



Quality Assurance Project Plan

USEPA Region 1 - New England
Regional Environmental Monitoring and Assessment
Program

**Assessment of Mercury in Hypolimnetic Lake-
bed Sediments of Vermont and New Hampshire**

Addendum, with detail concerning

Assessment of Hg in Zooplankton

Assessment of Hg in Avian Piscivores



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13.1 Collection, Processing and Analysis of Zooplankton for Hg

General Overview

In order to estimate the concentrations of HgT in large bodied zooplankton within the study lakes, plankton collections and analyses will be performed during the 2000 field season. Large-bodied (>200 μ) zooplankton will be collected from each of the 48 lakes on which fish tissue data are available. Collections will be performed using specially designed non-metal plankton nets. This element of the project is being carried out in concert with efforts by Dartmouth University to characterize the trophic transfer of Hg and other trace metals through the plankton webs of northern New England lakes.

Large-bodied zooplankton will be collected from composited vertical net tows of known length, acquired from the pre-established REMAP sampling stations. In the field, plankton are concentrated, transferred to pre-weighed, lot-certified vessels, and reconstituted to 50ml. In the laboratory, the plankton are concentrated by centrifugation, with supernatant water being discarded after spinning. The remaining material is then dried at 60°C, and weighed to determine total sample dry-weight biomass. The dried plankton is then processed following the method for sediment HgT described in Section 4.0 of this document.

Quality Assurance Objectives

Field and laboratory Quality Assurance objectives for the zooplankton HgT and biomass estimates are identical to those described for sediment percent solid and sediment HgT in section 4.0 (above). Please note that there is no practical method to collect 'blank' solid plankton material for blank analysis. As with sediment HgT, standard reference material is used in lieu of blanks.

As for precision and accuracy, initial analyses of samples from 6 lakes suggests the following target QA indicators:

Field accuracy - the target relative percent difference between duplicate field samples is: NA%

Analytical accuracy - the target relative percent difference between replicate analyses is: 9%

Analytical precision - the target recovery for laboratory matrix spikes is: 100 \pm 15%

Comparability - The experimental design of this project element was established in consultation with the Dartmouth trace metals project investigators. The methods used by this REMAP project are such that direct comparisons can be made with the Dartmouth project datasets. Project data can also be compared against recently published zooplankton HgT values (e.g. Lucotte et al., 1999; Garcia and Carignan 1998)

Site Selection and Sampling Procedures

All plankton samples are collected in the immediate vicinity of the pre-established REMAP sampling station. Field procedures are described in the following protocol:

Protocol for Acquiring Zooplankton for Mercury Analysis for the REMAP Assessment of Mercury in Vermont and New Hampshire Lakes Project

Collections of zooplankton within the \leq 201 μ size fraction are to be collected with specially designed project nets which are fabricated of non-metal materials. The dimensions of these nets are 30 cm by 125 cm, \geq 201 μ , equipped with a detachable 200ml 'Dolphin' reduction bucket ([®]Wildlife Supply

Company, Saginaw MI). Collections are to be performed during a constrained time period to control for seasonal variation in the zooplankton assemblage. In the present study, sampling will be performed during August.

Summary:

For the HgT sample, a minimum of 5 tows will be collected from the immediate vicinity of the lakes' REMAP project sampling station, and the contents composited, reduced, then decanted to a pre-weighed, graduated 50ml sample vial, after which the total volume of the sample will be constituted to 50ml. The length of each individual tow composited will be recorded on the field sampling sheet. This sample will be used to measure HgT in the plankton, as well as total planktonic biomass. A modified clean-hands dirty hands protocol will be used for this collection, which is described in the detailed steps below.

Two additional samples will be collected for the purpose of taxonomic analyses. The first sample, the 201 u size fraction, will be composited from two individual tows, which is then decanted to a 50ml sample vessel, narcotized with CO₂, and preserved with formalin solution. The second sample, the 45 - 200 u size fraction, will be composited from two individual tows, which is then decanted to another 50ml sample vessel, narcotized with CO₂, and preserved with formalin solution. The length of each composite contributing tow will be recorded on the field sampling sheet.

Zooplankton-HgT samples will be handled in the same method as sediment samples, and in accordance with the REMAP Quality Assurance Project Plan.

Equipment:

- 201 u zooplankton net described above
- 45 u zooplankton net
- 200 ml lot-certified PETE 'compositing vessel'
- 500ml acidcleaned squeeze bottle (this should be re-cleaned after every tenth sampling event).
- 500ml squeeze bottle for CO₂ water (seltzer)
- CO₂ (seltzer) water
- 1 pre-weighed, pre-coded, lot-certified 50ml polycarbonate sample vessel
- 2 non-weighed 50ml polycarbonate vessels
- powder-free vinyl gloves
- protective plastic sheet 4'x 4' or larger
- field sampling sheet

Preparatory Steps:

Prior to going out into the field, a pre-coded 50ml sample vessel is weighed to the nearest 0.1 mg, and the weight and code recorded.

In the field, after the vessel has arrived at station and has been securely anchored, 'clean hands' and 'dirty hands' are designated. 'Clean hands' and 'dirty hands' don regular-length powder-free vinyl gloves.

A plastic sheet is draped over the gunwale of the sampling boat, such that the net will not have the opportunity to contact the boat.

'Dirty hands' removes and assembles the non-metallic net, and 'clean hands' and 'dirty hands'

jointly backflush the net 3X in lake surface water. The dolphin bucket is similarly rinsed.

Tows for HgT and Biomass Determination:

'Dirty hands' lowers the net to within 1 meter of the lake bottom, and rests the net 30 seconds to allow the water column to recolonize.

'Dirty hands' records the depth of this tow on the field sampling sheet.

'Dirty hands' retrieves the net at a rate of < 1 m per second.

When the net-hoop breaches the surface, 'dirty hands' lifts the net, and rinses the contents down along the net-sides using lake water and an acidcleaned squeeze bottle.

Once the sample is condensed into the dolphin bucket, 'clean hands' removes the bucket, further reduces the sample, and decants it into the 100 ml 'compositing vessel.'

This tow collection procedure is repeated until a minimum of 5 tows are collected. The field coordinator will determine if additional tows are necessary to obtain sufficient material for biomass and HgT analyses.

The contents of the compositing vessel is decanted to the 201u dolphin bucket, and the contents reduced to < 50ml volume.

'Clean hands' opens the 50ml sample vessel, rinses it 3X with lake water, and decants the reduced composite plankton material into the vessel. The vessel is then filled to 50ml with lake water, and capped tightly¹

'Dirty hands' opens a zip-bag, and 'clean hands' drops the filled 50ml vessel into the bag.

