Total Mercury Concentrations in Mille Lacs Lake Yellow Perch

by

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Project Report

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GREAT LAKES INDIAN FISH & WILDLIFE COMMISSION
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Introduction

In September of 1999, 32 yellow perch were collected from Mille Lacs Lake (Minnesota) for total mercury analysis. Yellow perch were collected because eight bands of Chippewa have the opportunity to harvest around 100,000 pounds of perch annually from the lake and because perch from Mille Lacs Lake had not previously been comprehensively analyzed of total mercury.

The samples were delivered as whole fish to the Lake Superior Research Institute (LSRI) during October 1999. A chain of custody was started at the time of collection of the walleye can completed with the delivery of the walleye to the laboratory. Fish were frozen within 24 hours of collection. LSRI collected single skin-off fillets and analyzed them for total mercury (see Appendix 1).

Results

Yellow perch ranged in length from 5.5 to 13.1 inches and had total mercury concentrations of 0.028 to 0.182 µg Hg/g of fillet (Table 1). Total mercury concentrations in the fillets generally increased in a non-linear trend with increasing length of the fish (Figure 1). A two parameter non-linear power regression ($Y = aX^b$) was done using Sigma Plot software resulting in length explaining 75 percent (r^2=0.75, n=32) of the variability in the total mercury data.

Conclusions

The yellow perch from Mille Lac Lake are below 0.2 µg Hg/g of fish. This value of 0.2 0.2 µg Hg/g of tissue was a reference point used to model fish consumption data collected during the 3rd year of the GLIFWC Fish Consumption Study. At an average concentration 0.2 µg Hg/g of tissue, 100% of the participating family members were estimated to be below the EPA'a acceptable daily intake (i.e. reference dose) of 0.1 µg Hg/ kg of body weight/ day (Groetsch *et al.* 2001)

Thus based on this modeling, yellow perch from Mille Lacs Lake and other fish with mercury concentrations at or below 0.2 µg Hg/g of fish tissue, are believed to be safe for consumption by the most sensitive population segment (i.e., women of childbearing age, pregnant or breast feeding mothers, and young children).

References

Groetsch KJ, J Kreuger, N Kmeicik. 2001. Results from the third year of the Tribal Fish Consumption Study, 1999-2000. GLIFWC Project Report.

Table 1. The tag number, length, sex and total mercury concentration from fillets of perch collected from Mille Lacs Lake in September of 1999 and analyzed to mercury in 2000.

No.	Tag Number	Fresh Total Length (in)	Sex	Total Mercury Concentration (µg/g)[a]
1	219	5.5	Immature	0.0488*
2	220	6.8	Male	0.0497*
3	221	6.5	Immature	0.0420*
4	222	7.4	Immature - Female	0.0277*
5	223	6.7	Immature	0.0548*
6	224	8.3	Female	0.0487*
7	225	7.9	Female	0.0393*
8	226	5.6	Immature - Male	0.0342*
9	227	7.7	Female	0.0464*
10	228	13.1	Male	0.182
11	229	11.7	Female	0.119
12	230	10.4	Female	0.0631*
13	231	10.6	Female	0.120
14	232	10.0	Female	0.0799*
15	233	10.2	Female	0.0967
16	234	10.9	Female	0.123
17	235	10.7	Female	0.0922
18	236	11.1	Female	0.0815*
19	237	11.6	Female	0.106
20	238	9.8	Female	0.0484*
21	239	9.2	Female	0.0822
22	240	5.7	Immature	0.0362*
23	241	9.3	Female	0.0578*
24	242	9.0	Male	0.0624*
25	243	10.1	Female	0.102
26	244	8.8	Female	0.0540*
27	245	8.5	Female	0.0733*
28	246	8.6	Female	0.0793*
29	247	10.6	Female	0.0961
30	248	9.1	Female	0.0559*
31	249	9.5	Female	0.0597*
32	250	9.5	Male	0.0987

[a]: Concentrations denoted with an (*) are above the limit of detection but below the limit of quantification. These values are considered to have good accuracy, but values in this range may have a great degree of variation associated with the results.

