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Comparison of chlorine and chloramine in the release of mercury from dental amalgam

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ABSTRACT

The purpose of this project was to compare the ability of chlorine (HOCl/OCl^-) and monochloramine (NH_2Cl) to mobilize mercury from dental amalgam. Two types of amalgam were used in this investigation: laboratory-prepared amalgam and samples obtained from dental-unit wastewater. For disinfectant exposure simulations, 0.5 g of either the laboratory-generated or clinically obtained amalgam waste was added to 250 mL amber bottles. The amalgam samples were agitated by end-over-end rotation at 30 rpm in the presence of 1 mg/L chlorine, 10 mg/L chlorine, 1 mg/L monochloramine, 10 mg/L monochloramine, or deionized water for intervals of 0 h, 2 h, 4 h, 8 h, and 24 h for the clinically obtained amalgam waste samples and 4 h and 24 h for the laboratory-prepared samples. Chlorine and monochloramine concentrations were measured with a spectrophotometer. Samples were filtered through a 0.45 μm membrane filter and analyzed for mercury with USEPA standard method 245.7. When the two sample types were combined, the mean mercury level in the 1 mg/L chlorine group was 0.020 mg/L ($n=25$, $\text{SD}=0.008$). The 10 mg/L chlorine group had a mean mercury concentration of 0.59 mg/L ($n=25$, $\text{SD}=1.06$). The 1 mg/L chloramine group had a mean mercury level of 0.023 mg/L ($n=25$, $\text{SD}=0.010$). The 10 mg/L chloramine group had a mean mercury level of 0.024 mg/L ($n=25$, $\text{SD}=0.011$). Independent samples t-tests showed that there was a significant difference between the natural log mercury measurements of 10 mg/L chlorine compared to those of 1 mg/L and 10 mg/L chloramine. Changing from chlorine to chloramine disinfection at water treatment plants would not be expected to produce substantial increases in dissolved mercury levels in dental-unit wastewater.

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1. Introduction

Mercury is a potent neurotoxin and is of interest due to its continued presence at levels of concern in the environment. Exposure to high levels of mercury can irreversibly damage the brain, kidneys, and the developing fetus. Lower dose exposure to mercury can result in irritability, shyness, tremors, changes in vision or hearing, and memory problems (ATSDR, 1999). Amalgam, a mercury-containing restorative

material, is widely used by dentists to restore decayed tooth structure. As a consequence of the placement and removal of amalgam restorations, mercury can contaminate wastewater leaving dental facilities. Mercury concentrations in this waste stream can reach substantial concentrations and in one well documented instance, a large dental treatment facility was disconnected from a municipal wastewater treatment system as a result of exceeding mercury discharge limits (Stone et al., 1999). Dental-unit wastewater is increasingly being recognized

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as a source of anthropogenic mercury emission and this has resulted in an escalating trend to regulate the discharge of mercury from dental offices across the United States (Stone et al., 2008).

The ability of chlorine (and other oxidizing disinfectants) to mobilize mercury from dental amalgam is well recognized (Batchu et al., 2006). Chlorine has a long history in the disinfection of drinking water, with the first recorded use occurring in 1896 (AWWA, 2006). Its utilization was linked with a dramatic decrease in water-borne illnesses, including typhoid, dysentery, and cholera (AWWA, 2006). Recently, chlorine has come under scrutiny because its by-products such as trihalomethanes (THM) and haloacetic acids (HAA) – produced when surface waters are disinfected with chlorine – are thought to be carcinogenic (AWWA, 1998, 2006; USEPA, 2001; Yang and Shang, 2004). The U. S. Environmental Protection Agency (USEPA) has promulgated a regulation under the Disinfectants and Disinfection By-Products Rule that sets maximum levels for THM and HAA (USEPA, 2001). In an effort to meet the new drinking water regulations, many water treatment utilities have begun switching to chloramination because of the diminished tendency for chloramine to produce halogenated by-products (AWWA, 1998, 2006).