'Dirty hands' closes the bag and places it into the designated cooler for submission to the VTDEC LaRosa laboratory for analysis.

Tows for Taxonomic Analyses - 201 u:

Two additional tows are composited, using the 201 u net, into the compositing vessel using the procedure outlined above.

The contents of the compositing vessel is then covered with seltzer water, capped, and allowed to sit 60 seconds. At this time, the contents are returned to the dolphin bucket, reduced to the maximum extent possible, rinsed using the seltzer-squeeze bottle into a labeled 50 ml sample vessel, to approximately 25ml volume.

The sample is capped and allowed to sit 5 minutes. The sample is then opened, and filled to 50

¹Available REMAP project data indicates that epilimnetic water HgT concentrations are > 2 orders of magnitude smaller than plankton concentrations which were determined during the planktonHgT method demonstration. Such concentrations are unlikely to contaminate the zooplankton samples. Thus, it is recommended that the plankton sample be kept in the lake water from was obtained until the sample is dried for biomass determination and digestion in the laboratory.

ml with formalin-solution.

Tows for Taxonomic Analyses - 45-200 u:

Two tows are composited using the 45 u net, following the procedure outlined directly above.

While the 201 u dolphin bucket is held above the assembled 45 u net, the contents of the 45 u composite is passed through the 201 u dolphin bucket, and allowed to run out into the 45 u net. This step removes plankton in the >201 u fraction from the 45 -200 u fraction.

The 45u sample is then recondensed, and transferred back to the compositing vessel.

The contents of the compositing vessel is then covered with seltzer water, capped, and allowed to sit 60 seconds. At this time, the contents are returned to the dolphin bucket, reduced to the maximum extent possible, rinsed using the seltzer-squeeze bottle into a labeled 50 ml sample vessel, to approximately 25ml volume.

The sample is capped and allowed to sit 5 minutes. The sample is then opened, and filled to 50 ml with formalin-solution.

The taxonomy samples are submitted to Dartmouth University.

Analytical Procedures and Calibration

HgT is determined using the analytical procedures presented in Section 4 and Appendix B of the REMAP QAPP.

Biomass is determined gravimetrically as follows:

- The zooplankton HgT/biomass sample is removed from its plastic bag in the laboratory, and weighed to the nearest 0.1 mg.
- The sample tube is centrifuged, and dewatered of supernatant
- The sample is dessicated at 60°C
- The sample tube is re-weighed to the nearest 0.1 mg.
- Dry-weight biomass (% solid) is determined as the ratio of the sample weight to the sample wet-weight in 50ml water. This value is then converted to dry-weight biomass based on the total volume of water strained through the composite tows.

References:

Lucotte, M. R. Schetagne, N Therien, C. Langlois, and A Tremblay *eds.*, 1999. Mercury in the Biogeochemical Cycle. Natural Environments and Hydroelectric Reservoirs of Northern Quebec. Springer-Verlag

Garcia, E. and R. Carignan. 1998. Impact of wildfire and clear-cutting in the boreal forest on methyl mercury in zooplankton. Can J. Fish Aquat. Sci. 56:339-345

13.2 Collection , Processing, and Analysis of Avian Piscivores for Hg and for Waterbody Hg Risk Characterization

General Overview

We propose to use the Common Loon, Common Merganser (*Mergus merganser*), and Belted Kingfisher (*Ceryle alcyon*) as biological indicators of MeHg availability in New England lakes. Previous studies by the authors indicate each species is an adequate and logistically feasible indicator of MeHg availability in lake systems. Use of this piscivorous bird guild will provide (1) complete coverage of target REMAP lakes, (2) individual lake comparisons, (3) and an evaluation of the most at-risk species. Calculations of exposure from fish ingestion show the kingfisher ingests 40 μg of MeHg per kilogram of its body weight per day (EPA 1997), while our data indicate that the loon in northern Maine and New Hampshire lakes ingests 52 (male) and 57 (female) μg MeHg/kg of its body weight per day respectively.

Project Component Objectives:

1. Assess MeHg availability in eggs, blood, and feathers of target piscivorous birds using a random sampling outline established by the U.S. EPA for NH, VT, and ME.
2. Relate exposure data to multi-media sampling and apply to risk assessment models.
3. Evaluate MeHg impacts on the Common Loon, the species most likely to exhibit effects.

Justification for measuring MeHg effects on the Common Loon:

We propose to use the Common Loon as the target species for measuring MeHg effects because (1) it can be reliably captured and recaptured, (2) is easily monitored and observed throughout the breeding season, (3) exhibits high territorial fidelity, (4) can acclimate to moderate levels of human disturbance, (5) is long-lived and occupies a top trophic level, (6) has elaborate social, reproductive, and behavioral demands, and (7) has documented reproductive and behavioral impairments associated with elevated Hg levels.

Quality Assurance Objectives

Laboratory Quality Assurance objectives for bird HgT are provided below. Please note that there is no practical method to collect 'blank' tissue for blank analysis. Standard reference material is used in lieu of blanks.

As for precision and accuracy, analyses of bird matrices for two laboratories uses the following target QA indicators:

Field accuracy - the target relative percent difference between duplicate field samples is: NA%

Analytical accuracy - the target relative percent difference between replicate analyses is: 10% (UP) to 24% (UMO)

Analytical precision - the target recovery for laboratory matrix spikes is: $100 \pm 10\%$ (UP) to 24% (UMO)

Comparability - The experimental design of this project element was established in consultation with the Northeast Loon Study Working Group (a governmental and private collaborative of ecotoxicologists). The methods used by this REMAP project are such that direct comparisons can be made with recently published HgT values for loon matrices (e.g. Evers et al. 1998, Meyer et al. 1998,

Scheuhammer et al. 1998).

Site Selection And Sampling Procedures

Methods for collecting exposure information:

All sampling will use nonlethal methods. Blood and feathers are to be collected from captured adult and juvenile loons. Capture of loons uses nightlighting methods developed by Evers (1992). The technique of using vocalizations and playback recordings to attract a loon is most effective for capturing parental adults. Once within reach, the loon is scooped with a large dip net into the boat. The captured bird is then measured, banded, and blood and feathers taken before release in its territory 20-40 minutes later. Eggs are to be collected from abandoned nests. Flooding and human disturbance account for many loon nest failures.

Methods for evaluating effects:

In the past 8 years we have identified 6 behavioral, demographic, and physiological parameters that will best measure subtle MeHg effects. This proposal will directly contribute to determining the importance of developmental stability. Clarke (1995) considers the inability of an individual to develop bilateral characters to be one of the best estimates of developmental stability and suggested fluctuating asymmetry to be a sensitive measure (Polak and Trivers 1994). Second secondaries, used for Hg analysis, will be cut at a standardized location along the rachis and weighed on a digital scale.