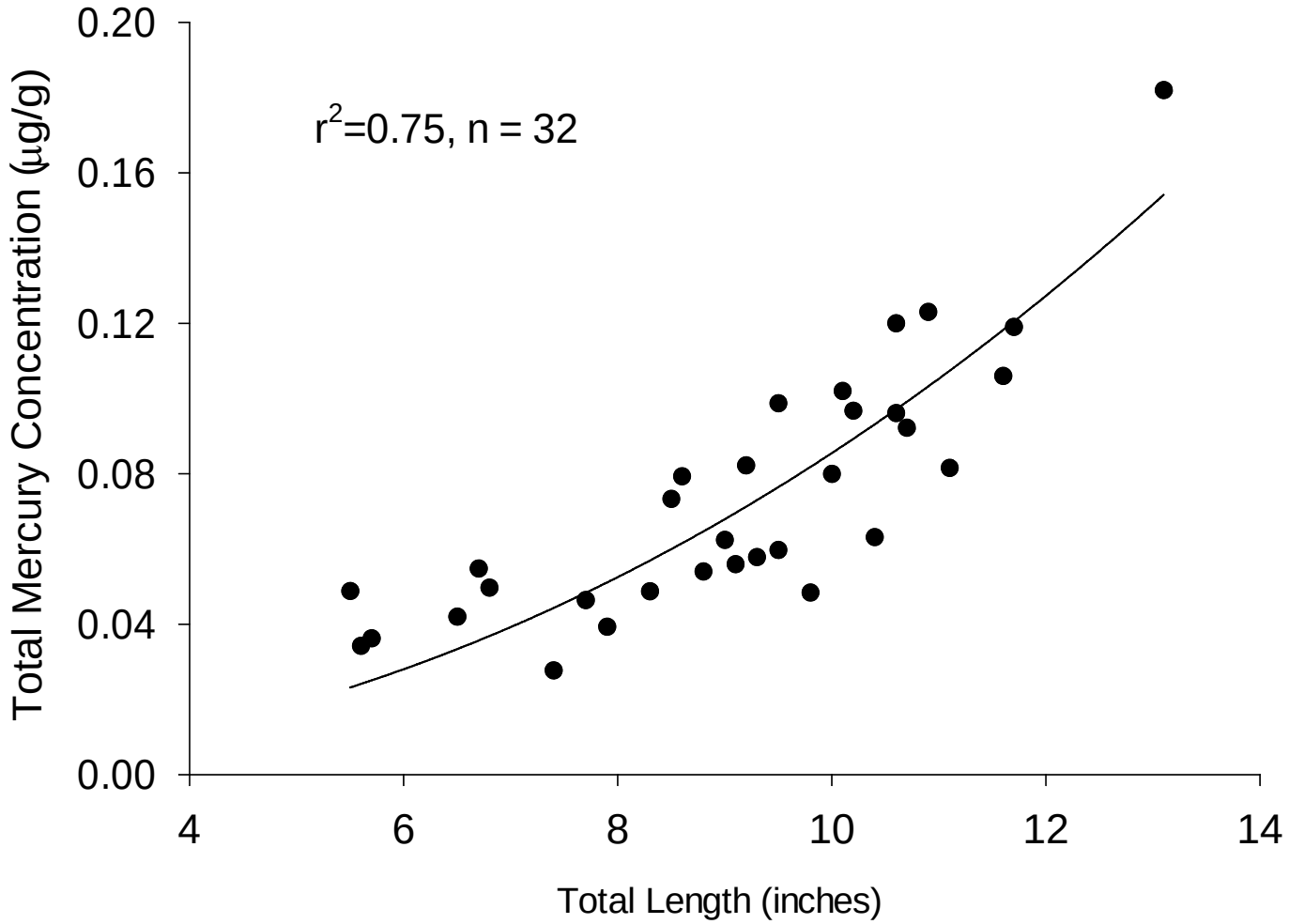

Figure 1. Total mercury concentration (µg/kg) in skin-of fillets versus the total length of Mille Lacs Lake perch.

APPENDIX 1

Lake Superior Research Institute Report entitled

Total Mercury Concentrations
in Yellow Perch from Mille Lacs Lake, Minnesota

Total Mercury Concentrations in Yellow Perch from Mille Lacs Lake, Minnesota

for

Great Lakes Indian Fish and Wildlife Commission
Odanah, Wisconsin

by

Environmental Health Laboratory
Lake Superior Research Institute
University of Wisconsin - Superior
Superior, Wisconsin

June 2000

Introduction

The fishing rights of the 1837 Treaty between the Chippewa Indian Nation and the United States Government were exercised in 1999. Health concerns for tribal members that eat fish taken from the various lakes requires that fish be monitored for chemicals of concern. Yellow perch (*Perca flavescens*) were captured by Great Lakes Indian Fish and Wildlife Commission fish assessment personnel and a sample was frozen and sent to the Environmental Health Laboratory at the University of Wisconsin - Superior, Superior, WI for analysis of total mercury.

Methods

The fish were captured with electrofishing gear between September 20 - 23, 1999 from Mille Lacs Lake, Aitkin County, Minnesota. They were placed in individual plastic bags and frozen the same day of capture. The fish were transported to the University of Wisconsin - Superior laboratory and kept frozen until processed for analysis. Processing included thawing the fish, fileting, and grinding of the skinless muscle tissue. A sample of the ground tissue was placed into a three dram glass vial and frozen until analysis for total mercury. When processing the fish, the fish were measured for total length and their sex determined.

Laboratory ware used in the analysis of tissues for mercury was cleaned prior to use according to the methods in Appendix A and B. Procedures for grinding the tissues are described in Appendix C. Weighing of the fish tissues in preparation for mercury analysis are described in Appendix D. Preparation of stock, standard, and spiking solutions are described in Appendix E. Analysis of the tissues for mercury was accomplished according to the method in Appendix F.

Quality Assurance

Several types of procedures and analyses were conducted to verify the quality of the mercury analysis with the fish tissues. A procedural blank was created from a commercially prepared can of tuna fish (*Thunnus* sp.). After opening the can containing the tuna fish, it was mixed and a portion removed for analysis without processing. The remainder of the tissue in the can was processed through the tissue grinder in the same manner as the walleye filet tissue, a portion was saved and analyzed for mercury. The result from a single sample was 76.8% agreement of analyses between the before and after processing. Acceptance criteria for agreement of this type of sample is 75% or greater. It is not unusual for the relative low agreement when the tissues contain low levels of mercury as did this tuna fish sample.

