with 128 filled surfaces.

4.6 The Proportion of Filled Tooth Surfaces That Are Restored With Dental Amalgam

Available data on the relative use of amalgam versus alternate dental restorative materials were reviewed from the US, Canada and the United Kingdom. These 3 countries have similar standards of living and all continue to maintain similar policies with respect to the promotion and use of dental amalgam as a dental material for the restoration of carious teeth. Data from

Sweden, Norway and Finland were also available, but were omitted from consideration due to the policies and bans against the use of amalgam in dentistry in those countries. Such bans and policies result in data that would not be relevant to the US situation. Similarly, data were available for Japan (Nakata, 1997). However, historical episodes of mass Hg contamination and poisoning in that country, such as at Minamata Bay, have resulted in a very low rate of amalgam use due to its known Hg content, despite the absence of any ban or policies against amalgam use in dentistry in Japan (Nakata, 1997).

Exposure to Hg^0 from dental materials only occurs from tooth surfaces restored with amalgam. Therefore, it is appropriate to discount the numbers of tooth surfaces restored with alternate dental materials such as composite resin, ceramics, gold alloys, etc. The NHANES surveys do not record the composition of the dental restorative materials used to fill tooth surfaces of survey participants.

The majority of filled surfaces are restored with amalgam. Amalgam is still the preferred dental restorative material of the US dental profession, and is still recommended by the American Dental Association. The preponderance of filled surfaces being restored with amalgam can also be deduced from the study of Dye et al (2005) who detected a significant association between urinary Hg concentration and the numbers of filled tooth surfaces reported for women aged 16 to 49 years examined during the 1999-2000 NHANES survey. Dye et al (2005) found a significant relationship indicating that urine Hg concentration increased by an average of 0.06 µg Hg/filled surface, consistent with numerous other similar studies (see Table 2). Dye et al (2005) detected this significant relationship despite only assuming that the filled surfaces were all restored with dental amalgam, thus confirming that the vast majority of extant filled teeth in the US general population have been restored with dental amalgam.

One assumption that Dye et al (2005) made, in addition to assuming that all filled surfaces were of dental amalgam, was that all five-surface fillings constituted crowns composed of ceramics, various metal alloys or any material other than amalgam. As a result, all 5-surface fillings were omitted from their analysis.

Kingman et al (1998) reported data on the relative abundance of amalgam versus alternate dental restorative materials in a specific study cohort of Americans. In that study of 1,127 male retired military service personnel, an average of 35.8 filled surfaces were reported, with an average of 19.9 surfaces filled with dental amalgam, for an average of 55.6% of filled surfaces containing amalgam. Unfortunately, the all-male, all adult study group of Kingman et al (1998) is not representative of the general US population, nor is the fact that this study group received much of its dental care via military dental services.

Rosenstiel et al. (2004) reported on a survey of US dentists' choices of dental materials for their own personal dental treatments. Surveyed dentists reported an average of 37% of existing teeth were restored with amalgam versus 41% restored with alternate materials and 22% of existing teeth being un-restored. Therefore, amalgam represented 47.4% of existing fillings. They also reported that surveyed dentists opted for amalgam to treat their own teeth in <17% of restorations placed in the year preceding the survey. For this latter statistic, use of amalgam was combined with use of gold such that the proportion of fillings placed specifically with amalgam could not be determined.

Adegbembo and Watson (2005) reported that dentists in the province of Ontario Canada, removed an estimated total of 2,855,178 amalgam restorations but replaced these with amalgam in only an estimated 1,163,665 cases; this represents the use of amalgam in 40.7% of situations where a previous amalgam filling was removed. It is likely that a similar pattern exists with the placement of new fillings into previously un-restored teeth, given that dentists' selection of restorative material will likely be similar whether placing new or replacing previously existing restorations.

Other sources of data on the relative proportion of filled teeth restored with amalgam versus alternate dental materials relate to the population in general and not to individuals. These include the following:

- Beazoglou et al (2007), considering use of amalgam and alternates in the US, through evaluation of dental insurance claims:

- Children aged 0 – 9 years: 59.5% of fillings placed are of amalgam;
- Children & teens aged 10-19 years: 45.1% of fillings placed are of amalgam;
- Adults aged ≥ 20 years: 36.8% of fillings placed are of amalgam.

- Haj-Ali et al. (2005), in a survey of US general practice dentists:
  - 32% reported being amalgam-free, but still placing amalgam in posterior teeth in 3% of cases;
  - 68% reported using amalgam, but only placing amalgam in posterior teeth in 39% of cases.
- Burke et al (2003) report on amalgam use by dentists in the United Kingdom:
  - 50% of UK dentists reported decreased use of amalgam over the 5 years preceding the survey, with 2% reporting not using amalgam at all;
  - Increased use of glass ionomer, resin modified glass ionomer, and composite materials were reported by 41%, 47% and 62% of UK dentists, respectively.

Although these latter studies provide information on the proportional use of different dental materials by dentists, they provide no information on the proportional use of different materials in individual dental patients, the latter being required for extrapolation to individual members of the US general population. There are individuals within the population with 100% of their filled teeth containing amalgam, and others with 100% of their filled teeth containing alternate materials.

4.7 Exposure Scenarios Evaluated within this Report

Based on the information reviewed above, it was decided to approach the assessment of exposure to Hg^0 from amalgam in 4 different ways:

1) It was assumed that all filled tooth surfaces were filled with dental amalgam.
2) Consistent with the assumption of Dye et al (2005), all 5-surface fillings were assumed to be composed of materials other than amalgam, and were thereby omitted from analysis. All remaining filled surfaces (1 surface filling to 4 surface fillings) were assumed to be composed of amalgam. It is likely that a significant proportion of 5-

surface fillings are crowns composted of ceramics, metal alloys other than amalgam, or of other materials, so we simply assumed that all 5-surface fillings constituted non-amalgam crowns.

3) In addition to the assumption for scenario 2, it was further assumed that, in each NHANES survey participant with restored tooth surfaces, only 50% of those filled tooth surfaces were composed of dental amalgam. This assumption was based on the reports of Kingman et al. (1998) and Rosenstiel et al. (2004) in which amalgam comprised approximately 50% of in-place restorations. In all cases where the total number of restored surfaces was an odd number ≥ 3, the assumed number of amalgam surfaces was rounded down to the nearest whole number (3÷2 was set to 1, for example). However, for persons with only 1 filled surface, these individuals were assigned a random number between 1 and 100, and all those assigned a random number between 1 and 50 were ascribed a number of amalgam filled surfaces of 0, and those with a random number between 51 and 100 were ascribed a number of amalgam filled surfaces of 1.
4) Finally, it was further assumed, in addition to the assumptions outlined for scenarios 2 and 3 above, that 30% of persons with restored tooth surfaces had all of those surfaces restored with a dental material other than amalgam. This assumption was made recognizing that approximately 30% of dentists in the US (Haj-Ali et al., 2005) reported being amalgam-free, and the possibility that all of their patients might have all existing fillings placed/replaced with materials other than amalgam. This assumes that dental patients are distributed equally across all dentists in the US.

4.8 Determining the Number of Amalgam Filled Surfaces that Will Not Exceed Reference Exposure Levels

Whereas dose can be derived employing Equation 4, above, this equation can be reversed to derive the number of filled tooth surfaces that will not exceed a known, 'safe' reference exposure level. This equation can be written as:

N_{Safe} = (REL-equivalent Hg dose) * P / [B * Cr] (Equation 5)

where,

N_{Safe} = the number of amalgam-filled surfaces that will not exceed, on average, the dose delivered by any given reference exposure level

REL-equivalent Hg dose = the absorbed dose of Hg associated with any given reference exposure level (µg/kg-day)

P = proportion of total Hg excretion via urine (unitless)

B = increase in UHg per amalgam-filled surface ([µg Hg/g creatinine]/N)

Cr = creatinine excreted per kg body weight in 24 hours (g creatinine/kg-day)

The reference exposure levels considered in this report, and their respective equivalent doses, are summarized in Table 3. Values for P, B and Cr were set at liberal (non-conservative) values. P was set at 0.15 (15%) for the proportion of Hg excreted via urine, consistent with expectations for low numbers of filled surfaces. B was set at the lower limit of the range applied to the various age groups (for toddlers, children and young adolescents B = 0.08 µg Hg/g creatinine/surface; for older adolescents, adults and seniors, B = 0.06 µg Hg/g creatinine/surface). Likewise, the variable Cr was set at the lowest value of the range (0.015 g creatinine/kg body weight/day) applied to dose calculations.

Table 3. Published reference exposure levels (REL) for Hg^0 and their equivalent doses.

Agency or Author	Year of publication	Terminology	REL (µg Hg^0/m^3)	REL-equivalent absorbed dose (µg Hg/kg-day) [1]
California EPA	2008	Chronic reference air concentration (RfC)	0.03	0.005
Richardson et al	2009	Chronic reference exposure level (REL)	0.06	0.01
Lettmeier et al	2010	Chronic reference air concentration (RfC)	0.07	0.011
US ATSDR	1999	Chronic minimal risk level (MRL)	0.2	0.032
US EPA	1995	Chronic reference air concentration (RfC)	0.3	0.048

1. Calculated as: REL (µg Hg^0/m^3)* 15.85 m^3/day * 80% Hg^0 absorbed ÷ 80 kg adult body weight. Body weight and inhalation rate from US EPA (2009); Hg^0 absorption rate after WHO (1991). Derived for adults because toxicological data underlying all RELs was drawn from studies on adults.

5 EXPOSURE ASSESSMENT RESULTS

5.1 Exposure to Hg from Dental Amalgam Fillings in the US Population

Based on the data collected during the 2001-2004 NHANES surveys, a total of 181.1 million Americans possess a grand total of 3.68 billion restored tooth surfaces, which equates to 1.46 billion restored teeth. Estimated Hg exposures for the general US population, resulting from the presence of amalgam fillings in their teeth, are summarized in Table 4. Estimates are presented both as weight-standardized doses (µg Hg/kg-day) and as µg Hg/day. Exposure estimates on a µg Hg/day per filled tooth basis are consistent with those reported by Health Canada in 1995 (see also Richardson and Allan 1996).

Considering the least conservative scenario (Scenario 4; predicts the lowest levels of exposure for any of the scenarios), whereby only those fillings covering 1 to 4 tooth surfaces, with 30% of citizens having all fillings composed of a dental restorative material other than amalgam, and only 50% of the restored surfaces in the remaining citizens being composed of amalgam, average exposures range from 0.04 µg Hg/kg-day for children and adolescents, to 0.07 µg Hg/kg-day for seniors. The differences in average doses by age group reflect differences in numbers of filled tooth surfaces (as determined from NHANES data), greater creatinine excretion in older versus younger age groups (see Table 2), and differences in body weights (as determined from NHANES data).

Comparison of amalgam dose levels to published reference exposure levels (typically considered as 'safe') for Hg^0, for non-occupational exposures, is presented in Table 5. Again, for the least conservative of the scenarios evaluated (Scenario 4), some 67.2 million Americans with amalgam fillings were predicted to exceed the dose associated with the US EPA's REL of 0.3 µg/m^3. With respect to the California EPA's recently published REL of 0.03 µg/m^3, a total of 122.3 million Americans are predicted to exceed that reference level.