Methods for handling samples:

Blood is drawn from the metatarsal vein through a leuc adapter directly into 5-10 cc vacutainers with sodium heparin (green tops). Vacutainers are opened once 10-14 hours later to add 10% buffered formalin (1:20 formalin-blood ratio) using USFWS protocols (Stafford and Stickel 1981, Wiemeyer et al. 1984). Each time, formalin is drawn from a sealed container with a new 1 cc syringe. The vacutainer with blood preserved by formalin is then placed in a refrigerator and not opened again until reaching the lab. Feathers are clipped at the calamus and placed in a polyethylene bag.

Methylmercury is locked in the keratin proteins in the feather and are not subject to degradation (Thompson 1996) and are cleaned in the lab to remove external contaminants. Whole eggs are frozen in a polyethylene bag after field removal. Frozen eggs are later cracked and the contents (including the inner shell membrane) are placed in I-Chem jars and are not opened until reaching the lab.

Methods for cataloging samples:

All samples are labeled in the field using a standard protocol which includes date, species, age, sex, band number, lake and territory name, and state. In the field lab, samples are listed on a form and another label is made based on the field form, compared with the field label, and added to the sample (therefore all samples are double labeled). A catalog accompanies the samples when sent to the analytical lab and samples are rechecked for errors before preparation for analysis.

Methods for analyzing piscivore tissues for Hg:

All loon samples are analyzed using cold-vapor atomic absorption spectroscopy at the University of Pennsylvania's toxicology lab (School of Veterinary Medicine, 382 W. St. Rd., Kennett Square, PA 19348) supervised by Dr. Bob Poppenga (610-444-5800 x2217). Analysis is for total Hg since methylmercury comprises 95% or more of the Hg in bird eggs, blood, and feathers (Thompson 1996). Preparation and analysis of egg contents for Hg concentrations are similar to those used for blood homogenizing and digestion. Detailed procedures from Dr. Poppenga's laboratory are included below.

All kingfisher and merganser samples are processed by the Leetown Science Center of the University of Maine, and analyzed for Hg using cold vapor atomic fluorescence spectrometry. Detailed procedures are included below.

Methods for data summary and analysis:

Field and lab data are placed in a database (Microsoft Access) and proofed by a field biologist. Data may be categorized or used as continuous points while making comparisons. Data are analyzed using SYSTAT 5.0 and Microsoft Excel Analysis ToolPak 5.0. Data are checked for normality using normal probability plots of residuals and for homoscedasticity with the Bartlett's Test. Data are frequently transformed to normalize variances. The type of transformation necessary is determined using the Box-Cox method.

Analytical Procedures And Calibration

Analytical procedures for total Hg in loon tissues (S.O.P's from the University of PA):

Organization:

Dr. Irine Rudik will oversee the laboratory mercury operations, including cleaning and preparation of sample containers, logging, inventory, and storage of samples, processing of samples, and analysis. Dr. Robert Poppenga will supervise quality assurance procedures and overall data interpretation.

Chemical Analyses:

All samples are analyzed for total Hg by UP SOP 06-02-00v2 (below)
All analyses are by atomic absorption spectrometry.

Data Management

Instrument operators are responsible for preliminary data reduction and validation. Instrument output is captured by appropriate software on desktop computers. Backup files are archived, and hard-copy print-outs are made of all output and placed in the project file. If the results of a matrix spike or standard reference material do not meet accuracy standards, all samples in that batch will be re-analyzed. If any sample does not meet precision standards, that sample will be re-analyzed. Dr. Bill Birdsall will review all preliminary data reduction and validation, and prepare draft data reports for review by Dr. Robert Poppenga. All project data will be recorded in computer spreadsheets for final analysis and reporting.

Assessment/Oversight

Surveillance: Analytical results are screened weekly by a supervisor to ensure that all QA procedures are being followed and that results are acceptable.

Peer Review: Sample splits are periodically submitted as blind samples to a second laboratory for confirmatory analysis.

Performance Evaluation: Instrument performance is evaluated through use of control charts. Analytical precision of total mercury will be calculated as the RSD for three or more replicate analyses. The precision objective is $\pm 10\%$ for total mercury. Accuracy for total mercury can presently be assessed by recovery of matrix spikes and standard reference materials (DORM 2, NRC, Canada). The accuracy objective is $100 \pm 10\%$ for spikes that are at least 10X the un-spiked sample concentration. Data quality indicators will be calculated as described in "Preparing Perfect Project Plans",

EPA/600/9-89/087, October 1989.

For mercury analyses, 10% of samples are split into laboratory duplicates. Random blanks will be included in each analytical run. During each day's operation, at least 5% of the samples analyzed will be distilled water blanks, matrix spikes, or known standards. The analytical detection limit for total mercury in whole blood, feathers and eggs is 25 ppb. Instrument performance is permanently recorded in instrument log books, which include a control chart for tracking instrument performance.

Audits: An audit during the first year of the project to demonstrate analytical precision and accuracy would be desirable. Random samples are interchanged with a least one other laboratory on a cooperative basis to provide independent checks on system performance. Routine analysis of standard reference materials (DORM -2, CNRC) certified for total mercury insures accuracy of the analytical systems.

Preventive Maintenance: Instrument manufacturer recommendations for service and maintenance are followed in all cases. Balances are serviced and certified annually. If performance of any instrument falls below norms, sample processing is halted and the manufacturer's trouble-shooting procedures are followed to identify and rectify the problem. Most instrument manufacturer's maintain a technical support program that is available for consultation on an as-needed basis.

Analytical Protocol for Mercury (and Selenium) in Avian Blood, Feather, and Egg

Sample Log-in, Organization and Storage: Samples are received via courier in plastic coolers. Sample identification sheets sent with the samples are copied and placed in the Loon Lab book. Blood samples are placed in the sample refrigerator at 4° C until analysis. Feathers are kept at room temperature until analysis. Fish fillets are kept at -24° C until analysis. On the day of analysis, sample identifications are checked with the copied master sheets and logged into the Loon Lab book. Amounts of each sample to be used for analysis are weighed and weights are recorded in the laboratory book.

Blood Sample Preparation

For each sample, assign one beaker for Se and one Tuftainer for Hg.

If the blood tube is over ½ full, empty some of the liquid into a plastic tube to overflow during grinding.

The remaining clot in the initial blood tube can now be ground with a small tissue grinder. Use a clean tissue grinder for each sample. Add the ground sample to the plastic over flow tube, if necessary, and grind again, making the entire sample homogeneous.

Check the sample for any remaining clots and continue grinding until a homogenous liquid is obtained.

