Total Mercury and Copper Concentrations in Lake Trout and Whitefish Fillets From Lake Superior

Activity: 19 -23

By
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INTRODUCTION

Mercury and copper are the two most toxic heavy metals to fish. Furthermore, methyl mercury can accumulate in fish muscle tissue to levels that, consumed on a regular basis, may pose a risk to human health. Both of these metals are released during copper mining and ore processing. Copper mining and processing were a major industry in the Keweenaw Peninsula of Michigan during the mid-1800's. Mining processes which involved heating the ore, released mercury as a bi-product into the air. Furthermore, the unused portion of the ore after the desired minerals were removed (i.e. tailings) were dumped off the shoals of the Keweenaw Peninsula into Lake Superior. These tailing have formed large sholes referred to as stamp sands. Because the extraction of copper from the ore is not a 100% efficient process, it is reasonable to assume that copper, as well as mercury, were in the tailings released into the waters around the Keweenaw Peninsula.

The waters around the Keweenaw Peninsula are the location of several lake trout and whitefish spawning reefs. The lake trout and whitefish populations around the Keweenaw Peninsula are sustained by the fish reproduction that occurs on these reefs. These lake trout and whitefish populations have significant cultural and economic importance to the Anishinabe.

The purpose of this study was to determine the total mercury and total copper concentrations in the muscle tissue (i.e. fillet) of lake trout and whitefish, in size ranges commonly caught by tribal fishermen around the Keweenaw Penisula.

METHODS

Fish Collection

The lake trout and whitefish were collected in conjunction with the GLIFWC fall Lake Superior fisheries assessment. Lake trout and whitefish were sampled using gangs of gill nets. The gangs were deployed in <50 ft of water above spawning reefs. Both ends of the gang were marked with orange flags stating "GLIFWC Assessment Net". The nets were removed 24 hours after deployment.

Lake trout were sampled using nylon and monofilament gill nets. Three gangs of gill nets (3 X 1000 ft/gang) were deployed at each lake trout sampling site. Each gang consisted of three 250 ft multifilament nylon net segments and one 250 ft monofilament net segment connected end-to-end to make a total length of 1000 ft. Within a single gang, one nylon net segment was a 4.5 inch stretch mesh, one nylon net segment was 5.0 inch stretch mesh, and one nylon net segment was 5.5 inch stretch mesh. The monofilament 250 ft segment within each gang were either 4.5, 5.0, or 5.5 inch stretch mesh; within a set of three gangs, one segment of each of the three monofilament stretch mesh sizes were used.

Whitefish were sampled using only monofilament gill nets. Three gangs of gill nets (3 X 750 ft/gang) were deployed at each sampling site. Each gang consisted of three 250 ft monofilament net segment connected end to end to make a total length of 750 ft. Within a single gang, one monofilament net segment was a 4.5 inch stretch mesh, one monofilament net segment was a 5.0 inch stretch mesh, and one monofilament net segment was a 5.5 inch stretch mesh.

Fish Selection

Total length of the fish was used to select the samples. Within each of four management units, three small lake trout (16.9 to 23.2 inches) and three large lake trout (29.6 to 36.0 inches) were attempted to be collected. Furthermore, three small (13.4 to 19.3 inches) and three large (25.1 to 30.9 inches) common whitefish were attempted to be collected from each of these four management units. Size ranges were chosen based on an approximately normal statistical distribution created with a 10 year GLIFWC Lake Superior data set of tribal commercial catch. For the small fish within a species, upper and lower limits within the size range represent - 1 and - 3 standard deviations of the mean, respectively. For the large fish within a species, upper and lower limits within the size range represent +1 and +3 standard deviations of the mean, respectively. Actual fish sizes are reported in the results section.

Descriptive Data Collection

Lake trout (*Salvelinus namaycush*) and common whitefish (*Coregonus clupeaformis*) collected were aged, sexed, measured for total length, and measured for round weight. The data were initially recorded on scale envelopes then later transferred to computer data sheets. The total length of the fish was from the anterior-most portion of the fish to the tip of the longest caudal fin rays when the lobes of the caudal fins were compressed dorso-ventrally. The round weight was collected using a spring scale and comprised the weight of the fish prior to removing any fish tissues. After the fillet was collected, the sex was determined by cutting open the peritoneal cavity and observing the gonads. For whitefish, a scale that was removed from the middle region of the side of the body was aged. For lake trout, the sagittal otolith was removed from behind the brain of the fish and aged.

Sample Collection

After the descriptive data were collected, one fillet was removed from the fish using a clean stainless steel fillet knife. The fillet consisted of the muscle from behind the pectoral fin to the tail.

Each skin-on fillet was placed into a separate plastic bag (1-gallon), along with a sample tag, labeled with a unique identification code. Each sample tag contained the unique identification code, sample site within a management unit, date/time of collection, laboratory sample number, and type of analysis.

The individual bags of fillets for each management unit were placed into a cooler on ice during transport to a freezer. The fillets were transported back to the lab within 24 hours of collection and were placed into a freezer and stored at a temperature below -10ºC. Upon completion of the sampling, the fillets were transported on ice to the analytical laboratory for analysis. Chain-of-custody forms were used to track samples and were updated appropriately.

Whitefish Scale Preparation and Aging

The raw scales were used for aging the whitefish. Three scales per whitefish were aged and the mean value was recorded as the estimated age. Three scales were placed, sculptured side up, between the glass plates of a microfiche reader (Micron 790A).