Muscle tissue from spiny dogfish shark (*Squalus acanthias*) that has a know concentration of mercury (4.64 µg/g) was also analyzed four times during the project to determine the accuracy of our analyses. The mean value from the analyses was 4.56 ± 0.487 µg/g for a 98.3% agreement. The agreement was considered to be very good agreement for analysis of the shark tissue.

Three fish (228, 235, 243) were analyzed twice to test for agreement of duplicate analyses. Duplicate sample analysis was of tissue that had been separately processed from raw tissue. Agreement ranged from 75.6 to 90.6% for a mean value of 85.0 ± 8.17%. The same three fish were spiked three times with known concentrations of mercury and analyzed for efficiency of recovery of mercury from the tissues (Table 1). Recovery of spikes averaged 93.09 ± 8.56%.

Table 1. Percent Recovery Efficiency of Yellow Perch Muscle Tissue Spiked with Known Concentrations of Mercury.

Fish Identification	Spike #1	Spike #2	Spike #3	Mean	Standard Deviation
228	91.4	94.7	93.4	93.2	1.66
235	96.2	100.4	78.7	91.8	11.5
243	80.3	99.1	103.6	94.3	12.4

Results

Thirty-two yellow perch ranging in total length from 13.8 to 33.5 cm were analyzed for total mercury concentrations (Table 2). Five of the fish were males and the remainder were female except for four that did not have their sex determined. Total mercury concentrations in the skinless muscle tissue ranged from 0.0277 to 0.182 µg/g wet weight. The Level Of Detection (LOD) was 7 ng for the instrument used to analyze the mercury content. The Level Of Quantization (LOQ) or the concentration that was measured with confidence above the baseline was 3.18 x LOD. For this data set, all values less than 0.0922 µg/g were below the LOQ. A relationship (Fig. 1) is apparent between the total mercury concentration in yellow perch muscle tissue and total length (cm). With the data available a linear regression was calculated ($y = -0.05841 + 5.70494x$; $r^2 = 0.6834$). Total mercury in the filet muscle of yellow perch is less than 0.2 µg/g (ppm) in all fish and is well below the FDA Action Level of 1.0 ppm.

Comparison of the yellow perch muscle concentrations of mercury was made with the walleye from the same lake that were captured and analyzed in 1998 (Fig. 2). Mercury concentrations in the muscle tissue increased with length (cm) and is described by the linear relationship $y = -0.1598 + 5.8318x$; $r^2 = 0.6703$. Walleye mercury concentrations ranged from 0.03 to 0.3 µg/g (ppm) wet weight. Walleye were approximately twice the size of the yellow perch and contained mercury at approximately 1.5 times that of yellow perch. Age of the walleye are known, but the yellow perch have not been aged. When aging of the yellow perch is completed, a comparison of the two species can be made on an equal age basis and the two species data points may coincide more closely.

Table 2. Total Mercury Concentrations in Yellow Perch Skinless Wet Muscle Tissue Captured in 1999 from Mille Lacs Lake, Minnesota.

Fish Identification	Fresh Total Length (in)	Frozen Total Length (cm)	Sex	Total Mercury Concentration (µg/g)
219	5.5	13.8	Immature	0.0488*
220	6.8	17.4	Male	0.0497*
221	6.5	16.7	Immature	0.0420*
222	7.4	18.9	Immature - Female	0.0277*
223	6.7	17.1	Immature	0.0548*
224	8.3	20.9	Female	0.0487*
225	7.9	20.3	Female	0.0393*
226	5.6	14.6	Immature - Male	0.0342*
227	7.7	19.2	Female	0.0464*
228	13.1	33.5	Male	0.182
229	11.7	29.6	Female	0.119
230	10.4	26.1	Female	0.0631*
231	10.6	26.8	Female	0.120
232	10.0	24.6	Female	0.0799*
233	10.2	25.9	Female	0.0967
234	10.9	27.7	Female	0.123
235	10.7	27.5	Female	0.0922
236	11.1	27.5	Female	0.0815*
237	11.6	29.4	Female	0.106
238	9.8	24.4	Female	0.0484*
239	9.2	23.7	Female	0.0822
240	5.7	14.6	Immature	0.0362*
241	9.3	24.2	Female	0.0578*
242	9.0	22.5	Male	0.0624*
243	10.1	25.8	Female	0.102
244	8.8	23.0	Female	0.0540*
245	8.5	21.6	Female	0.0733*
246	8.6	21.9	Female	0.0793*
247	10.6	26.4	Female	0.0961
248	9.1	23.2	Female	0.0559*
249	9.5	24.4	Female	0.0597*
250	9.5	24.2	Male	0.0987

* Value is below the LOQ.