Note and record the color and consistency of each sample.

Weigh a 1.0 g sample for Se analysis, and a 0.5 g sample for Hg analysis, and proceed with sample digestion and analysis.

Return any excess sample to the original blood tube for future use.

Feather Sample Preparation

Equipment:

Clean scissors

Colored tape

Permanent marker

Plastic feather washing containers

wrist action shaker

Two 250 ml beakers: one labeled HPLC grade acetone and the other labeled reagent grade acetone

Two 1000 ml beakers: one labeled milli-Q water and the other labeled waste acetone.

Reagent grade acetone

HPLC acetone

Millipore Type 1 water

Step A

Organize the samples according to animal # and mark a 50 ml plastic centrifuge tube with the corresponding number. On data sheet labeled with case # and date, write individual band #, animal #, and any unusual condition of feather such as “cut above down”.

Remove one secondary feather from the field bag and cut at the base of the vane or calamus at the beginning of the feather tract (this is the downy area of the feather that close-up looks like a “V”). This is to standardize the samples. Next, cut each feather in half by folding down barbules and cutting through the middle of the vane. Try not to lose any barbule sections in this process. This cut is necessary so the feather sections fit in the plastic washing containers. Transfer feathers into corresponding containers. Plastic tubes are then placed in a tube holder.

Pour reagent grade acetone into plastic containers to about 1/3 full and cap tightly. Agitate containers in holder for one minute and then decant carefully into waste container marked acetone. Be careful that no pieces of feather are lost.

1. Two repetitions with reagent grade acetone (includes steps already described).
2. Three repetitions with water.
3. Two repetitions with HPLC grade acetone.

Water can be decanted directly into sink.

After the last repetition, set samples in incubator with temperature of 37° C with the caps removed. Incubate overnight.

Refer to *Mercury by Atomic Absorption: Cold Vapor Hydride Generation Method* for further details of feather sample preparation prior to analysis.

Step B

Laboratory Clean-up

_ All glassware used for metals analyses must not be used for other purposes.

- _ Place dirty glassware in the container marked Dirty Metals Glassware.
- _ Wash this glassware in an Alconox solution and rinse well with tap water and then distilled water.
- _ Next, rinse glassware with a 30% nitric acid solution.
- _ Finally, rinse glassware with distilled water.
- _ Drain distilled water from glassware and air dry in container marked Clean Glassware.
- _ Store dry glassware in cabinet labeled Metals Glassware Only.

Egg Sample Preparation

Equipment

Permanent Marker

Blender with glass pitcher and stainless steel blades

Stainless steel or Teflon poker

Log-in book

Detergent

Distilled Water

1. Thaw eggs contents in I-Chem jars overnight.
2. Homogenize eggs in a blender (Whole chicks may have to be cut into smaller pieces to facilitate blending; poker might be needed to push “chunks” towards the blades).
3. When egg contents are thoroughly homogenized place contents back into the I-Chem jars they were removed from.
4. Freeze the homogenized egg contents making sure they are clearly labeled.
5. Thoroughly clean blender (pitcher and blades) and poker (if used) in hot soapy water. Alconox solution is best, but we can use household dishwashing solution.
6. Rinse blender with tap water removing all the soap
7. Rinse blender with distilled water obtained from a local college or university. (Distilled water from the store can be used, but university DDH₂O is better)
8. At the end of the day disassemble the blender and thoroughly wash and rinse with tap water and distilled water. Allow to air dry.

Protocol for Analysis of Samples

Set up the GBC 906 AA with H63000 hydride generation unit.

Weigh out 20 blood samples for Se analysis and 20 blood samples for Hg analysis. Include an SRM for

each batch of 20 samples as well as one sample duplicate for each set of ten samples.

Digest and prepare samples for analysis according to Se and Hg protocols (see above).

Employing the GBC FS3000 autosampler, analyze each set of 20 samples in turn on the AA. Run blank, four standards, sample blank, SRM and 10 samples plus one duplicate. Normalize the calibration curve with the 0.03 g/ml standard, run sample blank, SRM and the second set of 10 samples plus one duplicate. This method is described in detail below.

Mercury by Atomic Absorption: Cold Vapor Hydride Generation Method (UP SOP 06-02-00v2)

Purpose: The purpose of this analysis is the quantification of mercury in a variety of tissue matrices, including liver, kidney, and blood.

Reagents

- Concentrated H₂SO₄, reagent grade.
- Concentrated HNO₃, reagent grade
- Concentrated HCl, reagent grade
- Potassium permanganate solution: dissolve 10 g KMnO₄ (Fisher, P279-500) in 100 ml distilled water.
- Hydroxylamine hydrochloride solution: dissolve 12 g NH₂OH.HCl (Fisher, H330-100) in 100 ml distilled water.

- Mercury stock standard solution (10 mg/ml): dilute 1.0 ml mercury standard (1000 mg/ml, Fisher SM114-500) and 30 ml concentrated hydrochloric acid to 100 ml with distilled water.

- Mercury working standards (0.10 mg/ml, 0.05 mg/ml, 0.03 μ /ml, 0.02 μ /ml and 0.01 mg/ml).
 - 0.10 mg/ml: dilute 2.0 ml of the stock standard solution and 60 ml of concentrated HCl to 200 ml with distilled water.

 - 0.05 mg/ml: dilute 250 μ l of the stock standard solution and 16 ml concentrated HCl to 50 ml with distilled water.

 - 0.03 mg/ml: dilute 150 μ l of the stock standard solution and 16 ml concentrated HCl to 50 ml with distilled water

 - 0.02 mg/ml: dilute 100 μ l of the stock standard solution and 16 ml concentrated HCl to 50 ml with distilled water.

 - 0.01 mg/ml: dilute 50 μ l of the stock standard and 16 ml concentrated HCl to 50 ml with distilled water.

- Sodium borohydride solution: dissolve 3 g powdered NaBH₄ (Aldrich, 21-346-2) and 3 g NaOH in distilled water, and make up to 500 ml with distilled water. This solution is unstable, it may be stored for 3-4 days in a glass bottle in the refrigerator. Do not make the lid airtight, as hydrogen gas may evolve.

Sample Preparation

- Weigh out a 0.200 g sample of DORM-2 SRM into a tared Tuftainer. Weigh out 0.50 g quantities of the samples to be tested into tarred Tuftainers.
- Add 2.0 ml conc. H_2SO_4 and 1.0 ml conc. HNO_3 to each container and a blank, lightly tighten their tops, and place containers in the drying oven at 95°C for three hours. DO NOT USE PIPETS WITH INK LABELS - USE DISPENSING BOTTLES).
- Cool the samples, SRM, and blank to room temperature and add sufficient KMnO_4 to each container to ensure that color persists during the next heating step. Caution: this step may be exothermic.
- Heat samples, SRM, and blank at 95°C for 30 minutes.
- After cooling, reduce any remaining KMnO_4 using a slight excess of $\text{NH}_2\text{OH}\cdot\text{HCl}$ solution.
- Dilute each sample, SRM, and blank and 30 ml conc. HCl to 100 ml with distilled water.