5.2 Numbers of Filled Tooth Surfaces that Will Not Exceed Reference Exposure Levels

Based on the assumptions outlined in Section 4 (Methods), we were able to determine the maximum number of amalgam surfaces that could be possessed by each age group such that the Hg exposure would not, on average, exceed the dose associated with a reference exposure level for Hg^0. These results are presented in Table 6. Results have been calculated for each of the published RELs discussed herein (CalEPA 2008, Richardson et al. 2009, Lettmeier et al. 2010, US ATSDR 1999, and US EPA 1995). With respect to the US EPA's reference air concentration (RfC) of 0.3 $\mu g/m^3$, toddlers, children and young teens (<18 years) could possess up to 6 amalgam-filled tooth surfaces, and older teens, adults and seniors could possess up to 8 amalgam-filled surfaces. With an average of approximately 2 filled surfaces per filled tooth (determined from NHANES data), this equates to 3 filled teeth and 4 filled teeth, respectively.

Application of other RELs result in lower maximum numbers of filled tooth surfaces, with the lowest REL, that from CalEPA (2008), limiting the number of amalgam-filled surfaces to less than 1 for all age groups.

5.3 Discussion of Exposure Results

The number of Americans predicted to exceed the US EPA RfC-associated dose is considerably greater than the approximately 30 million estimate provided in a petition submitted to the FDA (see Moms Against Mercury et al, 2009) in response to the publication by FDA of the Final Rule on dental amalgam. The results presented herein are considered to represent a significant improvement in accuracy, compared to the dated basis of the Petitioners' estimates, due to the following factors:

- significantly greater supporting data, including 6 key studies, encompassing a total of 3871 subjects (studies involving related cohorts not double-counted), linking urine Hg concentration to numbers of amalgam surfaces (see Table 2);
- the ability to quantitatively differentiate adults from children with respect to the association of urinary Hg concentration to amalgam load (number of surfaces);
- recent data on the numbers of restored tooth surfaces specifically in the post-2000 American population;

- the ability to accurately extrapolate to the entire general population by application of population statistical weighting factors provided by NCHS with the NHANES data sets.

The Petitioners' estimate of approximately 30 million Americans exceeding the dose associated with US EPA's RfC was extrapolated from data and information presented in the report prepared by Health Canada (1995). That report employed Canadian dental health data from 1970-72. Access to dental health care has improved significantly in North America over the past 40 years, resulting in a greater number of individuals in the population with filled teeth now compared to 40 years ago.

The Health Canada report also estimated Hg dose from amalgam largely on the basis of a single study of 80 Swedish subjects reported by Skerfving (1991) that linked urine Hg concentration to the number of amalgam-filled teeth. The present report benefited from multiple studies of a total exceeding 3800 subjects, thereby greatly increasing accuracy and reliability of exposure estimates.

Despite using Canadian population data on the numbers of filled teeth across the population, collected as part of a Canadian national health survey conducted between 1970 and 1972, the Health Canada (1995) report failed to apply appropriate population weighting factors in that analysis, despite those weighting factors being available. For the present study, our ability to apply the weighting factors specifically formulated by the NCHS for the NHANES survey data greatly increases the accuracy and reliability of extrapolation of the results of our analysis to the entire US general population.

Table 4. Summary of Hg doses estimated for the US population with amalgam fillings

		Number with fillings		Number of filled surfaces			Dose as ug Hg/kg-day			Dose as ug Hg/day			Hg concentration (ug Hg/g creatinine) [4]	
		NHANES 2001-04	US population [2]	Mean [3]	Min	Max	Mean [3]	Min	Max	Mean [3]	Min	Max	Min	Max
Scenario 1 [1]	Toddlers	94	740,404	14.6	1	72	0.15	0.02	0.54	2.53	0.18	9.87	0.58	6.76
	Children	1181	12,806,364	9	1	72	0.11	0.01	0.45	3.72	0.27	22.9	0.58	6.48
	Adolescents	2059	17,671,696	7.1	1	84	0.09	0.01	0.37	5.79	0.49	33.53	0.56	6.13
	Adults	4454	120,199,880	20.2	1	128	0.16	0.01	0.49	12.98	0.44	58.79	0.56	8.82
	Seniors	2031	29,711,241	32.9	1	109	0.22	0.01	0.5	16.87	0.46	55.39	0.57	5.81
Scenario 2 [1]	Toddlers	87	667,166	7.8	1	36	0.1	0.01	0.37	1.63	0.18	6.51	0.58	3.38
	Children	1109	11,987,269	5.4	1	32	0.08	0.01	0.31	2.71	0.23	22.9	0.58	3.15
	Adolescents	2038	17,561,152	6.7	1	47	0.08	0.01	0.37	5.53	0.49	32.17	0.56	4.15
	Adults	4402	120,298,407	13.2	1	72	0.12	0.01	0.39	10.11	0.44	45.6	0.56	4.82
	Seniors	1972	28,902,381	14.9	1	67	0.13	0.01	0.39	10.43	0.46	39.55	0.56	4.62
Scenario 3 [1]	Toddlers	84	625,582	4	1	18	0.05	0.01	0.19	0.83	0.18	3.25	0.58	1.94
	Children	1025	11,064,670	2.8	1	16	0.04	0.01	0.15	1.37	0.23	11.45	0.58	1.83
	Adolescents	1898	16,362,871	3.4	1	23	0.04	0.01	0.18	2.8	0.44	15.22	0.56	2.33
	Adults	4315	118,460,911	6.5	1	36	0.06	0.01	0.19	4.94	0.43	22.31	0.56	2.66
	Seniors	1940	28,583,321	7.3	1	33	0.07	0.01	0.19	5.11	0.39	19.77	0.56	2.78
Scenario 4 [1]	Toddlers	57	379,004	4.4	1	16	0.06	0.01	0.19	0.95	0.2	3.25	0.58	1.89
	Children	714	7,714,637	2.7	1	16	0.04	0.01	0.15	1.37	0.24	8.22	0.58	1.83
	Adolescents	1341	11,289,979	3.3	1	23	0.04	0.01	0.18	2.77	0.44	15.14	0.56	2.33
	Adults	3003	82,524,655	6.6	1	31	0.06	0.01	0.19	5.05	0.43	22.31	0.56	2.54
	Seniors	1387	20,403,213	7.3	1	33	0.07	0.01	0.19	5.11	0.39	19.77	0.56	2.78

Footnotes to Table 4:

1. Scenario 1: all restored tooth surfaces, including 5 surface fillings, assumed to be amalgam; Scenario 2: 5-surface fillings omitted and all of remaining fillings (1-surface to 4-surface) assumed to be amalgam; Scenario 3: 5-surface fillings omitted and 50% of remaining restored tooth surfaces assumed to be amalgam; Scenario 4: 5-surface fillings omitted, 30% of remaining persons assumed to have no amalgam, and 50% of remaining restored tooth surfaces assumed to be amalgam.
2. Determined from the statistical weighting provided by NHANES.
3. Derived as the weighted US population mean, not the mean of NHANES participants.
4. Urine Hg concentration derived as: Background urine Hg concentration + (number of amalgam surfaces X incremental increase in urine Hg concentration per amalgam surface) (see Methods). Background urine Hg concentration set equal to 0.5 ug Hg/g creatinine, consistent with Dye et al (2005)

Table 5. Proportion and numbers of US citizens with amalgam fillings that exceed published reference exposure levels for Hg^0

		TODDLERS	CHILDREN	TEENS	ADULTS	SENIORS	Total population N > REL
Scenario 1	Total population with fillings	740,404	12,806,364	17,671,696	120,199,880	29,711,241	181,129,584
All filled surfaces assumed to be amalgam	% > CalEPA REL [1]	100	100	100	100	100	181,129,584
	% > Richardson et al REL [2]	100	100	99.4	99.5	99.7	180,400,644
	% > Lettmeier et al REL [3]	100	100	99.0	99.0	99.5	179,613,884
	% > US ATSDR REL [4]	84.3	81.5	74.3	92.0	95.4	163,078,979
	% > US EPA REL [5]	74.6	68.8	62.5	87.1	92.3	152,539,776
Scenario 2	Total population with fillings	667,166	11,987,269	17,561,152	120,298,407	28,902,381	179,416,376
Same as Scenario 1, but 5 surface fillings excluded	% > CalEPA REL	100	100	100	100	100	179,416,376
	% > Richardson et al REL	100	100	99.3	99.5	99.4	178,500,660
	% > Lettmeier et al REL	100	100	98.9	98.9	98.9	177,551,107
	% > US ATSDR REL	72.2	77.4	73.0	90.1	91.4	157,330,552
	% > US EPA REL	60.7	61.6	60.3	84.1	84.8	144,115,315
Scenario 3	Total population with fillings	625,582	11,064,670	16,362,871	118,460,911	28,583,321	175,097,356
Same as Scenario 2, but only 50% of filled surfaces assumed to be amalgam	% > CalEPA REL	100	100	100	100	100	175,097,356
	% > Richardson et al REL	100	100	97.6	97.7	97.7	171,253,842
	% > Lettmeier et al REL	100	99.8	95.7	95.6	96.0	168,068,173
	% > US ATSDR REL	48.6	50.0	50.5	74.7	77.6	124,708,512
	% > US EPA REL	37.1	29.6	31.7	58.0	62.1	95,120,044
Scenario 4	Total population with fillings	379,004	7,714,637	11,289,979	82,524,655	20,403,213	122,311,488
Same as Scenario 3, but 30% with fillings assumed to have no amalgam	% > CalEPA REL	100	100	100	100	100	122,311,488
	% > Richardson et al REL	100	100	97.2	98.0	97.9	119,908,745
	% > Lettmeier et al REL	100	99.7	95.3	96.2	96.0	117,784,675
	% > US ATSDR REL	60.0	48.8	49.4	75.7	77.3	87,852,641
	% > US EPA REL	45.2	29.2	30.8	59.0	61.9	67,220,662

Footnotes to Table 5:

1. CalEPA REL = 0.03 µg Hg^0/m^3; REL-equivalent dose = 0.005 µg/kg-day, calculated as: 0.03 µg Hg^0/m^3 * 15.85 m^3/day * 80% Hg^0 absorbed ÷ 80 kg adult body weight. Body weight and inhalation rate from US EPA (2009).
2. Richardson et al (2009) REL = 0.06 µg Hg^0/m^3; REL-equivalent dose = 0.010 µg/kg-day, calculated as: 0.06 µg Hg^0/m^3 * 15.85 m^3/day * 80% Hg^0 absorbed ÷ 80 kg adult body weight. Body weight and inhalation rate from US EPA (2009).
3. Lettmeier et al (2010) REL = 0.07 µg Hg^0/m^3; REL-equivalent dose = 0.011 µg/kg-day, calculated as: 0.07 µg Hg^0/m^3 * 15.85 m^3/day * 80% Hg^0 absorbed ÷ 80 kg adult body weight. Body weight and inhalation rate from US EPA (2009).
4. US ATSDR (1999) REL = 0.2 µg Hg^0/m^3; REL-equivalent dose = 0.032 µg/kg-day, calculated as: 0.2 µg Hg^0/m^3 * 15.85 m^3/day * 80% Hg^0 absorbed ÷ 80 kg adult body weight. Body weight and inhalation rate from US EPA (2009).
5. US EPA (1995) REL = 0.3 µg Hg^0/m^3; REL-equivalent dose = 0.011 µg/kg-day, calculated as: 0.07 µg Hg^0/m^3 * 15.85 m^3/day * 80% Hg^0 absorbed ÷ 80 kg adult body weight. Body weight and inhalation rate from US EPA (2009).