The focus or center of the cycloid scale was identified. The number of annuli consistent in formation around the focus were counted to determine the age of the whitefish. The annuli are made up of several circuli. Circuli develop during the year as the fish grows. Circuli laid down close together represents slow growth usually associated with the winter. The closely positioned circuli forms a dark ridge that represents the annuli. Special attention was given to avoid making common errors in aging scales such as missing the first annulus, aging regenerated scales, and counting anomalous rings.

Lake Trout Otolith Preparation and Aging

The sagittal otolith of the lake trout was ground down with a very fine-grain sand paper to more clearly reveal the annuli. The otolith was aged using a dissection scope (50X) (Nikon SMZ-2B) and reflected light. The nucleus of the otolith was identified. One year of growth is represented by one opaque (dark) and one translucent (light) band juxtaposed. The dark bands were counted starting from the nucleus to the outer edge and represent the estimated age of the lake trout. Only those dark bands which are consistent in formation around the periphery of the whole otolith were included in the counted number and recorded.

Chemical Analysis

Total mercury and total copper analysis on the lake trout and whitefish fillets (Appendix A) were conducted by the Environmental Health Laboratory, Lake Superior Research Institute at the University of Wisconsin - Superior in Superior, Wisconsin. Analysis procedures and quality control follow LSRI standard operating procedures and are described in the laboratory report in Appendix B. The quality control results were acceptable and support the accuracy and precision of this data.

Statistics

The Quatro Pro® statistical package was used to conduct descriptive statistics, regression analyses and ANOVA's.

RESULTS AND DISCUSSION

The size range of fish tested were representative of the size range caught and sold by tribal commercial fishermen. The mean, median, minimum and maximum concentration for each metal and species, regardless of management unit or size, were <1.0 ppm total mercury and <4.0 ppm total copper (Table 1 and Figure1).

Mean total mercury and total copper concentrations in fillet tissue of lake trout (*Salvelinus namaycush*) and common whitefish (*Coregonus clupeaformis*) appeared similar across management units within each size group (Tables 1 & 2). Due to the apparent similarity in chemical concentrations within size groups for a given fish species and the low sample size, data were pooled for each metal within each size group across management units (Tables 3 & 4)

Table 1. Lake trout fillet total mercury and total copper mean concentrations, standard deviations, and sample sizes by management unit and size group.

Lake Trout						
			Total Mercury		Total Copper	
Unit	**Size Group**	**Sample Size**	**Mean**	**Std. Dev.**	**Mean**	**Std. Dev.**
	16.9 to 23.2	3	0.14	0.03	0.59	0.04
MI-2	23.3 to 29.5	0	na	na	na	na
	29.6 to 36.0	3	0.57	0.31	0.65	0.36
	16.9 to 23.2	3	0.13	0.07	0.63	0.18
MI-3	23.3 to 29.5	1	0.33	na	0.68	na
	29.6 to 36.0	3	0.44	0.10	0.68	0.27
	16.9 to 23.2	3	0.18	0.02	0.76	0.52
MI-4	23.3 to 29.5	0	na	na	na	na
	29.6 to 36.0	3	0.42	0.10	0.71	0.11
	16.9 to 23.2	1	0.10	na	0.57	na
MI-5	23.3 to 29.5	4	0.23	0.07	0.74	0.42
	29.6 to 36.0	1	0.39	na	1.4	na

Table 2. Whitefish fillet total mercury and total copper mean concentrations, standard deviations, and sample sizes by management unit and size group.

Whitefish						
			Total Mercury		Total Copper	
Unit	**Size Group**	**Sample Size**	**Mean**	**Std. Dev.**	**Mean**	**Std. Dev.**
MI-2	13.4 to 19.3	0	na	na	na	na
	19.4 to 25.0	0	na	na	na	na
	25.1 to 30.9	1	0.11	na	0.65	na
MI-3	13.4 to 19.3	3	0.05	0.03	1.06	0.32
	19.4 to 25.0	0	na	na	na	na
	25.1 to 30.9	4	0.1	0.03	0.73	0.33
MI-4	13.4 to 19.3	3	0.05	0.01	0.70	0.25
	19.4 to 25.0	2	0.08	0.03	1.25	0.08
	25.1 to 30.9	3	0.10	0.02	1.69	0.02
MI-5	13.4 to 19.3	3	0.10	0.02	0.89	0.02
	19.4 to 25.0	0	na	na	na	na
	25.1 to 30.9	2	0.07	0.00	0.88	0.00

Table 3. Lake trout and whitefish fillet total mercury and total copper mean concentrations, standard deviations, and sample sizes by size group.

Lake Trout					
Size Groups	Sample Size	Total Mercury		Total Copper	
		Mean	Std. Dev.	Mean	Std. Dev.
16.9 to 23.2	10	0.147	0.04	0.650	0.26
23.3 to 29.5	5	0.250	0.07	0.728	0.33
29.6 to 36.0	10	0.468	0.17	0.752	0.30

Whitefish					
Size Groups	Sample Size	Total Mercury		Total Copper	
		Mean	Std. Dev.	Mean	Std. Dev.
13.4 to 19.3	9	0.052	0.007	0.912	0.634
19.4 to 25.0	2	0.074	0.021	1.046	0.264
25.1 to 30.9	10	0.108	0.023	1.037	1.012

Mean total mercury concentration in fillets were less than 0.5 ppm for all size groups of lake trout and less than 0.2 ppm for all size group of whitefish. Two individual lake trout, both > 29 inches in length, had > 0.5 ppm total mercury in the fillet tissue. All other individual lake trout fillets analyzed were <0.5 ppm. The 0.5 ppm value is the level above which young children, women of child bearing age, and pregnant women should limit their consumption according to the Wisconsin Department of Health.

