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Note

Changes in methylmercury concentration during storage: effect of temperature

Istvan Devai *, Ronald D. Delaune, William H. Patrick Jr., Robert P. Gambrell

Wetland Biogeochemistry Institute, Louisiana State University, Baton Rouge, LA 70803, USA

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Abstract

The widespread presence of methylmercury (MeHg) in the environment and the potential toxicity and bioaccumulation in the food chain has stimulated a demand for accurate and sensitive methods for the determination of mercury compounds in water, sediments, fish and other biological samples. A capillary gas chromatographic method followed by atomic fluorescence spectrometry (GC–AFS) was used to determine changes in MeHg concentration with time during storage in methylene chloride and as affected by storage temperature. Results of our laboratory experiments showed that significant changes in MeHg concentrations occurred during a 15-day storage period. Decreases in the MeHg concentration with time were uniform and at the end of the 15-day storage period only about half the initial concentration (recoveries were between 40.2 and 51.2%) remained in samples stored at various temperature. These results draw attention to the need for immediate analysis of MeHg samples following extraction. Temperature of storage was not a significant factor in the change in MeHg concentration. © 2001 Published by Elsevier Science Ltd.

Keywords: Methylmercury; Storage; Temperature

1. Introduction

Mercury is a widely distributed and persistent pollutant in the environment and is among the most highly bioconcentrated trace metals in the human food chain. Mercury has been used in numerous products and is present in coal, and its emission from combustion and other processes have resulted in widespread reports concerning its ecotoxicological importance. Mercury is found in elemental form and in various organic compounds and complexes (Alli et al., 1994; Cai et al., 1997a; Lindberg and Stratton, 1998). Methylmercury (MeHg) is one organic form of mercury and is far more toxic than elemental mercury. Several decades ago it was discovered that inorganic mercury compounds can undergo methylation in an aquatic environment, can bioaccumulate through the food chain, and can potentially result in severe (primarily neurodevelopmental) effects to humans (e.g. Minamata disease) if consumed in sufficient quantities (Vazquez et al, 1999).

The widespread presence of MeHg in the environment and the potential toxicity and bioaccumulation of MeHg in the food chain have stimulated a demand for accurate and sensitive methods for the determination of mercury compounds in water, sediment, fish and other biological samples (Alli et al., 1994; Cai et al., 1996, 1997a; Madson and Thompson, 1998; Beichert et al., 2000; Qian et al., 2000; Zhang and Lindberg, 2000). Most extraction methods and gas chromatographic detection procedures reported for the quantification of MeHg have shown one or more deficiencies (Alli et al., 1994; Hintelmann, 1999). The recommended species-specific detection method for MeHg analysis is capillary gas chromatography followed by atomic fluorescence spectrometry (GC–AFS) (Alli et al., 1994; Cai et al.,

* Corresponding author. Tel.: +1-225-578-8800; fax: +1-225-578-6423.
E-mail address: idevai@lsu.edu (I. Devai).

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1996, 1997a,b; Holz et al., 1999; Bloom, 2000). The most common solvent used in the final stage of the extraction is methylene chloride (Alli et al., 1994; Cai et al., 1996, 1997a,b; Bloom et al., 1997; Wagemann et al., 1997; Madson and Thompson, 1998).

Several studies in the literature reported the artificial formation of MeHg during sample preparation, derivatisation and detection (e.g. Falter et al., 1999). Concerns also exist regarding the stability of various mercury species in petroleum hydrocarbons (Bloom, 2000). Degradation of organomercury species has been reported by hydroxyl radicals and other reactive oxygen species (Suda et al., 1991; Suda and Takahashi, 1992). However, oxidative decomposition occurred only if strong hydroxyl radical producing agents (e.g. xanthine oxidase, copper-ascorbate) were present or if highly reactive peroxidase-hydrogen peroxide-halide systems were used. It has also been reported that there is a theoretical possibility for the photodecomposition of MeHg complexes (Tossel, 1998). However, there is no information reported for changes in MeHg concentration during storage, especially as influenced by storage temperature.

The purpose of this study was to determine changes in MeHg concentration during storage in methylene chloride as affected by storage temperature.

2. Materials and methods

2.1. Chemicals and materials used

Pesticide grade methanol and "GC RESOLV" methylene chloride (tested for use in high resolution gas chromatography) were obtained from Fisher Scientific. Crystalline methylmercury chloride (MeHgCl) (CAS# 115-09-3) and MeHgCl solution (1000 ppm Hg) in water were purchased from Alfa Aesar (Johnson Matthey Catalog Company, Inc.).

Forty milliliter precleaned vials for water sampling according to US EPA method 40CFR 136 (Cat. No. 2-3188) assembled with open-top screw caps with specially designed Teflon/silicone septa were obtained from Supelco, Inc. 12×32 mm (2 ml) amber autosampler vials and PTFE/silicone/PTFE aluminum seals and 10 μl 10R-GP SGE syringes were also purchased from Supelco, Inc.