Analysis

- Set up the mercury program -- see GBC Flame protocols. NOTE: this is a FLAMELESS method
 - Under Run Parameters, select flame OFF. DO NOT LIGHT THE FLAME IN THIS METHOD.
 - Set up hydride generation unit. Place the closed ended cell in its holder on the burner head, connect it to the white cap on the trap with the short section of tygon tubing, connect the two narrow bore teflon tubes to the trap, and also connect the drain tube.
 - Fill the labeled supply bottles with conc. HCl and NaBH_4 solutions and connect them to the system with their teflon tubes.
 - Starting from the back, stretch the three supply tubes over the pump head. Lock the pump in place with the upper rotating pin and the lower tension adjustment screws.
 - Turn on the nitrogen tank by opening the center valve; turn on the inert gas valve on the hydride generator.
- Turn on the red button on the hydride generator. Bubbles should be seen in the transparent block where the HCl and NaBH_4 solutions combine. Check the system for leaks.
- Employing the FS3000, aspirate blank (30% aqueous HCl) and 0.1 $\mu\text{g}/\text{ml}$ standard alternately until the absorbance for the 0.1 $\mu\text{g}/\text{ml}$ standard reads approximately 0.9 to 1.0 absorbance units.

- _ Run program. Aspirate blank (30% aqueous HCl) and five standards before the sample blank and samples. Allow at least 60 seconds before sampling to allow sufficient time for hydride generation and passage to the flame.
- _ Follow each sample and standard with a 20 second rinse using deionized water.

Reference: M. Sadiq, T. H. Zaidi, H. Al-Mohana, (1991), *Bull. Environ. Contam. Toxicol.* 47:335-341.

Preparation of Kingfisher and Merganser Tissue Samples

(Orono SOP: F10.9 Date Prepared: April 18, 2000)

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SOP for CEM MARS-X Microwave

I. EQUIPMENT

- _ CEM MARS-X microwave accelerated reaction system
- _ 14 HP-500 Plus digestion vessels (CEM corp.)
- _ Analytical balance (accurate to 0.001 g)
- _ Micropipette (up to 2000 mL)
- _ 14, 50 mL glass volumetric flasks
- _ 14 plastic funnels
- _ Plastic spatulas (for sample transfer)
- _ 14, 60 mL bottles of polyethylene composition (or suitable substitute)

II. REAGENTS

- _ 1000 ng Hg/mL 10% HNO₃ stock solution
- _ Concentrated Trace Grade Nitric Acid (HNO₃)
- _ 30 % Hydrogen Peroxide (H₂O₂)

III. PROCEDURE

- _ Set up a digestion log in the appropriate project book with the following format:
Acid Digestion of 99' DEP Wood Samples Using MARSX Microwave (Wood Method)

9-26-99 MP

| Sample ID | Digestate ID | Bomb ID | Sample Wt. (g) | Bomb Wt. (g) | Total Wt. (g) | Final Wt. (g) |
|-----------|--------------|---------|----------------|--------------|---------------|---------------|
| SRM 1575 | 9926401 | 2 | 0.264 | 162.3 | 178.2 | 177.7 |
| Blank | 9926402 | 3 | ----- | 158.6 | 173.5 | 174.4 |
| 011E/spk. | 9926403 | 4 | 0.485 | 157.3 | 175.7 | 174.8 |
| 011E | 9926404 | 5 | 0.492 | 157.4 | 175.4 | 175.2 |
| 012M | 9926405 | 6 | 0.475 | 158.3 | 175.0 | 174.6 |
| EtcI. | 9926406 | 7Uprobe | 0.479 | 157.6 | 176.2 | 175.3 |

1. Placed new rupture discs in each vessel
2. Added 25 mL of 30 % H₂O₂ and 10 mL of concentrated HNO₃ to each vessel
3. Spiked 9926403 w/ 0.250 mL of 1000 ng Hg/mL 10 % HNO₃
4. Placed in microwave and ran using Wood Method
5. Diluted to 50 mL w/ DIW and stored in 60 mL poly-bottles w/ green tape
6. Noticed unusual green coloration of 9926408
7. Particulate matter visible in 9926405 after digestion

Prep Notes (Keep these notes in mind as you read through and follow the actual procedure):

- Be sure to properly title each digestion with the sample type, microwave used and method used
- Be sure to initial and date each digestion
- Digestate ID's are derived from the Julian date on which the digestion is performed (9926401, 99 = year of digestion, 264 = Julian date, 01 = sample designator)
- Note the order of samples (SRM first, Blank second, Spike third followed by a duplicate of the sample that was spiked)
- Maximize each digestion, use all 14 vessels whenever possible which means a total of 11 samples can be run in addition to the necessary SRM, Blank and Spike for each run
- Record each step that is carried out in the digestion procedure and note any irregularities in the procedure such as unusual pressure or temp readings, unusual colors, particulate matter in the digestate, spilled sample, etc (see example above)
- Be sure to calibrate both the H₂O₂ and spike pipette to ensure that they are delivering 2.000 mL and 0.250 mL respectively (do this using Sartorius balance and DIW 1 mL water = 1 g)
- Take out spike before starting the digestion, this will ensure it has completely thawed by the time you need to use it
- Use no more than 0.500 g of any sample until you have established that greater sample will not damage the vessels (check to make sure pressure of a sample weight greater than 0.500 g does not exceed 325 psi)
- Always use a sample in the probe to protect against microwave damage from excess pressure
- Be sure to add the 2 mL of 30 % H₂O₂ and 10 mL of concentrated Trace Grade HNO₃ to all vessels (this includes the SRM, Blank, Spike and all samples) if additional purity is needed use Optima Grade HNO₃ and ULTREX H₂O₂ in place of above
- Bomb Weight is the weight of the empty bomb with no sample in (minus Kevlar liner), Total Weight is the weight of the bomb with sample and reagents in it (H₂O₂, HNO₃, spike, etc), Final Weight is the weight of the vessels once they have been removed from the microwave (post digestion) (these are all obtained using the OHAUS Scout Scale accurate to 0.1g). *Probe bomb weight should be taken without the load disc and with the Teflon cap over the pressure probe connection*

Digestion Procedure:

