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## Determination of methyl mercury in dental-unit wastewater

Mark E. Stone<sup>a,\*</sup>, Mark E. Cohen<sup>a</sup>, Lian Liang<sup>b</sup>, Patrick Pang<sup>b</sup>

<sup>a</sup>Naval Institute for Dental and Biomedical Research, Building 1-H, 310A B Street, Great Lakes, IL 60088-5259, USA

<sup>b</sup>Cebam Analytical Laboratories, 3927 Aurora Ave, Seattle, WA 98103, USA

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### Abstract

**Objective.** The objective of this investigation was to establish whether monomethyl mercury (MMHg) is present in dental-unit wastewater and if present, to determine the concentration relative to total mercury.

**Methods.** Wastewater samples were collected over an 18-month period from three locations: at the dental chair; at a 30-chair clinic, and at a 107-chair clinic. Total mercury determinations were completed using United States Environmental Protection Agency's (USEPA) method 1631. MMHg was measured utilizing modified USEPA method 1630.

**Results.** The total mercury levels were found to be: 45182.11  $\mu\text{g/l}$  ( $n = 13$ ,  $\text{SD} = 68562.42$ ) for the chair-side samples, 5350.74  $\mu\text{g/l}$  ( $n = 12$ ,  $\text{SD} = 2672.94$ ) for samples at the 30-chair clinic, and 13439.13  $\mu\text{g/l}$  ( $n = 13$ ,  $\text{SD} = 9898.91$ ) for samples at the 107-chair clinic. Monomethyl Hg levels averaged 0.90  $\mu\text{g/l}$  ( $n = 13$ ,  $\text{SD} = 0.87$ ) for chair side samples, 8.26 ( $n = 12$ ,  $\text{SD} = 7.74$ ) for the 30-chair facility, and 26.77  $\mu\text{g/l}$  ( $n = 13$ ,  $\text{SD} = 34.50$ ) for 107-chair facility. By way of comparison, the MMHg levels for the open ocean, lakes and rain are orders of magnitude lower than methyl mercury levels seen in dental wastewater (part per billion levels for dental wastewater samples compared to part per trillion levels for samples from the environment).

**Significance.** Environmentally important levels of MMHg were found to be present in dental-unit wastewater at concentrations orders of magnitude higher than seen in natural settings.

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**Keywords:** Amalgam; Dental materials; Dental wastewater; Methyl mercury

### 1. Introduction

While the environmental significance of mercury (Hg) release from the dental practice has become an important issue [1–3], it has often at times been dismissed with the assumption that the Hg in dental amalgam is unavailable for uptake by biologic organisms, i.e. that Hg is in the form of intermetallic compounds [4] and therefore not bioavailable. Our supposition is that biologically available species of Hg, specifically monomethyl mercury (MMHg), may be resident in dental-unit wastewater. In an attempt to resolve this issue, the concentrations of both total Hg and MMHg were measured in dental-unit wastewater samples obtained from three separate locations over 18 months.

Hg is generally found in three forms: elemental, inorganic and organic. Each form possesses its own

characteristic toxicokinetics and human health effects. Elemental Hg volatilizes at room temperature and human exposure is primarily through inhalation of the vapor. Hg vapor is lipid soluble and easily crosses alveolar membranes of the lungs; it is taken up by red blood cells and transported to the central nervous system [5].

Absorption of inorganic Hg (also known as ionic Hg) by the gastrointestinal tract in humans is relatively limited and approximates 7% of the ingested dose [5]. Kidney tissue contains the highest concentration of Hg after exposure to inorganic salts and elemental Hg. It has been demonstrated that elemental Hg in human saliva can be oxidized to ionic Hg [6], which may be protective since ionic Hg is a less toxic species: both MMHg and ionic Hg were present in saliva of all study subjects [6] but only patients with amalgam restorations had detectable elemental Hg levels in their saliva.

Organic Hg is the most important species in terms of toxicity to humans. It produces neurotoxic effects in adults and the developing nervous systems of fetuses and young

\* Corresponding author. Tel.: +1-847-688-1900x83619; fax: +1-847-578-3793.

E-mail address: [mark.stone@ndri.med.navy.mil](mailto:mark.stone@ndri.med.navy.mil) (M.E. Stone).

children are particularly susceptible [5]. Toxicity is manifested by: paresthesia, ataxia, neurasthenia, vision and hearing problems, tremor and spasticity, and in high enough concentrations, death. MMHg, a form of organic mercury, interacts with DNA and RNA and binds to sulfhydryl groups which play a major role in determining the secondary structure of proteins and other biological macromolecules. MMHg has been shown to disrupt the organization of microtubules, sub-cellular structural elements which play a defining role in the segregation of chromosomes during cell division [7].