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Table 6. Numbers of amalgam-filled surfaces that will not exceed doses associated with published reference exposure levels (REL) for Hg^0.

Age group [1]	REL source	REL ($\mu g\ Hg/m^3$)	REL-associated dose (ug/kg-d) [2]	No. of surfaces not exceeding REL dose [3]
Toddlers, children & young teens	California EPA (2008)	0.03	0.005	0.6
	Richardson et al (2009)	0.06	0.01	1.3
	Lettmeier et al (2010)	0.07	0.011	1.4
	US ATSDR (1999)	0.2	0.032	4
	US EPA (1995)	0.3	0.048	6
Older teens, adults & seniors	California EPA (2008)		0.005	0.8
	Richardson et al (2009)		0.01	1.7
	Lettmeier et al (2010)		0.011	1.8
	US ATSDR (1999)		0.032	5.3
	US EPA (1995)		0.048	8

3. Age groups combined as members of each have the same urinary Hg content per filled surface.
4. REL-associated doses derived as per footnote to Table 7.
5. Calculations employed non-conservative assumptions; alternate possible values would predict fewer numbers of fillings. Assumptions as follows:
    a. toddlers, children and young teens – 0.08 µg Hg/g creatinine/filled surface;
    b. older teens, adults & seniors – 0.06 µg Hg/g creatinine/filed surface;
    c. creatinine excretion per day set to 0.015 g/kg-day for all age groups;
    d. proportion of Hg excretion via urine set at 15% for all age groups.

The exposure estimates presented herein are considered accurate, reliable and scientifically defensible. To further evaluate this, we conducted the following evaluation:

For an adult, each amalgam-filled surface results in an increase of Hg in urine of 0.1 µg Hg/L or 0.06 to 0.07 µg Hg/ g creatinine (see studies summarized in Table 2). Therefore, a hypothetical average adult with 100 amalgam-filled tooth surfaces would have a predicted incremental increase of Hg in urine of 10 µg/L, or 6 to 7 µg/g creatinine. 10 µg Hg/L urine or 6 to 7 µg Hg/g creatinine in urine falls well within the range observed for the general US population. Urinary Hg concentrations measured as part of NHANES (2003-04) ranged up to 75.75 µg/L and 36.1 µg Hg/g creatinine (N=2538).

Following the methodology outlined herein, the dose of Hg resulting from 100 filled surfaces in an adult is approximately 0.4 µg/kg-day (range: 0.27 to 0.52 µg/kg-day).

In workers exposed to Hg^0 in room air, a urine Hg concentration of 10 µg/L results from exposure to an average room air Hg concentration of about 4 µg Hg/m^3 (conversion after Tsuji et al 2003). Considering that workers will inhale an average of 6.6 m^3 of air over 8 hours (US EPA, 1989a), will absorb an average of 80% of the inhaled Hg^0 (WHO, 1991) and weigh an average of approximately 70 kg (determined from papers reviewed by Tsuji et al 2003), the resulting dose is about 0.3 µg/kg-day.

Our hypothetical estimated Hg exposure associated with 100 amalgam-filled tooth surfaces (and a urine concentration of 10 µg Hg/L) agrees well with the dose to workers exposed to a level of Hg^0 that results in the same urinary Hg concentration.

Therefore, our estimates of exposure from amalgam fillings are accurate, valid and scientifically defensible.

6 TOXICITY OF MERCURY VAPOUR

The most recent regulatory review of the toxicology of Hg^0, to approximately mid 2007, is offered by Richardson et al. (2009), based on a more detailed report prepared by Health Canada (HC 2008a). Therefore, a further detailed evaluation of these studies is not included herein.

6.1 Recent Reference Exposure Levels for Protection of Public Health

From that Health Canada review, the most appropriate study identified upon which to base a reference exposure level for Hg^0 was the study of dentists in Singapore, conducted by Ngim et al. (1992). The selection of this key study was based, in large part, on the fact that concomitant exposure to chlorine gas would not have occurred in this cohort, interfering with Hg^0 absorption, kinetics and toxicity, as discussed in Section 3.3. Other studies involving workers in the chloralkali industry, such as Fawer et al. (1983), will be confounded by this concomitant Cl2 exposure. The Ngim et al (1992) study provided a LOAEL of 6 µg Hg/m^3 (adjusted to continuous exposure) and a resulting REL of 0.06 $µg/m^3$ after application of a UF of 100 (10 for use of a LAOEL X 10 for all of gender differences in pharmacokinetics and toxicity, potential genetic predisposition to toxicity and potential fetal sensitivity to CNS effects).

In 2010, Lettmeier et al (2010; authorship erroneously indicated as Beate et al. 2010 due to journal confusion of first and last name of lead author) published valuable new information on the toxicology of Hg^0. They reported on a study of small scale gold miners from Zimbabwe and Tanzania that provided the first occupational study of Hg^0 exposure that encompassed relatively low exposure (to < 0.2 µg Hg/g creatinine; although maximum in cohort was 547 µg/g creatinine).

This paper offers the opportunity for advancement in the assessment of the toxicity from exposure to Hg^0, and one basis for a revised reference air concentration (RfC) that does not rely on data collected decades ago. The data of Lettmeier et al (2010) present three significant advantages for the setting of a RfC (Richardson and Brecher, in press):

- The toxicological data relate to what are clinical signs and symptoms rather than sub-clinical measures of neurotoxicity, the latter often the cause for debate regarding significance for human health risk assessment;

- The mercury vapour exposures can be safely assumed to have been free of concomitant exposure to Cl_2, so that confounding is avoided with respect to the chemical form of Hg, the absorption and toxic effects of mercury vapour.

- A dose-response analysis was reported (but not presented) in which 'cut-off' exposure values or points of departure from the dose-response relationship were determined, rather than relying on simple group average exposure levels for definition of the LOAEL or NOAEL.

From their dose-response analysis, Lettmeier et al (2010) defined "cut-off" or point of departure exposure levels that defined a lowest-observed-adverse-effect-level (LOAEL) for ataxia of gait (4.7 µg Hg/g creatinine in urine) and for "sadness" (3.6 µg Hg/g creatinine in urine). These values were combined and converted to an equivalent airborne Hg^0 LOAEL concentration of 3.5 µg Hg^0/m^3 air. From this, the authors proposed addition of uncertainty factors ranging up to 50 (although UFs were not based on a *de novo* re-evaluation of the up-to-date toxicological database (Richardson and Brecher in press)), to arrive at possible REL value of 0.07 µg Hg^0/m^3.

6.2 Other Reference Exposure Levels

Prior to the publication by Richardson et al. (2009) and Lettmeier et al (2010), four national and international regulatory reference exposure levels (RELs) for protection of public (non-occupational) health had been defined for Hg^0:

- USEPA (1995): 0.3 µg/m^3 (reference air concentration (RfC);
- USATSDR (1999): 0.2 µg/m^3 (minimal risk level (MRL));
- California EPA (CalEPA 2008): 0.03 µg/m^3 (REL); and
- WHO (2000): 1 µg/m^3 (air quality guideline as annual average concentration).

It is apparent that RELs established by the USEPA and USATSDR are no longer valid. They rely on occupational studies of chloralkali workers whose exposure and effects would have been reduced by concomitant chlorine gas (Cl_2) exposure. The EPA also acknowledges in their IRIS listing for mercury, elemental, the outdated nature of their REL, indicating that significant new literature was identified during a 2002 contracted review of Hg^0 toxicological literature published since 1995. However, no revisions to the REL have been introduced since its formal publication in 1995.

The California EPA (CalEPA, 2008) employed the same chloralkali studies as the USEPA and USATSDR. However, recognizing the significant new literature on the toxicology of Hg^0, and in particular the paucity of data on the fetal effects of Hg^0, the CalEPA promulgated a REL for Hg^0 of 0.03 µg/m^3. Specifically, CalEPA recognized the need for an additional precaution to protect the "greater susceptibility of children and their developing nervous systems". CalEPA added an additional factor of 10 for a total uncertainty factor of 300 (compared to the USEPA's uncertainty factor of 30 (USEPA 1995)).

The RELs of USPEA, USATSDR, CalEPA and WHO were all based on the study of Fawer et al (1983) and similar studies (including: Piikivi and Tolonen, 1989; Piikivi and Hanninen, 1989; Piikivi, 1989 and Liang et al., 1993) of occupational Hg^0 exposure. The key studies identified were all predominantly focussed on neurological effects observed in workers in chloralkali plants. However, Richardson et al. (2009) pointed out that the concomitant exposure to chlorine gas (Cl_2) that occurs in chloralkali plants reduces airborne Hg^0 concentrations, reduces Hg respiratory absorption, reduces deposition of Hg^0 to the brain, and reduces the resulting toxicity of Hg^0 exposure.

Hg^0 converts to $Hg^{2+}Cl_2^{-1}$ in the presence of Cl_2 at room. Also, the inhalation absorption of $HgCl_2$ is only half or less of that of Hg^0. Hg deposition to the brain is also altered. Hg^{2+} (associated with $HgCl_2$) does not effectively cross the blood–brain barrier as does Hg^0. Following Hg^0 exposure, the red blood cell (RBC) to plasma Hg concentration ratio typically ranges between 1:1 and 2:1. However, much less Hg is associated with RBCs in the blood of chloralkali workers (with Cl_2 present). RBC to plasma Hg concentration ratios in chloralkali workers were only 0.02:1 whereas workers of the two other industries (with no concomitant exposure to Cl_2), had RBC to plasma Hg concentration ratios between 1.5:1 and 2:1. Rodents exposed to Hg^0 alone or in the presence of Cl_2, demonstrated reduced Hg absorption in the presence of Cl_2 and the deposition of Hg to the brain of rodents exposed concomitantly to Hg^0 and Cl_2 was only 1/5th of that when exposure was to Hg^0 alone.

With this confounding by Cl_2 in mind, and based on a review of other studies available to mid 2007, Richardson et al. (2009; and Health Canada 2008a) identified the study of Ngim et al. (1992) as the best available study of occupational exposure (of dentists is Singapore) that excluded concomitant exposure to Cl_2. From this study, a reference exposure level of 0.06 µg/m^3 was derived for public health protection and risk assessment of non-occupational Hg^0

exposures in the general population. This REL is now employed by Health Canada for environmental risk assessment of Hg^0 exposures.

6.3 The Children's Amalgam Trials

Recent studies of children receiving dental amalgams have suggested the absence of differences in neurotoxicity between groups of children receiving amalgam fillings as compared to children who received resin composites. The three major dental amalgam studies that were identified were:

- New England Dental Amalgam Study
- Portugal Dental Amalgam Study
- China Dental Amalgam Study

The major results of these studies, as reported by the authors, are summarized in Table 7. The discussion that follows relates only to the Casa Pia and New England CATs only. The Chinese study was a retrospective study with a less desirable study design that the others. Also, that study reported a median of only 2 amalgam surfaces per child, and a median duration of exposure (amalgam placement) of only 31 months.

