2005 Walleye Total Mercury Analyses

by

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INTRODUCTION

Walleye (*Stizostedion vitreum*) are targeted for harvest by Chippewa tribal members from many off-reservation inland lakes in Wisconsin each spring (Krueger 2006). 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 2005. 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 are safer to eat. 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 2005, 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 (GLIFWC 2005). The maps for the six Wisconsin Ojibwe tribes were updated in 2005 using a methodology described in DeWeese and Madsen (2006) 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 2005. 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), and EPA Science to Achieve Results (STAR) grant funds received to test mercury levels in walleye from 10 lakes in Minnesota during 2004 and Michigan during 2005.

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: 2005
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 Spring Walleye 2004 sample analysis (mean ± 2 times the standard deviation of all DORM-2 analyses). The calculated acceptable range was 3.27 to 5.31 µg Hg/g.

DORM-2 was analyzed in duplicate with each batch of 20 samples. The measured mean for the 2005 STAR grant funded analyses was 4.40 ± 0.35 µg Hg/g tissue (94.9 ± 7.4 percent of certified value) and 4.41 ± 0.42 µg Hg/g tissue (95.1 ± 9.0 percent of certified value) for the EPA Supplemental funded analyses. All analyses were within the acceptance range of 70.4 to 114 percent of the certified value.

Spikes

A total of 57 spike samples were analyzed (12.2 percent of total samples). Spike recovery was considered acceptable when it was in the range of 69.1 to 123 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 2004 sample analysis. Mean recovery for the 43 spiked samples analyzed with EPA Supplemental funds was 87.3 ± 12.8 percent. Five spike recovery values were outside of the acceptance range. The sample spiking was repeated and the results of the second analysis were within the acceptance range. The reported mean ± standard deviation includes percent recoveries from samples outside the acceptable range along with the re-analysis

of these samples. Mean recovery for the 14 spiked samples was 82.3 ± 22.9 percent. Two of the spiked samples had recoveries outside the acceptable range and were re-analyzed. The re-analysis resulted in recoveries within the acceptable range. Again, the reported mean ± standard deviation includes percent recoveries from samples outside the acceptable range along with the re-analysis of these samples. An asterisk denotes which walleye samples (for both the STAR and EPA Supplemental grants) were re-analyzed in duplicate because their associated spike relative percent agreement values were outside of the acceptable quality control range (Appendix 3).

Duplicates

Overall, 11.1 percent, or 52 out of 468 total samples were analyzed in duplicate. Fish tissues were analyzed in duplicate 12 times as part of the STAR and 40 times as part of the EPA Supplemental sample analyses. Two portions of the same tissue were digested and analyzed independently. Duplicate values were acceptable when having a relative percent agreement >75.9 percent. The acceptable value was calculated as the mean ± 2 times the standard deviation of all duplicate analyses conducted from Spring Walleye 2004 sample analysis at the LSRI laboratory.

Relative percent agreement between duplicate analyses averaged 94.5 ± 5.5 percent for the STAR and 94.1 ± 6.3 percent for the EPA Supplemental analyses. Two of the relative percent agreement values for the EPA Supplemental analyses were below the acceptance range and were analyzed a second time. The results of the second analysis were within the acceptance range. All other duplicates were above the acceptance value. An asterisk denotes which walleye samples were re-analyzed in duplicate because their associated duplicate relative percent agreement values were outside of the acceptable quality control range (Appendix 3).

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 were considered acceptable when the relative percent agreement was in the range of 63.8 – 100 percent. 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 2004 project. The procedural blanks were 90.3 ± 9.3 percent for the STAR and 87.7 ± 7.7 percent for the EPA Supplemental analyses. 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 158 quality control samples measured. Nine samples, or 5.7 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 2005, 342 and 126 skin-off walleye fillets were collected and analyzed for total mercury from 31 Wisconsin lakes and 11 Michigan lakes, respectively (Appendix 3). Samples from the 31 Wisconsin lakes and one Michigan lake (Bond Falls Flowage, Ontonagon County) were analyzed using EPA Supplemental funds and the remaining 10 lakes from Michigan were analyzed using STAR grant funds.

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

Table 1. Summary statistics for mercury concentration (ug Hg/g fish tissue) and fresh length (inches) for walleye collected from Wisconsin lakes during spring 2005.