The switch to disinfection by chloramination has been accompanied by reports of lead spikes exceeding the USEPA's action level for lead in drinking water (15 µg/L) (Renner, 2004; Switzer et al., 2006; Miranda et al., 2007). The chloramine-mediated mobilization of lead from plumbing lines raises the possibility that chloramine may mobilize mercury from amalgam trapped in plumbing lines. To investigate this issue, a study was designed to compare the capacity of chlorine and chloramine to mobilize mercury from dental amalgam.

2. Materials and methods

This study was designed to compare chlorine with chloramine in the mobilization of mercury from dental amalgam. Laboratory and clinically derived amalgam samples were exposed to 1 mg/L and 10 mg/L solutions of chlorine (HOCl/OCl⁻) and monochloramine (NH₂Cl). Exposure took place over 24 h with sampling intervals of 0 h, 2 h, 4 h, 8 h, and 24 h for the clinically obtained amalgam samples and 4 h and 24 h for the laboratory-prepared amalgam samples. Parameters measured at each sampling interval were: pH, free chlorine, monochloramine, and mercury concentrations.

2.1. Preparation of amalgam samples

Two types of amalgam were used in this investigation: laboratory-prepared amalgam and amalgam waste obtained from dental-unit wastewater. Laboratory samples were prepared in the following manner: double spill Megalloy®EZ capsules (Dentsply Caulk, Milford, DE, USA) were triturated with a ProMix™ amalgamator (Dentsply Caulk, Milford, DE, USA) for 12 s at 4200 cycles/m and then allowed to set for eight weeks at room temperature. The set amalgam was ground in a Janke & Kunkel Model A10 analytical mill (IKA Works, Inc., Wilmington, NC, USA) and dry sieved using standard testing sieves and a

model SS-3 sieve shaker to generate a particle size distribution of 212-to-710 µm (Gilson Inc., Lewis Center, Ohio, USA).

Clinically obtained amalgam samples were acquired from chairside wastewater. Five dental chairs were fitted with an air/water separator and wastewater samples were collected in polycarbonate containers, brought back to the laboratory, and filtered through 0.45-µm pore size mixed cellulose ester membrane filters (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) with positive pressure (argon gas). The filters were allowed to air dry in a fume hood and the amalgam containing debris was removed from the filters and stored in a borosilicate glass beaker. No attempt was made to characterize the particle size distribution of these clinically obtained samples.

2.2. Preparation of chlorine and chloramine solutions

Solutions of chlorine (HOCl/OCl⁻) and chloramine (NH₂Cl) were made daily (Switzer et al., 2006). The free residual chlorine solution was made from a stock solution of five percent by volume of sodium hypochlorite. Chloramine was prepared by reacting sodium hypochlorite with a fivefold molar excess of aqueous ammonia to minimize the formation of dichloramine (Switzer et al., 2006). Both chlorine and monochloramine solutions were prepared at concentrations of 1 mg/L and 10 mg/L at a pH of 8 at room temperature. Determinations of sample pH were completed using a Beckman Φ32 pH meter (Beckman Coulter, Inc., Fullerton, CA USA).

2.3. Exposure simulations

For disinfectant exposure simulations, 0.5 g of either the laboratory or clinically generated amalgam was added to amber-colored 250 mL bottles. Prior to exposure experiments, disinfectant stock solution concentrations were verified and then monitored throughout the experiment. The amalgam samples were agitated by end-over-end rotation at 30 rpm in the presence of 1 mg/L chlorine, 10 mg/L chlorine, 1 mg/L monochloramine, 10 mg/L monochloramine, or deionized water for intervals of 0 h, 2 h, 4 h, 8 h, and 24 h for the clinically obtained amalgam samples and 4 h and 24 h for the laboratory-prepared amalgam samples. A total of 25 samples were collected for each disinfectant group: 16 samples for clinically derived amalgam and 9 samples for the laboratory-generated amalgam samples. Samples were obtained at each time interval (in either three or six replicates) and the values then averaged.