The New England CAT the Casa Pia, Portugal CAT were omitted from the 2008 Health Canada evaluation, primarily due to the sub-chronic duration of exposure apparent from publications available at the time that Health Canada was compiling relevant publications for determination of its chronic REL. One of these, the Casa Pia CAT, has since reported results for up to 7 years post-recruitment (Lauterbach et al., 2008) or for 8 years (Townes et al 2008), depending on the outcome being investigated.

6.4 Weaknesses presented by the CAT studies

These are the first studies of their kind that focus on the lower end of the population spectrum in terms of Hg^0 exposure or dose, and focus very specifically on dental amalgam. That exposure has been expressed and reported as both the urinary Hg concentrations that were recorded, and the numbers of amalgam-filled tooth surfaces placed into study participants. However, the types and incidence of health effects have only been reported as a function of the presence/absence of amalgam fillings, reporting relative incidence between the amalgam and composite resin groups. No dose-response analysis has yet been reported that employs the

data on direct measurement of Hg in urine as the exposure metric. These studies have other weaknesses that are discussed below.

Recipients of amalgam fillings in the New England CAT received an average of approximately 4 amalgam-filled surfaces (mean number at year 5 follow up; Bellinger et al. 2006), which is less than the general population (see Table 8). Amalgam recipients in the Casa Pia CAT received an average of approximately 11 amalgam filled surfaces (mean number at year 7; Lauterbach et al. 2008), which is, in fact, greater than the average for the US population (see Table 8). However, neither the maximum number of filled surfaces nor the distribution of filled surfaces across the amalgam cohort of the Casa Pia study have been published for further comparison to the US population. A further comparison of the New England CAT and the US population is presented in Table 8, below.

The US EPA considers the minimum study duration to be 7 years for consideration as a chronic study (US EPA 1989b). The New England trial has been reported for a total of only 5 years and, therefore, cannot be considered to represent chronic exposure. As a result, it is not reliable for evaluation of potential chronic risks to the amalgam-bearing population. The Casa Pia Trial did report on follow ups for a total of 7 or 8 years post-recruitment (depending on outcome studied). However, given the known cumulative nature of Hg in the body, and particularly the brain (Mutter et al. 2007), it would not seem reasonable to accept a mere 7 or 8 years of exposure to Hg^0 as representative of chronic exposure applicable to the average 80 year lifespan currently realized in the US population. As noted for methyl Hg (Rice 2004), there is evidence from both human and experimental studies that developmental and adult exposure to moderate levels of methyl Hg cause delayed neurotoxicity that only appears years or decades after the cessation of exposure, often associated with aging. Since Hg^0 and methyl Hg affect the same critical organ (brain and CNS) and act by similar mechanisms (see Report Part 2), and given that delayed and/or persistent toxicity has also been observed in workers exposed to Hg^0 long after exposure ceased (Kishi et al. 1993), it cannot be simply assumed that cumulative and delayed effects due to Hg^0 exposure do not exist.

Table 7 Summary of the Major Children's Dental Amalgam Studies

Study	Group	Potential Effects Studied	Exposure Measure	Critical Effects
New England Dental Amalgam Study as Reported by Bellinger et al. (2006; 2007; 2008)	Children between the ages of 6 and 10 years were followed for 5 years; 267 receiving mercury amalgams and 267 receiving resin composites. Average amalgam load was 4.0 amalgam surfaces per child, which diminished as the study progressed due to loss of filled deciduous teeth.	Neuropsychological outcome (as 5-year change in full-scale IQ scores); memory and visuomotor ability; and renal glomerular function.	At year 5, treatment group had mean urinary concentrations of mercury that were approximately 0.3 µg/g creatinine than the control group (i.e., mean = 0.9, SD = 0.8, range = 0.1 to 5.7 versus mean = 0.6, SD = 0.5, range = 0.1 to 2.9).	Although elevated urinary concentrations of mercury were found in the treatment group, there were no differences in neurological or other effects observed in treatment group vs control group.
Portugal Dental Amalgam Study as Reported by DeRouen et al. (2006), Lauterbach et al. (2008)	Children between the ages of 8 and 12 years were followed for 7 years; 253 receiving mercury amalgams and 254 receiving resin composites. Average amalgam load was 10.7 amalgam surfaces per child, which diminished as the study progressed due to loss of filled deciduous teeth.	Neuropsychological outcome (as 7-year change in full-scale IQ scores); memory and visuomotor ability; and renal glomerular function.	Throughout the study, treatment group had mean urinary concentrations of mercury that ranged from 1 to 1.5 µg/g creatinine greater than the control group.	Although elevated urinary concentrations of mercury were found in the treatment group, there were no differences in neurological or other effects observed in treatment group vs control group.
China Dental Amalgam Study as Reported by Ye et al. (2009)	Children between the ages of 7 and 11 years were evaluated; 198 receiving mercury amalgams and 205 receiving resin composites. Median amalgam load was 2 amalgam surfaces per child (range 1 – 12).	Neuropsychological outcome (full-scale IQ scores); neuromotor; and renal glomerular function. Median duration of amalgam exposure was 31 months (2.6 years).	Treatment group had mean urinary concentrations of mercury that were approximately 0.2 µg/g creatinine than the control group (i.e., 1.6 versus 1.4 µg/g creatinine); however, difference was not considered to be statistically significant.	Although elevated urinary concentrations of mercury were found in the treatment group, there were no differences in neurological or other effects observed in treatment group vs control group.

Table 8. Comparison of the New England CAT amalgam cohort relative to the US population.

Cohort distribution	US Population (from NHANES 2001-2004)	New England CAT
Proportion with have ≤15 filled surfaces	69.9% (5 surface fillings excluded)	>90% (estimated from Maserejian et al. 2008)
Proportion with have >15 filled surfaces	30.1% (5 surface fillings excluded)	<10% (estimated from Maserejian et al. 2008)
Maximum number of filled surfaces	32 (children) 47 (adolescents) (5 surface fillings excluded)	24 (as reported by Maserejian et al. 2008)

The CAT studies employed inappropriate referent groups that were not free of Hg exposure. It is not the presence of amalgams, per se, that presents potential risk but the Hg exposure that arises from those amalgams. In fact, for any given level of exposure, the source of that exposure is irrelevant. At any given level of exposure, Hg^0 from any source will cause the same effect(s). It is not the source of that Hg that is the determinant of potential toxicity but the level of Hg exposure. It is apparent from both CATs that the Hg exposure in the referent groups is almost identical to that in the amalgam group. For the Casa Pia study, overall Hg exposure, as determined by urine Hg concentrations, was more or less equivalent at recruitment and at the end of year 7 (DeRouen et al. 2006). Within the New England CAT, the referent group (children receiving composite resin fillings) had mercury concentrations in urine that actually exceeded that of the group receiving amalgam fillings (maximum of 5.75 µg/g creatinine and 8.77 µg/g creatinine for the amalgam-exposed and referent groups, respectively; Bellinger et al. 2007). Therefore, given that any toxic effects would be related to Hg exposure, and given that the exposures in the control and referent groups are more or less identical, you would not expect to see any differences. A true reference group would have members whose urine Hg concentration was 0.5 µg/g creatinine or lower, the background level in the US for persons with no amalgams. The overlap in urinary Hg concentrations between amalgam and referent groups completely undermines the ability to differentiate any health consequences between these

groups that would be attributable to Hg exposure. If the referent groups are receiving the same level of Hg^0 exposure from a source(s) other than amalgam, then any health consequences of those exposures would be identical between the amalgam and referent groups. Therefore, differences between these two groups would not be expected, let alone quantifiable.

When occupational studies are conducted to investigate potential health effects in workers exposed to Hg^0, the referent or control group for comparison purposes is selected from another worker group that has significantly lower Hg exposure. The ratio of average Hg exposure in the exposed versus referent groups from a quick random selection of 14 occupational studies found that the average exposure is referent groups was generally 3 to 10 times lower than in the Hg exposed groups. In other words, the referent groups had Hg exposure demonstrably and significantly lower than the exposed groups. In the CATs, however, urine Hg monitoring results clearly show that these two groups had, for all intents and purposes, the same Hg exposure. This concurrence of Hg exposure levels in the amalgam and composite resin groups effectively negates any ability of these studies to differentiate health effects due to differential Hg exposure between these 2 groups. There exists no demonstrable and significant Hg exposure difference. Since no difference exists, we would not expect to find any differences in the types or incidence of effects. Therefore, by corollary, the absence of detected differences in the types or incidence of effects between these amalgam and composite resin groups cannot be used to validly or defensibly conclude that health effects are absent in the amalgam group.

No dose-response analyses of the CAT data have been conducted. All authors of these CAT studies report only the absence of statistical differences between the exposed and control groups. However, given the overlap in urine Hg concentrations between amalgam and composite resin groups (discussed above), this is not a sufficiently robust approach to conclude with any confidence that there is no association between Hg^0 exposure and neurological effects in these studies. A variety of other factors have been overlooked in the toxicological analyses published to date. These include:

- Statistical power: although efforts were made to ensure a study design with good statistical power (DeRouen et al. 2002), the uneven distribution of study participants across all dose groups greatly undermines that statistical power. As shown in Table X, vast majority of participants in the New England CAT had 10 or less amalgam-filled surfaces, and only a relatively few has >15 filled surfaces; none exceeded 24 filled surfaces. To ensure maximum statistical power to detect dose-response associations,

equal numbers of participants should be distributed across all dose groups. NTP rodent studies, for example, require equal sized groups of animals at all exposure levels (NTP 2006) in order to maximize statistical power for detecting differences among dose groups. Therefore, due to the small numbers of individuals with higher numbers of filled surfaces (versus lower numbers of surfaces) the power to detect statistically significant differences in incidence of toxic effects at higher dose levels versus lower levels is severely compromised, as is the ability to detect any dose-response association at a statistically significant level. A *post-hoc* calculation of the statistical power to detect significant differences between different dose groups in New England and Casa Pia studies is possible, though not completed herein due to insufficient published data.

- The analytical treatment did not effectively control for confounders, and in particular, the need to apply an exposure metric that incorporates both dose and duration. The analysis of mercury in urine data from the New England CAT (Maserejian et al. 2008) clearly demonstrated that an exposure metric that integrated both exposure level (number of amalgam surfaces) and exposure duration (years) explained greater variability in the urine Hg data than either of dose or duration alone. However, the various analyses of the toxicological consequences of Hg exposure (DeRouen et al. 2006; Bellinger et al. 2006; and other papers from these same studies) made no apparent attempt to consider such an integrative exposure metric. The potential implications of this can be illustrated with the recent re-analysis of the Casa Pia urine porphyrin data by Geier et al (in press). Woods et al (2009) reported an effect of amalgam on urinary porphyrin profile that they indicated as diminishing with time. However, Geier et al (in press) have shown this effect to be persistent and strongly Hg dose-dependent, when the exposure metric is properly controlled for all confounders and considers exposure duration as well as exposure level.

6.5 Changes in Urinary Prophyrin Profile as a Toxic Effect of Amalgam and Hg^0 Exposure

Porphyrins are formed in the production of heme, with redundant excess production being excreted via the urine in known concentrations and patterns (Geier et al, in press; Woods et al, 2009). Disruption of the heme synthesis pathway results in alteration of the concentrations and ratios (profiles) of the various porphyrins in urine. Changes in urinary porphyrin profiles result from metal-induced enzyme inhibition at various stages within the heme synthesis pathway (Geier et al, in press). These profile changes are largely metal specific (Geier et al, in press) and a number of studies have demonstrated that specific changes in porphyrin profile results from Hg^0 exposure (discussed by Woods et al. 2009).