- Set-up digestion procedure in the lab notebook
- Replace rupture discs in all vessels
- Remove 1000 ng Hg/mL 10 % HNO₃ from the freezer and place in hood to thaw
- Record bomb weight, then transfer just the top section of the vessel (flip vessel top upside down and place sample in depression) to the SETRA scale, tare and add appropriate sample. record sample weight (repeat for all vessels)
- Add 10 mL of HNO₃ and 2 mL of 30 % H₂O₂ to the vessel (add 0.250 mL of Spike for the Spiked sample at this time) (repeat for all vessels)
- Record Total Weight of the entire vessel (repeat for all vessels)
- Place Kevlar liner over Teflon vessel, then place in the vessel containment unit, hand tighten bolt on top and then turn an additional 1/3 turn using wrench (repeat for all vessels except **probe: before wrench tightening attach pressure sensor to the probe, rotate into correct position then wrench tighten**)
- Place in the carousel in the proper order
- Place top on carousel (optional, helps give vessels support, especially when using < 14 vessels)
- Insert temperature cable into the probe vessel (BE CAREFUL; this is a fiber optic probe which is fairly flexible however it can not withstand sharp bends and rough handling. KEEP IN STORAGE BOX WHEN NOT IN USE)
- Turn the microwave on (insert the exhaust hose into the hood at this point)
- Insert the carousel into the microwave being sure that the carousel is properly sitting on the carousel platform
- Rotate the carousel to where carousel position 6 is lined up with the arrows on the front of the microwave using the rotate button on the keypad
- Insert the pressure probe into right side of microwave using only the large diameter seal for hand

placement

- Insert the temperature probe into the probe opening on the top of the microwave (there is a blinking red light that can be seen through the correct opening—the probe will snap in)
- Rotate the carousel 360° in each direction to ensure that the pressure and temperature cables do not touch (perform this using the rotate carousel button on the microwave control panel)
- From “CEM Method Menu” (if this is not displayed press “Home”) select “load method”, then select “digestion directory”, then select the appropriate method to match the sample. The selected method will now be displayed at the bottom of the “Home” screen
- Press START
- Keep an eye on pressure and temperature readings throughout the procedure to ensure that the temperature does not exceed the maximum temperature for the method and that the pressure does not exceed 320 psi
- **ERROR MESSAGES;** the following are three common error messages that may be displayed throughout the procedure causing the system to shut down:
 - o **Pressure/Temperature Drop Error, Pressure or Temperature has dropped too rapidly**
 - o Press 9 (continue) if the problem persists the carousel may have to be removed, temp probe reinstalled and method run again.
 - o **Warning, cannot Zero ESP-1500 Plus, Run Aborted**
 - o Press 2 (zero and continue)
 - o **EST-300 Plus Error, Communications Failure**
 - o Press 1 (reset) if the problem persists there may be problems with the temperature probe. If you have to press 1 more than once throughout a method, abort method and consult a service representative from CEM
- When method has ended allow the temperature to cool below 80° C if internal pressure is high then allow the pressure to drop to at least 60 psi before removing
- To remove from the microwave, first disconnect the pressure sensor from the microwave, then CAREFULLY grasp the temperature probe on the black ring near its microwave insertion point and pull straight down (do not jerk the probe out). PLACE TEMPERATURE PROBE BACK IN STORAGE BOX
- Vent all vessels in the hood prior to unscrewing the tops
- Replace Teflon cap on the pressure probe connection
- Remove the Teflon vessels and record Final Weight
- Carefully pour contents of vessel (digestate) into a 50 mL volumetric flask (using plastic funnel) rinse both the inside of vessel bottom and tops 3 times with DIW using the low flow DIW squirt bottle being sure not to exceed 50 mL total volume, bring to 50 mL total volume with DIW then carefully transfer to a 60 mL poly-bottle (or other appropriate bottle) labeled with the appropriate digestate ID
- Place 5 mL of concentrated HNO₃ and 5 mL of DIW into empty vessel
- Run Clean Cycle, this is performed same as above, just select Clean Method instead of a sample method (no weights need to be recorded)
- Dump contents of “cleaned” vessels into waste container labeled 50 % Nitric Acid in the fume hood
 - o Rinse the Teflon vessels 3 times with DIW and place upside down on adsorbent bench paper to dry (KEEP KEVLAR SUPPORT SLEEVES DRY)

Analysis of Hg in Kingfisher and Merganser Tissue

(Orono SOP F-10.11 Revised 9/23/98)

TOTAL MERCURY ANALYSIS USING THE MERLIN COLD VAPOR ATOMIC FLUORESCENCE SPECTROMETER

1.0 Scope and Application:

This method is for the determination of total mercury in water and solid matrixes using the Merlin fluorescence spectrometer.

2.0 Reagents:

2.1 Reagent Water (DIW): Water in which mercury is not detected by this method; 18 M ultrapure deionized water starting from a prepurified source.

2.2 Stannous Chloride (2% SnCl₂): Dissolve 10 grams SnCl₂ in 500 mL of 10% trace metal grade hydrochloric acid (add 50 mL HCl to ~ 400 mL DIW) and bring to 500 mL total volume with DIW. This solution should be prepared daily.

2.3 Nitric and Hydrochloric Acids: Trace metal grade from Fisher Scientific.

2.4 10% Nitric Acid: Dilute 100 mL HNO₃ to 1000 mL with DIW.

2.5 Mercury Stock/Working Standards:

a. 100 ppb (100ng/mL) Hg in 10% HNO₃: Add 0.1 mL of the 1000 ppm stock standard from Aldridge Scientific and 100 mL HNO₃ to ~ 800 mL of DIW in a 1000 mL volumetric flask. Bring to 1000 mL final volume with DIW. Mix well before proceeding. This solution should be stable for at least 1 month.

b. Calibration Standards:

| <u>Standard Conc., ppb</u> | <u>Vol. of 100ppb std., mL</u> | <u>Final vol.10% HNO₃, mL</u> |
|----------------------------|--------------------------------|--|
| 0.1 | 0.1 | 100 |
| 0.5 | 0.5 | 100 |
| 1.0 | 1.0 | 100 |
| 2.5 | 2.5 | 100 |
| 3.0 | 3.0 | 100 |

Concentrations can be varied based upon samples to be run. These are stored in the hood in the volumetric flasks used to make them (volumetric flasks should not be washed in order to help maintain stability of the standards). Calibration standards should be prepared daily.

2.6 Secondary QC Check Standard:

a. 100 ppb (100ng/mL) Hg in 10% HNO₃: Add 0.1 mL of the 1000 ppm stock standard from Spex Industries and 100 mL HNO₃ to ~ 800 mL of DIW in a 1000 mL

volumetric flask. Bring to 1000 mL final volume with DIW. Mix well before proceeding.
This solution should be stable for at least 1 month.

b. 1 ppb (1ng/mL) Check Standard: Add 1 mL of the 100 ppb stock standard and 10 mL HNO₃ to ~ 90 mL of DIW in a 100 mL volumetric flask. Bring to 100 mL final volume with DIW. This solution should be re-made daily.

