2006 Walleye Total Mercury Analyses

by

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INTRODUCTION

Walleye (*Sander vitreus*) are targeted for harvest by Chippewa tribal members from many off-reservation inland lakes in Wisconsin each spring (Krueger 2007). Tribal representatives have expressed concern about the health risk that mercury in fish poses to tribal members. As a result of this concern, the Great Lakes Indian Fish and Wildlife Commission (GLIFWC) has been collecting walleye annually since 1989 during spring from various lakes routinely harvested by tribal members. Muskellunge (*Esox masquinongy*) and northern pike (*Esox lucius*) are collected occasionally, but were not collected in 2006. Several funding sources have been used for collection and analysis of the fish for total mercury concentration. The fish were measured for total mercury as a surrogate for methylmercury because most mercury (>95%) in top predator fish is in the form of methyl mercury (Bloom 1992, Lasorsa and Allen-Gil 1995).

The walleye data are used to prepare tribal and lake specific, color-coded GIS maps that include fish consumption advice (Appendix 1). These maps are intended to help tribal members reduce their risk to methyl mercury exposure by selecting lakes for harvest where walleye contain lower mercury concentrations. The maps have been updated every 2-3 years and made available to tribal members at offices where permits for off-reservation spearing are issued and recently, at health service provider offices. In 2006, updated, large, wall-sized maps were posted at these offices and in various public locations such as tribal administration buildings, grocery stores, school libraries, or community centers (DeWeese, personal communication). The maps for the six Wisconsin Ojibwe tribes were updated in 2005 using a methodology described in Madsen et al. (In review) and were expanded in 2006 to include walleye lakes within the 1837 ceded territory in Minnesota and select walleye lakes in the 1842 ceded territory in the Upper Peninsula of Michigan.

This report presents results of mercury testing of walleye collected from off-reservation lakes during 2006. Funding for the collection and analysis of these samples came from United States Environmental Protection Agency (EPA) Supplemental Funds, received to test for mercury levels in walleye from 25 lakes in each of three years (2004-2006).

METHODS

Collection of Samples

Walleye from inland lakes were collected during spring from tribal spearers and netters and by GLIFWC fishery assessment crews. Plans called for twelve walleye to be collected with three fish taken from each of four size ranges (12.0 to 14.9, 15.0 to 17.9, 18.0 to 22.0, and greater than 22.0 inches).

Upon collection, walleye were measured for total length and sex was determined. A metal identification tag with a unique number was attached to each fish. Fish were then placed on ice in a cooler and transferred to a freezer (at temperatures at or below -10 °C) within 36 hours. A chain-of-custody form was filled out to identify fish collected from individual lakes each night

(Appendix 2). The form also served as a record of who collected and transported the samples and when they were placed on ice or transferred to a freezer. A second chain-of-custody form was used when transferring fish to the Lake Superior Research Institute (LSRI) in Superior (Appendix 2).

Processing

Walleye were processed into skin-off fillets at GLIFWC using stainless steel knives and cutting surfaces. All surfaces and equipment were washed with a mild dish detergent then rinsed with tap water prior to processing each fish. The following descriptive data were collected from each fish: a second length measurement (denoted as frozen length), sex, round weight, fillet weight, and the second or third dorsal spine was removed for aging. A single skin-off fillet was removed from each walleye, weighed on a digital scale, and placed into a one-gallon plastic bag with an interlocking seal. A sample label containing the name of the lake, fish identification number, year, date of filleting, analytical processing lab, species, type of sample and title of study was placed into each bag with the fillet (Figure 1). The tag identification number was recorded on the outside of each bag. All descriptive data were recorded on a laboratory data sheet. All individually bagged fillets for a given lake were placed into a single 15-gallon plastic bag, sealed, and labeled with the name of the lake. Spines were placed into small envelopes with a label, similar to the fillet labels (Figure 1), affixed to the outside of the envelope. The age of the fish was determined by counting the number of annuli (translucent zones) in the spine cross-section consistent with Schram (1989). Experienced GLIFWC Inland Fisheries technicians aged the spines.

All chain-of custody forms and GLIFWC laboratory data sheets were filed and kept in a three-ring binder at GLIFWC's main office.

Figure 1. Example of a sample label placed into one-gallon walleye fillet bags.

Project: Spring Mercury Walleye	Client: GLIFWC
Species: Walleye	Tag No. 0551
Month/Day Collected: 4/23	Year: 2006
Lake Name: Sherman Lake (Vilas)	Sample Processing: Hg
Tissue type: Fillet	Processor: LSRI

Total Mercury Analyses

Walleye fillets were received by LSRI in good condition with chain-of-custody documentation. A complete description of fillet grinding, total mercury analysis and associated quality control and assurance is provided in the LSRI laboratory report (Appendix 3). Briefly, the fillets were partially thawed and ground three times with a stainless steel motorized meat grinder. An aliquot (200 mg) of the ground tissue was digested and analyzed for total mercury using a Cold Vapor

Atomic Absorption Spectroscopy (Perkin Elmer FIMS-100 Flow Injection Mercury Analysis System) method based on EPA Method 245.6.

Quality Control

Quality control at LSRI was monitored using four methods: 1) the analysis of a certified reference tissue (DORM-2, *Squalus acanthias*) to determine accuracy, 2) tissue spikes to test the extraction method for efficiency and interferences, 3) duplicate analyses to determine precision, and 4) procedural blanks to determine whether sample processing changed the mercury content of the samples.

A quality assurance report from an audit of the laboratory processing and analysis is included with the LSRI laboratory report in Appendix 3. An audit of the field collection of samples is included in Appendix 4.

RESULTS

Quality Control

Standard Reference Material

The DORM-2 reference tissue has a certified concentration of 4.64 ± 0.26 µg Hg/g tissue. An acceptable range of mercury concentrations for DORM-2 standard reference material samples was calculated for this study based upon the analyses conducted from the Spring Walleye 2003-2005 studies (mean ± 2 times the standard deviation of all DORM-2 analyses). The calculated acceptable range was 3.40 to 5.24 µg Hg/g.

DORM-2 was analyzed in duplicate with each batch of 20 samples. The recovery values ranged from 79.3 to 110% with the grand mean and standard deviation of the recoveries being 93.4 ± 7.2 percent of the certified value. All results were within the acceptable range of 73.3 to 113% of the certified value.

Spikes

A total of 43 spike samples were analyzed (11 percent of total samples). Spike recovery was considered acceptable when it was in the range of 60.0 to 122 percent of the expected value. This was based upon the mean ± 2 times the standard deviation of all analyses of the spiked samples conducted from Spring Walleye 2003-2005 sample analysis. Mean recovery for the 43 spiked samples was 91.0 ± 8.6 percent with the values ranging from 62.2 to 108%. All spike recovery values were within the acceptance range (60.0 to 122 %).

Duplicates

Fish tissues were analyzed for mercury in duplicate 43 times (11 percent of total samples). Two portions of the same tissue were digested and analyzed independently. Duplicate agreement values were acceptable when having a relative percent agreement > 79.4%. The acceptable value was calculated as the mean ± 2 times the standard deviations of all duplicate analyses conducted from Spring Walleye 2003-2005 sample analysis at the LSRI laboratory. Relative percent agreement between the duplicate analyses of the same tissue ranged from 79.1 to 100% with the average and standard deviation of the agreements being 95.6 ± 4.3 percent. One relative percent agreement value was below the acceptance range of > 79.4%.

Procedural Blanks

Procedural tissue blanks (canned tuna, *Thunnus* sp.) were split into two aliquots on each processing day. One aliquot was processed in the same manner as the walleye fillets and the second aliquot was directly digested without processing. Results for the procedural blanks were considered acceptable when the relative percent agreement was > 66.3%. This is based on the mean ± 2 times the standard deviation of all the relative percent agreement values determined for the procedural blanks from the Spring Walleye 2003-2005 projects. Four tuna procedural blanks were processed coincident with the grinding of walleye collected for the GLIFWC EPA Mercury/Mapping Grant. One of the four procedural blanks was analyzed with each set of mercury samples for a total of eleven analyses resulting in a mean of 91.5 ± 5.2 relative percent agreement (Table 1). The relative percent agreement values ranged from 80.2 to 98.8% which were all within the acceptable range of > 66.3%. The procedural blank percent agreement analyses suggest that processing did not change the mercury content of the samples.

Quality Control Data Completeness

An assessment of the overall acceptability of the quality control data was made by adding up the total number of quality control samples that were outside of control limits and dividing by the total number of quality control samples. The project QAPP suggests a goal of fewer than 10 percent of the total quality control samples should exceed quality control parameters. Overall, there were a total of 162 quality control samples measured. One sample, or 0.006 percent of the total samples, exceeded the quality control parameters. This percentage was less than the goal of <10 percent of the quality control samples not meeting project quality control parameters. Overall, the sample data were in good agreement with the quality assurance parameters, so the data were determined to be precise and accurate.

Sample Results

During 2006, skinless fillets of 389 walleye from 37 lakes in Wisconsin (365 walleye, 35 lakes), Michigan (12 walleye, 1 lake) and Minnesota (12 walleye, 1 lake) were analyzed for total mercury concentration. Overall, total mercury concentrations on a wet weight basis ranged from

0.056 to 1.49 µg Hg/g (parts per million) and from 0.056 to 1.49 µg Hg/g from Wisconsin lakes, 0.104 to 0.543 µg Hg/g from the Michigan lake and 0.079 to 0.298 µg Hg/g from the Minnesota lake. Walleye lengths ranged from 12.0 to 28.1 inches from Wisconsin lakes, 14.0 to 24.6 inches from the Michigan lake and 14.5 to 26.0 inches from the Minnesota lake.

Walleye length and mercury data are summarized for each lake in each state in Table 1 (Wisconsin), Table 2 (Michigan) and Table 3 (Minnesota).

Table 1. Summary statistics for mercury concentration (ug Hg/g fish tissue) and fresh length (inches) for walleye collected from Wisconsin lakes during spring 2006.

COUNTY	LAKE	# of Fish	Mean Conc.	St. Dev. Conc.	Median Conc.	Max. Conc.	Min. Conc.	Mean Length	St.Dev Length
BARRON	PRAIRIE L*	6	0.270	0.127	0.239	0.477	0.132	19.5	2.9
BURNETT	LITTLE YELLOW L	5	0.250	0.071	0.247	0.355	0.161	17.9	2.8
DOUGLAS	L MINNESUING	11	0.887	0.391	0.921	1.49	0.333	18.8	4.7
IRON	TRUDE L	10	0.587	0.217	0.693	0.824	0.291	16.6	3.4
IRON	TURTLE-FLAMBEAU FL	12	0.511	0.235	0.523	1.03	0.183	18.1	3.4
LANGLADE	ROSE L	8	0.279	0.124	0.297	0.420	0.112	16.4	1.7
LANGLADE	SAWYER L	9	0.387	0.221	0.277	0.765	0.163	16.8	2.3
LINCOLN	RICE R FL CHAIN	12	0.457	0.221	0.505	0.816	0.163	18.5	4.3
ONEIDA	BEARSKIN L	12	0.213	0.147	0.141	0.512	0.075	18.2	3.8
ONEIDA	BUCKSKIN L	12	0.646	0.180	0.680	0.867	0.327	18.6	3.7
ONEIDA	CLEAR L	8	0.586	0.239	0.555	0.919	0.312	16.0	2.8
ONEIDA	CRESCENT L	12	0.139	0.075	0.125	0.311	0.056	18.3	3.9
ONEIDA	KATHERINE L	12	0.506	0.317	0.429	1.43	0.195	18.5	4.2
ONEIDA	PELICAN L	12	0.305	0.149	0.256	0.582	0.139	18.6	4.0
PRICE	BUTTERNUT L	12	0.720	0.264	0.682	1.34	0.442	17.6	4.3
PRICE	ROUND L	5	0.396	0.200	0.380	0.679	0.197	14.1	1.7
SAWYER	L CHIPPEWA	8	0.406	0.188	0.381	0.668	0.209	15.9	2.1
SAWYER	L CHIPPEWA (CHIEF L)*	9	0.315	0.148	0.230	0.538	0.172	16.2	2.6
SAWYER	L CHIPPEWA (CRANE L)	12	0.331	0.292	0.215	1.11	0.110	17.6	4.4
SAWYER	LAC COURTE OREILLES	12	0.255	0.178	0.206	0.611	0.080	18.3	3.8
SAWYER	NELSON L	11	0.428	0.160	0.403	0.706	0.177	18.8	3.7
SAWYER	ROUND L	12	0.225	0.163	0.182	0.500	0.064	17.8	3.7
SAWYER	SAND L	12	0.678	0.427	0.491	1.33	0.243	18.0	4.1
SAWYER	SISSABAGAMA L	12	0.309	0.160	0.259	0.600	0.099	18.5	4.2
SAWYER	WINDFALL L	11	0.338	0.131	0.264	0.537	0.169	17.7	4.2
VILAS	BIG L (BOULDER JCT)	12	0.569	0.196	0.549	0.810	0.256	17.7	3.6
VILAS	BIG ST GERMAIN L	12	0.303	0.150	0.266	0.601	0.145	18.7	3.6
VILAS	CATFISH L	12	0.396	0.203	0.336	0.834	0.178	18.5	4.6
VILAS	HARRIS L	12	0.506	0.288	0.457	1.11	0.223	18.3	4.0
VILAS	HORSEHEAD L	8	0.270	0.136	0.267	0.499	0.112	16.0	3.1
VILAS	LAC VIEUX DESERT	12	0.204	0.103	0.202	0.445	0.085	18.6	4.2
VILAS	LITTLE JOHN L	8	0.114	0.060	0.087	0.224	0.062	17.0	4.1
VILAS	SHERMAN L	12	0.341	0.156	0.327	0.624	0.174	18.2	3.9
VILAS	SQUAW L	10	0.578	0.315	0.581	1.29	0.237	17.1	3.9
WASHBURN	STONE L	10	0.471	0.224	0.442	0.957	0.241	19.2	3.3

* Reported mean includes one or more fish measured as "frozen length" at GLIFWC laboratory.

Table 2. Summary statistics for mercury concentration (ug Hg/g fish tissue) and fresh length (inches) for walleye collected from Michigan lakes during spring 2006.

County	Lake	# of Fish	Mean Conc.	St. Dev. Conc.	Median Conc.	Max. Conc.	Min. Conc.	Mean Length	St.Dev Length
GOGEBIC	L GOGEBIC	12	0.288	0.153	0.258	0.543	0.104	18.3	3.7

Table 3. Summary statistics for mercury concentration (ug Hg/g fish tissue) and fresh length (inches) for walleye collected from Minnesota lakes during spring 2006.

County	Lake	# of Fish	Mean Conc.	Std. Dev. Conc.	Median Conc.	Max. Conc.	Min. Conc.	Mean Length	Std. Dev. Length
MILLE LACS	MILLE LACS L	12	0.161	0.076	0.157	0.298	0.079	18.9	3.8

Percent Moisture

Percent moisture was measured in 118 of the 389 walleye tissues. Walleye muscle tissue had a mean moisture value of 79.2 ± 1.0 percent (Appendix). Of the 118 tissues analyzed for moisture, fifteen were analyzed in duplicate, all yielding relative percent agreements of 99.2 percent or greater. Ten samples were also dried an additional 24 hours and reweighed to ensure dryness, all yielding agreements greater than 98 percent.

SUMMARY

Walleye total mercury results from 2006 are summarized in this report. Quality control results indicated that the measured total mercury concentrations were precise and accurate. Total mercury concentrations in walleye tended to vary within a lake by size (larger fish generally having higher mercury concentrations) and between lakes for similar size groups of fish. These data have been entered into GLIFWC's mercury database used to produce GIS-based mercury in walleye consumption advisory maps (DeWeese and Madsen 2006).