Although research has demonstrated that porphyrin profiles can be a predictor of Hg exposure and Hg-induced neurotoxic effect (discussed by Woods et al, 2009), the inhibition of enzymes

within the (essential) heme synthesis pathway can be viewed as a toxic effect in and of itself. Porphyrin concentrations and profiles in urine are a direct measure of that effect (i.e., of enzyme inhibition).

Evaluating heme synthesis disruption as a toxic endpoint in and of itself would be analogous to the US EPA's approach respecting zinc toxicity (US EPA 2005), for which the blood-borne enzyme, zinc superoxide dismutase, was significantly inhibited, relative to pretreatment levels, in adult women consuming zinc supplements at an average daily dose of between 0.81 to 0.99 mg/kg-day (average: 0.91 m/kg-day). The USEPA considered this average dose level to be a LAOEL to which a total uncertainty factor of 3 was applied. That UF was applied to account for variability in susceptibility in human populations, but recognizing that the dose associated with effects was very close to the background intake of this essential element in the general population.

With respect to Hg^0 exposure and inhibition of heme synthesis, the average exposure level observed in the Casa Pia children's trial of approximately 2 µg Hg/g creatinine in urine would be viewed as a LOAEL, to which an uncertainty factor of 3 to 10 might be applied, resulting in a reference exposure level, as a urine Hg concentration, of between 0.2 to 0.7 µg/g creatinine. However, the evident dose-response relationship demonstrated by Geier et al (in press) indicates that a proper dose response analysis of the Casa Pia porphyrin data will define a point of departure of the porphyrin-Hg relationship, likely defining an appropriate benchmark dose as recommended by the USNRC (2008). Such an analysis may well define the apparent threshold for Hg^0 effects on the heme synthesis pathway. This detailed analysis was not possible herein, given the short timeframe required to produce this report and the late acquisition of the requisite data.

Having a point of departure for this end point that is less than the average exposure for the general population without amalgams is also not unknown or without precedent. This situation arises with lead (Pb) for which the point of departure for detrimental impacts on children's IQ is near or equivalent to typical blood Pb levels found in children in the US (CalEPA, 1997; Health Canada 2008b).

7 FETAL AND INFANT EXPOSURE TO HG FROM AMALGAM

The fetus and young infant are considered to be vulnerable or 'sensitive' receptors for specific consideration during the assessment of exposure and risks to neurotoxic substances such as Hg (US EPA 1998). This is partly due to the incomplete development of blood-brain barrier which is only complete towards the middle of the first year of life (Rodier, 1995). The fetal and infant CNS is also considered vulnerable to neutotoxins due to its ongoing development and maturation, which continues well after birth. In fact, brain maturation and development continues beyond childhood, with specific regions undergoing significant development at sexual maturity, later adolescence and early adulthood (Lebel et al. 2007; Geidd, 2004; Thompson et al, 2000).

7.1 Exposure to the fetus

The placenta provides a partial barrier to Hg exposure from the mothers' blood as it aids the fetus as it absorbs and excretes nutrients and toxins by the umbilical cord (Tortora and Derrickson, 2007). Animal studies (on rats) have shown that the placenta provides partial protection against mercury through its metallothionein content which binds Hg (Yoshida et al., 2005) thereby retaining greater concentrations of mercury compared to maternal and fetal blood. The placenta intercepts and accumulates inorganic Hg up to approximately 4 times that in maternal blood and cord blood (Ask et al., 2002). However, the placenta is not a complete barrier with respect to exposure to Hg^0 or methyl Hg (Ask et al, 2002), which both cross the placenta as a result of their high lipid solubility. The mechanism for Hg^0 transport across the placenta is not specifically known, but is expected to be similar to methyl Hg for which active transport is achieved via a mechanism involving a neutral amino acid carrier (Kajiwara et al., 1996).

The fetal liver may also provide partial protection of the fetus from amalgam-related Hg exposure. Bood entering fetal circulation via the umbilical cord from the placenta passes through the liver (Guyton, 1976), which contains high concentrations of catalases which oxidize Hg^0 to Hg^{2+} (Magos et al, 1977). Takahashi et al (2001), in a study of rodents into which amalgam fillings had been placed, observed that the fetal liver had the highest Hg concentration followed by kidney and then the brain. The brain was still impacted; concentrations of Hg in fetal rat brain tissue were significantly greater than control animals, and those concentrations were significantly correlated to the number of amalgam fillings in the dam. However, brain concentrations were lower than either liver or kidney. This is supported by the work of Lutz et al

(1996), who studied fetuses terminated or aborted at second trimester. They found significantly more mercury in fetal liver than in fetal brain.

As previously stated, the fetus is exposed to Hg from amalgam in a pregnant woman's teeth. Hg is found in amniotic fluid, cord blood, placenta, and various fetal and neonatal tissues including liver, kidney and brain, in concentrations that increase with increasing maternal amalgam load (Palkovicova et al. 2008; Ursinyova et al. 2006; Luglie et al. 2005; Ask-Bjornberg et al. 2003; Lindow et al. 2003; Ask et al. 2002; Vahter et al. 2000; Lutz et al. 1996; Drasch et al. 1994).

For assessment of fetal exposure to Hg from maternal amalgam, cord blood is the best biological medium for quantifying that Hg exposure. In general, maternal blood Hg concentration increases as amalgam load increases, and cord blood Hg concentration increases as maternal blood Hg concentration increases. These associations and their application to estimating fetal Hg dose resulting from maternal amalgam load are discussed in detail below.

Numerous studies have demonstrated that Hg levels in blood are increased in persons, including pregnant women, with amalgam (see Table 9). Other studies demonstrate that the concentration of Hg in cord blood increases as a function of either maternal amalgam load or maternal blood Hg concentration (see Table 10), reflecting the transfer from maternal blood via the placenta to cord blood. Further studies demonstrate that maternal amalgam-related Hg is deposited to fetal tissues, other than cord blood, as well (see Table 10).

Over the years, several studies have indicated that an increase in the mercury concentration found in the fetus was observed as the number of amalgams in the mother increased. Drasch et al. (1994) found that mercury from amalgam accumulated in the kidneys of fetuses in a manner similar to that in children and adults. A study by Ask and al. (2002) demonstrated an association of both I-Hg (inorganic mercury, Hg) and MeHg concentrations in the placenta with concentrations in maternal blood. The placental concentration of I-Hg increased proportionally with the number of amalgam fillings. Although their analytical method did not make the distinction between Hg^0 and Hg^{2+} (divalent mercury, mercuric mercury), we can assume that most of the mercury crossing the placenta was in the form Hg^0, since the uptake was 10 to 40 times higher in Hg^0 than Hg^{2+} in studies on rats (Klaassen, 2001). A study from Ask-Bjornberg et al (2003) found a clear increase of I-Hg exposure in the fetus, by reporting increased cord blood concentrations as the number of amalgams in the mother increased. A study by Vahter et al.

(2000) showed Hg^0 as the principal form of mercury to which the fetus is exposed from dental amalgam.

Ramirez et al. (2000) compared levels of mercury in the mother with the fetus, and reported significantly higher levels of Hg in cord blood and meconium (fetus equivalent of feces) relative to Hg levels in the mother's blood. Based on these results, it can be presumed that the absorption of mercury in the fetus from the mother is greater than its excretion from the fetus. This means that during the gestation of the fetus, the concentration of mercury continues to increase or bio-accumulate in fetal tissues. The trapping of mercury in the fetus' tissues is most likely due to a metabolism similar to that in adult tissues: the Hg^0 is converted to Hg^{2+} through oxidation by catalases and then is bound covalently to glutathione (GSH) and protein cysteine groups (Lorscheider et al., 1995). Once transformed to Hg^{2+}, the mercury is unable to migrate out of fetal tissues; effectively creating a one-way migration of mercury from the mother to the fetus, leading to accumulation in the fetus throughout gestation.

In a manner similar to the blood-brain barrier, the placenta does not allow mercury to return to the mother's blood from the fetus once it is oxidized (and loses its lipid solubility) and is bound to sulfhydryl groups (such as GSH). Therefore, the Hg that is not metabolized in the placenta (Ask et al, 2002) reaches the fetus where it becomes bound and is retained in the fetus. With feces being a major route of excretion for mercury (Lorscheider et al., 1995), this would explain the high levels of mercury found in the meconium (Ramirez et al., 2000).

Table 9: Summary of studies linking amalgam load in adults to blood Hg concentrations.

Authors	Year	Group composition	Relationship	Comments
Studies specific to mothers				
Gerhardsson and Lundh	2010	100 pregnant women in prenatal care unit in Sweden.	Positive correlation between maternal blood concentration and number of fish meals, occlusal amalgam fillings, crab intake and age. Median concentration: 0.70 (0.27-2.1)	Information collected through interviews. Blood sampling during the pregnancy. Hg concentrations reported as ug/L.
Palkovicova et al	2008	99 mothers that were enrolled in the Early Childhood Development and PCB Exposure in Slovakia study	Positive association between number of amalgams and maternal blood concentration. Median concentration: 0.63 (0.14-2.9). Mean concentration: 0.79	Number of amalgams determined by a questionnaire answered by the subjects. Hg concentrations reported as ug/L.
Vahter et al	2000	225 women recruited in prenatal care unit in Sweden	Significant increase of maternal blood concentration with increased number of amalgam. At gestational week 36, median inorganic Hg: 0.32 (0.0-1.9)	Number of amalgams determined by a questionnaire answered by the subjects. Calculated for both total and inorganic mercury. Hg concentrations reported as ug/L.
Oskarsson et al	1996	30 lactating mothers in Sweden	0.14 x number of amalgam fillings + 0.79 = Total Hg concentration in blood; Mean concentration: 2.3 ±1.0(0.9-4.6). 0.06 x number of amalgam fillings – 0.05 = inorganic Hg concentration in blood. Mean concentration: 1.7±0.7	Number of amalgams obtained through drawings from the patients interpreted by a dentist. Hg concentrations reported as ng/g.

Table 9 continued				
Studies on adults in general				
Authors	Year	Group composition	Relationship	Comments
Melchart et al	2008	90 randomized persons with dental amalgam restorations who suspected that their health complaints were caused by dental amalgam; having reported at least 10 symptoms (including at least 3 of strong intensity) and whose age were 20 to 50 years.	Hg concentrations in blood were significantly lower after removal of amalgams.	Hg concentrations reported as ng/mL. As stated by the authors, the group is not representative of all persons with dental amalgams.
Akesson et al	1991	244 dental personnel in the public dental service in Sweden.	Number of amalgam significantly correlated to the Hg blood concentration. Mean concentration: 16.9	Hg concentrations reported as nmol/L.
Snap et al	1989	10 volunteer subjects within the Iowa university faculty, staff, and graduate students that were reported to eat little or no fish and seafood.	Hg mercury concentration in blood correlated to the number of occlusal amalgam surfaces. Mean concentration: 2.18 (1.55-4.09)	Hg concentration reported as ng/mL
Abraham et al	1984	47 male medical students with dental amalgam restorations on Iowa's university campus.	Hg mercury concentration in blood correlated with both amalgam surface area and numbers. Mean concentration: 0.7±0.6 (0-3.3)	Hg concentrations reported as ng/mL.