3.0 Equipment List:

- 3.1 Merlin Cold Vapor Atomic Fluorescence Spectrometer.
- 3.2 PSA 20.100 Serial Sampler.
- 3.3 PSA Automatic Hydride Generator.
- 3.4 Volumetric Flasks.
- 3.5 Graduated Cylinders.
- 3.6 Glass screw-top sample vials with Teflon-lined caps.
- 3.7 Gases:
 - a. Grade V Argon with moisture trap for sample purge. Main flow is regulated prior to the instrument by an external flowmeter.
 - b. Reagent grade nitrogen for Nafion tube purge.
- 3.8 12" Nafion tube to decrease moisture in the sample stream.
- 3.9 Computer:
- 3.10 Water System:

4.0 Sample Preparation:

See the appropriate sample preparation SOP.

5.0 Procedure:

- 5.1 Turn on argon and nitrogen flows.
- 5.2 Turn on the instrument and associated accessories. The detector is left on all of the time.
- 5.3 Load the windows software by double-clicking on the 'Avalon' icon. There is a DOS version as well - it is loaded by pressing '1' for the first option when the computer boots up in DOS.
- 5.4 Make reagents and standards at this point to allow the system to warm up.
- 5.5 Select or create a new method from the 'Library' option and ensure that instrumental parameters are correct.
- 5.6 Empty standard vials and re-fill with new standard solutions. These vials are labeled and stored in the hood filled with the corresponding standard concentration - they need not be washed or changed unless there is indication of a problem.
- 5.7 Standards can be run as part of the run 'batch' or as a separate analysis ('new cal. curve' option under 'Calibration').
- 5.8 Samples can be set up in a batch using the autosampler or run individually as single analyses.
- 5.9 Most samples will need to be diluted. Start with a level biased low to help avoid contaminating the sample train. If samples are of the same matrix, the dilution level can be assumed to be the same for all. Any results not bracketed by the standards should be re-run at the end.
- 5.10 Turn on the peristaltic pump by depressing the button on the front of the unit. Establish flow in the tubing by pulling the metal arms of the pump forward ~ 3/4 of the way. Too

much tension can close off the tubing as well as decrease tubing life. The flow from the reagent containers can be used to determine the optimum tension position - it wants to be smooth with the SnCl₂ channel ~ 1/2 the HNO₃ channel.

- It is recommended that the tubing be replaced before each run. Tubing used for solid matrices and/or high level samples should not be used for water analysis or low-level samples.

- 5.11 Connect dryer tube (sample line) to the back of the detector.
- 5.12 Check the waste container and empty if necessary (neutralize contents).
- 5.13 Allow the reagents to flush the system for 15-20 minutes prior to beginning analysis.
- 5.14 Type sample information into the 'batch' file option found under 'analysis' and print out.
- 5.15 Fill autosampler based upon the above printout.
- 5.16 Standard and sample vials must be filled to the same height in the vial. Capillary action in the sampling probe causes slight variations in the amount of sample taken up.
- 5.17 Analyze standards with either the 'new curve' option or as part of the batch run. A curve will be automatically graphed once all standards have been run. This should be very linear. If the standards need to be re-run, re-fill vials and flush probe with the 10% reagent blank for a few seconds to avoid carryover into the blank standard analysis.

Note: To print peaks: there is a very short window of time allowed for this after each standard. A command key must be pressed to send the peak scan to the printer during this window, otherwise the scan is lost. It is recommended to print out peaks occasionally for QC and troubleshooting purposes.

- 5.18 Once an acceptable calibration curve is generated and printed, the batch file created above can be run. Under 'analyze', choose the 'batch' option. Begin analysis by addressing each computer check prompt as it appears.
 - 5.19 Monitor the system periodically as it is running to ensure the probe is being inserted into the vials and that the reagents have sufficient volume.
 - 5.20 Make note of any sample that will need to be re-run. These should be run at the end as opposed to holding until another run. This is so that all information concerning this sample and its associated batch (blanks, etc.) are together, making calculations and QC related determinations easier.
- 5.21 Clean-up and shut down:
- a. Flush all 3 channels with DIW for few minutes.
 - b. Flush all channels with 20% sodium hydroxide (NaOH) for 15 - 20 minutes (this step is especially important as it is the only way to remove any SnCl₂ buildup. If this is not removed, it will scavenge mercury from the sample train, thus lowering results.)
 - c. Follow this step with a 5 minute DIW flush, then a 10 minute 10% HNO₃ rinse.
 - d. Lastly, flush 5 minutes with DIW and then allow the tubing to pump dry.
 - e. Periodically, the quartz U-tube assembly will need to be carefully removed and cleaned more rigorously (i.e. immersing the entire quartz assembly in 20% NaOH for 30 minutes, rinsing with DIW then soaking in 25-30% HNO₃ for at least an hour - this step may be extended to standing overnight if time is a problem).
 - f. Neutralize contents of the waste container and flush down the sink.
 - g. Autosampler vials are first washed with soap and water then immersed in a 50% HNO₃ acid bath

for 24 hours.

5.22 Print out Results.

5.23 Shut down the system by turning off all components except the detector.

7.0 Analytical Quality Control:

An analytical batch is a set of not more than 10 samples analyzed with the same instrument calibration and QC data. Calibration standards should bracket the expected sample concentration range. An analytical batch must contain the required QC in order for generated data to be considered valid. All daily activities and problems are to be entered in the log book.

7.1 QC Sample:

A calibration QC is a sample at a concentration between 25-75% of the maximum value of the calibration range and prepared from a source independent of the calibration standard. The QC sample is used to ensure the analytical process is in control. This sample is run immediately after calibration and is expected to be within accuracy objectives. Additionally, QC sample concentrations are plotted on a control chart.

7.3 Blanks:

a. Reagent Blanks: These blanks include all chemicals and reagents used in the analysis.

Purging

reagents with mercury-free nitrogen or argon can reduce contamination to acceptable levels.

b. Bottle Blanks: These are sample bottles filled with DIW at the time of sample splitting and/or receipt and are treated as a sample. These blanks are preserved as for the associated samples and allowed to stand not less than 12 hours prior to analysis.

c. Field Blanks: This is a bottle filled with DIW and shipped with each set of samples. If the mercury concentration is equal to or greater than the MDL or is greater than one-fifth the level found in associated samples, the source of contamination needs to be identified and corrective action taken.

