Mercury Levels in Crayfish from Voyageurs National Park

Description of Work

The Environmental Physics Laboratory at the University of Minnesota Duluth will analyze crayfish, shipped to the lab by the National Park Service, for mercury concentrations. About 20-30 crayfish from each of 5 lakes within Voyageurs National Park are expected. In addition, a subset of over 150 crayfish donated to the National Par Service will also be analyzed. The estimated total number of crayfish to be analyzed is 121.

All samples will be analyzed for total mercury. Samples will also be analyzed for methyl mercury, but the number analyzed will depend on the values observed. That is, if the methyl mercury-to-total mercury ratios are nearly unity for a subset of 6 samples, then no further methyl mercury analyses will be required. On the other hand, if the methyl mercury-to-total mercury ratio is significantly different from unity, then more analyses may be required. The number of analyses to be performed in that case will be determined through discussions between the National Park Service and University staff.

Procedures

Sample Preparation. Specimens will be kept frozen in original containers until processed. Processing will consist of drying (60°C, 24 hr) followed by grinding with mortar and pestle. Ground samples will be sealed in zip-loc bags and stored in a freezer until digestion for analyses. Bags will be labeled with the same identification numbers used on the original containers. Weights will be recorded before and after drying to determine percent moisture.

Total Mercury Analysis Method. The procedure for total mercury in tissue analysis utilizes acid digestion followed by cold vapor atomic absorption spectrometry (CVAA), described in USEPA Method 245.6. Analyses will be performed on dried aliquots where percent moisture will also be determined. Results will be reported in terms of both dry and wet tissue weight.

Briefly, the method involves digesting approximately 100 mg of dried sample with nitric and sulfuric acids, followed by oxidation with potassium permanganate and potassium persulfate in 250 mL boiling flask. This is followed by neutralizing the oxidants with hydroxylamine sulfate and reduction of Hg(II) to Hg(0) by the addition of tin sulfate. The amount of mercury is determined by sparging the flask with air through an atomic absorption cell.

The detection limit is 2 ng/g of ww.

Methyl Mercury Analysis Method. The procedure for methyl mercury analysis utilizes a methanolic/alkaline digestion followed by ethylation, headspace sampling, gas chromatography (GC) separation, and atomic emission detection (AED). Sample digestion and ethylation procedures are similar to methods described by Swan (1998), Jimenez and Sturgeon (1997), and Cai and Bayona (1995).

Briefly, the method involves adding approximately 50 mg of dried tissue to a 15 mL polyethylene bottle (Nalgene) containing 3 mL of methanolic KOH. The sample is sonified for 1 hr and stored overnight at room temperature for analysis the next day. Approximately 50 µL of digestate are added to a 20-mL headspace sample vial containing 10 mL of DIW. Next, buffer is added (to obtain a pH near 5) and the sample vial is capped with a PTFE faced septum. After preheating the sample vial at 80 °C, 20 µL of ethylating agent (sodium tetraethyl borate) are added to the vial which is then briefly shaken vigorously. The ethylating agent converts the methyl mercury and Hg(II) species to the volatile methylethyl mercury and diethyl mercury forms, respectively. The sample is then placed in the headspace sampler for analysis.

The detection limit for methyl mercury in tissue is 0.3 ng/g ww .

Reporting

Results will be presented to the National Park Service in the form of an electronic spreadsheet. A text file describing the laboratory methods (including QA/QC procedures) will also be provided.

QA/QC

Laboratory personnel will wear latex gloves whenever working with samples to avoid in advertent contamination.

Precision will be monitored by duplicate analyses. These will be run on at least 10 % of all samples analyzed

Analyte recovery will be checked by analyzing spiked samples for a minimum of one of every fifteen samples analyzed.

Accuracy will be determined by analyzing a minimum of one certified standard (NRC DORM-2 certified dogfish) with each batch of samples.

Timeline

Analyses and reporting will be completed by May 31, 2003.

Budget

Project costs are all on a per sample basis. All QA/QC related analyses are at no charge. Per sample costs given below include sample preparation and reporting of results.

Item	Analyte	Cost per Analysis	Estimated No. to Analyze	Total Cost
Crayfish	Total Hg	$ 100	121†	$ 12,100†
Crayfish	Methyl Hg	$ 150	6†	$ 900†
			Misc. Supplies	$ 43
			Direct Cost Total	$ 13,000
			15% Indirect Cost	$ 1,957
				$ 15,000

†If it is determined that more methyl mercury analyses of crayfish are needed, then the number of total mercury analyses will be reduced in order for the total project cost to remain at or below $14,950.