The absorption of MMHg from the gastrointestinal tract can be as high as 95%, and this form of Hg has a marked affinity for the central nervous system [5,8]. The human health effects of organic Hg exposure are well recognized and substantial data have been acquired from acute poisoning events in Japan, Iraq, and the United States [9,10]. In Minamata Japan, inorganic Hg was used as a catalyst in the production of acetaldehyde from acetylene at the Chisso chemical plant. Years of Hg release into Minamata Bay resulted in concentrations of Hg in sediments approximating 2 kg/mtonnes. The organic Hg released into the bay was incorporated into the food chain where it was accumulated by aquatic organisms and biomagnified across trophic levels, eventually reaching concentrations in fish upwards of tens of parts per million (mg/kg). Over 2200 Japanese citizens have been diagnosed with MMHg poisoning, now known as Minamata disease.

## 2. Materials and methods

*Sample collection.* Wastewater samples were collected over an 18-month period from three locations: a single dental chair, a 250-gallon holding tank at a 107-chair clinic, and from a holding tank at a 30-chair clinic. A total of 13 samples were obtained from the single dental chair and from the holding tank at the 107-chair clinic. Twelve samples were obtained from the holding tank at the 30-chair clinic. Samples at each location were collected daily in short contiguous sequences separated by 6-to-18 months.

For chair side collection, samples were obtained at the end of the working day after seven to eight patients were treated. All patients had old amalgam restorations removed and/or new amalgam restorations placed. Samples were obtained by installing a collection system (Fig. 1) at the end of the high volume evacuation (HVE) line. Care was taken to collect only the liquid portion of the sample (supernatant), leaving particulate settled at the bottom of the collection containers.

The second set of samples was collected directly from a 250-gallon holding tank that accumulates dental-unit wastewater from a 107-chair dental clinic. Grab samples were collected after the tank contents were agitated with a rotary mixer for 5 min.



Fig. 1. Apparatus used to collect chair side wastewater samples.

Samples from the 30-chair clinic represented the third sample set and were collected from the holding tank of the dental vacuum system used in this clinic.

All samples were collected in acid washed and double deionized water rinsed borosilicate glass containers utilizing ‘ultra clean’ sampling techniques [11]. The pH of each sample was determined in the laboratory prior to analysis. All samples were stored unpreserved at 4 °C.

*Analytical methods.* Total Hg was determined using EPA standard method 1631 [12] by oxidation with BrCl, reduction, purge, gold sand trap collection, and cold vapor atomic fluorescence (CVAf) detection. For determination of MMHg, samples were prepared by solvent extraction, and analyzed using modified EPA method 1630 [13] by aqueous phase ethylation, Tenax Trap™ collection, GC separation and CVAfS detection. A method for the simultaneous determination of MMHg, ionic Hg and total Hg in biologic materials has been published [14].

## 3. Results

Table 1 presents data and descriptive statistics showing total Hg and MMHg, from the three sample sets. The total Hg

Table 1  
Mean mercury determinations by location

Sample location	Mean total Hg (SD) <sup>a</sup>	Sample size, range <sup>b</sup>	Mean MMHg <sup>b</sup> (SD)	Sample size, range <sup>a</sup>
Chair side <sup>b</sup>	45182.1 (68562.4)	<i>n</i> = 13, 1779.5–173000.0	0.90 (0.87)	<i>n</i> = 13, 0.08–2.66
107 Chair clinic <sup>c</sup>	13439.1 (9898.9)	<i>n</i> = 13, 2395.0–35600.0	26.77 (34.50)	<i>n</i> = 13, 2.08–99.4
30 Chair clinic <sup>d</sup>	5350.7 (2672.9)	<i>n</i> = 12, 2360.0–12636.3	8.26 (7.74)	<i>n</i> = 12, 0.19–20.2

<sup>a</sup> Units of concentration are  $\mu\text{g/l}$  (ppb, parts per billion).

<sup>b</sup> Settled supernatant collected directly from dental chair.

<sup>c</sup> Samples obtained from the 250-gallon holding tank that collects wastewater from a 107-chair clinic.

<sup>d</sup> Samples collected from the holding tank at the 30-chair clinic.

levels from these locations were: 45182.11  $\mu\text{g/l}$  (*n* = 13, SD = 68562.42) for the chair-side samples, 13439.13  $\mu\text{g/l}$  (*n* = 13, SD = 9898.91) for the 107-chair clinic, and 5350.74  $\mu\text{g/l}$  (*n* = 12, SD = 2672.94) for the 30-chair clinic.

MMHg levels were relatively low for the chair side samples, 0.90  $\mu\text{g/l}$  (*n* = 13, SD = 0.87) compared to samples at the 107-chair and 30-chair facilities, 26.77  $\mu\text{g/l}$  (*n* = 13, SD = 34.50) and 8.26  $\mu\text{g/l}$  (*n* = 12, SD = 7.74), respectively. MMHg levels from the 107-chair clinic were almost 31 times higher than the chair side samples, and 9.5 times higher than samples from the 30-chair clinic. By way of comparison, the MMHg levels for the open ocean, lakes, and rain [11] are presented in Table 2 and are orders of magnitude lower than MMHg levels seen in dental wastewater (part per billion levels for dental wastewater samples compared to part per trillion levels for natural environmental samples).