The National Academy of Sciences (NAS) has recommended that 2-3 mg copper is a safe and adequate daily intake. This provides enough copper for adult nutrition. Total copper concentrations in fillets were < 1.5 ppm for 25 individual lake trout tested and < 4.0 ppm for 21 individual whitefish analyzed. Based on these data, the maximum amount in an 8 ounce (0.227 kg) portion of whitefish and lake trout would be 0.91 mg and 0.34 mg, respectively. Furthermore, these total copper concentration were 3-4 orders of magnitude less than copper concentrations that cause liver and kidney damage in rats and pigs when exposed for >21 days.

Table 4. Mean, median, mode, range, minimum, maximum, and standard deviation both metals in lake trout and whitefish fillet tissue.

	Lake Trout		**Whitefish**	
	Mercury	**Copper**	**Mercury**	**Copper**
Mean	0.30	0.71	0.08	0.99
Median	0.29	0.60	0.07	0.70
Mode	0.39	0.42	0.06	0.65
Range	0.87	0.10	0.98	3.45
Minimum	0.06	0.42	0.04	0.39
Maximum	0.93	1.40	0.14	3.84
Standard Deviation	0.19	0.30	0.03	0.77

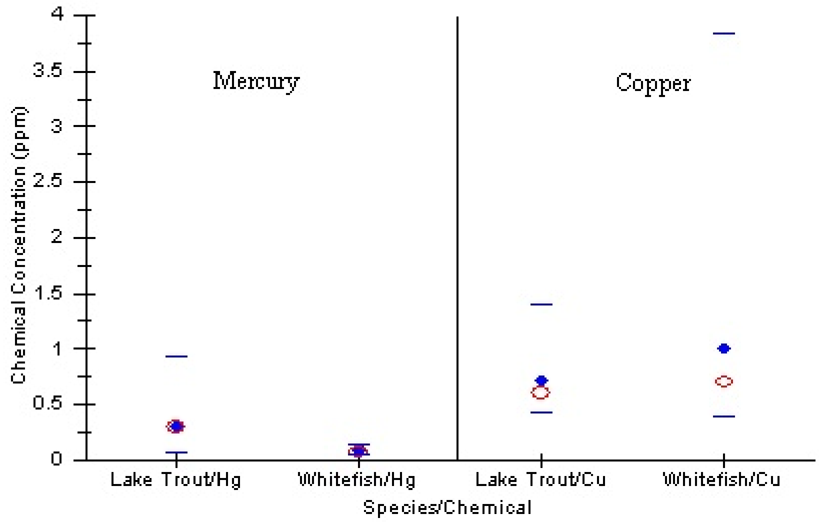

Figure 1. The mean (blue dot), median (red circle), maximum (upper blue line), minimum (lower blue line) mercury and copper concentrations in lake trout and whitefish fillet tissue.

ANOVA: Metals Concentrations vs. Age, Length, and Weight

Total Mercury and Copper in Lake Trout

Significant positive correlations were found between the total mercury concentration in lake trout fillets and each of the fish attributes (i.e. age, weight, length) (Table 5, Figure 2). No significant correlations were found for total copper (Table 5, Figure 2).

Table 5. Analysis of variance results (coefficient of determination (r^2), the p-value, and the sample size (n)) for the individual comparisons between age, weight, and length to the concentrations of total mercury and total copper in lake trout (*Salvelinus namaycush*).

	Lake Trout					
	Total Mercury			**Total Copper**		
	Age (years)	Weight (grams)	Length (inches)	Age (years)	Weight (grams)	Length (inches)
r^2	0.561	0.637	0.446	0.003	0.0003	0.016
p-value	<0.001*	<0.001*	<0.001*	0.839	0.942	0.571
n	19	23	25	19	23	25

*: Significant positive correlation (p-value = 0.05) between the chemical concentrations in the fillet tissue of lake trout (*Salvelinus namaycush*) and the corresponding attribute (i.e. age, weight, length).

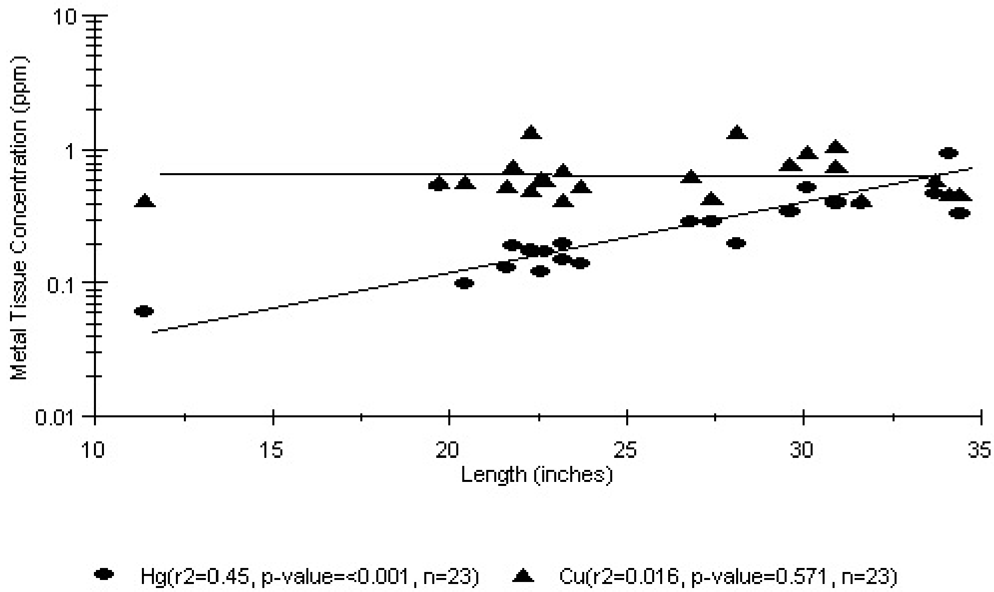

Figure 2. Total mercury and total copper fillet tissue concentrations (ppm) for lake trout regressed versus length (inches) with the r^2, p-value, and sample size (n) stated in the legend.