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LIST OF APPENDICES

Appendix 1

Example Great Lakes Indian Fish and Wildlife Commission (GLIFWC) Geographic Information System (GIS) - Based Mercury in Walleye Consumption Advisory Map

Appendix 2

Great Lakes Indian Fish and Wildlife Commission Chain of Custody Forms for Collection and Transport of Fish for Mercury Analysis

FIELD CHAIN-OF-CUSTODY/DATA FORM

Study Title: Spring Walleye Sampling For Mercury **Year:**

Name of Lake:_____ **County**_____ **Area** _____

SECTION A: SAMPLE COLLECTION

COLLECT WALLEYE IN THE FOLLOWING SIZE GROUPS				
Size Ranges	**12.0-14.9**	**15.0-17.9**	**18.0-22**	**>22**
Number of Walleye	**3**	**3**	**3**	**3**

No	Fish Tag No	Length (in.)	Sex (M/F/U)	No	Fish Tag No	Length (in.)	Sex (M/F/U)
1				7			
2				8			
3				9			
4				10			
5				11			
6				12			

SECTION B: SAMPLE STORAGE AND CUSTODY

Check (X) either Cooler or Freezer($<0^0C$)

1. Crew Leader/ Warden:_____ Date:_____ Time:_____ Cooler on Ice _____ Freezer_____
2. Custody given to : _____ Date:_____ Time:_____ Cooler on Ice _____ Freezer_____
3. Custody given to : _____ Date:_____ Time:_____ Cooler on Ice _____ Freezer_____

Comments:_____

OFFICE USE ONLY– DO NOT WRITE BELOW THIS LINE

3. 3rd Custody: _____ Date:_____ Time:_____ Cooler on Ice _____ Freezer_____
4. 4th Custody: _____ Date:_____ Time:_____ Cooler on Ice _____ Freezer_____
5. 5th Custody: _____ Date:_____ Time:_____ Cooler on Ice _____ Freezer_____
6. 6thCustody: _____ Date:_____ Time:_____ Cooler on Ice _____ Freezer_____
7. 7thCustody: _____ Date:_____ Time:_____ Cooler on Ice _____ Freezer_____

TRANSFER CHAIN-OF-CUSTODY FORM

Study Title: Spring Walleye Sampling For Mercury **Year:**

Purpose: Transfer Filets to UW-Superior, LSRI

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SECTION A: SAMPLE STORAGE

Container Type Enter: 1 = Cooler + Ice 2 = Freezer (≤-10°C)		Placed INTO Container				Taken OUT of Container			
		Date	Time	Initials	°C	Date	Time	Initials	°C
A	GLIFWC	placement into the freezer is recorded on the field COC forms.							
B									
C									
D									
E									
F									

SECTION B: SAMPLE COLLECTION

The individual samples for each lake are listed on the attached sheets.
The lakes being delivered are:

WALLEYE:

1. _____ _____ 11. _____ _____
2. _____ _____ 12. _____ _____
3. _____ _____ 13. _____ _____
4. _____ _____ 14. _____ _____
5. _____ _____ 15. _____ _____
6. _____ _____ 16. _____ _____
7. _____ _____ 17. _____ _____
8. _____ _____ 18. _____ _____
9. _____ _____ 19. _____ _____
10. _____ _____ 20. _____ _____

SECTION C: SAMPLE CUSTODIAN

1. **Collected by:** Collection information list on Field COC at GLIFWC Office.

2. **Transferred by:**_____ **Date:**_____ **Time:**_____

   Relinquished by:_____ **Date:**_____ **Time:**_____

3. **Received by:**_____ **Date:**_____ **Time:**_____

   Relinquished by:_____ **Date:**_____ **Time:**_____

4. **Received by:**_____ **Date:**_____ **Time:**_____

   Relinquished by:_____ **Date:**_____ **Time:**_____

5. **Received by:**_____ **Date:**_____ **Time:**_____

   Relinquished by:_____ **Date:**_____ **Time:**_____

Appendix 3

Lake Superior Research Institute Final Report: Total Mercury Concentrations in Muscle Tissue from Walleye Captured in Wisconsin, Minnesota and Michigan Ceded Territory Waters During Spring 2006

Total Mercury Concentrations in Muscle Tissue From Walleye Captured in Wisconsin, Minnesota and Michigan Ceded Territory Waters during Spring 2006

by

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Introduction

Skinless fillet samples from walleye (*Stizostedion vitreum*) captured during the spring of 2006 from waters in the 1837 and 1842 Treaty ceded territories were analyzed for total mercury (Hg) content at the University of Wisconsin-Superior's Lake Superior Research Institute (LSRI). Three hundred eighty nine skinless walleye fillets from thirty-seven lakes in Wisconsin, Michigan and Minnesota collected by tribal spearers and GLIFWC Inland Fisheries assessment crews were analyzed as part of the EPA Mercury/Mapping Grant Number GL-96540801.

Methods

At the time fish were captured, a tribal warden or biologist was present to measure the total length of each fish. Fish were tagged with a unique number (i.e., a fish identification number) and whole fish with chain-of-custody forms were transferred to the Great Lake Indian Fish and Wildlife Commission (GLIFWC) laboratory. The samples were immediately placed on ice and were frozen within 36 hours of capture. At the GLIFWC laboratory, one fillet was removed from each fish, the skin was removed from the fillet and the fillet was placed into a plastic bag along with a label containing the fish identification number. This fish processing followed SOPs developed by GLIFWC. Sex of the fish was determined during the filleting process. A dorsal fin spine was removed from each fish to determine its age. At the LSRI laboratories, the walleye were received frozen and in good condition with chain-of-custody documentation. Samples were stored in a freezer at approximately -20°C until they were removed and thawed for processing and analysis.

Before processing the fish tissues, all glassware, utensils, and grinders were cleaned according to the appropriate methods (SOP SA/8). Each day, the fish to be processed were removed from the freezer and allowed to warm to a flexible, but stiff, consistency. The skinless fillet was ground three times in a grinder. A small amount of the initial tissue that passed through the grinder was collected and discarded (SOP SA/10). A sub-sample of the ground tissue was placed into a clean glass vial and frozen until mercury analysis was conducted. The grinder was disassembled after each fillet was ground and the unit was washed according to the grinder cleaning procedure (SOP SA/8).

Commercial canned tuna fish (*Thunnus sp.*) were used as procedural blanks for this project. These procedural blanks consisted of one aliquot from a can of tuna that was transferred directly into a sample bottle after the liquid was squeezed out of the can. The second portion was ground in the same manner as the walleye fillets. This check was made to ensure that no contamination or loss of mercury was occurring in the grinding process. Four procedural blanks were prepared during this project. The initial procedural blank was prepared on the first day fish were ground for the project and the last procedural blank was generated on the next to the last day fish were processed. The other two were prepared on intermediate dates when fish were being ground.

Fish tissues were weighed for mercury analysis following standard laboratory procedure (SOP SA/11). Mercury solutions for making tissue spikes and preparing analytical standards were prepared by the procedures in SOP SA/42. Mercury analyses were performed using cold vapor

mercury analysis techniques on a Perkin Elmer FIMS 100 mercury analysis system (SOP SA/13). Sample analysis yielded triplicate absorbance readings whose mean value was used to calculate the concentration of each sample. If the relative standard deviation (RSD) of the three measurements was greater than 5%, additional aliquots of the sample were analyzed in an attempt to obtain an RSD of less than 5%. If an RSD of < 5% was not able to be achieved, the sample was redigested and reanalyzed. Mercury concentrations and quality assurance calculations were done in Microsoft Excel according to SOP SA/37. The biota method detection limit was 0.0042 µg Hg/g for a tissue mass of 0.2 g. The detection limit was determined using a whole fish composite of rainbow trout containing a low concentration of mercury (SOP SA/35).

Moisture content of tissue was calculated using the wet and dried tissue weights (SOP NT/15). A portion (1 to 4 g) of ground tissue was placed into a pre-dried and pre-weighed aluminum pan immediately following tissue grinding. The pan and wet tissue were immediately weighed and placed into an oven (60°C) and dried for various time intervals. Drying times varied from 24 to 96 hours. Approximately 30 percent of the walleye analyzed for mercury had moisture content determined. In general, 3 fish per lake were randomly selected for determination of percent moisture.

Quality Assurance

Data quality was monitored by four methods: analysis of similar fish tissues (Commercial canned tuna; *Thunnus* sp.) before and after the tissue grinding process (procedural blanks) to measure laboratory bias; analysis of dogfish shark (DORM-2, *Squalus acanthias*) from the Canadian government (certified reference material from National Research Council Canada, Ottawa, Ontario, Canada) that has a certified concentration of mercury to measure analytical accuracy; duplicate analysis of fish tissue from the same fillet to measure analytical precision; and analysis of tissue with known additions of mercury to determine spike recovery and analytical interferences. Two sets of standard solutions with known amounts of mercury (analytical standards) were analyzed with each group (maximum of 40 samples plus QA samples) of tissue samples. These analytical solutions contained 0, 50, 100, 500, 1000 and 6000 ng Hg/L. They were prepared from a purchased 1000 ± 10 ppm mercury (prepared from mercuric nitrate) reference standard solution (Fisher Scientific, Pittsburgh, PA). Summary tables of the mercury calibration curve data are provided (Appendix A).

Results for the procedural blanks were considered acceptable when the relative percent agreement was > 66.3%. This is based on the mean ± 2 times the standard deviation of all the relative percent agreement values determined for the procedural blanks from the Spring Walleye 2003-2005 projects.

Duplicate agreement values were acceptable when having a relative percent agreement > 79.4%. The acceptable value was calculated as the mean ± 2 times the standard deviations of all duplicate analyses conducted from Spring Walleye 2003-2005 sample analysis at the LSRI laboratory.

An acceptable range of mercury concentrations for DORM-2 standard reference material

samples was calculated for this study based upon the analyses conducted from Spring Walleye 2003-2005 sample analysis (mean ± 2 times the standard deviation of all DORM-2 analyses). The calculated acceptable range was 3.40-5.24 µg Hg/g.

Prior to digestion, tissues from ten percent of the fish samples were spiked, in duplicate, with a known quantity of mercury and analyzed for recovery of the spiked mercury. Spike recovery was considered acceptable when it was in the range of 60.0 to 122 percent of the expected value. This was based upon the mean ± 2 times the standard deviation of all analyses of the spiked samples conducted from Spring Walleye 2003-2005 sample analysis.

A quality assurance audit was conducted by the LSRI quality assurance officer during the Spring Walleye 2006 project. That report is provided in Appendix B.

Results from fish tissues analyzed for GLIFWC EPA Mercury/Mapping Grant (Number 96540801)

Quality Assurance – Four tuna procedural blanks were processed coincident with the grinding of walleye collected for the GLIFWC EPA Mercury/Mapping Grant. One of the four procedural blanks was analyzed with each set of mercury samples for a total of eleven analyses resulting in a mean of 91.5 ± 5.2 relative percent agreement (Table 1). The relative percent agreement values ranged from 80.2 to 98.8% which were all within the acceptable range of > 66.3%.

Analysis of the dogfish shark tissue (DORM-2) standard reference material was conducted in duplicate with all 11 sets of walleye tissues analyzed (Table 2). The certified mercury concentration for the dogfish tissue was 4.64 ± 0.26 µg Hg/g. The recovery values ranged from 79.3 to 110% with the grand mean and standard deviation of the recoveries being 93.4 ± 7.2 percent of the certified value. All results were within the acceptable range of 73.3 - 113%.

Fish tissues were analyzed for mercury in duplicate 43 times. Two portions of the same tissue were digested and analyzed independently. Relative percent agreement between the duplicate analyses of the same tissue ranged from 79.1 to 100% with the average and standard deviation of the agreements being 95.6 ± 4.3 percent (Table 3). One relative percent agreement value was below the acceptance range of > 79.4%.

Samples of tissue were spiked with known concentrations of mercury prior to digestion. Mean recovery for the 43 spiked samples was 91.0 ± 8.6 percent with the values ranging from 62.2 to 108% (Table 4). All spike recovery values were within the acceptance range (60.0-122 %).

Mercury Analysis – Skinless fillets of 389 walleye from 37 lakes in Wisconsin (35 lakes), Michigan (1 lake) and Minnesota (1 lake) were analyzed for total mercury concentration. Total mercury concentrations on a wet weight basis (Table 5) ranged from 0.056 to 1.49 µg Hg/g (parts per million).

Tissue Moisture Analysis – Percent moisture was measured in 118 of the 389 walleye tissues. Moisture analysis took place immediately following grinding for 111 of the fish, and after the fish had been ground and frozen for the additional seven fish from Little Yellow and Prairie Lakes. The later analysis was based on an additional request from the project sponsor. Walleye muscle tissue had a mean moisture value of 79.2 ± 1.0 percent (Table 6). Of the 118 tissues analyzed for moisture, fifteen were analyzed in duplicate, all yielding relative percent agreements of 99.2 percent or greater. Ten samples were also dried an additional 24 hours and reweighed to ensure dryness, all yielding agreements greater than 98 percent.

Table 1. Relative Percent Agreement of Total Mercury for Procedural Blank Samples (Before and After Grinding).

Date of Analysis	Grinding Date	Before Grinding µg Hg/g	After Grinding µg Hg/g	Mean µg Hg/g	Relative* Percent Agreement
7/10/2006	5/31/2006	0.213	0.183	0.198	84.8
7/12/2006	6/12/2006	0.110	0.118	0.114	93.0
7/18/2006	6/26/2006	0.090	0.098	0.094	91.5
7/19/2006	7/5/2006	0.115	0.107	0.111	92.8
7/20/2006	5/31/2006	0.166	0.180	0.173	91.9
7/26/2006	6/12/2006	0.085	0.091	0.088	93.2
7/27/2006	6/26/2006	0.076	0.074	0.075	97.3
7/28/2006	7/5/2006	0.089	0.073	0.081	80.2
8/1/2006	6/12/2006	0.104	0.096	0.100	92.0
8/2/2006	6/26/2006	0.077	0.070	0.074	90.5
8/3/2006	5/31/2006	0.173	0.171	0.172	98.8
			Mean ± Std. Dev.		91.5 ± 5.2

* Relative percent agreement is calculated by the equation $(1- |\text{before} - \text{after}|/\text{mean})100$

Table 2. Mercury Concentrations of Dogfish Tissue (Standard Reference Material DORM-2) Analyzed during Fish Analysis. The Standard Reference has a Certified Mercury Concentration of 4.64±0.26µg Hg/g Tissue.

Date of Analysis	Dorm 2-1 µg Hg/g	Percent of Expected Dorm 2-1	Dorm 2-2 µg Hg/g	Percent of Expected Dorm 2-2
7/10/2006	4.15	89.4	4.48	96.5
7/12/2006	5.10	110	5.11	110
7/18/2006	4.01	86.4	3.98	85.8
7/19/2006	4.65	100	4.25	91.6
7/20/2006	4.43	95.5	4.30	92.7
7/26/2006	4.47	96.3	4.15	89.4
7/27/2006	4.32	93.1	4.22	90.9
7/28/2006	3.68	79.3	4.00	86.2
8/1/2006	4.54	97.8	4.02	86.6
8/2/2006	4.40	94.8	4.26	91.8
8/3/2006	4.41	95.0	4.40	94.8
		Mean ± Std. Dev.	4.33 ± 0.34	93.4 ± 7.2

Table 3. Relative Percent Agreement for Duplicate Analysis of Total Mercury Content in Skinless Fillet Tissue of Walleye.