Table 10: Summary of studies linking amalgam load of the mother to Hg Levels in fetal tissues.

Authors	Year	Group composition	Relationship	Comments
Palkovicova et al	2008	99 mother-child pairs that were enrolled in the Early Childhood Development and PCB Exposure in Slovakia study	Positive association between number of amalgams and cord blood concentration. Median concentration in cord blood: 0.80 (0.15-2.54) µg/L. Mean concentration in cord blood: 0.86 µg/L.	Number of amalgams determined by a questionnaire answered by the subjects.
Ursinyova et al	2006	409 mother-infant pairs randomly selected in regional maternity hospitals in Slovakia.	The number of maternal amalgam fillings significantly increased Hg concentration in placenta. Cord blood concentration was significantly correlated to placenta concentration. Mean concentration in placenta: 4.5 µg/kg. Mean concentration in cord blood: 1.33 µg/L.	Number of amalgams determined by a questionnaire answered by the subjects.
Ask-Bjornberg et al	2003	123 women recruited in antenatal care clinics in Sweden.	Inorganic Hg in cord blood increased significantly with the number of maternal dental amalgam fillings. Median concentration in cord blood: 0.15 µg/L. (range: 0.03-0.53 µg/L.)	Number of amalgams determined by a questionnaire answered by the subjects.
Lindow et al	2003	53 healthy women who delivered healthy babies at term in North of England Maternity Hospital	Positive correlation between the number of fillings and the fetal hair mercury level.	Number of amalgams calculated through dental examination. Fetal hair mercury level reported as ug/g.
Ask et al	2002	119 women recruited in antenatal care units in Sweden	Placental inorganic mercury levels increased significantly with increasing number of amalgam fillings. Median concentration in placenta: 1.3 µg/kg.	Number of amalgams determined by a questionnaire answered by the subjects.
Vahter et al	2000	225 women recruited in prenatal care unit in Sweden	Significant increase of cord blood concentration with number of amalgams. Median concentration of inorganic mercury in neonate: 0.34 µg/L (range: 0.0-1.1 µg/L.)	Number of amalgams determined by a questionnaire answered by the subjects. Calculated for inorganic mercury.

Table 10 continued				
Authors	Year	Group composition	Relationship	Comments
Ramirez et al	2000	36 mothers within 5 km of Apokon Valley	The mercury concentration significantly higher in the cord blood than in the maternal blood. The Hg level in cord blood significantly higher than levels found in meconium. Mean maternal blood concentration 24±5.47 ppb (range: 20-30 ppb). Mean cord blood concentration 53±37.49 µg/L (range: 20-130). Mean meconium concentration 48.64±43.48 (20-200)	Mercury concentration reported as ppb generally interpreted as µg/L. or µg/kg, depending on medium. Only positive samples (> detection limit) considered (N=5 for maternal blood; N=12 for cord blood; N= 36 for meconium).
Lutz et al	1996	20 fetus at second trimester in Sweden.	The concentrations of Hg in kidneys were significantly higher than in brain. Median concentration in the brain: 4 (2-23). Median concentration in the kidney: 6 (5-34).	Mercury levels reported as ug/kg.

7.2 Estimating cord blood Hg levels from maternal amalgam load

Although several studies, among those mentioned in Tables 9 and 10, report a significantly higher concentration in cord blood than in maternal blood, or report significant increases in mercury concentrations with increasing number of amalgam, most of them do not quantify this relationship precisely. Furthermore, for some of those studies, the numbers of maternal amalgam fillings were based on self reported values collected through questionnaires, rather than through dental exam. Others of these studies have small sample sizes or selected to report, out of all their analytical samples, only those where Hg was measured above analytical method detection limits (such as in Ramirez et al. 2000).

Two recent studies, Palkovicova et al. (2008; conducted in Slovakia) and Rudge et al. (2009; conducted in South Africa), quantified statistically significant relationships between mercury concentrations in the mother and the fetus, plotting individual data (rather than means over combined ranges, etc). The linear regression formulas presented in these studies are both presented in Figure 4. Mean or median concentrations of Hg in cord blood ranged from approximately 1.2 (Palkovicova et al, 2008) to 2 times (as in Rudge et al, 2009) greater than Hg concentration in maternal blood. The incremental increase in cord blood was 0.76 µg Hg/L per amalgam filled tooth in the study of Palkovicova et al (2008), and was 1.4 Hg/L per amalgam filled tooth in the study of Rudge et al. (2009). It is apparent that the baseline cord blood Hg concentration, the concentration associated with no maternal amalgam fillings, was already elevated relative to maternal blood Hg levels, further demonstrating the cumulative or bioconcentrating nature of Hg exposure from mother to fetus.

In order to reduce potential confounding due to concomitant exposure to methyl Hg from fish consumption and Hg0 from amalgams, the formula from Palkovicova et al. (2008) was selected as the basis for fetal exposure analysis. In the Palkovicova et al study, the likelihood of methyl Hg exposure through fish consumption by the mothers involved in the study was considered low compared to the Rudge et al. South African study, since fish is an important source of protein in South Africa (WorldFish Center, 2005)). Therefore, the potential data variations and confounding due to dietary methyl Hg exposure are lower in the Palkovicova et al. study.

There are insufficient studies of Hg toxicokinetics between mother and fetus, quantifying absorption and excretion of Hg over time in the fetus, to permit the development of a fully quantitative physiologically-based pharmaco-kinetic (PBPK) model. Therefore, the regressions presented in Figure 4 are the best method currently available for estimation of fetal exposure to Hg from maternal amalgam load.

Nonetheless, it remains possible to approximate the concentration of mercury in the fetus after delivery from the number of amalgams in the mother with available literature. In 1996, Oskarsson et al. (1996) reported a statistically significant association between total mercury in blood of lactating women in relation to their number of. These authors' regression relationship is plotted in Figure 5, below. In the original graph, units were recorded in ng/g of blood. For Figure 5, below, Hg concentration was converted to units of µg Hg/L blood (assuming that 1 litre of blood is 1.06 kg (Cutnell et al., 1998)).

Based on the foregoing, the formula of Palkovicova et al. (2008) was combined with the formula of Oskarsson et al. (1996) to yield the plot in Figure 6, which gives the relationship between the number of amalgams in the mother and the Hg concentration in the cord blood of the fetus.

Using the slope from Figure 6, it can be estimated that for every amalgam filling in the mother, the Hg concentration in cord blood increases by an average of 0.11 µg Hg/L of cord blood. This is almost the same as the estimation of Oskarsson et al (1996) of the average increases of maternal blood Hg concentration with each filling (0.1 ng/g that can be converted to 0.1 µg Hg/L). Therefore, it can be assumed that the dose of Hg the fetus is exposed to from a single amalgam is equivalent to that of the mother. From this, daily dose of Hg to the fetus from the amalgam fillings can be considered approximately the same as the daily dose to the mother. This is supported by the fact that the placenta is not a complete barrier to Hg0, as previously discussed. Vimy et al (1990) have also reported a ratio for mother's blood to cord blood of 0.9, which is the same as determined herein (i.,e, 0.1 ÷ 0.11 = 0.91).

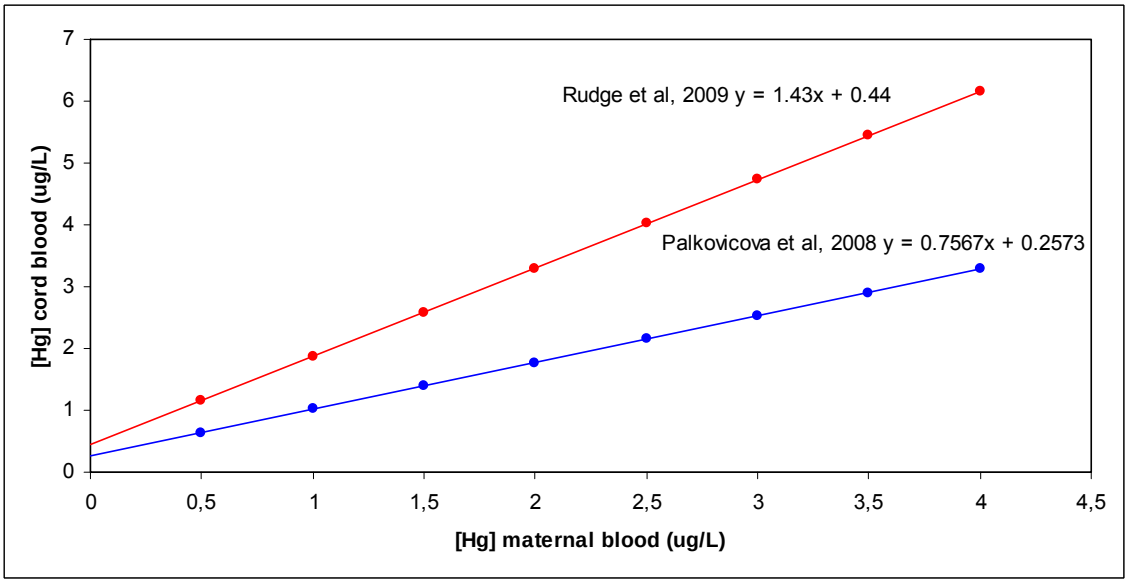

Figure 4: Linear regressions correlating mercury concentration in maternal blood and cord blood. Relationships from Rudge et al (2009) from 62 mother-child pairs from South Africa, and from Palkovicova et al (2008) from 99 mother-child pairs from *Slovakia*.

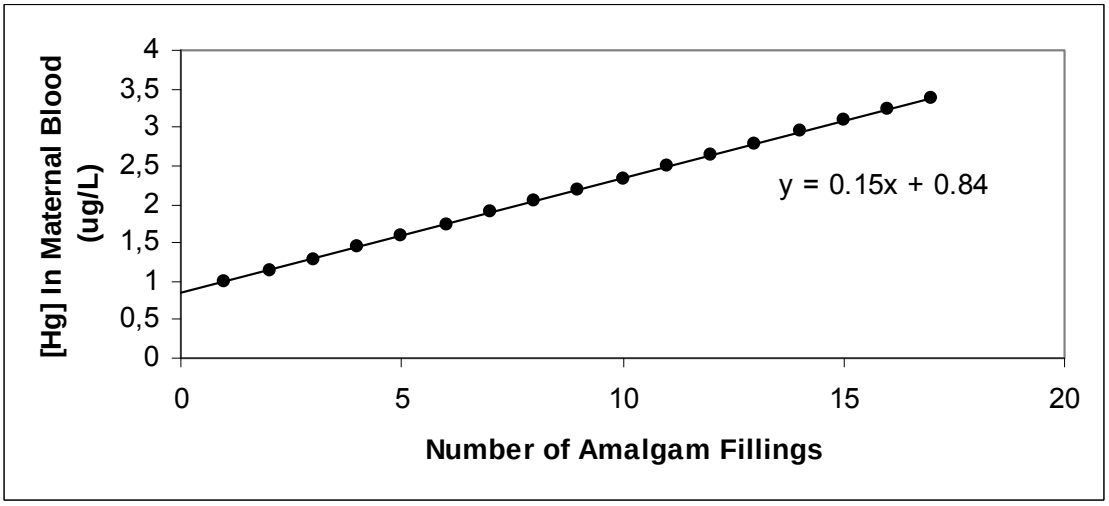

Figure 5: Linear regression correlating number of amalgam fillings and mercury concentration in maternal blood. From Oskarsson et al. (1996). Based on a sample size of 30 lactating mothers in Sweden.