Total Mercury and Copper in Whitefish

Significant positive correlations were found between the total mercury concentrations in whitefish fillets and each of the fish attributes (i.e. age, weight, length) (Table 6, Figure 3). No significant correlations were found for total copper (Table 6, Figure 3).

Table 6. Analysis of variance results (coefficient of determination (r^2), the p-value, and the sample size (n)) for the individual comparisons between age, weight, and length to the concentrations of total mercury and total copper in common whitefish (*Coregonus clupeaformis*).

	Whitefish					
	Total Mercury			**Total Copper**		
	Age (years)	Weight (grams)	Length (inches)	Age (years)	Weight (grams)	Length (inches)
r^2	0.420	0.389	0.463	0.035	0.061	0.033
p-value	0.002*	0.003*	<0.001*	0.43	0.295	0.433
n	20	20	21	20	20	21

*: Significant positive correlation (p-value = 0.05) between the chemical concentrations in the fillet tissue of common whitefish (*Coregonus clupeaformis*) and the corresponding attribute (i.e. age, weight, length).

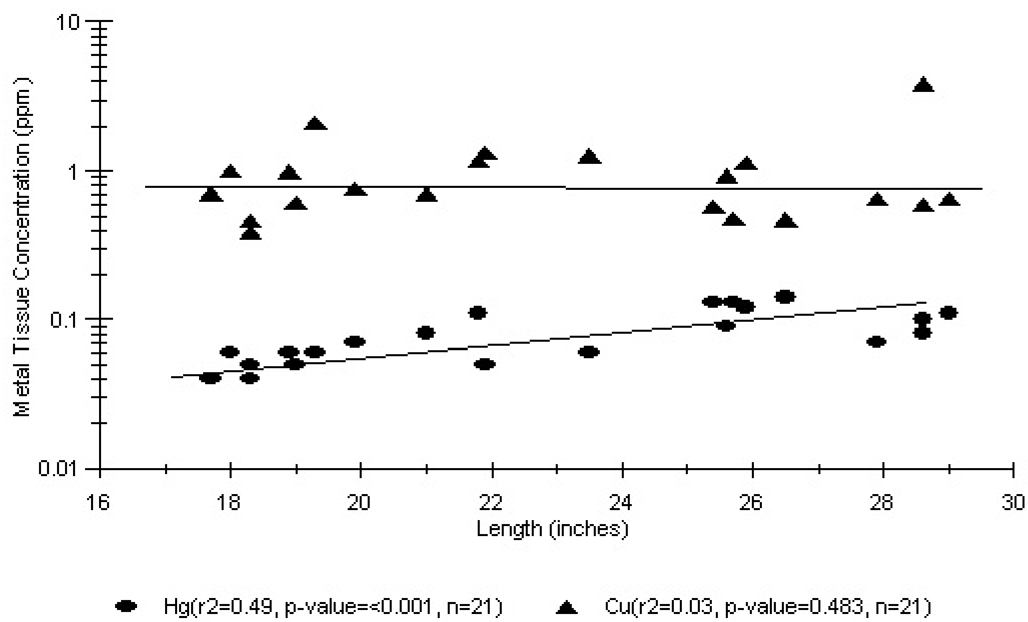

Figure 3. Total mercury and total copper fillet tissue concentrations (ppm) for whitefish regressed versus length (inches) with the r^2, p-value, and sample size (n) stated in the legend.

APPENDIX A
SUMMARY DATA

Title: Wet weight total copper and mercury data for lake trout and whitefish skin-off fillets in addition to collection date, sample code, location of collection, Lake Superior lake trout management unit (mgt. unit), length (inches), sex, age (years), and weight (grams).