2.4. Laboratory determinations

Immediately after collection of the agitated samples, chlorine and monochloramine levels were monitored by the DPD colorimetric method (Eaton et al., 2005) utilizing a Genesys 20 spectrophotometer at a wavelength of 515 nm (Thermo Electron Corporation, Madison, WI USA). Sample pH determinations were completed with a Beckman Φ32 pH meter (as above).

Mercury levels were determined by the USEPA standard method 245.7 (USEPA, 2005) utilizing a Millennium Cold Vapor Atomic Fluorescence mercury analyzer (PS Analytical, Deerfield Beach, FL USA). Immediately after collection, all samples were filtered through a 0.45 µm filter and digested by exposure to a solution of potassium bromate/potassium bromide.

Table 1 – Mean mercury levels (mg/L) and standard deviations (SD) from the exposure of amalgam waste to chlorine (HOCl/OCl⁻) and monochloramine (NH₂Cl) at concentrations of 1 mg/L and 10 mg/L

Sample	DI water control	Chlorine		Monochloramine	
		1 mg/L	10 mg/L	1 mg/L	10 mg/L
Clinic amalgam	0.013 (0.005)	0.020 (0.009)	0.17 (0.164)	0.020 (0.009)	0.021 (0.008)
Lab amalgam	0.012 (0.007)	0.021 (0.005)	1.3 (1.5)*	0.027 (0.009)	0.029 (0.013)*
Combined	0.013 (0.006)	0.020 (0.008)	0.59 (1.06)*	0.023 (0.010)	0.024 (0.011)*

For the chlorine and chloramine experimental groups: clinic amalgam, *n*=16; lab amalgam, *n*=9; combined clinic and lab amalgam, *n*=25. For the deionized (DI) water controls: clinic amalgam, *n*=13; lab amalgam, *n*=6; for combined clinic and lab amalgam, *n*=19. *Lab amalgam and combined mean mercury levels at 10 mg/L chlorine vs. 10 mg/L monochloramine are significantly different (*p*<0.05).

After oxidation by the potassium bromate/potassium bromide solution, the sample was sequentially pre-reduced with NH₂OH·HCl to destroy the excess bromine and then the ionic mercury was reduced with SnCl₂ to convert mercury (II) to volatile mercury (0). The mercury (0) was separated from solution by passing the sample through a gas/liquid separator and purged with high-purity argon gas into the cell of the PS Analytical cold-vapor atomic fluorescence spectrometer (CVAFS) for detection. The concentration of mercury was determined by atomic fluorescence spectrometry at 253.7 nm. Quality assurance/quality control, with blanks, duplicates, and spiked samples, was completed as required by method 245.7 (USEPA, 2005).

2.5. Statistical analysis

t-tests were done for each comparison between the chloramine and chlorine levels used in the study (i.e., 1 mg/L chloramine versus 1 mg/L chlorine; 1 mg/L chloramine versus 10 mg/L chlorine; 10 mg/L chloramine versus 1 mg/L chlorine; and 10 mg/L chloramine versus 10 mg/L chlorine). An ANCOVA was done to determine whether each one of the variables had a significant impact on the mercury levels.

3. Results

3.1. Descriptive statistics

Data describing the mercury levels leached from the four different experimental groups and the deionized water control are presented here and summarized in Table 1. Both laboratory-derived and clinically derived amalgam samples were grouped together for each concentration. The mean mercury level in the 1 mg/L chlorine group was 0.020 mg/L (*n*=25, SD=0.008). The mean mercury mobilized from clinically obtained amalgam was found to be 0.020 mg/L (*n*=16, SD=0.009). The mean mercury mobilized from laboratory-prepared amalgam was 0.021 mg/L (*n*=9, SD=0.005).