Methods Used for Analyzing Crayfish for Total and Methyl Mercury

Seventy-nine crayfish specimens were collected from five lakes within Voyageurs National Park by National Park Service personnel in September, 2002. After measuring size and determining sex, samples were shipped frozen to the Physics Department at the University of Minnesota Duluth (UMD) for mercury analyses.

At UMD length, wet/dry weights, and percent moisture were measured as part of the processing for mercury analyses. Whole individuals were dried at 70 ˚C for 24 hours, ground by mortar and pestle, and stored at -10 ˚C in sealed plastic bags until needed for analyses.

The method used for total mercury analyses of the dried/ground crayfish follows EPA Method 245.6. The detection limit achieved at UMD for total mercury analyses was 1.7 ng/g and 0.5 ng/g for results expressed in terms of dry and wet weights, respectively (based on 80 mg dw sample and 74% moisture of original sample). The method used for methylmercury analyses follows the methods described by Swan (1998), Jimenez and Sturgeon (1997), and Cai and Bayona (1995). The detection limit achieved at UMD for methyl mercury analyses was 3.5 ng/g and 0.9 ng/g for results expressed in terms of dry and wet weights, respectively (based on 60 mg dw sample). These methods are summarized below and include details specific to analyses performed by UMD.

Analysis of Total Mercury in Tissue

Preparation of Potassium Dichromate Preservative

(used for storing standards)

1. Add approximately 1/2 liter of deionized water (DIW) to a 1-L volumetric flask.
2. Add 25 g of potassium dichromate (EM Chemical) to the flask (caution, reagent is toxic).
3. Using a graduated cylinder transfer 250 ml of nitric acid (trace metal grade, Fisher) to the flask. Do this very slowly. Mix by swirling the solution until the dichromate solid is completely dissolved.
4. Add DIW to obtain final 1 L volume.

Standard Preparation

Stock Solution

1. Add 500 ml of DIW to a 1 L volumetric flask.
2. Add 20 ml of potassium dichromate preservative and 40 ml of nitric acid.
3. Add 12.21 mg of the mercuric chloride reagent (Alfa) into a weighing boat and transfer to the volumetric flask.
4. Swirl to mix the solution, until the solid has dissolved.
5. Add DIW to obtain final 1 L volume.

Working standard

1. Add about 500 ml of DIW to a1-L teflon bottle (keep note of exact amount).
2. Add 20 ml of potassium dichromate preservative and 40 ml of nitric acid (Fisher)
3. Add 10 ml of the 9 ppm stock solution to prepare a 90 ppb solution.
4. The working standard is then diluted using DIW (make sure to calculate the correct volume) to a total volume of 1 liter.

Quality Control Standards

Quality control standards used.

Standard	ID	Hg Conc. (ng/g)	Source†
Dogfish Muscle	DORM-2	4640 ng/g	NRC
Oyster Tissue	1566a	64.2 ng/g	NIST

†NRC – National Research Council Canada
NIST – National Institute of Standards and Technology

Sample Digestion

1. Add ≈ 80 mg of dry tissue to a 250 mL flat bottom boiling flask with a standard taper neck.
2. Add 5 mL of distilled DIW to each flask.
3. Add 5 mL of conc. HNO_3 (Fisher) - swirl to mix.
4. Add 10 mL of conc. H_2SO_4 (Fisher) - swirl to mix.

5. Heat in water bath at 95-100° C for 1 hour (swirl to mix every 1/2 hr).
6. Add 15 mL of 5% $KMnO_4$ (JT Baker)- swirl and mix. If purple color dissipates, add more $KMnO_4$ until color persists: keep track of total amount added, so this total can be added to an additional blank (besides the blanks necessary for detection limit and regression needs) to quantify any increase of mercury in the sample. Heat an additional hour (swirl to mix every 1/2 hr).
7. Add 8 mL of 5% $K_2S_2O_8$ (Fisher) - swirl to mix, heat for 5 min; swirl to mix; heat another 5 min.
8. Add 50 mL DIW to each flask and allow to cool overnight.
9. On day two, add 6 mL of 12% NaCl/12% $NH_2OH{\cdot}H_2SO_4$ (Fisher / JT Baker) solution. Add distilled DIW to achieve a total volume of 150 mL.
10. Add 5 mL of 10% $SnSO_4$ (Fisher) solution and immediately connected the flask to the aeration apparatus.