Date of Analysis	Sample ID	µg Hg/g	Duplicate µg Hg/g	Mean µg Hg/g	Relative Percent Agreement
7/10/2006	Bearskin 6676	0.381	0.388	0.385	98.2
7/10/2006	Big Lake 1880	0.253	0.259	0.256	97.7
7/10/2006	Big Lake 672	0.801	0.820	0.811	97.7
7/10/2006	Big St. Germain 6646	0.206	0.204	0.205	99.0
7/12/2006	Butternut 9242	0.436	0.448	0.442	97.3
7/12/2006	Catfish 9459	0.837	0.832	0.835	99.4
7/12/2006	Catfish 66440	0.397	0.382	0.390	96.2
7/12/2006	Pelican 1392	0.142	0.137	0.140	96.4
7/18/2006	Sherman 6609	0.552	0.543	0.548	98.4
7/18/2006	Trude 6563	0.738	0.910	0.824	79.1
7/18/2006	Sawyer 1871	0.272	0.281	0.277	96.8
7/18/2006	Round 2043	0.374	0.386	0.380	96.8
7/19/2006	Windfall 6694	0.216	0.220	0.218	98.2
7/19/2006	Squaw 6615	0.557	0.638	0.598	86.5
7/19/2006	Clear 2002	0.319	0.317	0.318	99.4
7/19/2006	Chippewa 6576	0.611	0.643	0.627	94.9
7/20/2006	Lac Vieux Desert 1894	0.265	0.293	0.279	90.0
7/20/2006	Lac Courte Oreilles 606	0.127	0.124	0.126	97.6
7/20/2006	Lac Courte Oreilles 615	0.531	0.548	0.540	96.9
7/20/2006	Round 6593	0.063	0.066	0.065	95.4
7/26/2006	Rose 7567	0.407	0.432	0.420	94.0
7/26/2006	Buckskin 2051	0.809	0.863	0.836	93.5
7/26/2006	Crescent 6652	0.056	0.055	0.056	98.2
7/26/2006	Little Yellow 12105	0.164	0.157	0.161	95.7
7/27/2006	Little John 1812	0.076	0.081	0.079	93.7
7/27/2006	Stone 1933	0.539	0.524	0.532	97.2
7/27/2006	Sand 10253	0.330	0.315	0.323	95.4
7/27/2006	Horsehead 1822	0.113	0.111	0.112	98.2

7/28/2006	Harris 6599	0.443	0.473	0.458	93.4
7/28/2006	Katherine 2019	0.402	0.379	0.391	94.1
7/28/2006	Gogebic 1831	0.516	0.569	0.543	90.2
7/28/2006	Gogebic 1844	0.498	0.482	0.490	96.7
8/1/2006	Mille Lacs 1851	0.248	0.255	0.252	97.2
8/1/2006	Nelson 2091	0.384	0.382	0.383	99.5
8/1/2006	Nelson 2100	0.539	0.573	0.556	93.9
8/1/2006	Rice River Flowage 9233	0.166	0.161	0.164	97.0
8/2/2006	Lake Chippewa (Chief) 9121	0.222	0.222	0.222	100
8/2/2006	Lake Chippewa (Crane) 7557	0.128	0.129	0.129	99.2
8/2/2006	Minnesuing 1370	1.08	1.11	1.10	97.3
8/2/2006	Prairie 5088	0.350	0.352	0.351	99.4
8/3/2006	Sissabagama 6550	0.101	0.120	0.111	82.9
8/3/2006	Turtle Flambeau Flowage 7527	0.586	0.604	0.595	97.0
8/3/2006	Turtle Flambeau Flowage 7536	0.472	0.499	0.486	94.4
				Mean ± Std. Dev.	95.6 ± 4.3

Table 4. Percent of Mercury Recovered from Skinless Walleye Fillet Samples Spiked with a Known Concentration of Mercury.

Date of Analysis	Sample ID	Spike #1	Spike #2	Mean	Std. Dev.
7/10/2006	Bearskin 6676	101	101	101	0.0
7/10/2006	Big Lake 1880	101	102	102	0.7
7/10/2006	Big Lake 672	76.3	76.5	76.4	0.1
7/10/2006	Big St. Germain 6646	94.5	92.8	93.7	1.2
7/12/2006	Butternut 9242	94.1	87.3	90.7	4.8
7/12/2006	Catfish 9459	82.4	62.2	72.3	14.3
7/12/2006	Catfish 66440	84.4	85.5	85.0	0.8
7/12/2006	Pelican 1392	92.7	99.9	96.3	5.1
7/18/2006	Sherman 6609	80.3	79.2	79.8	0.8
7/18/2006	Trude 6563	89.3	66.0	77.7	16.5
7/18/2006	Sawyer 1871	83.3	98.0	90.7	10.4
7/18/2006	Round 2043	99.5	99.1	99.3	0.3

7/19/2006	Windfall 6694	89.0	71.1	80.1	12.7
7/19/2006	Squaw 6615	67.1	85.1	76.1	12.7
7/19/2006	Clear 2002	99.0	89.4	94.2	6.8
7/19/2006	Chippewa 6576	92.5	94.8	93.7	1.6
7/20/2006	Lac Vieux Desert 1894	102	99.8	101	1.6
7/20/2006	Lac Courte Oreilles 606	94.0	94.7	94.4	0.5
7/20/2006	Lac Courte Oreilles 615	85.0	94.4	89.7	6.6
7/20/2006	Round 6593	99.7	96.0	97.9	2.6
7/26/2006	Rose 7567	95.4	100.1	97.8	3.3
7/26/2006	Buckskin 2051	98.3	92.5	95.4	4.1
7/26/2006	Crescent 6652	97.1	108	103	7.7
7/26/2006	Little Yellow 12105	90.7	89.9	90.3	0.6
7/27/2006	Little John 1812	96.3	96.4	96.4	0.1
7/27/2006	Stone 1933	77.3	79.8	78.6	1.8
7/27/2006	Sand 10253	86.1	87.1	86.6	0.7
7/27/2006	Horsehead 1822	100	97.3	98.7	1.9
7/28/2006	Harris 6599	78.3	96.5	87.4	12.9
7/28/2006	Katherine 2019	80.6	85.5	83.1	3.5
7/28/2006	Gogebic 1831	81.4	90.6	86.0	6.5
7/28/2006	Gogebic 1844	83.2	80.9	82.1	1.6
8/1/2006	Mille Lacs 1851	94.0	97.8	95.9	2.7
8/1/2006	Nelson 2091	94.0	96.5	95.3	1.8
8/1/2006	Nelson 2100	90.9	88.4	89.7	1.8
8/1/2006	Rice River Flowage 9233	93.3	88.8	91.1	3.2
8/2/2006	Lake Chippewa (Chief) 9121	105	105	105	0.0
8/2/2006	Lake Chippewa (Crane) 7557	101	104	103	2.1
8/2/2006	Minnesuing 1370	101	95.2	98.1	4.1
8/2/2006	Prairie 5088	94.6	99.5	97.1	3.5
8/3/2006	Sissabagama 6550	97.7	100	98.9	1.6
8/3/2006	Turtle Flambeau Flowage 7527	86.7	68.7	77.7	12.7
8/3/2006	Turtle Flambeau Flowage 7536	84.3	87.4	85.9	2.2
				Mean ± Std. Dev.	91.0 ± 8.6

Table 5. Total Mercury Concentration (Wet Weight) in Walleye Fillets from Fish Captured during the Spring of 2006.

Analysis Date	Lake	Tag Number	Fresh Length (in)	Sex	Age (Spine)	µg Hg/g tissue
7/10/2006	Bearskin Lake	621	14.6	Male	5	0.139
7/10/2006	Bearskin Lake	622	19.7	Female	6	0.138
7/10/2006	Bearskin Lake	6673	16.2	Female	5	0.159
7/10/2006	Bearskin Lake	6674	13.6	Male	4	0.075
7/10/2006	Bearskin Lake	6675	14.9	Male	5	0.133
7/10/2006	Bearskin Lake	6676	23.8	Female	10	0.384
7/10/2006	Bearskin Lake	6677	20.1	Female	5	0.157
7/10/2006	Bearskin Lake	6678	23.6	Female	11	0.512
7/10/2006	Bearskin Lake	6680	23.3	Female	12	0.455
7/10/2006	Bearskin Lake	6681	18.0	Female	6	0.143
7/10/2006	Bearskin Lake	6682	15.8	Female	7	0.134
7/10/2006	Bearskin Lake	6683	15.0	Female	4	0.126
7/10/2006	Big Lake(Boulder Jct)	662	15.9	Male	6	0.475
7/10/2006	Big Lake(Boulder Jct)	672	17.0	Male	9	0.810
7/10/2006	Big Lake(Boulder Jct)	1879	13.7	Male	5	0.436
7/10/2006	Big Lake(Boulder Jct)	1880	14.3	Male	5	0.256
7/10/2006	Big Lake(Boulder Jct)	6661	12.0	Male	4	0.343
7/10/2006	Big Lake(Boulder Jct)	6663	22.0	Female	8	0.771
7/10/2006	Big Lake(Boulder Jct)	6665	18.2	Male	10	0.803
7/10/2006	Big Lake(Boulder Jct)	6666	22.7	Female	9	0.800
7/10/2006	Big Lake(Boulder Jct)	6667	15.7	Male	9	0.578
7/10/2006	Big Lake(Boulder Jct)	6668	20.6	Female	8	0.643
7/10/2006	Big Lake(Boulder Jct)	6671	18.5	Female	7	0.520
7/12/2006	Big Lake(Boulder Jct)	66440	22.3	Female	9	0.390
7/10/2006	Big St. Germain	1877	22.4	Female	8	0.298
7/10/2006	Big St. Germain	6638	20.7	Male	9	0.566
7/10/2006	Big St. Germain	6639	14.7	Male	4	0.158
7/10/2006	Big St. Germain	6640	23.3	Male	11	0.601
7/10/2006	Big St. Germain	6641	24.6	Female	10	0.337
7/10/2006	Big St. Germain	6642	20.3	Male	12	0.390
7/10/2006	Big St. Germain	6643	14.9	Male	7	0.204
7/10/2006	Big St. Germain	6644	14.4	Male	5	0.145

7/10/2006	Big St. Germain	6645	16.8	Male	7	0.246
7/10/2006	Big St. Germain	6646	15.2	Male	6	0.205
7/10/2006	Big St. Germain	6647	17.6	Male	9	0.201
7/10/2006	Big St. Germain	6648	19.4	Male	7	0.285
7/28/2006	Buckskin Lake	1828	20.5	Female	8	0.543
7/26/2006	Buckskin Lake	2046	14.0	Male	5	0.399
7/26/2006	Buckskin Lake	2047	12.4	Male	5	0.327
7/26/2006	Buckskin Lake	2048	14.6	Male	5	0.452
7/26/2006	Buckskin Lake	2049	17.0	Male	9	0.684
7/26/2006	Buckskin Lake	2050	17.4	Male	10	0.802
7/26/2006	Buckskin Lake	2051	17.4	Male	8	0.836
7/26/2006	Buckskin Lake	2052	22.7	Female	9	0.867
7/26/2006	Buckskin Lake	2053	21.9	Female	8	0.620
7/26/2006	Buckskin Lake	2054	23.4	Female	13	0.789
7/26/2006	Buckskin Lake	2055	22.6	Female	8	0.762
7/26/2006	Buckskin Lake	2060	19.0	Male	8	0.675
7/12/2006	Butternut Lake	9235	15.7	Male	5	0.447
7/12/2006	Butternut Lake	9236	12.1	Male	6	0.468
7/12/2006	Butternut Lake	9237	12.5	Male	6	0.481
7/12/2006	Butternut Lake	9238	19.8	Female	8	0.649
7/12/2006	Butternut Lake	9240	15.0	Male	7	0.683
7/12/2006	Butternut Lake	9242	13.4	Male	5	0.442
7/12/2006	Butternut Lake	9243	23.8	Female	9	0.950
7/12/2006	Butternut Lake	9244	23.8	Female	9	1.34
7/12/2006	Butternut Lake	9245	22.7	Female	8	0.681
7/12/2006	Butternut Lake	9247	18.4	Male	9	0.766
7/12/2006	Butternut Lake	9248	19.1	Male	9	0.800
7/12/2006	Butternut Lake	9249	15.3	Male	11	0.928
7/12/2006	Catfish Lake	424	23.8	Female	10	0.454
7/10/2006	Catfish Lake	6669	19.7	Female	10	0.578
7/12/2006	Catfish Lake	9459	27.0	Female	12	0.834
7/12/2006	Catfish Lake	9460	12.1	Male	7	0.276
7/12/2006	Catfish Lake	9465	21.1	Female	9	0.447
7/12/2006	Catfish Lake	9466	23.5	Female	10	0.638
7/12/2006	Catfish Lake	9467	18.7	Female	8	0.395
7/12/2006	Catfish Lake	9468	15.8	Male	7	0.218
7/12/2006	Catfish Lake	9469	14.4	Male	5	0.204
7/12/2006	Catfish Lake	9470	16.4	Female	5	0.274
7/12/2006	Catfish Lake	9471	13.6	Male	5	0.178

7/12/2006	Catfish Lake	9473	15.8	Male	5	0.257
7/19/2006	Clear Lake	2001	17.3	Male	10	0.651
7/19/2006	Clear Lake	2002	15.0	Male	5	0.318
7/19/2006	Clear Lake	2003	12.5	Male	4	0.312
7/19/2006	Clear Lake	2004	13.0	Male	5	0.421
7/19/2006	Clear Lake	2005	14.4	Male	7	0.458
7/19/2006	Clear Lake	2006	16.3	Male	10	0.809
7/19/2006	Clear Lake	2007	18.4	Male	9	0.919
7/19/2006	Clear Lake	2008	20.8	Female	9	0.797
7/26/2006	Crescent Lake	6649	19.8	Male	10	0.181
7/26/2006	Crescent Lake	6650	14.5	Male	5	0.101
7/26/2006	Crescent Lake	6651	12.6	Male	5	0.064
7/26/2006	Crescent Lake	6652	13.6	Male	5	0.056
7/26/2006	Crescent Lake	6653	16.7	Male	6	0.093
7/26/2006	Crescent Lake	6654	15.3	Male	5	0.072
7/26/2006	Crescent Lake	6655	17.6	Male	11	0.163
7/26/2006	Crescent Lake	6656	24.3	Female	11	0.230
7/26/2006	Crescent Lake	6657	21.8	Female	8	0.145
7/26/2006	Crescent Lake	6658	18.1	Male	8	0.104
7/26/2006	Crescent Lake	6659	22.4	Female	9	0.147
7/26/2006	Crescent Lake	6660	22.5	Female	10	0.311
7/28/2006	Harris Lake	1881	17.9	Female	8	0.456
7/28/2006	Harris Lake	1882	13.4	Male	5	0.226
7/28/2006	Harris Lake	1883	12.9	Male	5	0.251
7/28/2006	Harris Lake	6551	22.2	Female	9	0.580
7/28/2006	Harris Lake	6557	17.7	Female	6	0.331
7/28/2006	Harris Lake	6558	23.4	Female	8	0.861
7/28/2006	Harris Lake	6598	21.5	Male	13	0.827
7/28/2006	Harris Lake	6599	18.3	Male	10	0.458
7/28/2006	Harris Lake	6600	18.3	Male	10	0.460
7/28/2006	Harris Lake	6679	15.2	Male	6	0.284
7/28/2006	Harris Lake	6684	25.0	Female	10	1.11
7/28/2006	Harris Lake	6700	14.3	Male	5	0.223
7/27/2006	Horsehead Lake	1816	20.3	Female	10	0.337
7/27/2006	Horsehead Lake	1817	14.5	Male	8	0.279
7/27/2006	Horsehead Lake	1818	21.1	Male	8	0.393
7/27/2006	Horsehead Lake	1822	13.3	Male	4	0.112
7/27/2006	Horsehead Lake	1823	15.1	Male	8	0.499
7/27/2006	Horsehead Lake	1825	15.5	Male	6	0.147