For the 10 mg/L chlorine group, the mean mercury level was 0.59 mg/L (*n*=25, SD=1.06). The mean mercury mobilized from clinically obtained amalgam was found to be 0.17 mg/L (*n*=16, SD=0.16) and the mean mercury mobilized from laboratory-prepared amalgam was 1.3 mg/L (*n*=9, SD=1.5).

For the 1 mg/L chloramine group, the mean mercury was 0.023 mg/L (*n*=25, SD=0.010). The mean mercury mobilized from clinically obtained amalgam waste was found to be 0.020 mg/L

(*n*=16, SD=0.009) and the mean mercury mobilized from laboratory-prepared amalgam was 0.027 mg/L (*n*=9, SD=0.009).

For the 10 mg/L chloramine group the mean mercury level was 0.024 mg/L (*n*=25, SD=0.011). The mean mercury mobilized from clinically obtained amalgam was found to be 0.021 mg/L (*n*=16, SD=0.008), and the mean mercury mobilized from laboratory-prepared amalgam was 0.029 mg/L (*n*=9, SD=0.013).

For the deionized water control, the mean mercury concentration was 0.013 mg/L (*n*=19, SD=0.006). The mean mercury mobilized from clinically obtained amalgam waste was found to be 0.013 mg/L (*n*=13, SD=0.005) and the mean mercury mobilized from laboratory-prepared amalgam was 0.012 mg/L (*n*=6, SD=0.007).

Free residual chlorine data are presented here and summarized in Table 2. The mean free residual chlorine for the 10 mg/L chlorine group was 3.5 mg/L (*n*=16, SD=1.9) for the clinically obtained samples and 9.0 mg/L (*n*=9, SD=3.9) for the laboratory-obtained amalgam. The free residual chlorine for the 1 mg/L chlorine group was 0.70 mg/L (*n*=9, SD=0.60) for the laboratory-obtained amalgam samples. The residual free chlorine for the remaining groups and sample types was below the method detection limit (MDL=0.1 mg/L).

The monochloramine data are presented here and summarized in Table 3. The mean monochloramine level for the 10 mg/L chlorine group was 0.18 mg/L (*n*=16, SD=0.09) for the clinically obtained samples and 2.1 mg/L (*n*=9, SD=1.4) for the laboratory-obtained amalgam. The monochloramine level for the 1 mg/L chlorine group was 0.29 mg/L (*n*=16, SD=0.18) for the clinically obtained samples. The mean monochloramine level for the 10 mg/L monochloramine group was 2.2 mg/L (*n*=16, SD=0.80) for the clinically obtained samples and

Table 2 – Mean free residual chlorine levels (in units of mg/L) from the exposure of amalgam waste to chlorine (HOCl/OCl⁻) and monochloramine (NH₂Cl) at concentrations of 1 mg/L and 10 mg/L

Sample	DI water control	Chlorine		Monochloramine	
		1 mg/L	10 mg/L	1 mg/L	10 mg/L
Clinic amalgam	N/D	N/D	3.5 (1.9)	N/D	N/D
Lab amalgam	N/D	0.71 (0.61)	9.0 (3.9)	N/D	N/D

For clinically obtained samples, *n*=16; for laboratory prepared samples, *n*=9. The method detection limit for free residual chlorine determination is 0.1 mg/L. N/D means non-detectable. Standard deviations are in parentheses.

Table 3 – Mean residual monochloramine levels (in units of mg/L) from the exposure of amalgam waste to chlorine (HOCl/OCl⁻) and monochloramine (NH₂Cl) at concentrations of 1 mg/L and 10 mg/L

Sample	DI water control	Chlorine		Monochloramine	
		1 mg/L	10 mg/L	1 mg/L	10 mg/L
Clinic amalgam	N/D	0.29 (0.18)	0.18 (0.09)	0.41 (0.25)	2.2 (0.80)
Lab amalgam	N/D	N/D	2.1 (1.4)	0.35 (0.35)	2.4 (0.59)

For clinically obtained samples, $n=16$; for laboratory prepared samples, $n=9$. The method detection limit for monochloramine determination is 0.1 mg/L. N/D means non-detectable. Standard deviations are in parentheses.