Collection Date	Sample Code	Location	Mgt. Unit	Species	Length (inch)	Sex	Age (yrs)	Weight (g)	Total Hg (ppm)	Total Cu (ppm)
10\17\97	MI2LTSM2	Union Bay	MI-2	Lake Trout	22.7	M	7	1520	0.17	0.60
10\16\97	MI2LTLG2	Union Bay	MI-2	Lake Trout	34.1	F		4500	0.93	0.46
10\17\97	MI2LTSM3	Union Bay	MI-2	Lake Trout	21.6	M	9	1450	0.13	0.54
10\16\97	MI2LTLG1	Union Bay	MI-2	Lake Trout	30.9	F	13	3629	0.39	1.06
10\17\97	MI2LTLG3	Union Bay	MI-2	Lake Trout	31.6	F	11	4500	0.39	0.42
10\16\97	MI2LTSM1	Union Bay	MI-2	Lake Trout	22.6	M		1550	0.12	0.62
10\24\97	MI3LTSM2	Copper Harbor	MI-3	Lake Trout	11.4			200	0.06	0.42
10\29\97	MI3LTSM4	Copper Harbor	MI-3	Lake Trout	21.8	M	8	1300	0.19	0.75
10\24\97	MI3LTLG1	Copper Harbor	MI-3	Lake Trout	33.7	F	9	4350	0.46	0.60
10\28\97	MI3LTSM3	Copper Harbor	MI-3	Lake Trout	23.2	M	9	1600	0.15	0.72
10\24\97	MI3LTLG2	Copper Harbor	MI-3	Lake Trout	34.4	M	15	4650	0.33	0.47
10\24\97	MI3LTSM1	Copper Harbor	MI-3	Lake Trout	24.6	M		1800	0.33	0.68
10\24\97	MI3LTLG3	Copper Harbor	MI-3	Lake Trout	30.1	M	18	3980	0.52	0.98
10\31\97	MI4LTSM3	Buffalo Reef	MI-4	Lake Trout	23.2	M	8	1800	0.20	0.42
10\31\97	MI4LTLG3	Buffalo Reef	MI-4	Lake Trout	30.9	F	9	3500	0.40	0.77
10\31\97	MI4LTLG2	Buffalo Reef	MI-4	Lake Trout	29.6	M	10	3920	0.34	0.78
10\31\97	MI4LTLG1	Buffalo Reef	MI-4	Lake Trout	29.7	F	13	3980	0.53	0.58
10\31\97	MI4LTSM2	Buffalo Reef	MI-4	Lake Trout	22.3	M	7	1350	0.18	1.36
10\31\97	MI4LTSM1	Buffalo Reef	MI-4	Lake Trout	22.3	M	6	1660	0.17	0.50
11\20\97	MI5LTLG2	Big Bay Reef	MI-5	Lake Trout	26.8	M		3400	0.29	0.64
11\19\97	MI5LTSM1	Big Bay Reef	MI-5	Lake Trout	27.4	F		2800	0.29	0.43
11\20\97	MI5LTSM3	Big Bay Reef	MI-5	Lake Trout	23.7	M	8	2200	0.14	0.53
11\20\97	MI5LTSM2	Big Bay Reef	MI-5	Lake Trout	20.4	F	7	1600	0.10	0.57
11\19\97	MI5LTLG1	Huron River Reef	MI-5	Lake Trout	28.1	M	8	3050	0.20	1.36
11\20\97	MI5LTLG3	Huron River Reef	MI-5	Lake Trout	33.0	M	14	5000	0.39	1.40
10\16\97	MI2WFLG1	Union Bay	MI-2	Whitefish	29.0		13	1764	0.11	0.65
11\5\97	MI3WFLG1	Eagle Shoal	MI-3	Whitefish	26.5	M	12	3250	0.14	0.47
11\7\97	MI3WFLG4	Eagle Shoal	MI-3	Whitefish	25.7	F	15	2800	0.13	0.48
11\5\97	MI3WFSM3	Eagle Shoal	MI-3	Whitefish	18.3	M		850	0.05	0.46
11\7\97	MI3WFLG3	Eagle Shoal	MI-3	Whitefish	21.0	M	10	2250	0.08	0.70
11\6\97	MI3WFSM1	Eagle Shoal	MI-3	Whitefish	19.0	M	10	1450	0.05	0.61
11\6\97	MI3WFSM2	Eagle Shoal	MI-3	Whitefish	19.3	M	8	1400	0.06	2.11
11\7\97	MI3WFLG2	Eagle Shoal	MI-3	Whitefish	23.5	M	9	2050	0.06	1.27
11\26\97	MI4WFLG3	Betsy	MI-4	Whitefish	28.6	F	10	4080	0.10	3.84
11\26\97	MI4WFSM1	Betsy	MI-4	Whitefish	21.8	F	9		0.11	1.17
11\26\97	MI4WFSM2	Betsy	MI-4	Whitefish	21.9	F	7	2300	0.05	1.33
11\26\97	MI4WFLG1	Betsy	MI-4	Whitefish	27.9	F	12	4350	0.07	0.65
11\26\97	MI4WFLG2	Betsy	MI-4	Whitefish	25.4	F	11	3500	0.13	0.58
12\3\97	MI4WF735	Comm. harvest	MI-4	Whitefish	18.0		6	610	0.06	1.00
12\3\97	MI4WF728	Comm. harvest	MI-4	Whitefish	17.7		7	670	0.04	0.70
12\3\97	MI4WF729	Comm. harvest	MI-4	Whitefish	18.3		6	710	0.04	0.39
11\18\97	MI5WFLG2	Huron River Reef	MI-5	Whitefish	25.9	M	8	2700	0.12	1.14
11\18\97	MI5WFLG3	Huron River Reef	MI-5	Whitefish	25.6	M	8	3000	0.09	0.93
11\18\97	MI5WFLG1	Huron River Reef	MI-5	Whitefish	28.6	M	12	3800	0.08	0.59
11\19\97	MI5WFSM2	Huron River Reef	MI-5	Whitefish	18.9	M	8	1050	0.06	0.99
11\19\97	MI5WFSM3	Huron River Reef	MI-5	Whitefish	19.9	M	9	1200	0.07	0.76

APPENDIX B
CHEMISTRY REPORT

Analysis of Fish Tissue Collected from Michigan Waters of Lake Superior in the Fall of 1997

for

Great Lakes Indian Fish and Wildlife Commission
P.O. Box 9
Odanah, WI 54861

by

Environmental Health Laboratory
Lake Superior Research Institute
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15 July 1998

Introduction

Whitefish (*Coregonus clupeaformis*) and lake trout (*Salvelinus namaycush*) were collected by gill net from the Michigan waters of Lake Superior during October, November, and December 1997. The fish were collected by tribal fisherman and samples representing large and small commercial sizes were purchased by tribal biologists. Most of the fish were weighed, measured for total length, examined for fin clips, and sexed. The fish were filleted and frozen within eight hours of capture. The purpose of the sampling was to measure the mercury and copper concentrations in the fillets of the fish and to determine if size (i.e., age) influences the concentrations.