7/27/2006	Horsehead Lake	1826	12.8	Male	5	0.141
7/27/2006	Horsehead Lake	1827	15.1	Male	8	0.254
7/28/2006	Katherine Lake	2016	18.3	Female	7	0.298
7/28/2006	Katherine Lake	2017	14.6	Male	7	0.358
7/28/2006	Katherine Lake	2018	14.7	Male	8	0.358
7/28/2006	Katherine Lake	2019	15.9	Male	8	0.391
7/28/2006	Katherine Lake	2020	14.7	Male	5	0.195
7/28/2006	Katherine Lake	2021	15.1	Male	8	0.444
7/28/2006	Katherine Lake	2022	28.1	Female	13	1.43
7/28/2006	Katherine Lake	2025	22.5	Female	11	0.534
7/28/2006	Katherine Lake	2027	19.9	Female	10	0.414
7/28/2006	Katherine Lake	2028	22.3	Female	11	0.695
7/28/2006	Katherine Lake	2029	16.2	Male	6	0.510
7/28/2006	Katherine Lake	2030	19.7	Female	6	0.446
7/28/2006	L Gogebic	1831	22.4	Female	9	0.543
7/28/2006	L Gogebic	1832	18.3	Male	7	0.213
7/28/2006	L Gogebic	1833	22.0	Female	10	0.341
7/28/2006	L Gogebic	1835	18.0	Male	9	0.333
7/28/2006	L Gogebic	1836	16.3	Male	6	0.179
7/28/2006	L Gogebic	1837	13.2	Male	5	0.104
7/28/2006	L Gogebic	1839	17.2	Male	5	0.293
7/28/2006	L Gogebic	1840	14.2	Male	4	0.127
7/28/2006	L Gogebic	1841	14.0	Male	4	0.126
7/28/2006	L Gogebic	1842	17.0	Male	7	0.222
7/28/2006	L Gogebic	1843	22.0	Female	10	0.488
7/28/2006	L Gogebic	1844	24.6	Female	12	0.490
7/20/2006	Lac Courte Oreilles	605	22.0	Female	6	0.289
7/20/2006	Lac Courte Oreilles	606	16.4	Male	5	0.125
7/20/2006	Lac Courte Oreilles	607	22.9	Female	8	0.342
7/20/2006	Lac Courte Oreilles	608	23.5	Female	8	0.326
7/20/2006	Lac Courte Oreilles	609	26.0	Male	10	0.611
7/20/2006	Lac Courte Oreilles	610	20.8	Male	7	0.280
7/20/2006	Lac Courte Oreilles	611	13.5	Male	4	0.115
7/20/2006	Lac Courte Oreilles	612	16.1	Male	5	0.129
7/20/2006	Lac Courte Oreilles	613	13.0	Male	4	0.087
7/20/2006	Lac Courte Oreilles	614	14.5	Male	4	0.080
7/20/2006	Lac Courte Oreilles	615	20.9	Male	10	0.540
7/20/2006	Lac Courte Oreilles	616	15.5	Male	5	0.131
7/20/2006	Lac Vieux Desert	1887	18.2	Female	9	0.187

7/20/2006	Lac Vieux Desert	1889	24.7	Female	13	0.277
7/20/2006	Lac Vieux Desert	1891	19.4	Male	10	0.246
7/20/2006	Lac Vieux Desert	1892	13.9	Male	6	0.086
7/20/2006	Lac Vieux Desert	1893	17.9	Male	7	0.217
7/20/2006	Lac Vieux Desert	1894	23.3	Female	9	0.279
7/20/2006	Lac Vieux Desert	1895	22.0	Female	10	0.237
7/20/2006	Lac Vieux Desert	1896	15.9	Male	7	0.140
7/20/2006	Lac Vieux Desert	1897	15.3	Male	8	0.142
7/20/2006	Lac Vieux Desert	1898	25.0	Female	11	0.445
7/20/2006	Lac Vieux Desert	1899	13.5	Male	4	0.085
7/20/2006	Lac Vieux Desert	1900	14.4	Male	4	0.107
7/19/2006	Lake Chippewa	6572	16.1	Male	9	0.521
7/19/2006	Lake Chippewa	6573	14.4	Male	5	0.238
7/19/2006	Lake Chippewa	6574	15.2	Male	5	0.220
7/19/2006	Lake Chippewa	6575	13.9	Male	4	0.209
7/19/2006	Lake Chippewa	6576	16.7	Male	8	0.627
7/19/2006	Lake Chippewa	6577	19.2	Male	8	0.668
7/19/2006	Lake Chippewa	6579	13.3	Male	6	0.298
7/19/2006	Lake Chippewa	6583	18.1	Male	7	0.464
8/2/2006	Lake Chippewa (Chief)	9116	16.5	Male	5	0.220
8/2/2006	Lake Chippewa (Chief)	9117	15.9	Male	5	0.230
8/2/2006	Lake Chippewa (Chief)	9118	15.5	Male	6	0.270
8/2/2006	Lake Chippewa (Chief)	9119	19.0	Male	11	0.497
8/2/2006	Lake Chippewa (Chief)	9120	18.0	Male	9	0.491
8/2/2006	Lake Chippewa (Chief)	9121	14.3	Male	4	0.222
8/2/2006	Lake Chippewa (Chief)	9122	12.0	Male	5	0.172
8/2/2006	Lake Chippewa (Chief)	9123	14.3	Male	5	0.192
8/2/2006	Lake Chippewa (Chief)	9124	20.2*	Male	12	0.538
8/2/2006	Lake Chippewa (Crane)	7553	13.0	Male	4	0.109
8/2/2006	Lake Chippewa (Crane)	7554	15.0	Male	5	0.177
8/2/2006	Lake Chippewa (Crane)	7555	22.5	Male	10	0.606
8/2/2006	Lake Chippewa (Crane)	7556	13.3	Male	4	0.110
8/2/2006	Lake Chippewa (Crane)	7557	12.1	Male	4	0.129
8/2/2006	Lake Chippewa (Crane)	7558	18.1	Female	6	0.247
8/2/2006	Lake Chippewa (Crane)	7559	15.2	Male	4	0.183
8/2/2006	Lake Chippewa (Crane)	7560	15.1	Male	5	0.151
8/2/2006	Lake Chippewa (Crane)	7561	20.6	Female	7	0.442
8/2/2006	Lake Chippewa (Crane)	7562	25.6	Female	11	1.11
8/2/2006	Lake Chippewa (Crane)	7563	18.5	Male	7	0.266

8/2/2006	Lake Chippewa (Crane)	7564	22.6	Female	8	0.444
8/2/2006	Lake Minnesuing	897	12.7	Male	4	0.475
8/2/2006	Lake Minnesuing	1370	24.5	Female	11	1.10
8/2/2006	Lake Minnesuing	1394	24.4	Female	11	0.929
8/2/2006	Lake Minnesuing	1395	19.8	Female	8	0.855
8/2/2006	Lake Minnesuing	1396	20.8	Female	8	1.49
8/2/2006	Lake Minnesuing	1397	25.8	Female	11	0.921
8/2/2006	Lake Minnesuing	10292	13.8	Male	5	0.333
8/2/2006	Lake Minnesuing	10295	17.4	Male	7	0.643
8/2/2006	Lake Minnesuing	10297	13.3	Male	4	0.454
8/2/2006	Lake Minnesuing	10298	18.5	Female	11	1.48
8/2/2006	Lake Minnesuing	10299	15.9	Male	6	1.08
7/27/2006	Little John	1807	25.9	Female	10	0.224
7/27/2006	Little John	1808	15.7	Male	7	0.116
7/27/2006	Little John	1809	20.2	Male	10	0.188
7/27/2006	Little John	1810	16.3	Male	6	0.096
7/27/2006	Little John	1811	14.6	Male	6	0.062
7/27/2006	Little John	1812	15.2	Male	4	0.078
7/27/2006	Little John	1813	14.6	Male	6	0.076
7/27/2006	Little John	1814	13.8	Male	6	0.074
7/26/2006	Little Yellow Lake	12102	15.9	Male	5	0.247
7/26/2006	Little Yellow Lake	12104	21.5	Female	7	0.217
7/26/2006	Little Yellow Lake	12105	15.4	Male	6	0.161
7/26/2006	Little Yellow Lake	12106	20.4	Female	7	0.268
7/26/2006	Little Yellow Lake	12108	16.2	Male	6	0.355
8/1/2006	Mille Lacs L	1846	16.6	Male	4	0.093
8/1/2006	Mille Lacs L	1847	23.5	Female	7	0.157
8/1/2006	Mille Lacs L	1848	15.6	Male	4	0.085
8/1/2006	Mille Lacs L	1849	20.3	Female	7	0.226
8/1/2006	Mille Lacs L	1850	18.3	Male	6	0.174
8/1/2006	Mille Lacs L	1851	20.0	Male	11	0.252
8/1/2006	Mille Lacs L	1853	19.1	Female	5	0.156
8/1/2006	Mille Lacs L	1854	23.2	Female	13	0.298
8/1/2006	Mille Lacs L	1855	14.5	Male	4	0.083
8/1/2006	Mille Lacs L	1857	14.9	Male	4	0.096
8/1/2006	Mille Lacs L	1858	15.0	Male	4	0.079
8/1/2006	Mille Lacs L	1860	26.0	Female	10	0.231
8/1/2006	Nelson Lake	900	14.1	Male	5	0.177

8/1/2006	Nelson Lake	2091	18.2	Male	11	0.383
8/1/2006	Nelson Lake	2092	23.5	Female	12	0.613
8/1/2006	Nelson Lake	2093	17.0	Male	11	0.476
8/1/2006	Nelson Lake	2094	18.0	Male	8	0.403
8/1/2006	Nelson Lake	2095	18.3	Male	11	0.468
8/1/2006	Nelson Lake	2096	17.1	Male	7	0.360
8/1/2006	Nelson Lake	2097	17.5	Male	6	0.358
8/1/2006	Nelson Lake	2098	24.8	Female	14	0.706
8/1/2006	Nelson Lake	2099	14.2	Male	5	0.203
8/1/2006	Nelson Lake	2100	23.9	Female	13	0.556
7/12/2006	Pelican Lake	1378	14.5	Male	5	0.169
7/12/2006	Pelican Lake	1382	26.7	Female	16	0.582
7/12/2006	Pelican Lake	1383	23.1	Female	13	0.539
7/12/2006	Pelican Lake	1384	21.5	Female	9	0.375
7/12/2006	Pelican Lake	1385	22.4	Female	10	0.426
7/12/2006	Pelican Lake	1386	18.4	Male	9	0.332
7/12/2006	Pelican Lake	1387	18.2	Female	8	0.220
7/12/2006	Pelican Lake	1388	17.1	Male	7	0.239
7/12/2006	Pelican Lake	1389	17.0	Male	10	0.272
7/12/2006	Pelican Lake	1391	13.3	Male	7	0.143
7/12/2006	Pelican Lake	1392	14.8	Male	6	0.139
7/12/2006	Pelican Lake	1393	16.5	Male	6	0.219
8/2/2006	Prairie Lake	5088	22.8	Male	12	0.351
8/2/2006	Prairie Lake	6596	21.3	Male	14	0.477
8/2/2006	Prairie Lake	6688	16.0	Male	4	0.132
8/2/2006	Prairie Lake	9100	18.1	Male	6	0.180
8/2/2006	Prairie Lake	10200	16.9	Male	6	0.202
8/2/2006	Prairie Lake	Evidence Fish	21.9*	Male	9	0.276
8/1/2006	Rice R. Fl. Chain	9220	17.9	Male	10	0.497
8/1/2006	Rice R. Fl. Chain	9221	21.0	Female	10	0.439
8/1/2006	Rice R. Fl. Chain	9222	20.7	Male	9	0.816
8/1/2006	Rice R. Fl. Chain	9223	24.7	Female	12	0.765
8/1/2006	Rice R. Fl. Chain	9224	22.0	Female	11	0.538
8/1/2006	Rice R. Fl. Chain	9225	25.5	Female	12	0.539
8/1/2006	Rice R. Fl. Chain	9229	18.0	Male	13	0.597
8/1/2006	Rice R. Fl. Chain	9230	16.5	Female	8	0.513
8/1/2006	Rice R. Fl. Chain	9231	14.1	Male	5	0.224
8/1/2006	Rice R. Fl. Chain	9232	15.0	Male	6	0.188

8/1/2006	Rice R. Fl. Chain	9233	12.3	Male	5	0.163
8/1/2006	Rice R. Fl. Chain	9234	14.4	Male	7	0.203
7/26/2006	Rose Lake	1868	14.9	Male	5	0.158
7/26/2006	Rose Lake	1869	14.9	Male	6	0.229
7/26/2006	Rose Lake	1870	13.8	Male	6	0.112
7/26/2006	Rose Lake	7565	18.3	Male	10	0.407
7/26/2006	Rose Lake	7566	16.0	Male	5	0.172
7/26/2006	Rose Lake	7567	18.4	Male	9	0.420
7/26/2006	Rose Lake	7577	17.3	Male	10	0.370
7/26/2006	Rose Lake	7580	17.6	Male	7	0.364
7/18/2006	Round Lake (Price)	2031	16.3	Female	7	0.679
7/18/2006	Round Lake (Price)	2032	15.1	Male	7	0.197
7/18/2006	Round Lake (Price)	2043	12.9	Male	5	0.380
7/18/2006	Round Lake (Price)	2044	13.9	Male	8	0.498
7/18/2006	Round Lake (Price)	2045	12.2	Male	8	0.226
7/20/2006	Round Lake (Sawyer)	6584	22.1	Female	12	0.449
7/20/2006	Round Lake (Sawyer)	6585	23.8	Female	9	0.315
7/20/2006	Round Lake (Sawyer)	6586	18.7	Male	7	0.226
7/20/2006	Round Lake (Sawyer)	6587	16.9	Male	8	0.156
7/20/2006	Round Lake (Sawyer)	6588	15.0	Male	4	0.074
7/20/2006	Round Lake (Sawyer)	6589	14.0	Male	4	0.089
7/20/2006	Round Lake (Sawyer)	6590	18.8	Male	9	0.445
7/20/2006	Round Lake (Sawyer)	6591	15.3	Male	5	0.094
7/20/2006	Round Lake (Sawyer)	6592	14.0	Male	4	0.083
7/20/2006	Round Lake (Sawyer)	6593	13.6	Male	5	0.064
7/20/2006	Round Lake (Sawyer)	6594	18.1	Male	8	0.207
7/20/2006	Round Lake (Sawyer)	6595	23.6	Female	11	0.500
7/27/2006	Sand Lake	10246	20.0	Male	11	1.06
7/27/2006	Sand Lake	10250	16.0	Male	5	0.293
7/27/2006	Sand Lake	10251	13.5	Male	5	0.243
7/27/2006	Sand Lake	10252	13.0	Male	6	0.294
7/27/2006	Sand Lake	10253	15.0	Male	6	0.323
7/27/2006	Sand Lake	10254	21.0	Female	10	1.22
7/27/2006	Sand Lake	10255	15.0	Male	6	0.300
7/27/2006	Sand Lake	10256	18.5	Male	8	0.645
7/27/2006	Sand Lake	10257	14.0	Male	5	0.337
7/27/2006	Sand Lake	10258	22.5	Female	9	0.997
7/27/2006	Sand Lake	10259	24.0	Female	9	1.09
7/27/2006	Sand Lake	10260	23.5	Female	10	1.33