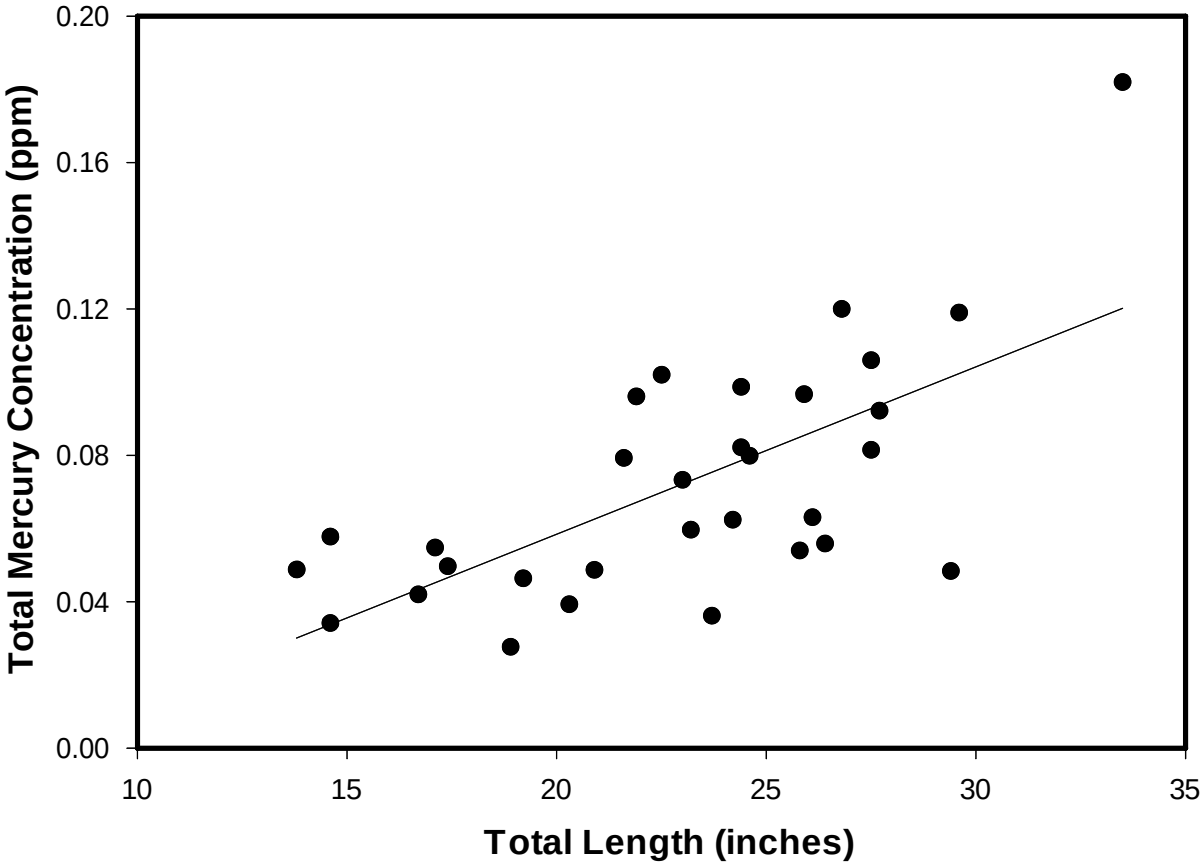

Figure 1. Relationship of total mercury concentration in skinless muscle tissue of yellow perch from Mille Lacs Lake, Minnesota in 1999 with total length of fish.. Regression line is $y = - 0.05841 + 5.70494x$; $r^2 = 0.6834$.