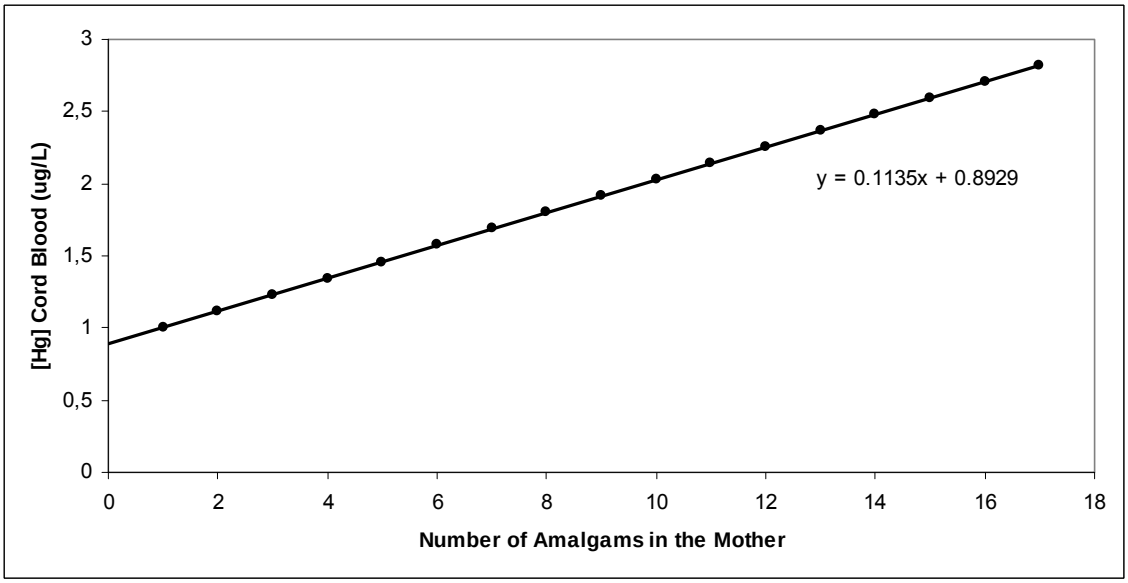

Figure 6: Derived relationship of Hg in cord blood as a function of maternal amalgam load. Graph plotted by calculating Hg concentration in the mother using the formula in Figure 5 and then using it in the formula from Palkovicova et al. (Figure 4) to get the Hg concentration in the cord.

Employing the equation presented in Figure 6, the estimated Hg concentrations in cord blood for varying numbers of maternal amalgam-filled teeth are presented in Table 11. The average number of filled teeth in women aged 16 to 49 years of age was 6.3, as determined from the 2001-2004 NHANES surveys. Based on the relationship depicted in Figure 6, the average cord blood concentration associated with that average number of filled teeth, assuming that all were composed of dental amalgam, is 1.6 µg Hg/L cord blood.

Uncertainties

There are a variety of uncertainties associated with the regression presented in Figure 6. These uncertainties include:

- The combination of regression equations derived from data on two different studies that investigated different populations (one in Slovakia and the other in Sweden)

- The form of Hg in maternal and cord blood employed herein was not differentiated between inorganic and organic Hg. However, employing the study of Palkovicova et al. (2008) controlled for this somewhat due to its focus on a population with lower fish consumption.

- The relative proportions of methyl Hg versus Hg^{2+} and Hg^{0} in maternal and cord blood confound any ability to precisely estimate fetal dose from maternal amalgam load. Kingman et al (1998) found that inorganic Hg in adult blood was 21% of total Hg. Vahter et al (2000), which is more specific to women, found 72% of blood Hg was in the form of methyl Hg, with the remainder (28%) as inorganic Hg. The relative abundance of these different Hg species in maternal and cord blood will likely be very population-specific, such that broad extrapolations from the existing data are difficult.

Table 11. Predicted cord blood Hg concentrations versus number of maternal amalgam-filled teeth.

Number of maternal amalgam filled teeth	Estimated maternal blood Hg concentration (based on Oskarsson et al. (1996)	Estimated cord blood concentration (based on Figure 6)
1	0.99	1.01
2	1.14	1.20
5	1.59	1.46
10	2.34	2.03
20	3.84	3.16
23 [1]	4.29	3.50
6.3 [2]	1.79	1.61

2. maximum for US female population aged 16-49 yrs; determined from NHANES 2001-02 and 2003-04; omits 5-surface fillings.
3. average for US female population aged 16-49 yrs; determined from NHANES 2001-02 and 2003-04; omits 5-surface fillings.

7.3 Amalgam-Related Hg Exposure to Infants via Breast Milk

For a breast-feeding infant, Hg is found in colostrum and breast milk in concentrations that increase with increasing maternal amalgam load (Ursinyova et al. 2006; Ask-Bjornberg et al. 2005; Da Costa et al. 2005; Drexler and Schaller, 1998; Drasch et al. 1998; Oskarsson et al. 1996). Several of these studies are summarized in Table 12.

Although amalgam-related Hg concentrations in breast milk are higher immediately after delivery, these appear to decrease over time (Drexler and Schaller, 1997), likely due to the dilution of the Hg in an increasing volume of milk production which increases as infant growth and food demand increase. However, assuming a constant rate of migration of amalgam Hg to breast milk, the total mass of Hg ingested by the infant on a daily basis would remain the same. Therefore, the simple dilution of the Hg in breast milk does not immediately negate the potential for risks associated with this ingestion exposure pathway.

Drasch et al (1998) compared mercury concentrations in breast milk and infant formula. They found that Hg concentration increased in the following progression: Mothers with no amalgam < mothers with 1 to 7 amalgam fillings < mothers with more than 7 amalgams. Infant formulas had Hg concentrations equivalent to mothers with 1 to 7 amalgams. Therefore, infants of mothers with >7 amalgam fillings will receive an ingestion dose greater than infants of mothers with fewer amalgam fillings or infants who are formula fed.

Although several studies report the correlation between breast milk Hg concentration and amalgam load, the actual absorption of inorganic Hg from the mother's breast milk by the newborn is not well documented. Ask-Bjornberg et al (2005) did not find a significant correlation between inorganic Hg concentration in infants' blood and breast milk Hg concentration, suggesting that absorption from the infant gastro-intestinal tract was low. In adults and animals, inorganic Hg has a relatively low absorption rate when compared to methyl Hg (up to 15% of Hg^{2+} against 90-95% of methyl Hg in adults; Klaassen, 2001), or in comparison to absorption of Hg^0 from the lungs (approximately 80%; discussed elsewhere in this report).

Some studies have speciated the Hg in breast milk to methyl Hg and inorganic Hg . However, no studies to date have reported the further speciation of the inorganic Hg to Hg^{2+} and Hg^0. The absorption of Hg^{2+} from the gastro-intestinal tract is known to be low, generally in the range of 5% to 15% (reviewed by Richardson and Allan 1995; Health Canada 1995). The gastro-intestinal absorption of Hg^0 is not well known. It has been reported to be as low as 0.01% (WHO 1991), however increased tissue burdens of Hg have been noted following accidental ingestion of gram quantities of metallic Hg (WHO 1991). Proportional absorption

generally increases at lower ingestion doses or concentrations of chemicals in ingested water, foods, etc. However, the rate of absorption of Hg^0 is unknown at the low levels of breast milk Hg concentration and infant oral dose that would be associated with breast feeding by mothers with amalgams.

Overall risks posed to breast-feeding infants cannot be determined with any degree of certainty until data on the further speciation of inorganic Hg (as Hg^{2+} and Hg^0) in breast milk are available, and the gastro-intestinal absorption rate of the Hg^0 from ingested breast milk is better understood. However, based on currently available information, this pathway is not considered to be particularly problematic relative to fetal exposure, and there are no data or information to suggest that the continued promotion of breast feeding, for its significant health and developmental benefits, should be altered for mothers possessing amalgam fillings.

7.4 Potential Developmental Effects Associated with Hg Exposure from Dental Amalgams

There is a paucity of information on the potential developmental effects that may be associated with Hg exposure, let alone from Hg associated with dental amalgams. As discussed in the exposure assessment section, the exposures that may result from dental amalgams could occur from two main sources: (1) exposures to the fetus from amalgams that pre-dated pregnancy; and (2) exposures to the fetus from any amalgams that an expecting mother may receive during pregnancy. To address either scenario, it would be ideal to have toxicity information in humans that provides possible outcomes to the fetus (e.g., blood or urine concentrations in expecting mothers that have been associated without adverse effects). At the current time, sufficient toxicological information was not identified to adequately address either exposure scenario. The lack of information on the potential developmental effects of mercury amalgams on the fetus is a concern since other forms of mercury are well known to cause developmental effects.

One study was identified which evaluated potential developmental toxicity from expecting mothers with pre-existing amalgams. In a study of 72 pregnant women, Luglie et al. (2005) found that mercury concentrations were elevated, but reported them as being not statistically significantly different in the amniotic fluid of women with greater numbers of amalgams and/or greater surface area of amalgams versus the control group. However, significance (p) values were reported as all being less than 0.05. Although the authors reported that there were no adverse outcomes in the pregnancies or newborns, no specific details were provided on this part of the analysis.

Table 12: Summary of studies linking amalgam load in mothers and mercury concentration in breast milk.

Authors	Year	Group composition	Relationship	Comments
Ask-Bjornberg et al	2005	20 women at delivery recruited in Sweden. Sampling at 4 days and 13 weeks after delivery.	Maternal blood I-Hg correlated significantly with the number of amalgam-filled surfaces. Total Hg in breast milk at 13 weeks correlated significantly to maternal blood I-Hg but not to infant blood I-Hg. Median total Hg-M at day 4: 0.29 µg/L (range: 0.06-2.1 µg/L). Median total Hg-M at 6 weeks postpartum: 0.14 µg/L (range: 0.07-0.37 µg/L)	Number of amalgam-filled surfaces recorded by a dentist.
Da Costa et al	2004	23 lactating mothers recruited in Brasilia during the first month after birth.	Significant correlation between number of amalgam surfaces and total Hg in breast milk. Mean concentration in breast milk: 5.73 ug/g (range: 0-23.07 ug/g)	Number of amalgams determined by clinical examination.
Ramirez et al	2000	36 mothers within 5 km of Apokon Valley	Mean maternal blood concentration 24±5.47 ppb (range: 20-30 ppb). Mean breast-milk concentration 36±18.16 ppb.	Only samples with Hg measured above detection limit included (N=5 for maternal blood; N= 5 for breast-milk).
Drasch et al	1998	46 mothers after delivery in Munich. Sampled on the 2nd and 7th day after delivery. 9 infant formula samples were also analysed.	Hg concentration in milk (Hg-M) is significantly correlated to the mother's number of teeth with amalgam. Hg-M in mothers without amalgam is significantly lower than in the infant formula. Hg-M in mothers with over 7 amalgams is significantly higher.	The mothers' dental statuses were recorded. Hg concentrations reported as ug/L.
Drexler and Schaller	1997	Women who gave birth in a rural area of Bavaria, Germany. First sample (one week after birth): 118. Second sample (2 months later): 86	The Hg concentration in breast milk was dependent on number of amalgam and surfaces in the first sample but not in the second. Concentration in breast milk for first sample: Median = 0.90 µg/L; mean = 1.37 µg/L. Second sample: median = 0.25 µg/L; mean = 0.64 µg/L	Number of amalgams determined by a dentist. Difference between the two samples attributed by authors to dilution as milk production increased 2 months after birth.
Oskarsson et al	1996	30 lactating mothers in Sweden	Significant correlation between total and inorganic mercury in milk and number of amalgams. Total mercury mean concentration: 0.6±0.4 ng/g.	Number of amalgams obtained through drawings from the patients interpreted by a dentist.