7.4 Analytical Duplicates:

Analytical duplicates are samples that have been collected, preserved, and prepared as one sample, but analyzed at separate times. These are analyzed at a frequency of 10% of the total sample number and are used to establish analytical precision and error. The relative percent difference (RPD) of each sample is calculated and recorded on the appropriate control chart. %RPD should be +/- 24.

7.5 Matrix Spikes (MS) and Matrix Spike Duplicates (MSD):

These are used to assess problems that may be associated with a given matrix. A sample (blanks may not be used) is spiked, in duplicate as follows:

- a. If the analysis is to be checked against a regulatory concentration limit, the spike level shall be at that limit or at 1-5 times higher than the background concentration of the sample, whichever is higher.
- b. If the analysis is not to be checked against a limit, the spike shall be at the concentration of the low-level working standard or at 1-5 times the background concentration, whichever is higher.

Calculate the relative percent difference (RPD) between the MS and MSD using the concentrations found in the MS and MSD. If the RPD does not meet the acceptance criteria of +/- 24%, the system is judged to be out of control. The problem must be identified and corrected, and the analytical batch reanalyzed.

$$RPD = 200 \times \frac{(|D1 - D2|)}{(D1 + D2)}$$

Where:

D1 = conc. in the MS sample

D2 = conc. in the MSD sample

Express the accuracy assessment as a percent recovery interval from P-2s to P+2s, where 'P' is the average percent recovery and 's' is the standard deviation of the percent recovery. This should be updated after every 5 to 10 new accuracy measurements.

7.6 Method Detection Limit (MDL):

Measurement of the method detection limit on a yearly basis is necessary in order to provide regular assessment of instrument/method performance, as well as a quantifiable concentration that will indicate when a measured value is above zero and is in fact detectable by the instrument. The MDL is defined as the minimum concentration of a substance that can be measured and reported with a 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte. The concentration of the low-level check standard should be 3 to 5 times the required detection limit. The detection limit is determined by analysis of seven replicates at a concentration 3 to 5 times the estimated detection limit and calculated by the student T-test as outlined in the 40 CFR part 136, Appendix B.

The laboratory must produce an MDL less than or equal to 0.2 ng/L or one-third the regulatory compliance limit, whichever is greater.

7.7 Control Charts:

Control charts are used to track variation of a process or analysis over time. The variation from the expected mean should exhibit a random distribution. Variation from the expected value may indicate instrument or human error. Persistent variation in one direction indicates bias. Control charts are expected to be within ± 2 standard deviations from the expected mean. The following general guidelines are useful for interpreting control charts. The results are expected to be distributed randomly about the mean or central line.

One point outside of the control limits is acceptable. Two consecutive points require investigation. Three or more points outside of the control limits mandate corrective action.

7.8 Laboratory Practices:

These include:

- a. Analytical balances are serviced yearly by a factory-authorized representative.
- b. Automated pipettors are checked on a monthly basis and prior to making primary standards.
- c. When a new calibration standard is made, it is compared to the standard being replaced to ensure consistency. Acceptance: 5%.
- d. Purified water system quality is monitored and conductivity is checked prior to use. There is a conductivity meter built into the still for continuous, on-line verification.
- e. Fume hood efficiency is metered and verified annually by University of Maine Office of Facilities Management.
- f. The drying oven temperature is documented daily. Adjustments are made as necessary. Acceptance: $75^{\circ}\text{C} \pm 3^{\circ}\text{C}$ or $50^{\circ}\text{C} \pm 3^{\circ}\text{C}$ if used for sample prep.
- g. All containers are labeled. Chemicals are dated and coded when received and expiration contents, date prepared, and initials of analyst. The chemicals and solutions are also entered in the appropriate log books; solutions are given a unique code based upon the date made.
- h. Routine paperwork and reagent tracking: Reagents are coded when received. When used, this code is noted in the 'comments' column in the reagent/standard log book.
- i. A log is kept for gas tanks. The date changed and tank ID number are recorded.

8.0 Instrument Maintenance:

All maintenance is recorded in the instrument log book. Routine maintenance is performed according to the instrument manufacturers recommendation. All maintenance is recorded in the maintenance logbook and in the runlog for the particular day.

9.0 Calculations:

9.1 Percent Relative Difference (%RPD) (for samples other than MS / MSD):

$$((\text{Sample} - \text{Duplicate}) / ((\text{Sample} + \text{Duplicate})/2)) \times 100 = \%RPD$$

9.2 Spike Recovery (%R):

$$((\text{Spiked Aliquot} - \text{Sample}) / \text{True Spike Value}) \times 100 = \%R$$

9.3 Response Factor (RF):

$$(\text{SnCl}_2 \text{ corrected area} / \text{standard conc.})$$

9.4 RSD:

$$100 \times (\text{std. dev.} / \text{ave. RF})$$

10.0 Notes:

10.1 Low or variable Peak Heights:

- a. Check U-tube for any deposits or cloudiness. This, in all likelihood, is due to SnCl₂. Clean as described in 5.21.
- b. Check tubing and connections for gas and/or liquid leakage.
- c. Check that the pump tubing has not slipped off the rollers of the pump. Tubing will travel if the tension is not tight enough.
- d. Check that standard concentrations are correct.
- e. Check for blockage in the tubing, especially at connections.

10.2 Sample probe freezes:

- a. Re-initialize the autosampler using the 'autosampler' option under 'accessories'.
- b. If 'a' does not work, turn off the autosampler and computer and re-load the software as in the beginning.
- c. Check that the sampler tubing is long enough for the sample locations being analyzed.

10.3 Calibration blank high:

- a. Flush tubing with 10% HNO₃ for a few minutes prior to re-analyzing.
- b. Replace tubing and/or sample probe.
- c. Vial has become contaminated.

10.4 Paying close attention to technique, cleanliness of the analytical environment, and regular routine

maintenance of the instrument will alleviate many progressive, long-term problems.

References:

Evers, D. C., J. D. Kaplan, M. W. Meyer, P. S. Reaman, A. Major, N. Burgess, and W. E. Braselton. 1998. Bioavailability of environmental mercury measured in Common Loon

feathers and blood across North American. Environ. Tox. Chem. 17:173-183.

Meyer, M. W., D. C. Evers, and J. Hartigan. 1998. Patterns of Common Loon (*Gavia immer*) mercury exposure, reproduction, and survival in Wisconsin, USA. Environ. Tox. Chem. 17:184-190.

Scheuhammer, A. M., C. M. Atchison, A. H. K. Wong, D. C. Evers. 1998. Mercury exposure in breeding Common Loons (*Gavia immer*) in central Ontario. Environ. Tox. Chem. 17:191-196.