#### 4. Discussion

To our knowledge, this is the first time MMHg has been reported in dental-unit wastewater, and this report establishes the existence of bioavailable Hg species in the wastewater effluent from dental treatment facilities. MMHg was found to be present, in important concentrations, in dental wastewater from three locations in two different treatment facilities from sampling over the 18-month study period. The highest concentrations of MMHg were found to be present in the holding tanks of the 107 and 30-chair clinics (Table 1). Lower, but still environmentally significant concentrations of MMHg were measured at the dental

Table 2  
Total and MMHg concentrations from various environmental locations

Location	Total Hg <sup>a</sup> (ng/l)	MMHg <sup>a</sup>
Open ocean	0.1–1.0	0.01–0.05
Pristine Lake	0.5–5.0	0.01–1.00
Polluted Lake	5.0–2000.0	0.10–10.00
Pristine rain	1.0–10.0	0.01–0.20
Urban rain	3.0–100.0	0.01–2.00

Ref.[11].

<sup>a</sup> Concentrations are in ng/l (ppt, part per trillion).

chair. The amount of MMHg is low in comparison to total Hg, (Table 1), yet noteworthy when the toxicity of MMHg is considered. The concentration of MMHg in dental wastewater samples is orders of magnitude greater than in environmental samples (Table 2).

Within a series of samples collected over several days, MMHg concentrations are relatively consistent; but large differences between values from samples taken several months apart are sometimes recorded (Fig. 2). Conversely, MMHg values from chair side samples were much more consistent throughout the study. Variability seen in holding tank samples may be associated with the residence time of wastewater in the tanks, the amount of rinse water used in the clinics as well as wash down cycles used in some dental vacuum systems.

The higher concentration of MMHg in samples from holding tanks compared to chair side samples is likely the result of methylation of inorganic Hg by bacteria and fungi. The biochemical mechanism of methylation is only superficially understood, but both biotic and abiotic mechanisms are known to occur [15]. Methylation of inorganic Hg has been shown in sediments and found to be the result of

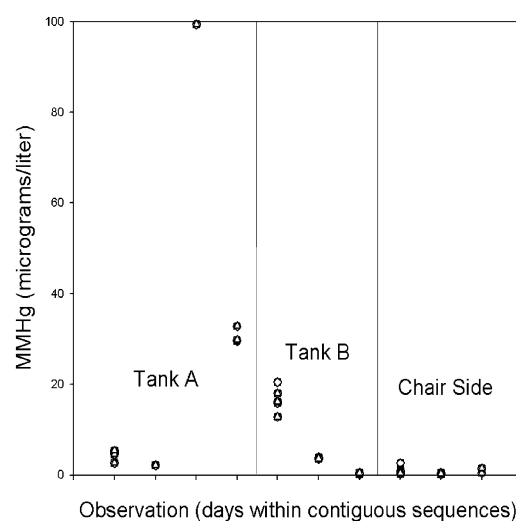


Fig. 2. Plot of MMHg concentrations over time from the three sites sampled for this study. Tank A (107-chair clinic): 5, 2, 2, and 4 measurements in daily sequences separated by four to six months. Tank B (30-chair clinic): 5, 4, and 3 measurements in daily sequences separated by six to eighteen months. Chair side: 5, 4 and 3 measurements in daily sequences separated by 5 to 9 months.

sulfate reducing bacteria (SRB) [16–21]. The higher concentrations of MMHg seen in holding tanks where large bacterial populations grow and prosper strongly suggest bacteria to be the source of the MMHg (although abiotic methylation via the coenzyme methylcobalamine, a Vitamin B-12 analog, cannot be ruled out). The source of MMHg formation in the dental-unit wastewater stream remains an area of ongoing research.

SRB are known to populate the oral cavity of healthy patients as well as patients with periodontal disease [22,23]. A recently published report [24] correlates MMHg levels in human mouths to the number of Hg containing restorations present, suggesting methylation may occur in the oral cavity. An earlier paper demonstrated the methylation of inorganic Hg by oral streptococci in vitro [25]. SRB in the oral cavity may provide a mechanism by which dental wastewater is 'inoculated' with these bacteria.

The presence of Hg in dental wastewater is an obvious concern for dental clinics, but also an important issue for municipally owned wastewater treatment plants. These facilities use bacteria in the treatment process and Hg provides a substrate for microbial mediated methylation. Significant concentrations of Hg have been measured in the influent of wastewater treatment facilities. A 9-week study tracking Hg pathways at a large Midwestern plant revealed an average daily Hg loading of 284 g. The Hg removal efficiency was determined to be 96%; resulting in the release of 4% of the Hg (approximately 10 g per day) into the Mississippi River [26].

## 5. Conclusion

Results underscore the importance of limiting the release of all forms of Hg into wastewater streams, as the potential exists for Hg to be transformed into more toxic species. Systems to remove Hg from dental-unit wastewater should be designed to minimize the production of organic Hg.

## 6. Disclaimer

The views expressed here are those of the authors and do not necessarily reflect the official policy or position of the Departments of the Navy or Defense, nor the US Government. The authors of this study believe in the clinical safety and efficacy of dental amalgam and are working to make its use less burdensome to the environment.

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