Elghany et al. (1997) reported an increase in congenital abnormalities in pregnant women exposed to mercury vapours in the workplace. In this retrospective epidemiological study of occupational exposure, 46 pregnant women were occupationally exposed to an estimated median exposure of 90 $\mu g/m^3$ and a range of 25 to 600 $\mu g/m^3$ while 19 women worked at the same location but no exposed to mercury vapours were identified as controls. Although not statistically significant, the women exposed to mercury vapour had a slightly greater rate of giving birth to a child with a congenital abnormality. As this study was completed as a review of medical records, there was no information on urine levels of mercury.

Although one study has found no association with amalgam placement during pregnancy and reduced birth weight (i.e., Hujoel et al., 2005), the potential for other effects remain a concern. In a recent presentation at the Society for Pediatric and Perinatal Epidemiologic Research (July 2010) Dr. Lisa DeRoo, an epidemiologist with the National Institute of Environmental Health Sciences, reported on a case-control study involving 1,336 infants born in Norway during a 7-year period. In that presentation, the odds of giving birth to a child with cleft palate was reported to be quadrupled for women who had fillings placed in the first or second month of pregnancy. Even greater odds were associated if multiple procedures took place during the first trimester. Nevertheless, personal communications with Dr. DeRoo have indicated that her team has not formally published the results since they are in the process of obtaining additional data (from another Nordic country) to try and replicate the findings. Pending replication, they intend to publish both sets of data in a single paper in 2011. Dr. DeRoo noted that the results from Norway were interesting but based on small numbers of exposed women and she stressed her team's caution and desire to replicate those results before publishing.

Although developmental effects have not been reliably reproduced in the few rat studies that have been completed (i.e., Morgan et al. 2002; Davis et al., 2001), such laboratory animal data would not be considered to be adequately sensitive to identify the more subtle neurological concerns that may be associated with elemental mercury.

An alternate approach for determining the potential for Hg^0 to cause developmental effects would be a quantitative comparison of the relative potency of Hg^0 to that of methyl Hg. The neurotoxicological and fetal potency of methyl Hg is well researched and could serve as a benchmark against which to rank the potency of Hg^0. Children born to women exposed to methyl Hg during pregnancy have elevated rates of a variety of neurological outcomes that

include decreased memory, decreased intelligence scores and poorer neuromotor abilities (measured when the children attain an age of seven years). The potential concern for developmental effects in general is enunciated in the USEPA's Guidelines for Neurotoxicity Risk Assessment (USEPA 1998), and is clearly demonstrated in the USEPA (2001) reference dose for methyl Hg. In developing the reference dose of 0.1 µg/kg bw/day, the US EPA provided an estimate of the $BMDL_{05}$ for methyl Hg of 1 µg/kg bw/day (i.e., $BMDL_{05}$ refers to the benchmark dose associated with the 95% lower confidence limit for a 5% response rate). This $BMDL_{05}$ has been further associated with a blood concentration of 46 to 80 ppb and was derived from women consuming fish and/or whale meat during pregnancy.

Nevertheless, at the current time, it remains unclear if Hg0 would have more, similar or less developmental toxicity than methyl Hg. Clearly, Hg0 has not received as much research attention as methyl Hg. To estimate the relative potency, it would be most useful to have more detailed information on both the toxicodynamics and toxicokinetics of these two species of Hg. If Hg0 was equally potent as methylmercury, it could be estimate that a $BMDL_{05}$ of 1 µg/kg bw/day would be equivalent to 3 $\mu g/m^3$ (using typical exposure assumptions of 20 m^3 of air inhaled per day and a body weight of 60 kg for women). However, given time and other constraints associated with the preparation of this report, this potency analysis was not undertaken.

Overall, the potential for the Hg0 from dental amalgams to cause developmental effects remains unclear for both women with existing amalgams and for women receiving amalgam placement during pregnancy. Studies on both of these exposure scenarios are required before risk-based conclusions can be made on the safety of such exposures. IN the interim, however, application of the Precautionary Principle will be warranted.

The Precautionary Principle is perhaps best enunciated within the Wingspread consensus statement (Wingspread 1998), as follows:

> *"Where an activity raises threats of harm to the environment or human health, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically."*

This statement on the Precautionary Principle is consistent with those of Health Canada, the Rio Declaration and other international agencies. It is the basis of the Canadian Chemicals

Management Plan and the European Union's REACH Program, both of which were established to address the thousands of essentially untested (toxicologically) chemicals currently in commerce.

This principle is embodied in the uncertainty factors developed by regulatory agencies in their goal to establish regulatory reference exposure levels (such as USEPA's reference air concentration; USATSDR's minimal risk level; etc.) that protect the health of all members of the general population. Uncertainty factors are specifically introduced to address those aspects of chemical exposure and toxicity for which insufficient data and information are available. Hg0 is such a case. Given this paucity of neurotoxicity and development toxicity data, the California EPA (2008) applied additional precaution in the application of uncertainty factors (total UF of 300, versus USEPA total UF of 30) within their derivation of a reference exposure level for Hg0 of 0.03 µg Hg/m3. Until further data are available on developmental and neurological outcomes associated with Hg0 exposure in humans, it is essential that precaution be applied in the determination of updated and revised reference exposure levels for the protection of public health.

8 RECOMMENDATIONS FOR FURTHER WORK AND RESEARCH

Based on the foregoing report, we have formulated a number of recommendations for further work and research that we believe would benefit the ongoing debate regarding the presence or absence of health effects associated with the Hg^0 exposure arising from dental amalgam. These recommendations are:

1. As part of a future NHANES survey, compile data on the specific restorative materials used to fill tooth surfaces within the US population. At the very least, recording whether the material used was amalgam versus some other material should be relatively simple. This distinction is relatively easy as it can be based solely on restoration color (silver versus other).
2. The USEPA and USATSDR should immediately initiate the review of Hg^0 toxicology, including all studies conducted in the past 2 decades, towards updating and revising their RELs for Hg^0. This review and update should include consideration of heme synthesis enzyme inhibition as one of the toxic endpoints.
3. A post-hoc analysis should be undertaken of the statistical power offered by the Casa Pia and New England children's amalgam trials to quantify precisely the degree of difference in incidence of neurological impairments that can be statistically differentiated between higher exposure subgroups and lower exposure subgroups within the amalgam cohorts of each study.
4. Quantitatively determine the impact of urinary Hg concentrations in the CAT referent groups (those that received composite resin fillings) relative to the amalgam groups to determine if non-amalgam sources and levels of Hg^0 exposure in the referent groups negate any ability to rely on these studies as a means of demonstrating the absence of health effects due to Hg exposure from amalgam. This could include a *post-hoc* re-screening of referent group members to re-examine inter-group differences employing those referents with a urine Hg concentration ≤ 0.5 µg Hg/g creatinine.
5. Combine the New England and Casa Pia studies in a meta-analysis, thereby providing increased statistical power for detecting differences in incidence of neurological effects between higher dose and lower dose members of the combined amalgam cohorts.
6. Conduct a dose-response analysis of both (and combined) amalgam trials data on neurological and other outcomes that appropriately controls for confounders and

employs a dose metric that reflects both exposure level and exposure duration, analogous to methods employed to assess porphyrin profiles conducted by Geier et al (in press). Dose-response data must be presented and analyzed with respect to individual CAT participants, and not simply as overall averages for exposed and referent cohorts.

7. Consider future follow up of both cohorts to increase the data available on duration of exposure, thereby extending the exposures to more effectively represent true chronic exposure, particularly given Hg's accumulation in the brain and other tissues over time (i.e., to exceed 5 and 7 years for the New England and Casa Pia amalgam trials, respectively).
8. Clarify the average numbers of amalgam filled tooth surfaces possessed by the different cohort groups that should be considered as in-place for the full duration of the CAT studies. It is apparent that members of these cohorts had varying numbers of amalgam fillings throughout the duration of these studies. A more detailed dose response analysis of these data, as described in point 4, could make this unnecessary, however.
9. Explicit publication of the urine Hg concentration data from the Casa Pia study, with an analysis of the association of urine Hg concentration with amalgam load.
10. Efforts should be expended to find an appropriate reference group for future CAT studies that are free of mercury exposure, not just free of amalgam.

9 DISCLAIMER

The statements made in this report are based solely on the information obtained to date as part of the above referenced study. SNC-Lavalin Environment, Division of SNC-Lavalin Inc. (SLE) has used its professional judgement in assessing this information and formulating its opinion and recommendations. New information may result in a change in this opinion. The mandate at SLE is to perform the tasks prescribed by the Client with the due diligence of the profession. No other warranty or representation, expressed or implied, as to the accuracy of the information or recommendations is included or intended in this report.

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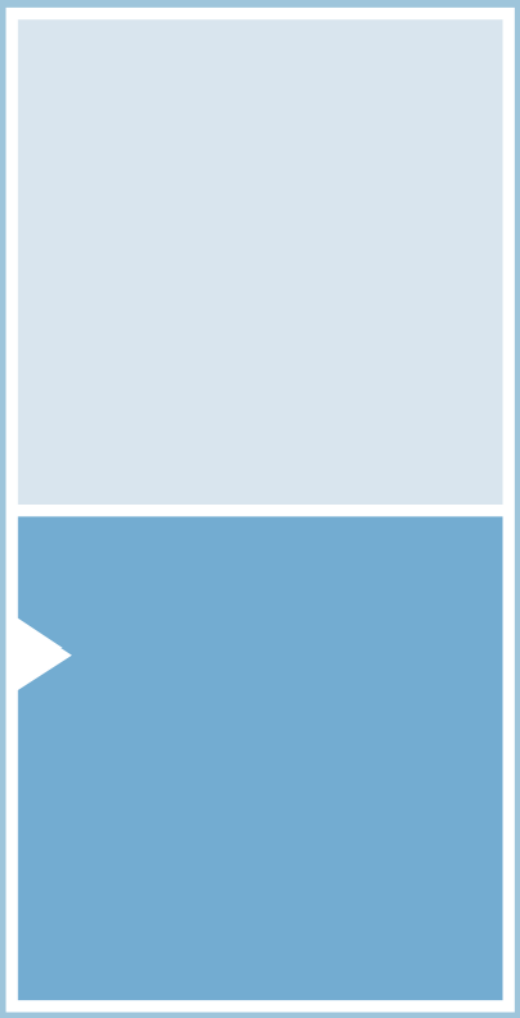

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