

# **Mercury in Plankton**

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**October 8, 1996**



# Mercury in Plankton

## 1.0 Subsample Collection

Samples were collected from the sampler cup at the base of the plankton net or the phyto-vibe nets by backwashing with lake water. The portion of the plankton suspension to be analyzed for mercury was then transferred to a 500 mL PFA Teflon jar and covered with its screw-on lid. This sample was carried into the clean room where a 20-30 mL subsample of the plankton suspension was collected using cleanroom techniques. A 10 mL automatic pipetter was used to transfer the sample to a cleaned, tared 30 mL PFA Teflon sample vial, which was sealed in a pre-marked polyethylene zipper bag. The bagged sample was then placed in a second polyethylene zipper bag which had been marked with the sample identifier. Once the log book had been filled out with sample number, location, type, date, initials, and observations, the sample was frozen.

## 2.0 Sample Containers

Samples were collected and stored in PFA Teflon vials and jars. These containers produce negligible Hg contamination of samples and can withstand extremely rigorous cleaning methods. New containers were purchased for this study.

## 3.0 Container Labeling

PFA Teflon is difficult to mark: laboratory ink markers do not produce permanent markings and adhesive labels do not stick well to PFA Teflon. Consequently, sample bottles were marked by engraving a unique sample number on both the bottle and the cap. These unique identifying numbers consisted of a letter followed by a two digit number (e.g., A07). The unique identifying number was used to track samples through all phases of sample collection, storage, processing, and analysis, and was cross-referenced to all sample attributes.

Polypropylene containers were used for storage of some reagents and solutions, and polyethylene bags were used for sample bottle storage; all were marked with permanent laboratory markers.

## 4.0 Cleaning of Sample Containers

Sample containers and other plasticware that may come in contact with samples were cleaned by one of the following two methods, depending on their composition.

### 4.1 Cleaning Procedure A (PFA Teflon and Other Fluoropolymers)

Items (sample bottles, stirring rods, large sample containers) were:

- Washed in hot tap water with a laboratory detergent (Alconox or similar product) in combination with a laboratory surfactant (Vers-A-Kleen or similar product).
- Rinsed repeatedly in hot tap water.

- Rinsed thoroughly in doubly-deionized water.
- Acid-washed in 50% HCl maintained at 70°C overnight.
  
- Rinsed thoroughly in doubly-deionized water.
- Soaked for four hours (minimum) in doubly-deionized water.
  
- Either:
  - dried in an inverted position on clean polystyrene racks in a dust-free environment;  
or
  - filled with 1% (v/v) HCl and sealed until use.
  
- Sealed with its own cap.
  
- Sealed in a polyethylene zipper bag subjected to cleaning Procedure B below. This bag was marked with the unique sample bottle identifier.
  
- Once sealed in the "inner" bag, the sealed sample bottle plus inner bag was sealed in a second, "outer", zipper bag subjected to cleaning Procedure B. This bag was also marked with the unique sample bottle number.
  
- Sample bottles sealed in inner and outer bags were stored in large polyethylene bags in cabinets in the laboratory. They were stored in clean coolers (ice chests) or PVC dry bags (the kind kayakers use) for transfer to, and during use on, the EPA lakes sampling vessel.

#### 4.2 Cleaning Procedure B (Polypropylene, Polyethylene)

Items (pipette tips, polyethylene zipper bags) were:

- Washed in hot tap water with a laboratory detergent (Alconox or similar product) in combination with a laboratory surfactant (Vers-A-Kleen or similar product).
  
- Rinsed repeatedly in hot tap water.
  
- Rinsed thoroughly in doubly-deionized water.
  
- Acid-washed overnight in room temperature 30% HCl.
  
- Rinsed thoroughly in doubly-deionized water.
  
- Soaked four hours minimum in reagent-grade water.
  
- Dried in an inverted position on clean polystyrene racks in a dust-free environment.
  
- Sealed in polyethylene bags that have been similarly cleaned.



## 5.0 Example Sample Label

Samples were uniquely coded at the time of collection including the unique sample bottle number, date, location, phase collected, sample volume, and whether they are duplicates or field blanks. An example of a sample label would be the following:

<u>A07</u>	<u>LM</u>	<u>5</u>	<u>6-16-94</u>	<u>Z</u>	<u>30 mL</u>	<u>a</u>
identifier	lake	station	date	phase	volume	replicate

The above example indicates a sample placed in sample bottle A07, from lake Michigan, Station 5, taken on June 16, 1994, zooplankton, a 30 mL subsample, and is replicate "a". All pertinent station information were cross-referenced and recorded in the field notebook. Identification of preservation methods on the label were not necessary as all samples were preserved in the same manner.