Drying Tube Preparation

1. The drying tube is prepared by first plugging the bottom with glass wool.
2. Add approximately 15 to 20g of magnesium perchlorate (Aldrich) until it reaches the top of the tube. Tap the tube lightly on a firm surface until the drying agent reaches the shoulder of the top portion of the tube.
3. Plug the top of the tube with glass wool and attach top and bottom caps.

Atomic Absorption Analysis

Analyses apparatus is a closed loop consisting of a diaphragm air pump (Cole Parmer), an analysis flask, a drying tube, and an atomic absorption cell (within spectrometer – model 3200 LDC Analytical). All components are connected using Teflon tubing.

1. After the flask is attached to the aeration apparatus, the air pump is turned on and the elemental mercury is sparged from the flask into the air of the closed circulating system until the absorption peak has reached a maximum.
2. The pump is turned off and the sample flask is purged by drawing room air through the system (using a disconnect in the tubing) using a 2^{nd} pump.
3. Peak heights are recorded by a chart recorder (Linear) and are measured by hand with a ruler.

Analysis of Methyl Mercury in Tissue

Preparation of Standard Solutions

A standard solution of 1000 ppm methyl mercury in water was purchased (Alfa Aesar). This standard is diluted to create a stock standard of 400 ug/L which contains 10%

methanol (Alfa Aesar) for preservation. Working standards of 1 ng/g are prepared fresh for each analytical run from the stock standard. Both standards were kept in 250 ml Teflon bottles at 4 ˚C.

Quality Control Standard

The quality control standard was dogfish muscle (DORM-2, National Research Council Canada). The methyl mercury concentration is certified at 4470 ng/g.

Preparation of Ethylating Agent

A 1% solution of ethylating agent is prepared by adding about 250 mg of sodium tetraethyl borate (Strem) to a 40 mL Teflon bottle. Next, about 25 g of cold DIW (containing 65µL of 25% KOH) are added. The bottle is briefly swirled to dissolve the powder and then poured out in equal portions to 5 cold10-mL headspace vials. The vials are immediately capped with Teflon faced septa and placed in a freezer until needed. Caution: vapors are harmful – these steps should be carried out under a hood.

Sample Digestion

1. Add ≈ 60 mg of dry tissue to a 15 mL polypropylene centrifuge tube.
2. Add about 4 mL (record weight added) of a methanolic (metnanol from Alfa Aesar) solution containing 25% KOH (w/v).
3. Shake well and sonify tubes for one hour (shaking tubes by hand every 1/2 hour). Lay tubes on their sides in a dark area overnight at room temperature.

Sample Analyses

1. On day 2, shake each tube again and centrifuge for about 1/2 minute.
2. Prepare a 20 mL headspace vial (Agilent) by adding 9.75 mL DIW and 0.25 mL of a buffer solution consisting of 2M acetic acid (Sigma) and 2M potassium acetate (Sigma).
3. Tare the vial and add about 25 mg of the liquid digestate from step 4 (record actual weight).
4. Cap the headspace vial with a teflon faced septum and store at room temperature in the dark until analysis later the same day.
5. When ready for analysis, approximately 10 µL of ethylating agent are injected through the septum using a syringe and the sample is gently mixed. To accomplish this the vial containing the agent is slightly warmed in the hand in order to thaw the agent enough for this purpose. The agent is kept in the freezer between analyses of each sample.
6. After allowing reaction time of 15 min the vial is shaken vigorously for 1/2 min and placed into the headspace sampling unit.
7. The headspace of the vial is automatically sampled and injected into a gas chromatograph.
8. After separation, detection is accomplished using an atomic emission detector.

9. The areas of the chromatograph peaks are measured using Chemstation software (Agilent). All integration baselines are checked by hand and modified as needed to ensure accuracy.

The following is a list of instrument conditions used for these analyses:
Headspace sampler (model 7694, Agilent)

Oven, loop, and transfer line temperatures – 50˚C
Vial equilibration time – 0.0 min
Vial pressurization time – 1.0 min
Vial pressurization – 15 psi
Loop fill time – 0.1 min
Loop equilibration time – 0.05 min
Inject time – 0.4 min

Gas chromatograph (model 5890 series II plus, Agilent)

Column – HP-1 30 m x 0.53 mm x 3 μm
Carrier gas – He
Initial pressure – 15 psi
Initial time – 0.4 min
Then constant flow of 9.6 mL/min for 2.9 min
Then 25 psi for 10 min
Inlet – 100˚C
Initial column temperature - 40˚C for 0.4 min
Then column temperature ramps to 250˚C at 50˚C/min; hold 1 min

Atomic emission detector (model 5921A, Agilent)

Transfer line – 250˚C
Cavity block – 250˚C
Make-up flow – low
Reagent gasses – O_2 and H_2
Detection wavelength – 253.7 nm

QA/QC Procedures

The following are QA/QC procedures followed for both total and methyl mercury runs.