7/18/2006	Sawyer Lake	1871	16.2	Female	6	0.277
7/18/2006	Sawyer Lake	1872	16.8	Male	8	0.488
7/18/2006	Sawyer Lake	1880	17.5	Female	10	0.765
7/18/2006	Sawyer Lake	1882	14.9	Male	4	0.163
7/18/2006	Sawyer Lake	1883	15.0	Male	4	0.218
7/18/2006	Sawyer Lake	1884	14.8	Male	6	0.210
7/18/2006	Sawyer Lake	1885	15.2	Male	5	0.240
7/18/2006	Sawyer Lake	1887	19.5	Male	9	0.434
7/18/2006	Sawyer Lake	1897	21.5	Female	6	0.691
7/18/2006	Sherman Lake	6601	20.2	Male	7	0.300
7/18/2006	Sherman Lake	6602	21.0	Female	8	0.465
7/18/2006	Sherman Lake	6603	20.2	Female	7	0.437
7/18/2006	Sherman Lake	6604	15.3	Female	4	0.234
7/18/2006	Sherman Lake	6605	22.3	Female	8	0.353
7/18/2006	Sherman Lake	6609	23.9	Female	9	0.548
7/18/2006	Sherman Lake	6610	16.3	Female	5	0.182
7/18/2006	Sherman Lake	6611	22.1	Male	10	0.624
7/18/2006	Sherman Lake	6612	17.7	Female	6	0.404
7/18/2006	Sherman Lake	6685	12.3	Male	7	0.192
7/18/2006	Sherman Lake	6686	13.8	Male	4	0.179
7/18/2006	Sherman Lake	6687	13.4	Male	4	0.174
8/3/2006	Sissabagama Lake	617	22.1	Female	11	0.391
8/3/2006	Sissabagama Lakc	618	17.6	Female	7	0.256
8/3/2006	Sissabagama Lake	619	23.9	Female	9	0.544
8/3/2006	Sissabagama Lake	6548	19.3	Female	8	0.362
8/3/2006	Sissabagama Lake	6549	18.1	Female	7	0.239
8/3/2006	Sissabagama Lake	6550	13.4	Male	4	0.110
8/3/2006	Sissabagama Lake	6552	12.8	Male	4	0.099
8/3/2006	Sissabagama Lake	6553	14.7	Male	6	0.261
8/3/2006	Sissabagama Lake	6554	22.2	Female	9	0.429
8/3/2006	Sissabagama Lake	6555	16.2	Male	7	0.174
8/3/2006	Sissabagama Lake	6556	16.0	Male	5	0.245
8/3/2006	Sissabagama Lake	6559	25.9	Female	10	0.600
7/19/2006	Squaw Lake	6613	22.2	Female	9	0.563
7/19/2006	Squaw Lake	6614	15.3	Female	6	0.243
7/19/2006	Squaw Lake	6615	16.3	Female	8	0.598
7/19/2006	Squaw Lake	6617	13.4	Male	5	0.385
7/19/2006	Squaw Lake	6618	12.0	Male	4	0.237
7/19/2006	Squaw Lake	6620	17.7	Male	5	0.349

7/19/2006	Squaw Lake	6621	22.5	Female	10	1.29
7/19/2006	Squaw Lake	6622	17.3	Female	10	0.684
7/19/2006	Squaw Lake	6623	20.8	Female	9	0.814
7/19/2006	Squaw Lake	6624	19.2	Female	8	0.616
7/27/2006	Stone Lake	887	15.6	Male	4	0.249
7/27/2006	Stone Lake	888	16.2	Male	4	0.241
7/27/2006	Stone Lake	889	16.8	Male	5	0.345
7/27/2006	Stone Lake	1931	22.9	Female	6	0.529
7/27/2006	Stone Lake	1932	20.7	Female	6	0.475
7/27/2006	Stone Lake	1933	19.6	Male	7	0.531
7/27/2006	Stone Lake	1934	20.3	Female	6	0.408
7/27/2006	Stone Lake	1935	23.2	Female	9	0.957
7/27/2006	Stone Lake	1936	14.0	Male	4	0.281
7/27/2006	Stone Lake	1942	22.5	Female	6	0.696
7/18/2006	Trude Lake	6560	23.2	Female	12	0.707
7/18/2006	Trude Lake	6563	18.5	Female	9	0.824
7/18/2006	Trude Lake	6566	19.6	Male	9	0.709
7/18/2006	Trude Lake	6568	18.3	Female	9	0.776
7/18/2006	Trude Lake	6569	13.5	Male	5	0.291
7/18/2006	Trude Lake	6570	12.7	Male	5	0.319
7/18/2006	Trude Lake	6571	12.7	Male	5	0.429
7/18/2006	Trude Lake	10261	16.0	Male	6	0.805
7/18/2006	Trude Lake	10262	15.0	Male	5	0.334
7/18/2006	Trude Lake	10263	16.3	Male	8	0.678
8/3/2006	Turtle-Flambeau Fl.	7523	14.0	Male	4	0.194
8/3/2006	Turtle-Flambeau Fl.	7527	18.2	Female	6	0.595
8/3/2006	Turtle-Flambeau Fl.	7528	22.4	Male	9	0.560
8/3/2006	Turtle-Flambeau Fl.	7529	22.0	Female	10	1.03
8/3/2006	Turtle-Flambeau Fl.	7530	23.0	Female	13	0.701
8/3/2006	Turtle-Flambeau Fl.	7531	19.5	Female	8	0.593
8/3/2006	Turtle-Flambeau Fl.	7532	21.4	Female	11	0.620
8/3/2006	Turtle-Flambeau Fl.	7533	14.6	Male	6	0.407
8/3/2006	Turtle-Flambeau Fl.	7534	15.8	Male	6	0.277
8/3/2006	Turtle-Flambeau Fl.	7535	13.8	Male	4	0.183
8/3/2006	Turtle-Flambeau Fl.	7536	16.7	Male	8	0.485
8/3/2006	Turtle-Flambeau Fl.	7537	16.0	Male	8	0.481
7/19/2006	Windfall Lake	6689	18.7	Female	9	0.243
7/19/2006	Windfall Lake	6690	24.8	Female	8	0.371

7/19/2006	Windfall Lake	6691	22.8	Female	9	0.447
7/19/2006	Windfall Lake	6692	12.5	Male	4	0.169
7/19/2006	Windfall Lake	6693	19.0	Female	9	0.537
7/19/2006	Windfall Lake	6694	13.8	Male	5	0.218
7/19/2006	Windfall Lake	6695	22.4	Female	10	0.483
7/19/2006	Windfall Lake	6697	13.4	Male	6	0.262
7/19/2006	Windfall Lake	6698	15.4	Male	5	0.237
7/19/2006	Windfall Lake	6699	17.0	Male	6	0.491
7/19/2006	Windfall Lake	96990	15.3	Female	6	0.264
* Frozen length reported because no fresh length was available.						

Table 6. Percent Moisture in Walleye Fillets (Measured Immediately After Grinding).			
Lake	Tag ID	Percent Moisture	Relative Percent Agreement
Bearskin	621	79.3	
Bearskin	6673	80.7	
Bearskin	6674	79.6	
Big Lake	1879	78.2	
Big Lake	1880	79.6	
Big Lake	6667	79.3	
Big St. Germain	6638 *	78.5	
Big St. Germain	6639 *	79.3	
Big St. Germain	6642 *	78.9	
Buckskin	2048	79.5	
Buckskin	2051	81.8	
Buckskin	2051 Dup	82.0	99.8
Buckskin	2053	80.4	
Buckskin	2055	80.3	
Butternut	9235	78.1	
Butternut	9240	78.5	
Butternut	9242	78.8	
Catfish	9469	80.6	
Catfish	9470	79.5	
Catfish	66440	81.7	
Clear	2003	79.9	
Clear	2004	78.5	
Clear	2005	79.0	
Clear	2005 Dup	79.1	99.8
Crescent	6653	78.6	
Crescent	6654	80.3	
Crescent	6657	80.5	
Gogebic	1836	79.3	
Gogebic	1837	79.2	
Gogebic	1841	80.0	
Harris	1882	80.6	
Harris	1883	80.2	
Harris	6700	80.3	
Horsehead	1816	79.9	

Horsehead	1817	78.4	
Horsehead	1823	79.6	
Katherine	2017	78.9	
Katherine	2017 Dup	79.0	99.9
Katherine	2021	78.7	
Katherine	2029	79.4	
Lake Chippewa (Crane)	7553	77.1	
Lake Chippewa (Crane)	7555	77.3	
Lake Chippewa (Crane)	7556	78.2	
Lac Courte Oreilles	606 *	77.3	
Lac Courte Oreilles	606 Dup *	77.8	99.4
Lac Courte Oreilles	608 *	77.9	
Lac Courte Oreilles	609 *	77.6	
Lac Vieux Desert	1887	79.7	
Lac Vieux Desert	1889	80.0	
Lac Vieux Desert	1892	79.1	
Lac Vieux Desert	1893	79.4	
Lake Chippewa	6572	78.2	
Lake Chippewa	6572 Dup	78.3	99.9
Lake Chippewa	6573	77.6	
Lake Chippewa	6575	78.0	
Lake Chippewa (Chief)	9116	78.7	
Lake Chippewa (Chief)	9120	79.8	
Lake Chippewa (Chief)	9121	79.8	
Little John	1808	79.1	
Little John	1807	79.2	
Little John	1810	80.1	
Little Yellow	12102	80.1	
Little Yellow	12104	79.2	
Little Yellow	12105	79.3	
Little Yellow	12106 **	79.4	
Little Yellow	12108 **	78.7	
Little Yellow	12108 Dup **	78.6	99.8
Mille Lacs	1846	79.2	
Mille Lacs	1846 Dup	78.6	99.2
Mille Lacs	1848	78.0	
Mille Lacs	1849	80.2	
Minnesuing	897	77.8	

Minnesuing	10292	78.1	
Minnesuing	10295	79.1	
Nelson	900	77.6	
Nelson	900 Dup	78.2	99.2
Nelson	2096	78.2	
Nelson	2099	77.6	
Pelican	1378	79.0	
Pelican	1378 Dup	78.7	99.6
Pelican	1388	79.9	
Pelican	1391	79.8	
Prairie	5088	77.3	
Prairie	6596 **	78.6	
Prairie	6596 Dup **	78.3	99.6
Prairie	6688	79.0	
Prairie	9100 **	77.9	
Prairie	10200	79.8	
Prairie	Ev.Fish **	78.4	
Rice River Flowage	9229	79.5	
Rice River Flowage	9229 Dup	79.3	99.9
Rice River Flowage	9230	80.1	
Rice River Flowage	9232	79.1	
Rose	1868 *	77.7	
Rose	1870 *	77.9	
Rose	7565 *	77.4	
Round (Price)	2031	80.1	
Round (Price)	2032	79.6	
Round (Price)	2044	80.3	
Round (Sawyer)	6585	78.9	
Round (Sawyer)	6587	79.2	
Round (Sawyer)	6587 Dup	79.1	99.9
Round (Sawyer)	6595	80.8	
Sand	10248	80.4	
Sand	10253	80.2	
Sand	10253 Dup	80.2	100.0
Sand	10256	79.9	
Sawyer	1871	79.2	
Sawyer	1872	78.8	
Sawyer	1872 Dup	78.3	99.4
Sawyer	1880	80.5	

Sherman	6604	78.7	
Sherman	6686	78.4	
Sherman	6687	78.3	
Sissabagama	6549	81.4	
Sissabagama	6553	81.3	
Sissabagama	6555	79.5	
Squaw	6614	80.4	
Squaw	6618	78.7	
Squaw	6620	78.6	
Stone	887	78.8	
Stone	888	78.1	
Stone	1931	79.7	
Stone	1931 Dup	79.4	99.6
Trude	6569	81.3	
Trude	6571	79.1	
Trude	10261	80.0	
Turtle Flambeau Flowage	7523	80.1	
Turtle Flambeau Flowage	7533	80.4	
Turtle Flambeau Flowage	7535	79.0	
Windfall	6689	81.3	
Windfall	6694	79.8	
Windfall	6697	78.3	

* Sample was returned to the oven and reweighed after an additional 24 hours of drying time.

** Moisture analyses conducted on samples of fish after they had been ground and frozen.

Appendix A

Standard Curve Data Run Coincident with The GLIFWC EPA Mercury/Mapping Grant Fish Analysis.

Analysis Date	Standard Conc. ng Hg/L	Blank Corrected Abs 1	Blank Corrected Abs 2	Blank Corrected Abs 3	Blank Corrected MEAN	Std. Dev.	Correlation	Slope	Intercept
7/10/06	0	0.0014*	0.0020*	0	0.0000	0.0004			
7/10/06	50	0.0018	0.0012	0	0.0015	0.0004			
7/10/06	100	0.0033	0.0027	0	0.0030	0.0004			
7/10/06	500	0.0150	0.0142	0	0.0146	0.0006			
7/10/06	1000	0.0257	0.0283	0	0.0270	0.0018			
7/10/06	6000	0.1639	0.1465	0	0.1552	0.0123	1.000	2.58E-05	0.0007
7/12/06	0	0.0010*	0.0014*	0	0.0000	0.0003			
7/12/06	50	0.0013	0.0012	0	0.0013	0.0001			
7/12/06	100	0.0025	0.0022	0	0.0024	0.0002			
7/12/06	500	0.0112	0.0107	0	0.0110	0.0004			
7/12/06	1000	0.0227	0.0220	0	0.0224	0.0005			
7/12/06	6000	0.1316	0.1302	0	0.1309	0.0010	1.000	2.18E-05	0.0002
7/18/06	0	0.0013*	0.0012*	0	0.0000	0.0001			
7/18/06	50	0.0015	0.0014	0	0.0015	0.0001			
7/18/06	100	0.0028	0.0035	0	0.0032	0.0005			
7/18/06	500	0.0141	0.0149	0	0.0145	0.0006			
7/18/06	1000	0.0279	0.0286	0	0.0283	0.0005			
7/18/06	6000	0.1621	0.1783	0	0.1702	0.0115	1.000	2.83E-05	0.0001
7/19/06	0	0.0017*	0.0020*	0.0012	0.0000	0.0004			
7/19/06	50	0.0014	0.0014	0.0014	0.0014	0.0000			
7/19/06	100	0.0027	0.0028	0	0.0028	0.0001			
7/19/06	500	0.0132	0.0152	0	0.0142	0.0014			
7/19/06	1000	0.0233	0.0296	0	0.0265	0.0045			
7/19/06	6000	0.1601	0.1650	0	0.1626	0.0035	1.000	2.71E-05	3.7E-05
7/20/06	0	0.0014*	0.0020*	0	0.0000	0.0004			
7/20/06	50	0.0014	0.0010	0	0.0012	0.0003			
7/20/06	100	0.0028	0.0024	0	0.0026	0.0003			
7/20/06	500	0.0141	0.0129	0	0.0135	0.0008			
7/20/06	1000	0.0285	0.0268	0	0.0277	0.0012			
7/20/06	6000	0.1641	0.1577	0	0.1609	0.0045	1.000	2.68E-05	0.0001
7/26/06	0	0.0016*	0.0017*	0	0.0000	0.0001			
7/26/06	50	0.0015	0.0013	0	0.0014	0.0001			
7/26/06	100	0.0030	0.0022	0	0.0026	0.0006			
7/26/06	500	0.0144	0.0131	0	0.0138	0.0009			
7/26/06	1000	0.0289	0.0262	0	0.0276	0.0019			
7/26/06	6000	0.1640	0.1568	0	0.1604	0.0051	1.000	2.67E-05	0.0002
7/27/06	0	0.0020*	0.0021*	0	0.0000	0.0001			
7/27/06	50	0.0014	0.0013	0	0.0014	0.0001			
7/27/06	100	0.0028	0.0027	0	0.0028	0.0001			
7/27/06	500	0.0150	0.014	0	0.0145	0.0007			