Custody of the fillets was recorded and the documents demonstrating this are attached to the end of this report. Fillets were received by the Environmental Health Laboratory on three occasions - November 17, 1997, January 12, 1998, and February 5, 1998. They arrived frozen and were stored in a freezer (0° Fahrenheit) until processed for copper and mercury analysis. Upon arrival at the university the fillets had the skin attached. After thawing for analysis, the skin was removed from the fillet and not included in the analysis. Analyses were performed by Christine Polkinghorne.

Standard Operating Procedures (SOPs) were used to process the fish in the field (Appendix A) and in the laboratory (Appendices B- G). During the analysis of the samples in the laboratory, several quality control procedures were conducted to ensure accuracy of analyses. Four tests of quality were conducted during the mercury analyses (Tables 1 - 4) and the same tests were conducted during the copper analyses (Tables 5 - 8). Duplicate agreement between repeated analyses were 91.8 and 91.4 for mercury and copper, respectively. Recovery of mercury and copper spiked into tissue samples averaged 80.7% and 99.4%, respectively. Samples of known concentrations were analyzed for each metal to test analytical accuracy. Dogfish shark with a concentration of 0.798±0.074 µg Hg/g of tissue was analyzed and mean daily accuracy ranged from 98.4 to 106.4%. An analytical standard solution of copper with a known concentration of 0.438 mgCu/L was analyzed and mean daily accuracy ranged from 99.1 to 102.3%. Tuna fish purchased from a local food store was analyzed for mercury and copper before grinding and after grinding using the same procedure done for the whitefish and lake trout. Agreement between the two analyses ranged from 83.1 to 93.7% for mercury and 54.8 to 96.4% for copper.

Results of the mercury and copper analyses for the whitefish (Tables 9 and 11) and mercury and copper analyses for whitefish and lake trout (Tables 10 and 12) are not corrected for spike recovery and are reported in ppm (µg/g) units.

Table 1. Results of Duplicate Analysis for Mercury Content in Whitefish and Lake Trout from Lake Superior.

Date of Analysis	Sample Identification Code	Percent Agreement
2/19/98	MI5-LT-SM-01	96.2
2/19/98	MI5-WF-LG-02	91.4
2/27/98	MI3-LT-SM-02	79.4
2/27/98	MI3-WF-LG-04	96.3
3/6/98	MI2-LT-SM-03	95.4
3/6/98	MI4-LT-SM-02	92.1
Mean (SD)		91.8 (6.4)

Table 2. Percent of Mercury Recovered from Fish Samples Spiked with Known Quantities of Mercury.

Spike Date	Sample Identification Code	Spike #1	Spike #2	Spike #3	Mean (%)	Std. Dev.
2/19/98	MI5-LT-SM-01	72.0	86.7	61.7	73.5	12.6
2/19/98	MI5-WF-LG-02	69.3	58.2	84.3	70.6	13.1
2/26/98	MI3-LT-SM-02	95.8	98.6	101.2	98.5	2.7
2/26/98	MI3-WF-LG-04	90.4	71.7	79.1	80.4	9.4
3/6/98	MI2-LT-SM-03	65.8	71.0	69.0	68.6	2.6
3/6/98	MI4-LT-SM-02	99.9	92.5	85.9	92.8	7.0
	Grand Mean (SD)				80.7	(13.8)

Table 3. Results of Mercury Analysis of Dogfish Shark sample (DORM-1) with Known Concentration of Mercury. (Actual Value 0.798±0.074 µgHg/g tissue).

Date of Analysis	Mercury (µg Hg/g)			Mean	Std. Dev.	Accuracy (%)
2/19/98	0.807	0.887	0.852	0.849	0.040	106.4
2/27/98	0.806	0.814	0.803	0.808	0.006	101.3
3/6/98	0.823	0.811	0.722	0.785	0.055	98.4

Table 4. Comparison of Mercury Analysis (µg/g) from Canned Tuna Fish Before and After Grinding.

Date of Analysis	Before Grinding (µg/g)	After Grinding (µg/g)	Percent Agreement
2/19/98	0.089	0.074	83.1
2/27/98	0.074	0.069	93.7

Table 5. Results of Duplicate Analysis in Whitefish and Lake Trout Tissue for Copper Content.

Date of Analysis	Sample Identification Code	Percent Agreement
1/14/98	MI2-WF-LG-01	79.0
1/14/98	MI3-LT-SM-03	98.9
1/22/98	MI3-WF-LG-01	97.7
2/9/98	MI5-LT-SM-02	98.7
2/9/98	MI4-WF-LG-03	82.5
Mean (SD)		91.4 (9.8)

Table 6. Percent of Copper Recovered from Whitefish and Lake Trout Samples Spiked with Known Quantity of Copper.

Date of Spike	Sample ID	Percent Spike Recovery
12/30/97	MI2-WF-LG-01	99.7
12/30/97	MI3-LT-SM-03	95.5
12/23/97	MI4-LT-LG-02	93.5
1/20/98	MI3-WF-LG-01	91.1
2/6/98	MI5-LT-SM-02	86.6
2/6/98	MI4-WF-LG-03	130.2
Mean (SD)		99.4 (15.7)

Table 7. Results of Copper Analysis for a Known Standard Solution. (ERA 3416 Sample with an Actual Value 0.438 mg/L.)