2.4 mg/L ($n=9$, $SD=0.59$) for the laboratory obtained amalgam. The mean monochloramine level for the 1 mg/L monochloramine group was 0.41 mg/L ($n=16$, $SD=0.25$) for the clinically obtained samples and 0.35 mg/L ($n=9$, $SD=0.35$) for the laboratory-prepared amalgam. The monochloramine level for the remaining groups and sample types was below the method detection limit ($MDL=0.1$ mg/L).

The skewness statistic – grouping all mercury measurements together – was found to be very large (5.342), suggesting that the measurements are not normally distributed. For this reason, a natural logarithm was used to transform the mercury variable.

3.2. t-tests

The first independent samples t-test was the comparison between 1 mg/L chlorine and 1 mg/L chloramine. The average natural log mercury value for 1 mg/L chlorine was -3.99 with a standard deviation of 0.42. The average natural log mercury value for 1 mg/L chloramine was equal to -3.90 with a standard deviation of 0.54. There was no statistically significant difference between 1 mg/L chlorine and 1 mg/L chloramine in terms of the amount of the natural log mercury leached.

The second independent samples t-test was the comparison between 10 mg/L chlorine and 1 mg/L chloramine. The average natural log mercury value for 10 mg/L chlorine was -1.52 , with a standard deviation of 1.34. The average natural log mercury value for 1 mg/L chloramine was equal to -3.90 with a standard deviation of 0.54. There is a statistically significant difference between 10 mg/L chlorine and 1 mg/L chloramine in terms of the amount of natural log mercury leached ($p<0.001$). On average, there was a higher concentration of mercury present for the 10 mg/L chlorine variable when compared with the 1 mg/L chloramine variable.

The third independent samples t-test looked at the comparison between 1 mg/L chlorine and 10 mg/L chloramine. The average natural log mercury value for 1 mg/L chlorine was -3.99 with a standard deviation of 0.42. The average natural log mercury value for 10 mg/L chloramine was equal to -3.87 with a standard deviation of 0.56. There is not a statistically significant difference between 1 mg/L chlorine and 10 mg/L chloramine in terms of the amount of mercury leached.

The fourth independent samples t-test was the comparison between 10 mg/L chlorine versus the 10 mg/L chloramine. The average natural log mercury value for 10 mg/L chlorine was -1.52 , with a standard deviation of 1.34. The average natural log mercury value for 10 mg/L chloramine was equal to -3.87 with a standard deviation of 0.56. There is a statistically significant difference between the 10 mg/L chlorine and 10 mg/L chloramine in terms of the amount of natural log mercury leached ($p<0.001$). There was a higher concentration of mercury present on average for the 10 mg/L chlorine variable when compared with the 10 mg/L chloramine variable.

3.3. ANCOVA

The independent samples t-tests showed that there was a significant difference between the natural log mercury measurements of 10 mg/L chlorine compared to 1 mg/L and 10 mg/L chloramine. This did not take into consideration any of the other variables in the dataset: time, sample type and pH level. In order to determine whether there is an impact on the mercury levels after accounting for these variables, an ANCOVA was fitted to the data.

It was determined that each one of the independent variables was significantly related to the mercury concentration: treatment, sample (laboratory-generated vs. clinical amalgam), time and pH were all highly significant ($p<0.001$).

After controlling for the other variables, it was found that for every unit increase in the pH level, the natural log mercury concentrations would decrease by -0.522 U. For the time variable, it was found that there was a statistically significant difference between time 0 and 24 h ($p<0.001$), 2 h and 24 h ($p=0.040$) and 8 h and 24 h ($p=0.004$), but there was not a significant difference between 4 h and 24 h. For the sample type, there was a significant difference between the laboratory and clinically derived amalgam variables ($p<0.001$). Clinically obtained amalgam on average would have less mercury leached when compared to the laboratory-prepared amalgam samples.