2.2. Sample preparation

MeHgCl standard was prepared by dissolving appropriate amounts of MeHgCl in methanol in a 40 ml precleaned vial. This solution was subsequently diluted with methylene chloride to achieve the required concentrations necessary for a five-point calibration assay (between 0.2 ppb and 10.0 ppb Hg) and for the storage experiment (6.40 ppb Hg). Immediately after the dilution 43 amber autosampler vials were filled with 1.0 ml (6.40 ppb) solution and sealed immediately. Following this procedure the initial MeHg concentration in three replicates of these vials was analyzed by the GC–AFS method described later. Vials were placed into incubators or refrigerators and maintained in the dark (to prevent photodecomposition) at -25 ± 2, $+3\pm2$, $+21\pm3$ and $+38\pm1$°C for 15 days. Following incubation duplicate samples were analyzed and MeHg concentrations were determined after 1, 2, 4, 7 and 15 days of incubation at each preselected temperature levels. MeHg concentrations determined at the beginning of the experiment and after 1, 2, 4, 7 and 15 days of incubation were used to determine changes in MeHg concentration over time during storage.

2.3. Analysis and instrumentation

MeHg analysis was performed using a GC–AFS system. An integrated gas chromatography-mercury atomic fluorescence spectrometer included a Hewlett-Packard model HP 6890 Series Plus gas chromatograph and coupled to a PSA Merlin Detector via a pyrolysis oven maintained at 810°C. A fused silica analytical column with dimensions of 15 m×0.53 mm i.d. (Megabore) coated with a 1.5 μm film thickness of DB-1 (J&W Scientific) was used. The column oven temperature was maintained at 50°C for 1.0 min, programmed at 30°C/min to 140°C which was held for 3.0 min, then programmed at 30°C/min to 250°C, which was held for 3.0 min. A split/splitless injector was used in the splitless mode and maintained at 200°C. The carrier gas flow was 4.0 ml/min of high purity argon and make-up gas flow was 120 ml/min of high purity argon. The column eluate was passed through a pyrolyzer (P.S. Analytical) — positioned inside the oven of the gas chromatograph — via a deactivated fused silica tubing into a Merlin Mercury Fluorescence Detector System (AFS) Model 10.023 (P.S. Analytical) which was used for mercury detection. For the PSA Merlin Mercury Fluorescence Detector system, the sheath gas flow was 200 ml/min of argon (Alli et al., 1994; Cai et al., 1996). A real time chromatographic control and data acquisition system (Hewlett-Packard ChemStation) was interfaced with the GC and AFS detector system for the analysis.

Quantitative MeHg analyses were obtained using a five-point (between 0.2 ppb and 10.0 ppb) calibration curve forced to zero ($R=0.999$) generated using standard solutions which were prepared by dissolving appropriate amounts of MeHgCl powder in methanol and then subsequently diluting with methylene chloride to achieve the required concentrations. The calibration curve was checked using a secondary standard solution source (5.0 ppb) diluted from **a** MeHgCl solution (1000 ppm Hg) in water. The recovery was 97.7%. The

absolute detection limit calculated as three times standard deviation of the baseline noise (Cai et al., 1996) was 0.15 pg Hg for MeHg. The detection limit was determined by analyzing over 300 noise peaks of five separate baseline runs. During the calibration and the storage experiment, samples were analyzed at selected times by injecting 5.0 μl of MeHg solutions in methylene chloride into the chromatograph using 10 μL SGE syringes.

3. Results and discussion

The laboratory experiments showed that significant changes in MeHg concentrations occurred during the 15-day storage period (Table 1). After only one day of storage at each temperature the recoveries of the initial 6.40 ppb MeHg were between 88.7 and 89.6%. It is interesting to note that the observed concentration changes measured over time were not influenced significantly by the storage temperature. The largest change or decrease in MeHg concentration was observed at the lowest storage temperature. Decreases in the MeHg concentrations were uniform, and at the end of the 15-day storage period only about half the initial concentration (recoveries were between 40.2 and 51.2%) remained in samples stored at various temperatures. Contrary to expectation, the highest recovery after 15 days of storage (51.2%) was found at the highest storage temperature ($+38\pm1$°C) which was very close to the recovery rate of the "room temperature" storage (50.9%). The lowest recovery after 15 days of storage (40.2%) was found at the lowest storage temperature (-25 ± 2°C). Samples were not stored under inert atmospheric condition, therefore vials' headspace potentially may have contributed to losses of methylmercury observed over time.

These results draw attention to the need for immediate analysis of MeHg samples following extraction. Temperature of storage was not a significant factor in change in MeHg concentration.

Table 1
Changes in methylmercury concentration over time during a 15-day storage period as affected by temperature

Temperature (°C)	Days	Average (ppb)	Standard deviation (ppb)	Recovery (%)
-25 ± 2°C	0	6.40	±0.18	N/A
	1	5.68	±0.02	88.7
	2	5.50	±0.02	85.9
	4	4.43	±0.33	69.2
	7	4.41	±0.08	68.9
	15	2.57	±0.23	40.2
$+3\pm2$°C	0	6.40	±0.18	N/A
	1	5.70	±0.03	89.0
	2	5.41	±0.18	84.6
	4	4.84	±0.27	75.7
	7	4.28	±0.14	66.9
	15	3.04	±0.15	47.5
$+21\pm3$°C	0	6.40	±0.18	N/A
	1	5.70	±0.21	89.0
	2	5.33	±0.36	83.3
	4	4.49	±0.12	70.1
	7	4.36	±0.06	68.1
	15	3.26	±0.09	50.9
$+38\pm1$°C	0	6.40	±0.18	N/A
	1	5.74	±0.13	89.6
	2	5.39	±0.01	84.2
	4	5.02	±0.21	78.5
	7	4.74	±0.33	74.1
	15	3.28	±0.04	51.2

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