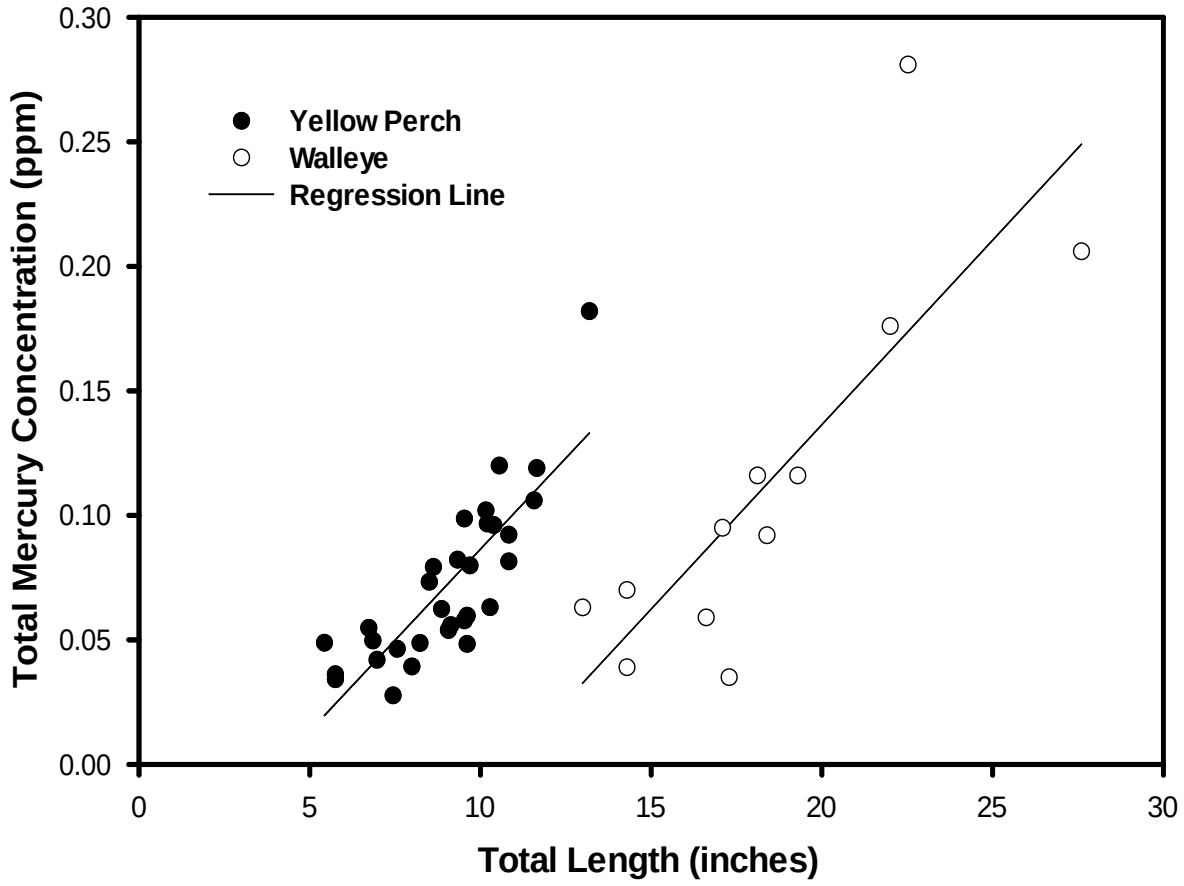

Figure 2. Comparison of skinless filet muscle total mercury in yellow perch captured in 1999 from Mille Lacs Lake with the total mercury concentration in walleye filets from the same lake captured in 1998.

Table 3. Total Mercury Concentration in Skinless Wet Muscle Tissue of Walleye (*Stizostedion vitreum*) Captured from Mille Lacs Lake in 1998.

Location of Capture	Fish Identification	Total Length (cm)	Sex	Total Mercury Concentration (µg/g)
Mille Lacs L.	855	42.2	M	0.059
Mille Lacs L.	856	43.4	M	0.095
Mille Lacs L.	857	43.9	M	0.035
Mille Lacs L.	858	46.7	M	0.092
Mille Lacs L.	859	49.0	M	0.116
Mille Lacs L.	860	46.0	M	0.116
Mille Lacs L.	861	36.3	M	0.039
Mille Lacs L.	862	36.3	M	0.070
Mille Lacs L.	863	33.0	M	0.063
Mille Lacs L.	865	70.1	F	0.206
Mille Lacs L.	867	55.9	M	0.176
Mille Lacs L.	868	57.2	M	0.281

APPENDIX A

STANDARD OPERATING PROCEDURE

COLD VAPOR MERCURY ANALYSIS - ROUTINE LABWARE CLEANING

INTRODUCTION

This cleaning procedure is used for the routine cleaning of labware being used during any cold vapor mercury analysis procedures. The proper safety equipment must be worn during the entire cleaning procedure. This includes gloves, goggles, and lab coat.

EQUIPMENT LIST

- Deionized Water
- Gloves
- Lab Coat
- Micro or Liquinox Detergent
- Various Labware Washing Brushes
- Plastic Dish Rack
- Plastic 14"x10"x10" HPDE tank with cover
- Ammonium Hydroxide, 30% (reagent grade)
- Nitric Acid, Concentrated (Reagent grade)
- Dish Pan
- Goggles
- Labware to be Washed
- pH Indicator Strips
- Wash Bottle

PROCEDURE: LABWARE CLEANING

1. Scrub the labware thoroughly in hot water containing Micro or Liquinox detergent.
2. Rinse the labware with hot water until there is no presence of soap.
3. Rinse the labware once with deionized water.
4. Place the labware in the plastic tank containing 10% nitric acid. Be sure the labware is completely filled with acid. Allow the labware to soak for a minimum of 60 minutes.
5. Remove the labware from the tank, emptying the acid back into the tank.
6. Rinse the labware three times with deionized water.
7. Place the clean labware in a plastic rack to air dry. When the labware is dry, cover the labware with a lid, stopper, or aluminum foil. Place the labware in a proper storage location until used.

PROCEDURE: PLASTIC TANK CONTAINING 10% (V/V) NITRIC ACID

1. Fill the tank with 14.4 liters of deionized water. Then add 1.6 liters of concentrated nitric acid and stir. The tank is now ready to be used to soak labware.
2. Every few months change the acid in the tank. Neutralize the acid with ammonium hydroxide until a pH of between 6 and 10 is achieved. Measure the pH in the tank with pH indicator strips.
3. Pour the neutralized acid down the drain with running cold water. Run the cold water for an additional 10 minutes.
4. Rinse the tank with warm tap water and then with deionized water. Fill the tank with 10% nitric acid as in step 1.

APPENDIX B

STANDARD OPERATING PROCEDURE

COLD VAPOR MERCURY ANALYSIS - MEAT GRINDER CLEANING

INTRODUCTION

This cleaning procedure is only required for meat grinder and labware being used for grinding of fish samples for cold vapor mercury analysis. The proper safety equipment must be worn during the entire cleaning procedure. This includes gloves, goggles, and lab coat.