4. Discussion

Chlorine has been used to disinfect drinking water since the 1860s, but only recently has the significance of chlorine-mediated production of disinfectant by-products been appreciated (AWWA, 2006). The presence of THM and HAA in chlorine-treated water led the USEPA to promulgate a regulation aimed at reducing THM and HAA levels in drinking water (USEPA, 2001). The new regulation has prompted some water treatment plants to switch to chloramine disinfection to decrease the amount of halogenated by-products in treated surface waters (AWWA, 1998, 2006; Renner, 2004). The USEPA's maximum disinfection concentration (for both chlorine and chloramine) is 4 mg/L (AWWA, 2006).

An unforeseen consequence of the switch to chloramine disinfection has been an increase in lead levels in drinking water (Renner, 2004; Switzer et al., 2006; Miranda et al., 2007). This led to the speculation that chloramine may mobilize mercury from amalgam in wastewater (Hammarback et al., 2007), as a substantial increase in dissolved mercury levels in the wastewater from one clinic seemed to coincide with the switch to

chloramine disinfection at that facility (Stone et al., 2006; Hammarback et al., 2007; Stone et al., 2007). This study was designed to investigate this hypothesis by comparing the ability of chlorine and chloramine to mobilize mercury from amalgam samples in a laboratory simulation.

The results demonstrate that chloramine does not mobilize mercury to the same degree as chlorine. Chloramine, while persisting for longer periods in water distribution systems, has a reduced oxidizing potential compared to chlorine (AWWA, 2006). This study demonstrates that, even at concentrations 2.5 times higher than what is deemed permissible by the USEPA, chlorine mobilizes 24.8 times more mercury than chloramine. The results suggest that the switch to chloramine was not the likely cause of the mercury spikes at the dental clinic, as chlorine disinfection used prior to the switch would have produced higher mercury levels in wastewater leaving the clinic.

This study used two different types of dental amalgam: laboratory-generated amalgam samples with a well defined particle size range (212–710 μm) and clinically obtained amalgam with an undetermined particle size distribution. The particle size distribution of amalgam in dental-unit wastewater is variable (Letzel et al., 1997) with 90% of the mercury located in amalgam particles greater than 10 μm in diameter (Cailas et al., 1994). A principal difference between the two types of amalgam used in this study may be the presence of finer amalgam particulate in clinically obtained wastewater samples.

There was a striking difference in mercury levels in the extracts from the clinically obtained amalgam samples and the laboratory-prepared amalgam in the 10 mg/L chlorine group. In this group, mercury levels in extracts from the laboratory-prepared samples were 7.6 times higher than the clinically obtained ones. This difference is likely due to the higher free residual chlorine levels in the extracts from the laboratory prepared samples. Extracts from the laboratory-prepared samples had a mean free residual chlorine concentration of 9.0 mg/L, compared to 3.5 mg/L for the extracts from the clinically obtained samples (2.6 times more free residual chlorine). The lower chlorine level in the extracts from the clinically obtained samples is likely due to larger amounts of organic materials in these samples. Organic material can consume residual chlorine, thus leaving less chlorine available to oxidize amalgam particles.

5. Conclusion

Data presented here do not support the suggestion (Hammarback et al., 2007) that the change from chlorine to chloramine disinfection triggered the dramatic increase in dissolved mercury levels seen in the one large dental treatment facility. Even at a concentration 2.5 times higher than the USEPA's maximum disinfection level, chloramine is less vigorous in mobilizing mercury than chlorine. While chloramine does appear to be able to increase lead levels in drinking water in some locations, and may even be responsible for increases in blood lead levels in exposed children (Miranda et al., 2007), chloramine is not as active as chlorine in mobilizing mercury from amalgam.

Disclaimer

The views expressed herein 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 U.S. Government. The use of commercially available products does not imply endorsement.

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