Date of Analysis	Copper Concentration (mg/L)			Mean	Std. Dev.	% Accuracy
1/14/98	0.434	0.445	0.424	0.434	0.011	99.1
1/22/98	0.443	0.438	0.445	0.442	0.004	100.9
2/9/98	0.431	0.450	0.464	0.448	0.017	102.3

Table 8. Comparison of Copper Analysis from Canned Tuna Fish Before and After Grinding.

Date of Analysis	Before Grinding (μg/g)	After Grinding (μg/g)	Percent Agreement
1/14/98	0.8125	0.7829	96.4
1/22/98 *	0.5882	1.0725	54.8
2/9/98 *	0.6436	0.9744	65.9

* The same fish sample was analyzed on two different dates.

Table 9. Results of Total Mercury Analysis for Whitefish from Michigan Waters of Lake Superior.

Sample ID	Fish Length (cm)	Sex	Date Collected	Mercury (µg/g)
MI2-WF-LG-01	73.7	-	10/16/97	0.11
MI3-WF-SM-01	48.3	M	11/5/97	0.05
MI3-WF-SM-02	49.0	M	11/5/97	0.06
MI3-WF-SM-03	46.5	M	11/6/97	0.05
MI3-WF-LG-01	67.3	M	11/6/97	0.14
MI3-WF-LG-02	59.7	M	11/7/97	0.06
MI3-WF-LG-03	61.0	M	11/7/97	0.08
MI3-WF-LG-04	65.3	F	11/7/97	0.13
MI4-WF-SM-01	55.4	F	11/26/97	0.11
MI4-WF-SM-02	55.6	F	11/26/97	0.05
MI4-WF-LG-01	70.9	F	11/26/97	0.07
MI4-WF-LG-02	64.5	F	11/26/97	0.13
MI4-WF-LG-03	72.6	F	11/26/97	0.10
MI5-WF-SM-01	-	-	11/18/97	0.09
MI5-WF-SM-02	48.0	M	11/19/97	0.06
MI5-WF-SM-03	50.5	M	11/19/97	0.07
MI5-WF-LG-01	-	-	11/18/97	0.08
MI5-WF-LG-02	-	-	11/18/97	0.12
MI5-WF-LG-03	-	-	11/18/97	0.09
MI4-WF-728	45.0	-	12/3/97	0.04
MI4-WF-729	46.5	-	12/3/97	0.04
MI4-WF-735	45.7	-	12/3/97	0.06

Table 10. Results of Total Mercury Analysis for Lake Trout Collected from Michigan Waters of Lake Superior.

Sample ID	Fish Length (cm)	Sex	Date Collected	Mercury (µg/g)
MI2-LT-SM-01	-	-	10/16/97	0.12
MI2-LT-SM-02	57.7	M	10/17/97	0.17
MI2-LT-SM-03	54.9	M	10/17/97	0.13
MI2-LT-LG-01	78.5	F	10/16/97	0.39
MI2-LT-LG-02	86.6	-	10/16/97	0.93
MI2-LT-LG-03	80.3	F	10/17/97	0.39
MI3-LT-SM-01	-	-	10/24/97	0.33
MI3-LT-SM-02	29.0	-	10/24/97	0.06
MI3-LT-SM-03	58.9	M	10/28/97	0.15
MI3-LT-SM-04	55.4	M	10/29/97	0.19
MI3-LT-LG-01	85.6	F	10/24/97	0.46
MI3-LT-LG-02	82.3	M	10/24/97	0.33
MI3-LT-LG-03	76.5	M	10/24/97	0.52
MI4-LT-SM-01	56.6	M	10/31/97	0.17
MI4-LT-SM-02	56.6	M	10/31/97	0.18
MI4-LT-SM-03	58.9	M	10/31/97	0.20
MI4-LT-LG-01	75.4	F	10/31/97	0.53
MI4-LT-LG-02	75.2	M	10/31/97	0.34
MI4-LT-LG-03	78.5	F	10/31/97	0.40
MI5-LT-SM-01	69.6	F	11/19/97	0.29
MI5-LT-SM-02	51.8	F	11/20/97	0.10
MI5-LT-SM-03	60.2	M	11/20/97	0.14
MI5-LT-LG-01	71.4	M	11/19/97	0.20
MI5-LT-LG-02	68.1	M	11/20/97	0.29
MI5-LT-LG-03	-	-	11/20/97	0.39

Table 11. Results of Total Copper Analysis for Whitefish from Michigan Waters of Lake Superior.

Sample ID	Fish Length (cm)	Sex	Date Collected	Copper (µg/g)
MI2-WF-LG-01	73.7	-	10/16/97	0.65
MI3-WF-SM-01	48.3	M	11/5/97	0.61
MI3-WF-SM-02	49.0	M	11/5/97	2.11
MI3-WF-SM-03	46.5	M	11/6/97	0.46
MI3-WF-LG-01	67.3	M	11/6/97	0.47
MI3-WF-LG-02	59.7	M	11/7/97	1.27
MI3-WF-LG-03	61.0	M	11/7/97	0.70
MI3-WF-LG-04	65.3	F	11/7/97	0.48
MI4-WF-SM-01	55.4	F	11/26/97	1.17
MI4-WF-SM-02	55.6	F	11/26/97	1.33
MI4-WF-LG-01	70.9	F	11/26/97	0.65
MI4-WF-LG-02	64.5	F	11/26/97	0.58
MI4-WF-LG-03	72.6	F	11/26/97	3.84
MI5-WF-SM-01	-	-	11/18/97	0.44
MI5-WF-SM-02	48.0	M	11/19/97	0.99
MI5-WF-SM-03	50.5	M	11/19/97	0.76
MI5-WF-LG-01	-	-	11/18/97	0.59
MI5-WF-LG-02	-	-	11/18/97	1.14
MI5-WF-LG-03	-	-	11/18/97	0.93
MI4-WF-728	45.0	-	12/3/97	0.70
MI4-WF-729	46.5	-	12/3/97	0.39
MI4-WF-735	45.7	-	12/3/97	1.00

Table 12. Results of Total Copper Analysis for Lake Trout from Michigan Waters of Lake Superior.