EQUIPMENT LIST

- Plastic Pan
- Dish Pan
- Goggles
- Liquinox Detergent
- Various Labware Washing Brushes
- Meat Grinder
- Deionized Water
- Gloves
- Lab Coat
- pH Indicator Strips
- Wash Bottle
- Labware to be Washed
- Ammonium Hydroxide, 30% (Reagent grade)
- Hydrochloric Acid, Concentrated (Reagent grade)

PROCEDURE: MEAT GRINDER AND LABWARE CLEANING

1. Dismantle the meat grinder before washing.
2. Scrub the meat grinder components and labware thoroughly in hot water containing Liquinox detergent.
3. Rinse the meat grinder components and labware with hot water until there is no presence of soap.
4. Rinse the meat grinder components and labware with deionized water.
5. Place the meat grinder components and labware in a plastic pan containing 0.1 M HCl. Be sure that the meat grinder components and labware are completely immersed in the acid. Allow the meat grinder components and labware to soak for 30 seconds.
6. Rinse the meat grinder components and labware with deionized water.
7. Assemble the meat grinder which is ready to be used.

PROCEDURE: PLASTIC PAN CONTAINING 0.1 M HYDROCHLORIC ACID

1. Fill the plastic pan with 4 liters of deionized water. Then add 33 mL of concentrated hydrochloric acid and stir. The pan is now ready to be used to soak.
2. Periodically change the acid in the plastic pan. Neutralize the acid with ammonium hydroxide until a pH of between 6 and 10 is achieved. Measure the pH in the plastic pan with pH indicator sticks.
3. Pour the neutralized waste down the drain with running cold water. Run the cold water for an additional five minutes.
4. Rinse the plastic pan with warm tap water and then with deionized water. Fill the plastic pan with 0.1 M hydrochloric acid as in step 1.

APPENDIX C

STANDARD OPERATING PROCEDURE

COLD VAPOR MERCURY ANALYSIS - FISH GRINDING

INTRODUCTION

This procedure is for the grinding of fish filets into homogeneous samples. The meat grinder and labware used to grind the fish is cleaned by the "Cold Vapor Mercury Analysis - Meat Grinder Cleaning (SA/9)" procedure. The jars the ground fish samples are placed in are cleaned by the "Cold Vapor Mercury Analysis - New Labware Cleaning (SA/15)" procedure. The proper safety equipment must be worn during the entire grinding procedure. This includes gloves, goggles, and lab coat.

EQUIPMENT LIST

- ♦ Fish Filets Samples
- ♦ Gloves
- ♦ Lab Coat
- ♦ Spatula
- ♦ Aluminum Foil
- ♦ Tuna fish
- ♦ Food Processor with Grinding Attachments
- ♦ Filet Knife
- ♦ Goggles
- ♦ Grinder
- ♦ Beaker
- ♦ Scintillation Vials

PROCEDURE: GRINDING FISH Filet SAMPLES

1. Cut the fish filets into small pieces that will fit through the grinder feed tube or food processor with grinding attachments.
2. Pass the fish through the grinder or food processor, discarding the first few grams of tissue that come through. Collect the fish tissue in a beaker.
3. Mix the fish tissue with a spatula.
4. Repeat steps 2 and 3 an additional two times.
5. Place the fish in a previously acid-cleaned container. Seal securely with the screw top lid. Label the vial with the appropriate information and place in a freezer until analyzed.
6. Wash the grinder (or food processor) and labware by the "Cold Vapor Mercury Analysis - Meat Grinder Cleaning " procedure before grinding the next fish sample.
7. Continue to grind each fish sample by steps 1 - 7.

PROCEDURE: PREPARING THE PROCEDURAL BLANK

1. Drain a can of tuna fish to be used as the procedural blank. Grind half the tuna fish as a procedural blank by use of steps 2 - 7. Label the tuna fish as "ground" and include with the analysis set.
2. The other half of the tuna is left not ground and handled like a sample by use of steps
3. Label the tuna fish as "unground" and include with the analysis set.

APPENDIX D

COLD VAPOR MERCURY ANALYSIS - FISH SAMPLE WEIGHING

INTRODUCTION

This procedure is for the weighing of ground fish tissue for cold vapor mercury analysis. The fish should be ground by use of the "Cold Vapor Mercury Analysis - Fish Grinding" procedure. The labware used in this procedure should be cleaned by the "Cold Vapor Mercury Analysis - Routine Labware Cleaning" procedure. The proper safety equipment must be worn during this entire procedure. This includes gloves, safety glasses or goggles, and lab coat.

EQUIPMENT LIST

- Ground Fish Samples
- Goggles or Safety Glasses
- Nitric Acid (10%)
- Glass Bottles with Ground Glass Stoppers
- Balance Capable of Reading to the Nearest 0.001 g
- Gloves
- Lab Coat
- Spatula
- Kimwipes

PROCEDURE

1. Remove the fish to be analyzed from the freezer and allow to partially thaw.
2. Check the level of the balance and adjust if necessary. Clean the top of the balance of any foreign materials with a soft brush.
3. Zero the balance with the zero adjustment to read 0.000 g.
4. Place a clean glass bottle on the balance and measure weight. Tare the balance.
5. Weigh approximately 0.2 g - 0.3 g of fish tissue into the glass bottle.
6. Weigh and record the total weight of the glass bottle and fish tissue.
7. Rinse the spatula with water, 10% nitric acid and deionized water. Wipe the spatula clean with a Kimwipe.
8. Label and record each glass bottle and fish sample. Be sure that none of the fish tissue adheres to the side of the glass bottle.