1. Standards and blanks covering the full range of samples are included with each analytical run.
2. Detection limits for each run are determined by 3 x the standard deviation of the blanks for the run. The average values for those are given above.
3. A minimum of two certified standards are included with each analytical run
4. Samples are run in duplicate at a minimum of once every 10 samples.
5. Spike recoveries are determined at a minimum of once every 15 samples.
6. All data entries to spreadsheets are checked for errors.
7. UMD determined lengths were checked against NPS determined lengths to verify

sample identification and/or to check for errors in that measurement.
8. Laboratory personnel wore latex gloves whenever working with samples to avoid contamination.

Mercury in Crayfish Survey within Voyageurs National Park

The objective of this study was to determine the importance of crayfish as a mercury source to crayfish consumers, such as otters, birds, and game fish, within Voyageurs National Park (VNP). Because crayfish can consume material both low (insects) and high (dead game fish) on the food chain it is not obvious what their mercury concentrations might be.

In the fall of 2002, 79 crayfish were collected from five of the smaller lakes (Brown, Jorgens, Oslo, Ryan, and Tooth) within VNP. Whole specimens were analyzed for total mercury (all samples) and methylmercy (majority of samples) concentrations. Methylmercury (MeHg) was included for analyses because it is the mercury form that is the most bioaccummulative and toxic. Although MeHg exists as only a small fraction (typically 1%) of the total mercury in lake water and sediments, it has been well documented that that form of mercury comprises over 95% of the mercury in game fish as a result of bioaccumulation. Thus, it is important know how much of the mercury in crayfish is in the bioaccummulative form.

Results of this survey showed an average total mercury concentration of 60 (± 22 std. dev.) ng/g. This is comparable to levels observed for young-of-year yellow perch in Rainy Lake (see Aquatic Synthesis for Voyageurs National Park, 2003). It was found that mercury concentrations are strongly correlated with crayfish size for Brown Lake ($r=0.83$, $p=0.0001$), while moderately correlated for Oslo and Ryan Lakes ($r=0.40$, 0.42; $p = 0.087$, 0.065), and not correlated for Tooth Lake ($r=0.26$, $p=0.76$). Only two specimens where captured from Jorgens Lake.

Significant differences in average mercury concentrations in crayfish occurred among the survey lakes. Averages ranged from a low of 36 ng/g for Brown Lake to highs of 70 ng/g for Tooth and Ryan Lakes. It is interesting to note that Tooth and Ryan lakes also exhibit the highest mercury concentrations in standard sized northern pike in the entire state of Minnesota.

Results of MeHg analyses showed, like fish, that most of the mercury is in the MeHg form. The average portion of the total mercury which is MeHg averaged 88% (± 9% std. dev.) across all analyzed specimens. This indicates that the mercury present in crayfish will be efficiently accumulated in crayfish consumers. The fraction of MeHg in crayfish was independent of crayfish size and total mercury concentration.

References

Cai, Y. and Bayona, J.M. **1995**. Determination of Methylmercury in Fish and River Water Samples Using in situ Sodium Tetraethylborate Derivatization Following by Solid-Phase Microextraction and Gas Chromatography-mass Spectrometry. *J. Crom. A 696*:113-122.

Jimenez, M.S. and Sturgeon, R.E. **1997**. Speciation of Methyl- and Inorganic Mercury in Biological Tissues Using Ethylation and Gas Chromatography with Furnace Atomization Plasma Emission Spectrometric Detection. *J. Anal. Atomic. Spectr. 12*:597-601.

Swan, H.B. **1998.** Aqueous Phase Ethylation Atomic Emission Spectroscopy for the Determination of Methylmercury in Fish Using Permeated Dimethylmercury Calibration. *Bull. Environ. Contam. Toxicol. 60*:511-518.

USEPA. **1994**. Methods for the Determination of Metals in Environmental Samples. EPA/600/R-94/111. Supplement I to USEPA 1991 document.

USEPA. **1992**. Code of Federal Regulations. 40 CFR Ch. 1 (7-1-92 edition) Pt. 136, Appendix B. pp. 565-567.

USEPA. **1991**. "Determination of Metals in Environmental Samples" EPA 600/4-91-010, Cincinnati, OH.