7/27/06	1000	0.0289	0.0280	0	0.0285	0.0006			
7/27/06	6000	0.1717	0.1616	0	0.1667	0.0071	1.000	2.78E-05	0.0002
7/28/06	0	0.0017*	0.0018*	0	0.0000	0.0001			
7/28/06	50	0.0015	0.0015	0	0.0015	0.0000			
7/28/06	100	0.0029	0.0023	0	0.0026	0.0004			
7/28/06	500	0.0145	0.0121	0	0.0133	0.0017			
7/28/06	1000	0.0289	0.024	0	0.0265	0.0035			
7/28/06	6000	0.1638	0.1415	0	0.1527	0.0158	1.000	2.54E-05	0.0004
8/1/06	0	0.0014*	0.0015*	0	0.0000	0.0001			
8/1/06	50	0.0015	0.0013	0	0.0014	0.0001			
8/1/06	100	0.0028	0.0027	0	0.0028	0.0001			
8/1/06	500	0.0137	0.0128	0	0.0133	0.0006			
8/1/06	1000	0.0272	0.0242	0	0.0257	0.0021			
8/1/06	6000	0.1555	0.1438	0	0.1497	0.0083	1.000	2.49E-05	0.0004
8/2/06	0	0.0018*	0.0017*	0	0.0000	0.0001			
8/2/06	50	0.0013	0.0015	0	0.0014	0.0001			
8/2/06	100	0.0026	0.0024	0	0.0025	0.0001			
8/2/06	500	0.0130	0.0129	0	0.0130	0.0001			
8/2/06	1000	0.0262	0.0255	0	0.0259	0.0005			
8/2/06	6000	0.1528	0.1473	0	0.1501	0.0039	1.000	2.50E-05	0.0003
8/3/06	0	0.0015*	0.0014*	0	0.0000	0.0001			
8/3/06	50	0.0011	0.0012	0	0.0012	0.0001			
8/3/06	100	0.0026	0.0037	0	0.0032	0.0008			
8/3/06	500	0.0128	0.0139	0	0.0134	0.0008			
8/3/06	1000	0.0250	0.0263	0	0.0257	0.0009			
8/3/06	6000	0.1465	0.1464	0	0.1465	0.0001	1.000	2.43E-05	0.0006

* Absorbance values for 0 ng/L standards are actual absorbances measured. Zero is used as value for blank concentration in calculating the standard curve.

Appendix B

Quality Assurance Audit Report on the Spring 2006 Walleye Project

Audit Date: June 2006
Report Date: July 25, 2006

Auditor: Dianne Brooke

1. Description and Scope of Audit

As part of a contaminant environmental monitoring study that was begun due to increased concerns about health risks and the consumption of fish, LSRI biologists and chemists are analyzing fish samples for contaminant levels. This audit report contains a review of the sample grinding methodology, data recording, data entry, and QA/QC training exercises. The sample grinding methodology for the Spring 2006 Walleye Project (date of contract = May 1 - October 31, 2006) was observed by the LSRI QA Manager. The primary staff members involved with the project are: Ms. Christine Polkinghorne (chemist), Ms. Heidi Saillard (chemist), and Mr. Tom Markee (chemist). Two LSRI students have assisted with the grinding and cleaning processes this past year. This audit outlines the QA/QC observations that occurred on June 20, 2006, where one staff member and one student were grinding the fish samples. The findings are listed under the subheadings.

2. Major Findings

Spring 2006 Walleye Project (Grinding Methodology/Lab Notebook Recording of Data)

On June 20, 2006, Dianne Brooke (LSRI QA Manager) observed one staff member and one student processing some of the walleye samples from Big Lake. The fish had been properly defrosted prior to grinding and were in appropriately labeled Ziploc® bags. The smaller fish were ground first because it took less time to defrost them. Fish Numbers 6663, 6665, and 6667 from Big Lake were observed being ground and their respective tissue being placed into vials. The following observations were made and discussed with the project staff.

- All personnel wore lab coats, safety glasses, and gloves.
- The student had a record of formal project SOP training, for SOPs: *SA/8 Routine Labware Cleaning for Metals Analysis; SA/10 Sample Grinding for Metals Analysis*; and *NT/15 Procedures for Determining Percent Moisture in Tissue Samples*. The three SOPs had last been revised in October, 2005. The staff member and student had received training in Good Laboratory Practices. Training certificates were on file for the staff member and student.
- The laboratory SOP notebook contained older versions of the project SOPs (this was remedied prior to the grinding process).
- The Balance PB303#3 was calibrated according to the procedures outlined in *SOP*

GLM/12 Procedures for Calibrating Laboratory Balances. The balance was tared each time prior to the adding of the Class 1 weights. Class 1 weights were used and the calibration information was recorded in the *Balance Calibration Notebook 05-9-27 BAL*, and referenced in the *Project Notebook 04-10-14-HS GLIFWC.*

- Observed the grinding process for Big Lake samples 6663, 6665, and 6667. The vials had attached labels containing the project code, lake code, and ID number. The vial cap contained the three-letter code and ID number. The labels were color-coded according to lake. The box containing the processed ground samples was also labeled.
- The *Project Notebook 04-10-14-HS GLIFWC* was well organized and complete. The Table of Contents had been filled out, researchers names and initials were recorded on the front inside cover, project/subcontract labels with the project ID number and year were affixed to each page of the notebook, and a copy of the subcontract had been Xeroxed and pasted into the notebook. Copies of the transfer chain-of-custody forms had also been pasted into the notebook. Although the notebook contained multiple sampling years' information, each year was clearly delineated by using plastic tab dividers.
- On page 93 of the *Project Notebook 04-10-14-HS GLIFWC* the LSRI SOPs used for the project were listed by category, number, and title.
- For the three Big Lake samples, the first few grams of ground sample was discarded and the remaining tissue was ground a second and a third time, all the while being mixed with a spatula when it was in the bowl. The three-time ground tissues were then placed into appropriately labeled vials. The procedures were conducted according to *SOP SA/10 Sample Grinding for Metals Analysis.*
- The weighing pans used for determining percent moisture content had been placed in the desiccator after being in a 60° C oven overnight (the minimum drying time is 16 hours according to *SOP NT/15 Procedures for Determining Percent Moisture in Tissue Samples).* It was not clear when the pans had been placed into the oven.
- The grinding equipment was cleaned according to *SOP SA/8 Routine Labware Cleaning for Metals Analysis.* The student technician was observed cleaning the pieces of the grinder as was the LSRI staff member. Each person washed the equipment in Liquinox® detergent, rinsed with tap water, then soaked in the 0.1 M HCl solution and finally rinsed in deionized water. Two sets of grinding equipment were cleaned simultaneously to keep up with the process of grinding tissue.
- On page 100 of the *Project Notebook 04-10-14-HS GLIFWC*, it was noted there were some discrepancies between the list of samples and the labels on the ground fish vials. The explanation of corrective action was well detailed and resulted in appropriate resolution.

<u>Spring 2006 Walleye Project - Bench Sheets for Analysis of Big Lakes Samples on 7/11/06</u>

Reviewed the three-ring binder entitled *GLIFWC Spring Walleye 2006.*

- The study ID number appeared on all output sheets.
- The data in the binder appeared to be thoroughly proofed, both for entry errors and calculation errors. The person checking the data initialed the rechecks and recorded the date when the data were proofed.

- In analyzing the samples for tissue moisture analyses, approximately 30% (118/389 samples) were chosen for this parameter. The contract stated that 94 fillets would be tested for percent moisture, so the researchers analyzed 24 more samples for this parameter. Of the 118 samples, 12.7% (n = 15 samples) were analyzed in duplicate and checked for relative percent agreement. The percent duplicate agreement for tissue moisture analyses ranged from 99.2 - 100%. Of the 118 samples, 8.5% (n = 10 samples) were placed back into the oven and reweighed after an additional 24 hours to ensure dryness. The QA/QC drying exercise yielded values that were above 98.0% duplicate agreement.
- Typically an analysis set consists of 36 samples being analyzed for mercury content. For each data set, the following QA/QC samples were analyzed: two dorm samples in duplicate, four duplicate agreement samples, and four spike recovery samples in duplicate. A calibration blank and five standards were also analyzed with the data set. One set of standards was run at the beginning of the analyses and the other set interspersed throughout the sampling series. This was recorded on preprinted bench sheets for the analyses dates of: 7/10/06, 7/12/06, 7/18/06, 7/19/06, 7/20/06, 7/26/06, 7/27/06, 7/28/06, 8/1/06, 8/2/06, and 8/3/06.
- The lowest values recorded for the QA/QC analytical parameters were: percent recovery for the dorm samples - 79.3%; relative percent agreement between duplicates - 79.1% (this sample will be re-analyzed according to project staff); mean percent spike recovery - 72.3% and the relative percent agreement for procedural blanks - 80.2%

3. Recommendations

The overall reviews of the methodology and data recording indicate that study personnel are highly organized and intentional in their QA/QC protocols for conducting research. The time/date when the weighing pans are initially placed into the oven (and removed from the oven) should be recorded in the notebook. The *SOP NT/15 Procedures for Determining Percent Moisture in Tissue Samples* should be amended to reflect the recording of time/date for drying aluminum pans. The *SOP SA/8 Routine Labware Cleaning for Metals Analysis* should be amended to include a statement regarding the frequency of changing the Liquinox® detergent water (according to the LSRI staff member, the cleaning water is changed every fourth time when cleaning the grinding equipment). Not all of the grinding equipment pieces fit into the 0.1 M HCl solution bath, necessitating the rotation of the stainless steel bowls so they can be acid rinsed. Perhaps a larger 0.1 M HCl solution bath could be used to fully submerge in equipment in the acid solution. If this change is made, it would also need to be reflected in *SOP SA/8 Routine Labware Cleaning for Metals Analysis*. The 0.1 M HCl solution had not been changed weekly according to the label on the 2½ gallon carboy (February, June, and July dates for 2006 had been written on the label). The *SOP SA/8 Routine Labware Cleaning for Metals Analysis* may need to be amended to include wording that the 0.1 M HCl solution should be remade prior to grinding the fish tissue samples. The percent moisture spreadsheet could be amended to include the "n" values for total number of samples, number of samples reweighed for the drying exercise, and number of samples analyzed twice for relative percent agreement.

PROCEDURES FOR DETERMINING PERCENT MOISTURE IN TISSUE SAMPLES

INTRODUCTION

This SOP includes general guidelines for the analysis of tissue samples for moisture content. It is a gravimetric technique requiring careful weighing techniques.

EQUIPMENT LIST

- Balance (i.e., Mettlers AG245, PB303, AB204, H34, H72 and H80)
- Aluminum Weighing Pans
- Drying Oven (60° C)
- Desiccation Container
- Spatula

PROCEDURE

1. Calibrate balance using Class 1 weights. Label the aluminum weighing pans and dry at 60° C for 16 hours.

2. Place dried weighing pans in desiccator until cool.

3. Weigh the dried and cooled weighing pans on balance to the 0.001 g.

4. Weigh approximately 1.0 g of thawed tissue and place in the labeled weighing pan.

5. Weigh the pan and the tissue on balance to the nearest 0.001 g.

6. Dry pan and tissue in drying oven at 60° C for 16 hours or until constant dry weight is achieved.

7. Remove dried pans and tissue from the oven and place in desiccator until cool.

8. Weigh the pan with the tissue on balance to the nearest 0.001 g.

9. It may be necessary to dry the pan and tissue a second time when the tissue is a large mass. Desiccate and re-weigh to prove that an equilibrium dry weight has been achieved.

10. Calculations:

    Aluminum pan with wet tissue- Dry Aluminum Pan = Wet weight of tissue

    (Aluminum pan and wet tissue weight - Aluminum pan and dry tissue / Wet tissue weight) X 100 = Percent moisture of tissue

ROUTINE LABWARE CLEANING FOR METALS ANALYSIS

INTRODUCTION

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

EQUIPMENT LIST

- Deionized Water
- Dish Pan
- Gloves
- Goggles
- Lab Coat
- Labware to be Washed
- Liquinox Detergent
- pH Indicator Strips
- Various Labware Washing Brushes
- Wash Bottle
- Plastic Dish Rack
- Grinder
- Plastic Tank with Cover
- Stainless Steel Bowls
- Ammonium Hydroxide, 30% (VWR Reagent)
- Fillet Knife
- Nitric Acid, Concentrated (Fisher Reagent)
- Spatula (Stainless Steel)
- Hydrochloric Acid, Concentrated (Fisher Reagent)
- Nalgene 2½ Gallon Carboy
- Sodium Bicarbonate
- Stainless Steel Bowls

PROCEDURE: CLEANING EQUIPMENT USED FOR FISH GRINDING [Grinder, Stainless Steel Bowls, Fillet Knife, Spatula]

1. Dismantle the meat grinder before washing.
2. Scrub equipment in hot water containing Liquinox detergent.
3. Rinse equipment with tap water until there is no presence of soap.
4. Rinse equipment once with deionized water.
5. Soak equipment in 0.1 M HCl for 30 seconds (be sure the equipment is completely immersed).
6. Rinse equipment three times with deionized water.
7. Upon drying, cover equipment with aluminum foil to store until used.

PROCEDURE: LABWARE CLEANING [Scintillation Vials]

1. Scrub the labware thoroughly in hot water containing 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 5 and 9 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.

PROCEDURE: 0.1 M HYDROCHLORIC ACID

1. Fill a 2½ gallon carboy to the 10-L mark with the deionized water. Add 83 mL concentrated hydrochloric acid. Cover the solution and mix. The 0.1 M hydrochloric acid is now ready to be used to soak the labware.
2. Remake the 0.1 M hydrochloric solution once a week. Neutralize the acid with ammonium hydroxide or sodium bicarbonate until a pH of between 5 and 9 is achieved. Measure the pH in the tank with pH indicator strips.
3. Pour the neutralized acid down the drain with running cold water.

SAMPLE GRINDING FOR METALS ANALYSIS

INTRODUCTION

This procedure is for the grinding of biological tissues into homogeneous samples. The grinder and labware used to grind the tissue is cleaned by the "Routine Labware Cleaning for Metals Analysis (SA/8)" procedure. The proper safety equipment must be worn during the entire grinding procedure. This includes gloves, goggles, and lab coat.

EQUIPMENT LIST

- Tissue Samples
- Fillet Knife
- Gloves
- Goggles
- Lab Coat
- Grinder
- Spatula
- Scintillation Vials or Jars
- Aluminum Foil
- Procedural Blank (i.e., Tuna Fish)
- Beaker or Stainless Steel Bowls
- Food Processor with Grinding Attachments

PROCEDURE: GRINDING TISSUE SAMPLES

1. Cut the tissue sample into small pieces that will fit through the grinder feed tube or food processor with grinding attachments.

2. Pass the tissue through the grinder or food processor, discarding the first few grams of tissue that come through. Collect the tissue in a beaker or bowl.