APPENDIX E

COLD VAPOR MERCURY ANALYSIS - STOCK, STANDARD AND SPIKE PREPARATION

INTRODUCTION

This procedure is used for the preparation of the stock, analytical standards, blanks and spikes for cold vapor mercury analysis. The fish used for the spike should be weighed by use of the "Cold Vapor Mercury Analysis - Fish Sample Weighing (SA/11)" procedure. The labware used in this procedure should be cleaned by the "Cold Vapor Mercury Analysis - Routine Labware Cleaning" (SA/8) procedure.

EQUIPMENT LIST

- Ground Fish Samples for Spikes
- Class "A" Pipets
- Wash Bottle
- Pipet Bulb
- Mercuric Chloride, Reagent Grade
- Nitric Acid, Concentrated (TraceMetal Grade)
- Deionized Water
- Mercury Waste Container
- 1,000 mL Plastic Graduated Cylinder
- Kimwipes
- Glass Bottles with Ground Glass Stoppers

PROCEDURE: STOCK PREPARATION

1. Weigh out 0.1355 g ± 0.0050 g of mercuric chloride into a 100-mL volumetric flask.
2. Add 10 mL of concentrated nitric acid (trace metals grade).
3. Dilute to volume with deionized water.
4. Calculate concentration of the mercury stock solution. Use the following calculation:

$$\frac{\text{mass of } HgCl_2 \text{ (g)}}{271.50 \text{ g/mol } HgCl_2} \times \frac{200.59 \text{ g mol Hg}}{100 \text{ mL}} \times \frac{\text{purity (\%)}}{100\%} \times$$

$$\frac{10^6 \text{ µg}}{\text{g}} = \text{concentration (µg Hg/mL)}$$

PROCEDURE: STANDARD AND SPIKE PREPARATION

1. Pipet 10 mL of the ~1000 µg/mL mercuric chloride stock solution into a 100-mL volumetric flask containing 10 ml HNO_3 and diluting to 100 mL with deionized water to prepare a ~100 µg/mL mercury sub-stock.
2. Pipet 5.0 mL of a ~100 µg/mL mercuric chloride stock solution into a 100-mL volumetric flask containing 0.5 mL of concentrated nitric acid and dilute to volume with deionized water to prepare a ~5000 ng/mL Hg sub-stock.
3. Pipet 1.0 mL of the ~5000 ng/mL mercuric chloride stock solution into a 100-mL volumetric flask containing 0.5 mL of concentrated nitric acid and dilute to volume with deionized water to prepare a ~50 ng/mL Hg sub-stock.

4. Calculate the concentration of the mercury sub-stocks using the following equation: $C_1 V_1 = C_2 V_2$ where: C_1 = conc. of Hg stock solution; C_2 = conc. of diluted solution; V_1 = volume of stock solution; V_2 = volume of diluted solution.
5. Prepare standards with the approximate concentrations: 25, 50, 100, 200, and 300 ng of mercury by pipetting 0.5, 1.0, 2.0, 4.0, and 6.0 mL of the ~50 ng/mL Hg sub-stock into separate bottles. Determine the amount of Hg added to each bottle in ng. Use the following calculation:
   ng of Hg = conc. of Hg sub-stock (ng/mL) X mL of sub-stock used.
6. Add deionized water to the bottles with mercury standards so that each bottle has an equivalent volume of liquid (i.e., pipet 5.5 mL of deionized water into the 25 ng mercury standard bottle).
6. Each standard should be prepared in triplicate.
7. Label and record the bottle and concentration of mercury added for each of the standards prepared.
8. Additional standards can be prepared if necessary, as mercury has a linear response curve up to 2000 ng.
9. Three to five reagent blanks (containing 6 mL of deionized water) should be prepared with each analysis set.

PROCEDURE: 1% (V/V) NITRIC ACID PIPET SOAKING SOLUTION

1. Place enough glass wool in the bottom of a previously cleaned 1,000-mL plastic graduated cylinder to cover the bottom.
2. Fill the graduated cylinder with approximately 800 mL of deionized water.
3. Add 8 mL of concentrated nitric acid to the graduated cylinder and stir.
4. Pipets used for mercury analysis should be soaked in this solution when not in use.

APPENDIX F

STANDARD OPERATING PROCEDURE

COLD VAPOR MERCURY DETERMINATION

INTRODUCTION

This procedure is used for the determination of total mercury in hair, fish, and other tissue samples. Do not use this procedure for analyzing human blood.

REFERENCES

"Determination of Mercury in Tissues by Cold Vapor Atomic Absorption Spectrometry", Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268, April 1991.