Sample ID	Fish Length (cm)	Sex	Date Collected	Copper (µg/g)
MI2-LT-SM-01	-	-	10/16/97	0.62
MI2-LT-SM-02	57.7	M	10/17/97	0.60
MI2-LT-SM-03	54.9	M	10/17/97	0.54
MI2-LT-LG-01	78.5	F	10/16/97	1.06
MI2-LT-LG-02	86.6	-	10/16/97	0.46
MI2-LT-LG-03	80.3	F	10/17/97	0.42
MI3-LT-SM-01	-	-	10/24/97	0.68
MI3-LT-SM-02	29.0	-	10/24/97	0.42
MI3-LT-SM-03	58.9	M	10/28/97	0.72
MI3-LT-SM-04	55.4	M	10/29/97	0.75
MI3-LT-LG-01	85.6	F	10/24/97	0.60
MI3-LT-LG-02	82.3	M	10/24/97	0.47
MI3-LT-LG-03	76.5	M	10/24/97	0.98
MI4-LT-SM-01	56.6	M	10/31/97	0.50
MI4-LT-SM-02	56.6	M	10/31/97	1.36
MI4-LT-SM-03	58.9	M	10/31/97	0.42
MI4-LT-LG-01	75.4	F	10/31/97	0.58
MI4-LT-LG-02	75.2	M	10/31/97	0.78
MI4-LT-LG-03	78.5	F	10/31/97	0.77
MI5-LT-SM-01	69.6	F	11/19/97	0.43
MI5-LT-SM-02	51.8	F	11/20/97	0.57
MI5-LT-SM-03	60.2	M	11/20/97	0.53
MI5-LT-LG-01	71.4	M	11/19/97	1.36
MI5-LT-LG-02	68.1	M	11/20/97	0.64
MI5-LT-LG-03	-	-	11/20/97	1.40

APPENDIX A

PROCEDURES FOR COLLECTING, PREPARING AND TRANSPORTING FISH SAMPLES

INTRODUCTION

This SOP includes general guidelines for the collection of fish samples at the study sites, preparing the specimens as samples, wrapping and labeling samples, preservation, and transportation to the laboratory for further studies. Species of fish collected may vary, and the preparation of each species may vary slightly, depending on the needs for the analysis to be performed. The objective of this SOP is to provide to the analytical laboratory samples of fish tissue that is properly identified, labeled, wrapped, preserved, and comparable from one sample to the next.

EQUIPMENT LIST

- Permanent Ink Marker
- Solvent Rinsed Aluminum Foil
- Gallon-Size Freezer Bags
- Knives Sufficient to Fillet Fish
- Freezer Space for Storage of Samples
- Coolers for Shipment
- Ice for Coolers
- Log Sheet to Record Data
- Label Tape
- Pencil

PROCEDURE

1. Collect fish samples in a manner appropriate for the study.
2. Identify the species of fish for sampling.
3. Prepare a waterproof label to identify each sample (use pencils or indelible ink only).
   a. Label the species.
   b. Label the date of capture.
   c. Label the place (lake) of capture.
   d. Total length and weight of whole fish.
   e. Sex of fish (when necessary or possible).
   f. Other data as required.
4. Prepare the fish as a sample (i.e., whole animal, entrails removed, fillet with skin or without skin, etc.).
5. Place sample in acetone- or hexane-rinsed aluminum foil if the sample is to be analyzed for organic materials. Place sample in a plastic bag if the sample is to be analyzed for metals.
6. Dual labels are recommended. Place a waterproof label in the package with the sample and another label on the outside of the package.
7. Place the sample on ice in the field as soon as possible (within two hours) and deliver to a freezer within the same 24-hour period.
8. Record on a separate log (sheet of paper or log book) the data that was included on the labels with the fish samples.
9. Transport sample to the laboratory in frozen condition (do not let samples thaw until ready for

analysis).

Example of Label

Name of Study:	Date:
Species:	Location of Capture:
Total Length (units):	Weight (units):
Sex:	Name of Investigator:
Other Information:	

APPENDIX B

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 C

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
- Ammonium Hydroxide, 30% (Reagent grade)
- Hydrochloric Acid, Concentrated (Reagent grade)
- Deionized Water
- Gloves
- Lab Coat
- pH Indicator Strips
- Wash Bottle
- Labware to be Washed

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 D

STANDARD OPERATING PROCEDURE

COLD VAPOR MERCURY ANALYSIS - FISH GRINDING

INTRODUCTION

This procedure is for the grinding of fish fillets 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 Fillets Samples
- ♦ Gloves
- ♦ Lab Coat
- ♦ Spatula
- ♦ Aluminum Foil
- ♦ Tuna fish
- ♦ Food Processor with Grinding Attachments
- ♦ Fillet Knife
- ♦ Goggles
- ♦ Grinder
- ♦ Beaker
- ♦ Scintillation Vials

PROCEDURE: GRINDING FISH FILLET SAMPLES

1. Cut the fish fillets 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 unground and handled like a sample by use of steps 5 + 6. Label the tuna fish as "unground" and include with the analysis set.

APPENDIX E

COLDS 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 F

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:

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

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

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 G

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 Spectrophotometer
- Electric 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 80-90ºC 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

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

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.