3. Mix the tissue with a spatula.

4. Pass the collected tissue through the grinder or food processor a second and third time and collect in the same beaker or bowl.

5. Mix the tissue with a spatula to insure homogeneity.

6. Place the tissue in a scintillation vial or jar previously washed (use procedure as described in SA/8). Seal securely with the screw top lid. Label the vial with the appropriate information and place in a freezer until analyzed.

7. Wash the grinder (or food processor) and labware by the "Routine Labware Cleaning for Metals Analysis (SA/8)" procedure before grinding the next sample.

8. Continue to grind each sample by repeating steps 1 - 7.

PROCEDURE: PREPARING THE PROCEDURAL BLANK

1. Prepare a procedural blank. When using the tuna, drain the liquid from the can. Grind half the procedural blank tissue as a procedural blank by use of steps 2-7. Label the procedural blank as "ground" and include with the analysis set.

2. The other half of the procedural blank is left unground and handled like a sample by use of steps 5 + 6. Label the procedural blank as "unground" and include with the analysis set.

SAMPLE WEIGHING FOR METALS ANALYSIS

INTRODUCTION

This procedure is for the weighing of biological tissue for metals analysis. The tissue should be ground according to the "Sample Grinding for Metals Analysis SA/10" or "Preparation of Tissues for Analytical Determinations Using Liquid Nitrogen SA/38" procedures. The labware used in this procedure should be cleaned using the "Routine Labware Cleaning for Metals Analysis (SA/8)" 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 Samples
- Gloves
- Goggles or Safety Glasses
- Lab Coat
- Kimwipes
- Spatula
- Deionized Water
- Nitric Acid (10%)
- Balance Capable of Reading to Nearest 0.001 g
- Polypropylene Digestion Vessels (Environmental Express)

PROCEDURE

1. Remove the sample to be analyzed from the freezer and allow to 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. Check balance calibration, if not previously done today, following "Procedures for Calibrating Laboratory Balances (GLM/12)".

4. Place a clean sample container on the balance and tare the balance.

5. With a spatula, stir the sample to insure homogeneity. Weigh the appropriate quantity (approximately 0.2 - 0.3 g for mercury analyses and 1.0 g for other metals analyses) of tissue into the sample container.

6. Record the weight of the sample.

7. Rinse the spatula with water, 10% nitric acid and deionized water. Wipe the spatula clean with a Kimwipe.

8. Label and record each sample container and sample. Be sure that none of the tissue adheres to the side of the sample container.

COLD VAPOR MERCURY DETERMINATION IN BIOTA

INTRODUCTION

This procedure is used for the determination of total mercury in fish, hair 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
- Hydrochloric Acid, Trace Metals Grade
- Nitric Acid, Fisher, Trace Metals Grade
- Mercury Cold Vapor Analyzer
- Hollow Cathode Mercury Lamp
- Variable Autotransformer
- Neptune Dyna-Pump Model 4K
- Hot Block (Environmental Express)
- Varian SpectrAA 200 Spectrophotometer
- FIMS-100 (Perkin Elmer) Mercury Analyzer
- Labindustries Repipet II Dispenser, 3 - 10 mL and 1 - 5 mL
- Wheaton Instruments Socorex Dispenser Model 511, 10 mL
- Polypropylene Digestion Cups and Covers
- Pipets/Pipettors
- Beakers
- Spatulas
- 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 for Spectra AA Analysis
- 0.05M Potassium Permanganate-5% (v/v) Sulfuric Acid
- 1000 ug/mL Mercuric Nitrate Stock
- 5 ug/mL Mercuric Nitrate Substock for Spectra AA Analysis
- 50 ng/mL Mercuric Nitrate Substock for Spectra AA Analysis
- 10 mg/L Mercuric Nitrate Substock for FIMS-100 Analysis

- 100 ug/L Mercuric Nitrate Substock for FIMS-100 Analysis
- Silicon Defoaming Agent (Perkin Elmer)
- Deionized Water in Teflon Squirt Bottle

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.

2. Place the digestion cups in Hot Block at 110°C and allow to digest for approximately 15 minutes or until all the fish tissue is dissolved.

3. Turn off the Hot Block and allow the digestion cups to cool to room temperature.

4. Add 5.0 mL of 5% potassium permanganate to each bottle in 1.0 mL increments swirling the digestion cups after each addition.

5. Add 10.0 mL of 5% potassium permanganate to each digestion cup in 5.0 mL increments, swirling the digestion cup after each addition. Additional 5% potassium permanganate solution (maximum of 5 mL) or solid potassium permanganate should be added to the samples if necessary so that the samples remain purple in color for at least 15 minutes. If extra potassium permanganate is added to a sample, an equal amount should be added to one set of standards and a blank.

6. Add 8 mL of 5% potassium persulfate to each digestion cup, and cover and swirl.

7. Allow the digestion cup to set overnight to oxidize organic mercury compounds to inorganic mercury ions.

8. The samples will remain stable for several days before analysis.

Sample Analysis Using Varian SpectraAA 200

Instrument Conditions

Current = 3.0 mA	Wavelength = 253.7 nm
Atomic Absorption Mode (AA)	Double Beam Mode (DB)
Statistics = 99	Integration = 1.0 seconds

D_2 Background Correction with diffraction grating filter
Circulating Pump autotransformer = 70% power

Instrument Conditions for Varian SpectrAA 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	Cal. Zero Rate = 0
Expansion Factor 1.0	Measurement Time = 4.5 s
Minimum Reading = Disabled	Pre-Read Delay = 0 s
Smoothing = 9 pt	Vapor Type = Cold Vapor
Conc. Units = ng	Burner Height = 16.0 mm
Conc. Decimal places = 2	

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. Autozero the AA by aerating deionized water through the cold vapor mercury analyzer.
3. Transfer the sample from the digestion cup to a glass bottle. Add 10 mL of hydroxylamine hydrochloride/10% sodium chloride to the digestion cup, then transfer to the glass bottle with the sample. Swirl sample until no purple or brown color remains. Rinse the digestion cup with three portions of deionized water, adding the rinse to the sample in the glass bottle each time. Be careful not to end up with the bottle more than two-thirds full.
4. Add 5.0 mL of 10% stannous chloride/0.5 M sulfuric acid 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 and record the maximum absorbance as the response.
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. Record the volume of waste neutralized in the Acid/Base Waste Log.
10. Collect the exhausted stocks and standards in a glass bottle identified as "Hazardous Waste - Mercuric Nitrate in % acid solutions. Corrosive Toxic." Note the start date. Each waste bottle will require an analysis before it will be accepted for disposal.

Sample Analysis Using Perkin Elmer FIMS-100 Flow Injection Mercury Analysis System

1. Prepare the following:
   Carrier Solution (3% HCl)
   Reductant Solution (5% $SnCl_2$, 1% Silicon Defoaming Agent, in 3%HCl)
   Weigh 50g $SnCl_2$ and add to 990 mL 3% HCl. Add 10 mL Silicon Defoaming Agent using 5 mL micropipettor.
2. Turn on computer and printer.
3. Turn on Nitrogen (400 psi).
4. Turn on FIMS 100 mercury analyzer and allow to warm up for 10 minutes minimum.
5. Press Ctrl+Alt+Del (on computer).
6. Username: administrator.
7. Leave password field blank. Click on "OK".
8. Open appropriate project Excel file prepared from Hg Calculations-Master and minimize the Excel window.
9. Double click on AA Winlab Analyst icon.
10. Choose "Use a custom designed workspace".
11. Choose "Hg.fms" > "file" > "open" > "method" > "Hg Analysis".
12. Click on "Browse" in Results Data Set window and enter a new data set name (DateProject). Be sure that the save data and print log boxes are both checked.
13. Turn clamps on the peristaltic pump rollers in order to allow pump to work.
14. Check filter compartment cover to see that it has been tightened.
15. Attach tubing from filter compartment to cell.
16. Click on Manual button (on top toolbar).
17. Click on FIAS button (on top toolbar). Run FIAS once using clean deionized water (Click on the "FIAS on/off" button). Place collection tubes into appropriate solution bottles (Red = Reductant solution, Yellow = Carrier Solution) and run FIAS two more times checking the flow of the instrument and the lines for bubbles while it is running. Remember while running a sample set to periodically check carrier and reductant volumes, so they do not deplete.
18. Just prior to analysis of all blanks, standards and samples (steps 19-22), add 10 mL of 10% (w/v) Hydroxylamine Hydrochloride - 10% (w/v) Sodium Chloride in two 5 mL aliquots, mix sample until no purple or brown color remains. Dilute to 50 mL with deionized water using the correct line on the digestion cup.
19. Rinse the collection tube with deionized water and place in the blank solution. Click on "analyze blank" and allow instrument time to complete triplicate analysis.
20. Rinse the collection tube with deionized water and place in the lowest standard. Choose appropriate standard concentration and click on "analyze standard" and allow instrument time to complete triplicate analysis. In the appropriate Excel file for that project, enter 0.000 for the blank absorbance and enter the mean Blank Corrected Signal

value for the standard. Repeat this step for each of the five standards to be run in order of lowest to highest to develop the standard curve.

21. Rinse the collection tube with deionized water and place in appropriate sample. Enter sample ID code into the appropriate field. Rinse the collection tube with DI water and place in appropriate sample. Click on "analyze sample" and allow instrument time to complete triplicate analysis. Enter the mean Blank Corrected Signal value into the appropriate Excel file for that project. Repeat this step for each of the samples to be analyzed.
22. The second Blank, second set of standards, and Dorm-2 samples should be run as they were above, sometime in between samples, to check the precision of the instrument. For example, if the sample set contains 52 samples, including duplicates and spikes, run the first set of standards (~13 samples), the Blank and the lowest standard (50 ng/L), Dorm 2-1 (1) and (2) (~13 samples), the next two standards (100 ng/L and 500 ng/L), Dorm 2-2 (1) (~13 samples), the last two standards (1000 ng/L and 6000 ng/L) and finally Dorm 2-2 (2). It is best to try to analyze the duplicates and spikes without interruption, so more or less than 13 samples may be analyzed between standards in order to keep the samples together and in order.

WHEN ANALYSIS OF ALL SAMPLES AND STANDARDS IS COMPLETE:

23. Place sample collection tube, and lines from reductant and carrier solutions into beaker of deionized water.
24. Flush/clean tubing with deionized water by running FIAS two times.
25. Lift collection tubing out of deionized water and run FIAS one more time to allow air to pass through all tubing. When FIAS is finished running, place collection tubing back into beaker of DI water for storage.
26. Raise waste lines out of liquid in waste container so liquid does not back up.
27. Release the peristaltic pump rollers so that tubing is not compressed.
28. Detach line from cell.
29. Unscrew the filter compartment cover and, using forceps to handle filter, dry filter with a Kimwipe.
30. Print report. Choose "file" > "utilities" > "reporter" > "Open Design". Choose "WR01 Mussel" (double-click), then double-click on the number 1 under result name and choose the data set for that day. Click "OK" > "Print Report" and close the reporter window.
31. Save Excel file to floppy disk.
32. Turn off FIMS instrument, computer, nitrogen, gas and printer.
33. Record the date, project, analyst, number of injections, and time run in FIMS-100 usage record book located on top of instrument.

PROCEDURES FOR DETERMINING DETECTION LIMITS

INTRODUCTION

Detection limits should be calculated by the following procedure for analytical methods utilizing a calibration curve. Examples of instruments that would provide data used to generate calibration curves are: gas chromatograph, organic carbon analyzer, high pressure liquid chromatograph, atomic absorption instrument, and the specific ion electrodes.

EQUIPMENT

- Standard or sample estimated to be within 5 times of the detection limit
- Calculator capable of doing standard deviations
- Student t chart

PROCEDURE

1. Select a low level standard that is estimated to be within 1-5 times the detection limit.
2. Analyze the standard a minimum of 7 times in the same manner as the samples.
3. Determine a mean and standard deviation, $SD_{(n-1)}$, for the response of the 7 replicates.
4. Calculate the instrument detection limit by multiplying the standard deviation by the student t value for the number of replicates (n-1):

$$DL = SD \times t_{(n-1)}$$

Student's t:

# Observations	$t_{(n-1)}$
7	3.143
8	2.998
9	2.896
10	2.821
11	2.764

5. Calculate the detection limit concentration using the calibration curve.
6. Compare the detection limit to the mean concentration. If the mean concentration is greater than 5-10X the calculated detection limit, repeat steps 1-7 using a lower concentration for the replicates.
7. Compare the calculated response of the detection limit concentration. During some procedures the calculated response at the detection limit will be a fictional number below the instrument's sensitivity. This may indicate that the calibration curve is not representative at that level. These procedures should be evaluated on a case-by-case basis with the project director.

PROCEDURES FOR CALCULATING MERCURY CONCENTRATIONS USING COLD VAPOR MERCURY ANALYSIS

INTRODUCTION

The following equations are used in calculating mercury concentrations.

PROCEDURE

Concentration of Mercury Stock Solution:

$$\frac{\text{mass } HgCl_2 \text{ (g)}}{271.50 \text{ g/mol } HgCl_2} \times \frac{200.59 \text{ g/mol Hg}}{100 \text{ mL}} \times \frac{\text{purity (\%)}}{100\%} \times \frac{10^6 \text{ µg}}{\text{g}} = \text{conc. Hg(µg/mL)}$$

Concentration of Mercury Sub-Stocks:

$$C_1V_1 = C_2V_2$$

where C_1 = concentration of mercury stock solution
C_2 = concentration of diluted solution
V_1 = volume of stock solution used
V_2 = volume of diluted solution

Amount of Hg in Each Standard:

ng of Hg = concentration of Hg sub-stock (ng/mL) x mL of sub-stock used

Calibration Curve:

ng of Hg (x) vs. maximum response (y)
Results in a linear regression with an intercept and slope. Using the equation for the regression:

$y = mx + b$ where m = slope and b = intercept

and inserting the response for any given sample, the concentration of Hg or y can be determined.

Calculation of µg Hg/g Tissue:

Divide the µg Hg calculated using the calibration curve by the mass of tissue analyzed.

FIMS MERCURY ANALYSIS - STOCK, STANDARD AND SPIKE PREPARATION

INTRODUCTION

This procedure is used for the preparation of the stock, analytical standards, blanks and spikes for analysis using the Perkin Elmer FIMS-100 Mercury Analyzer. The fish/tissue used for the spikes should be weighed by the use of the "Sample Weighing for Metals Analysis (SA/11)" procedure. The labware used in this procedure should be cleaned by the "Routine Labware Cleaning for Metals Analysis" (SA/8) procedure.

EQUIPMENT LIST

- Ground Tissue Samples for Spikes
- Class A Pipettes (1 mL and 3 mL)
- Deionized Water
- Pipette Bulb
- 1000 mg/L Mercuric Nitrate Stock/Reference Solution
- Concentrated Hydrochloric Acid (Trace Metal Grade)
- 5% (w/v) Potassium Permanganate ($KMnO_4$)
- Micropipettes and Tips
- Teflon Beakers for Making Substocks
- Mercury Waste Container
- 2 Volumetric Flasks (100 mL)
- Polypropylene Digestion Cups (Environmental Express)

PROCEDURE

1. Pipet 1 mL of a 1000 mg/L mercuric nitrate stock solution into a 100 mL volumetric flask containing ~60 mL of deionized water, 1 mL trace metal grade concentrated HCl, and 100 µL 5% $KMnO_4$. Dilute to 100 mL with deionized water to prepare a 10 mg/L Hg substock. Label this solution with the concentration, date and initials as it must be remade once a month.