EQUIPMENT LIST

- Stannous Chloride, Analytical Reagent
- Magnesium Perchlorate, Anhydrous for Elemental Analysis
- Potassium Persulfate, Reagent Suitable for Mercury Determination
- Hydroxylamine Hydrochloride, Reagent Suitable for Mercury Determination
- Potassium Permanganate, Certified A.C.S.
- Sodium Chloride, Certified A.C.S.
- Sulfuric Acid, A.C.S. Reagent, Suitable for Mercury Determination
- Nitric Acid, Fisher, Trace Metals Grade
- Mercury Cold Vapor Analyzer
- Hollow Cathode Mercury Lamp
- Variable Autotransformer
- Neptune Dyna-Pump Model 4K
- Hot Plate
- Instrumentation Laboratory Video 12 aa/ae or Varian SpectraAA 200 Spectrophotometer
- Meat Grinder
- Labindustries Repipet II Dispenser, 3 - 10 mL and 1 - 5 mL
- Wheaton Instruments Socorex Dispenser Model 511, 10 mL
- Glass Bottles with Ground Glass Stoppers
- Pipets/Pipettors
- Beakers
- Volumetric Flasks
- Spatulas
- Water Bath 18"x30"
- 5% (w/v) Potassium Permanganate
- 5% (w/v) Potassium Persulfate

- 10% (w/v) Hydroxylamine Hydrochloride-10%(w/v) Sodium Chloride
- 10% (w/v) Stannous Chloride-0.5M Sulfuric Acid
- 0.05M Potassium Permanganate-5% (v/v) Sulfuric Acid
- 1000 µg/mL Mercuric Chloride Stock
- 5 µg/mL Mercuric Chloride Sub-stock
- 50 ng/mL Mercuric Chloride Sub-stock

PROCEDURE

Digestion

1. Add 4.0 mL of concentrated sulfuric acid and 1.0 mL of concentrated nitric acid to each sample, standard, spike, duplicate and blank and stopper.
2. Place the bottles in hot water bath at 80-90^{o}C and allow to digest for approximately 15 minutes or until all the fish tissue is dissolved.
3. Vent the bottles occasionally during the heating process.
4. Turn off the hot plate and allow the bottles to cool to room temperature.
5. Add 5.0 mL of 5% potassium permanganate to each bottle in 1.0 mL increments swirling the bottles after each addition.
6. Add 10.0 mL of 5% potassium permanganate to each bottle in 5.0 mL increments, swirling the bottles after each addition. Additional 5% potassium permanganate solution should be added to the samples if necessary to that the samples remain purple in color for at least 15 min.
7. Add 8 mL of 5% potassium persulfate to each bottle, and stopper and swirl.
8. Allow the bottles to set overnight to oxidize organic mercury compounds to inorganic mercury ions.
9. The samples will remain stable for several days before analysis.

Sample Analysis

Instrument Conditions for IL Video 12

Current = 3.0 mA
Wavelength = 253.7 nm
Atomic Absorption Mode (AA)
Double Beam Mode (DB)
Statistics = 90
Integration = 1.0 seconds
D_2 Background Correction with diffraction grating filter
Circulating Pump autotransformer = 70% power

Instrument Conditions for Varian SpectraAA 200

Sampling Mode = AutoMix
Wavelength = 253.7 nm
Calibration Mode = Scale Expansion
Slit Width = 1.0 nm
Measurement Mode = Integrate
Lamp Current = 3.0 mA
Replicates Standard = 20
Background Correction = BC on

Replicates Sample = 20
Expansion Factor 1.0
Minimum Reading = Disabled
Smoothing = 9 pt
Conc. Units = ng
Conc. Decimal Places = 2

Cal. Zero Rate = 0
Measurement Time = 4.5 s
Pre-Read Delay = OS
Vapor Type = Cold Vapor
Burner Height = 16.0 mm

1. Set the AA to the instrument conditions listed above and allow instrument warm-up time. Prepare the 10% stannous chloride/0.5 M sulfuric acid solution and the magnesium perchlorate drying tube. Attach the drying tube in the cold vapor mercury analyzer.
2. Auto-zero the AA by aerating deionized water through the cold vapor mercury analyzer.
3. Add 10.0 mL of 10% hydroxylamine hydrochloride/10% sodium chloride solution and deionized water to each sample so that all samples contain the same volume (this is to adjust for any additional 5% potassium permanganate added to samples). Swirl the sample until no purple or brown color from the potassium permanganate remains.
4. Add 5.0 mL of 10% stannous chloride to a sample and immediately attach to the mercury analyzer.
5. Measure the absorbance of the sample until the maximum absorbance is reached and begins to decline.
6. Change the valves of the mercury analyzer to draw the mercury into a 0.05 M potassium permanganate/5% sulfuric acid trap. Purge the mercury analyzer of mercury until the absorbance reaches a minimum similar to the background absorbance.
7. Return the valves to the "analyze" position and rinse the aerator with deionized water before analyzing the next sample. Dispose of the analyzed and purged sample into an Acid Waste container.
8. Alternate analyzing the samples, standards and blanks by use of steps 3-7.
9. Neutralize the "Acid Waste" in a fume hood with ammonium hydroxide until the pH is between 6 and 10. Pour the neutralized waste down the drain with running cold water.
10. Dispose of the unused stocks and standards in a glass bottle identified as "Hazardous Waste - Mercuric Chloride in % acid solutions. Corrosive Toxic." The start date. Each waste bottle will require an analysis before it will be accepted for disposal.