## 6.0 Calibration Procedures and Frequency

All field equipment, with the exception of the plankton nets, was maintained and cleaned by U of MN personnel. Field notebooks noted problems with field equipment. The cold vapor atomic fluorescence spectrometer (CVAFS) used for Hg analysis in this study was subjected to checks for resolution, sensitivity, and reproducibility of response factors (mV/mass Hg) before and during analysis of each batch of samples. Instrument calibration curves were produced before and during analysis of each batch of samples. All standards were obtained from commercial sources. Logbooks recorded dates of calibration, names and concentrations of standards used, result of calibration, and any corrective action needed and taken.

## 7.0 Sample Preparation

Prior to analysis, samples were prepared by lyophilization (freeze-drying). The operational status of the freeze-dryer was checked before sample preparation began. Double-bagged sample bottles were removed from the freezer and carried into the cleanroom. Label information was recorded for each sample placed in the freeze-dryer. Using clean-hands/dirty-hands techniques, the samples were removed from their outer bags and the sample and its inner bag were placed in a sample tray. In the cleanroom the inner bags were opened and the lids of the samples were loosened but remained on top of the samples. The status of the dryer and the samples was checked at least once during the first half hour of the process and then daily thereafter.

Upon removal from the freeze-dryer (usually five days) the sample bottles were tightly sealed and the inner bags closed. The samples were prepared for digestion back in the cleanroom.

## 8.0 Sample Digestion

A portion of the lyophilized sample ( $20 \pm 10$  mg) was transferred to a clean, pre-weighed 5 mL conical-bottom PFA Teflon digestion vessel and the weight recorded to  $\pm 0.1$  mg. For zooplankton samples, 4 mL of a 1:1 concentrated sulfuric ( $\text{H}_2\text{SO}_4$ ) and nitric ( $\text{HNO}_3$ ) acid mixture was added to the digestion vessel which was tightly sealed. For phytoplankton samples and zooplankton samples

weighing less than 10 mg, 2 mL of a 1:1 acid mixture was added. The digestion vessels were triple-bagged, and then placed in a hot (70°C) water bath overnight.

## 9.0 Sample Analysis

Samples were analyzed by cold vapor atomic fluorescence spectroscopy (CVAFS) using the double amalgamation technique of Bloom and Creelius (1983) as described in Claas (1996). The oxidizing acids, H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub>, were pre-reduced by reaction with hydroxylamine hydrochloride, which was added to the bubbler flasks prior to the introduction of the sample.

One mL of the digestate was added to the bubbler and allowed to react with the hydroxylamine hydrochloride for five minutes prior to the addition of 0.5 mL of the stannous chloride (SnCl<sub>2</sub>) reducing agent. A sample gold trap was placed on the bubbler behind a soda-lime trap and 0.5 mL of stannous chloride to the sample. The bubbler was sealed and the N<sub>2</sub> gas was turned on at 350 mL min<sup>-1</sup>. The sample was then allowed to bubble for 20 minutes.

The sample gold trap was then removed from the bubbler and placed in the analytical system upstream of the analytical gold trap. The argon (Ar) flow was turned on and calibrated to 50 mL min<sup>-1</sup>. The automatic sequence controller was then turned on, which heats the sample trap to thermally release its Hg onto the analytical trap, then heated in turn to release the Hg from the analytical trap into the CVAFS analyzer. The peak height and area were recorded for each sample.

## 10.0 QA

Standards, blanks, and bubbler blanks were run at the beginning and during each set of samples. Knowns (NIST standard reference materials, generally citrus leaves or apple leaves) were run along with the standards. Blanks, duplicates, and spiked samples were also interspersed with the samples such that the total number of QA samples constituted 25% of all samples run.

## 11.0 Data Reduction

Bubbler blank peak areas were subtracted from the peak areas of the standards and a response factor (ng peak area<sup>-1</sup>) was calculated for each of the standards (0.2, 0.4, and 0.8 ng Hg). The mean response factor was then used to convert peak areas for the samples into quantities of Hg analyzed. Values were then converted to concentrations of Hg per dry weight of plankton (ng g<sup>-1</sup>) by using the dilution factors and sample weights.