2. Pipet 1 mL of the 10 mg/L Hg substock solution into a 100 mL volumetric flask containing ~60 mL of deionized water, 0.5 mL trace metal grade concentrated HCl, and 100 µL 5% $KMnO_4$. Dilute to 100 mL with deionzed water to prepare a 100 µg/L Hg substock. Label this solution with the concentration, date and initials as it must be remade once a week.

3. Pipet the following volumes of deionized water and 100 µg/L Hg substock into digestion cups labeled with the appropriate concentrations which are based on the final volume (50 mL) of standard at time of analysis. Use a micropipette to deliver all water volumes and stock Hg volumes less than 1 mL. Use a class A pipet to deliver 3 mL 100 µg Hg/L substock.

Concentration (ng/L)	Amount of 100 µg/L substock	Amount of DI water
Blank	0	3 mL
50	25 µL	2975 µL
100	50 µL	2950 µL
500	250 µL	2750 µL
1000	500 µL	2500 µL
6000	3 mL	0 mL

4. Each blank and standard should be prepared in duplicate.

5. A total of 10% of samples analyzed for mercury should be spiked in duplicate. Spiking is accomplished by pipetting a known volume of the 100 µg/L Hg substock into a digestion cup containing a known weight of fish tissue. A micropipette may be used to deliver two 750 µL aliquots onto pre-weighed tissue to give a total spiking volume of 1.5 mL.

6. All mercury waste from rinsing pipettes, beakers, etc. should be disposed of in mercury waste container. Volume and concentration placed in waste container should be recorded on the hazardous waste container inventory form for that bottle.

Appendix 4

Quality Assurance Report: 2006 Field Data Collection for EPA Grant # 96540801-0

Quality Assurance Report: 2006 Field data collection for EPA Grant # 96540801-0

By:

Matt Hudson
Environmental Biologist
Great Lakes Indian Fish and Wildlife Commission
Field Manager, EPA Grant # 96540801-0

Introduction

The following report satisfies quality assurance reporting requirements outlined in section 14.1 of the Quality Assurance Project Plan entitled "Great Lakes Indian Fish and Wildlife Commission (GLIFWC) Testing of Fish for Mercury Using EPA Supplemental Funds - EPA Grant # 96540801-0".

Quality Assurance Summary

1. System and Performance Audits - Results from the field audit, which included an audit of field walleye collections and an audit of GLIFWC laboratory tissue processing and data collection, are described in Appendix 4A. In general, protocols for data collection and sample handling were followed well by staff observed during the audits. Minor comments were made on improving the completion of chain of custody forms, but no major problems or deviations were noted.

2. Completeness and Quality of Field Sampling Process and Data - Funds were available to analyze 300 walleye for mercury from 25 lakes in 2005 under EPA Grant # 96540801-0. Plans called for twelve walleye to be collected, with three fish taken from each of four size ranges (12.0 to 14.9, 15.0 to 17.9, 18.0 to 22.0, and greater than 22.0 inches). Because twelve fish are not typically collected from all lakes, additional lakes were selected to reach the goal of 300 fish. A total of 40 lakes were selected for sampling and a total of 390 walleye samples from 37 lakes were collected (Table 1). One sample from Lake Minnesuing was not able to be processed. This sample was in a separate bag from the rest of the samples from this lake and was found during creation of the fish compost pile in a condition too decomposed to be useful for mercury analysis. Thus, only 389 of the 390 collected samples were analyzed. In addition, 12 samples of materials used to create a compost pile with fish waste from the walleye mercury project were analyzed, bringing the total number of mercury analyses conducted in 2006 to 401. Results from the compost pile samples will be submitted in a separate administrative report.

Overall, sample collection and analysis exceeded project goals. Observed collection of field samples and tissue processing and data collection was adequately followed according to QAPP guidelines. Therefore, no problems are seen with the quality of field data for this project.

3. Deviations - No deviations were reported during the 2006 walleye mercury project.

4. Significant Quality Assurance Problems and Recommended Solutions - No significant quality assurance problems were noted during the 2006 field sample and data collection process.

Table 1. Summary of completeness of mercury walleye collections during spring 2006 as part of EPA Grant # 96540801-0.

			Size Group					
State	County	Lake Name	12.0 to 14.9	15.0 to 17.9	18.0 to 22.0	> 22.0	Tot Collected	% of Goal
WI	Vilas	Sherman Lake	3	3	3	3	12	100%
WI	Vilas	Squaw Lake	3	3	2	2	10	83%
WI	Oneida	Bearskin Lake	3	3	3	3	12	100%
WI	Vilas	Big St.Germain Lake	3	3	3	3	12	100%
WI	Oneida	Crescent Lake	3	3	3	3	12	100%
WI	Vilas	Big L. (Boulder Jct.)	3	3	3	3	12	100%
WI	Sawyer	Sissabagama Lake	2	3	3	4	12	100%
WI	Vilas	Harris Lake	3	3	3	3	12	100%
WI	Sawyer	Windfall Lake	3	3	2	3	11	92%
WI	Iron	Trude	3	3	3	1	10	83%
WI	Sawyer	Lake Chippewa	3	3	2	0	8	67%
WI	Sawyer	Lake Chippewa (Chief Lake)	3	3	3	0	9	75%
WI	Sawyer	Lake Chippewa (Crane Lake)	3	3	3	3	12	100%
WI	Sawyer	Round Lake	3	3	3	3	12	100%
WI	Sawyer	Lost Land Lake	0	0	0	0	0	0%
WI	Sawyer	Lac Courte Oreilles	3	3	3	3	12	100%
WI	Washburn	Stone Lake	1	3	3	3	10	83%
WI	Washburn	Birch Lake	0	0	0	0	0	0%
WI	Sawyer	Sand Lake	3	3	3	3	12	100%
WI	Iron	Turtle-Flambeau Flowage	3	3	3	3	12	100%
WI	Lincoln	Rice R. Flowage Chain	3	3	3	3	12	100%
WI	Iron	Gile Flowage	0	0	0	0	0	0%
WI	Price	Butternut Lake	3	3	3	3	12	100%
WI	Oneida	Clear Lake	3	3	2	0	8	67%
WI	Oneida	Katherine Lake	3	3	3	3	12	100%
WI	Price	Round Lake	3	2	0	0	5	42%
WI	Oneida	Buckskin Lake	3	3	3	3	12	100%
WI	Oneida	Blue Lake	0	0	0	0	0	0%
WI	Oneida	Carrol Lake	0	0	0	0	0	0%
WI	Vilas	Little John Lake	3	3	1	1	8	67%
WI	Vilas	Horsehead Lake	3	3	2	0	8	67%
WI	Vilas	Catfish Lake	3	3	3	3	12	100%
WI	Oneida	Pelican Lake	3	3	3	3	12	100%
WI	Vilas	Lac Vieux Desert	3	3	3	3	12	100%
WI	Langlade	Sawyer Lake	3	3	3	0	9	75%
WI	Langlade	Rose Lake	3	3	2	0	8	67%
WI	Sawyer	Nelson Lake	2	3	3	3	11	92%
WI	Douglas	Lake Minnesung	3	3	3	3	12	100%
WI	Burnett	Little Yellow Lake	0	3	2	0	5	42%
WI	Barron	Prairie Lake	0	2	3	1	6	50%
MI	Gogebic	Gogebic	3	3	3	3	12	100%
MN	Mille Lacs	Mille Lacs	3	3	3	3	12	100%
		TOTAL COLLECTED	101	109	99	81	390	
		% OF REQUESTED	80.2%	86.5%	78.6%	64.3%	77.4%	

Appendix 4A

Field audits of walleye collection and tissue processing data collection for EPA Grant # 96540801-0

Audit of Fisheries Assessment Crew collection of fish for Hg Analysis

GLIFWC Procedure No. AD.005
Revision No. 1
Revision Date. 6/4/2004
Initial Date. 8/3/2001

Field Audit Form

Section 1: Data Collection

Data Type	(+/-)[a]	Comments	Date Observed
Biota sample	+	Conscious effort to read & follow directions in packet	4/17/06
Age[b]			

[a]: + = in compliance, - = out of compliance
[b]: Age will be determined at lab and not in the field, only scales or spines will be collected.

General Comments:

Section 2: Tissue Collection

Data Type	(+/-)[a]	Comments	Date Observed

[a]: + = in compliance, - = out of compliance

General Comments: Not part of field collection - separate audit

Section 3: Sample Packaging

Data Type	(+/-)[a]	Comments	Date Observed

Biota sample	+		4/17/06

[a]: + = in compliance, - = out of compliance

General Comments: Samples placed in black plastic bag as instructed

Section 4: Storage

Data Type	(+/-)[a]	Temp (°C)[b]	Comments	Date Observed
Biota sample	+	<-10°C		4/17/06

[a]: + = in compliance, - = out of compliance
[b]: Temperature of storage container

General Comments: Samples kept on ice in cooler in field & immediately placed in freezer upon returning to hotel that evening

Section 5: Custody (Chain-of-Custody Forms)

Data Type	(+/-)[a]	Comments	Date Observed
Walleye data	+		4/17/06

[a]: + = in compliance, - = out of compliance

General Comments: Looks good - included necessary info

Section 6: Transport

Data Type	(+/-)[a]	Comments	Date Observed
Biota samples	+		4/17/06

[a]: + = in compliance, - = out of compliance

General Comments: See #4

Auditor Name: Matt Hudson

Auditor Signature: Matt Hudson

Date Signed: 4/18/06

Audit of Warden Purchase & Collection of Fish for Hg Analysis

Field Audit Form

Section 1: Data Collection

Data Type	(+/-)[a]	Comments	Date Observed
Biota	+	Requested data was recorded properly	4/24/06
Age[b]			

[a]: + = in compliance, - = out of compliance
[b]: Age will be determined at lab and not in the field, only scales or spines will be collected.

General Comments:

Section 2: Tissue Collection

Data Type	(+/-)[a]	Comments	Date Observed

[a]: + = in compliance, - = out of compliance

General Comments: Not applicable

Section 3: Sample Packaging

Data Type	(+/-)[a]	Comments	Date Observed

~~Biota~~ Biota	+	Placed in plastic bags as instructed	4/24/06

[a]: + = in compliance, - = out of compliance

General Comments:

Section 4: Storage

Data Type	(+/-)[a]	Temp (°C)[b]	Comments	Date Observed
Biota	+	Cooler on ice	Overall good - make sure samples are on ice for all transport	4/24/06

[a]: + = in compliance, - = out of compliance
[b]: Temperature of storage container

General Comments: Not all frozen samples were in a cooler on ice (warden transferred custody of all his current fish to me - 5 lakes). This wasn't a big deal because air temps were below freezing, but shouldn't happen during day or warmer weather transfers. Fresh samples were put on ice as instructed.

Section 5: Custody (Chain-of-Custody Forms)

Data Type	(+/-)[a]	Comments	Date Observed
Biota physical data	+	Well-done	4/24/06

[a]: + = in compliance, - = out of compliance

General Comments:

Section 6: Transport

Data Type	(+/-)[a]	Comments	Date Observed
			4/24/06

[a]: + = in compliance, - = out of compliance

General Comments: See #4
Fresh samples were placed on ice immediately after purchase

Auditor Name: Matt Hudson

Auditor Signature: Matt Hudson Date Signed: 4/25/06

Audit lab processing of Walleye @ GLIFWC by Shane Crumb, Env Biologist Aide

GLIFWC Procedure No. AD.005
Revision No. 1
Revision Date. 6/4/2004
Initial Date. 8/3/2001

Field Audit Form

Section 1: Data Collection

Data Type	(+/-)[a]	Comments	Date Observed
Whole fish wt	+	No problems	5/1/06
fillet wt.	+	Good ~~placed~~ taved scale w/ fillet bag, put fillet in bag before weighing	↑
			5/1/06
Age[b]	+	Collected spine for aging & placed in labeled envelope	5/1/06

[a]: + = in compliance, - = out of compliance
[b]: Age will be determined at lab and not in the field, only scales or spines will be collected.

General Comments: Has good system together for systematically collecting required data for each fish

Section 2: Tissue Collection

Data Type	(+/-)[a]	Comments	Date Observed
Removal of fillet	+	Good - wore nitrile gloves (needs more ordered)	5/1/06

[a]: + = in compliance, - = out of compliance

General Comments: Make sure stainless steel table is completely cleaned between samples

Section 3: Sample Packaging

Data Type	(+/-)[a]	Comments	Date Observed

~~Biota~~ Biota	+	Placed in plastic bags as instructed	4/24/06

[a]: + = in compliance, - = out of compliance

General Comments:

Section 4: Storage

Data Type	(+/-)[a]	Temp (°C)[b]	Comments	Date Observed
Biota	+	Cooler on ice	Overall good - make sure samples are on ice for all transport	4/24/06

[a]: + = in compliance, - = out of compliance
[b]: Temperature of storage container

General Comments: Not all frozen samples were in a cooler on ice (warden transferred custody of all his current fish to me - 5 lakes). This wasn't a big deal because air temps were below freezing, but shouldn't happen during day or warmer weather transfers. Fresh samples were put on ice as instructed.

Section 5: Custody (Chain-of-Custody Forms)

Data Type	(+/-)[a]	Comments	Date Observed
Biota physical data	+	Well-done	4/24/06

[a]: + = in compliance, - = out of compliance

General Comments:

Section 6: Transport

Data Type	(+/-)[a]	Comments	Date Observed
			4/24/06

[a]: + = in compliance, - = out of compliance

General Comments: See #4
Fresh samples were placed on ice immediately after purchase

Auditor Name: Matt Hudson

Auditor Signature: Matt Hudson Date Signed: 4/25/06

Appendix 5

Lake Superior Research Institute Laboratory Limit of Detection (LOD) and Limit of Quantitation (LOQ) Study for Mercury in Biota, 2005

Detection limit for Mercury in Biota- 2006

# of replicates	Degrees of Freedom	t value							
7	6	3.143		When calculating detection limits a minimum of seven replicates is required. The analyte should not exceed ten times the expected detection limit.					
8	7	2.998							
9	8	2.896							
10	9	2.821							
11	10	2.764							
16	10	2.602							
21	20	2.528							
26	25	2.485		t-value x std. Dev. = detection limit (LOD)					
31	30	2.457							
61	60	2.39		LOQ = 10/3 x LOD					
0	0	2.326							
Analyzed May 4, 2006									
Sample	Tissue Type	ng/l	ng Hg	g sample		ug Hg/g			
GP-RT-HRC-3 #1	whole fish composite	237.39856	11.87	0.272	0.043639	0.044			
GP-RT-HRC-3 #2	whole fish composite	210.5955	10.53	0.256	0.041132	0.041			
GP-RT-HRC-3 #3	whole fish composite	210.5955	10.53	0.243	0.043332	0.043			
GP-RT-HRC-3 #4	whole fish composite	245.05658	12.25	0.292	0.041962	0.042			
GP-RT-HRC-3 #5	whole fish composite	179.96343	9.00	0.214	0.042048	0.042			
GP-RT-HRC-3 #6	whole fish composite	176.13442	8.81	0.219	0.040213	0.04	**Std. Dev.**	**DL (ug/g)**	**LOQ**
GP-RT-HRC-3 #7	whole fish composite	195.27946	9.76	0.238	0.041025	0.041	0.001408	0.004221	0.014069
GP-RT-HRC-3 #8	whole fish composite	187.62145	9.38	0.232	0.040436	0.04			
					0.001265	0.001408			
		2006	**Hg LOD =0.0042ug/g LOQ=0.0141ug/g**						
		2005	**Hg LOD =0.01128 ug/g LOQ=0.03676ug/g**						
		2004	**Hg LOD =0.00126ug/g LOQ=0.004194ug/g**						