Lake	# of Fish	Mean Conc.	Std. Dev. Conc.	Median Conc.	Max. Conc.	Min. Conc.	Mean Length	Std. Dev. Length
ANNABELLE L	12	0.558	0.172	0.498	0.944	0.383	14.2	2.7
ANVIL L	10	0.368	0.149	0.296	0.628	0.220	16.9	3.0
BASS-PATTERSON L	12	0.426	0.143	0.380	0.720	0.271	18.6	4.7
BIG FORK L	11	0.783	0.179	0.760	1.08	0.578	17.8	3.6
BIG L (MI BORDER)	12	0.250	0.102	0.260	0.405	0.071	18.3	4.2
BIG MUSKELLUNGE L	12	0.402	0.207	0.346	0.927	0.174	17.0	4.0
BUTTERNUT L	12	0.272	0.128	0.272	0.568	0.115	18.1	3.0
DAM L	12	0.566	0.205	0.542	0.946	0.278	18.5	3.9
ENTERPRISE L	9	0.370	0.144	0.363	0.602	0.211	17.0	3.1
ISLAND L	10	0.193	0.119	0.162	0.513	0.099	17.2	2.8
KAWAGUESAGA L	12	0.224	0.159	0.176	0.576	0.061	18.0	4.3
KENTUCK L	12	0.376	0.364	0.238	1.19	0.137	18.5	5.5
L CHETAC	12	0.192	0.095	0.208	0.393	0.066	17.5	3.2
L CHIPPEWA	12	0.641	0.311	0.546	1.36	0.258	17.5	3.8
LONG L	11	0.309	0.112	0.317	0.445	0.132	17.7	3.4
MINOCQUA L	12	0.308	0.175	0.262	0.712	0.165	18.7	4.6
N TWIN L	12	0.340	0.215	0.258	0.893	0.168	19.4	4.8
NAMEKAGON L	12	0.415	0.125	0.438	0.630	0.260	18.2	3.3
PIKE L CHAIN	12	0.310	0.204	0.214	0.795	0.133	18.1	4.1
PLUM L	12	0.367	0.096	0.357	0.522	0.180	18.5	3.6
PRESQUE ISLE L*	12	0.350	0.205	0.315	0.877	0.132	18.7	4.8
RAZORBACK L	8	0.305	0.122	0.300	0.437	0.105	16.0	2.8
RED CEDAR L	9	0.342	0.160	0.260	0.617	0.175	16.6	2.4
SHERMAN L	12	0.328	0.115	0.320	0.543	0.192	18.4	3.2

SISKIWIT L	7	0.549	0.265	0.564	0.877	0.235	15.4	1.7
SQUASH L	7	0.375	0.102	0.336	0.558	0.278	17.2	2.7
SQUIRREL L	13	0.360	0.199	0.295	0.856	0.137	18.6	3.9
STAR L	12	0.315	0.169	0.289	0.680	0.102	18.2	4.4
TEAL L	9	0.289	0.097	0.284	0.467	0.158	16.4	3.3
UPPER TURTLE L*	10	0.230	0.119	0.206	0.497	0.092	17.3	3.3
WILLOW FL	12	0.724	0.304	0.726	1.25	0.336	17.9	3.2

* 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 2005.

Lake	# of Fish	Mean Conc.	Std. Dev. Conc.	Median Conc.	Max. Conc.	Min. Conc.	Mean Length	Std. Dev. Length
BEATONS L	12	0.524	0.118	0.548	0.695	0.305	21.3	1.0
BOND FALLS FL	12	0.574	0.209	0.569	1.10	0.286	17.9	4.0
BRULE L	12	0.606	0.240	0.524	0.999	0.194	15.0	1.9
CISCO L CHAIN	12	0.361	0.207	0.343	0.882	0.109	18.7	3.9
INDIAN L	10	0.361	0.237	0.292	0.815	0.141	19.9	2.4
JAMES L	12	1.26	0.461	1.35	1.78	0.324	21.2	2.4
MARION L	8	0.624	0.268	0.579	1.00	0.370	18.3	2.6
OTTAWA L	12	0.240	0.122	0.212	0.489	0.083	17.7	3.6
STE KATHRYN L	12	0.311	0.136	0.277	0.660	0.191	18.4	2.4
TAMARACK L	12	0.557	0.214	0.569	0.896	0.212	18.5	4.0
WINSLOW L	12	0.497	0.202	0.481	0.752	0.245	16.7	3.0

Walleye lengths ranged from 12.0 to 28.9 inches from Wisconsin lakes and 12.0 to 27.3 inches from Michigan lakes. Total mercury concentrations on a wet weight basis ranged from 0.061 to 1.36 µg Hg/g from Wisconsin lakes and 0.083 to 1.78 µg Hg/g from Michigan lakes.

SUMMARY

Walleye total mercury results from 2005 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°C)

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 and Michigan Ceded Territory Waters During Spring 2005

Total Mercury Concentrations in Muscle Tissue From Walleye Captured in Wisconsin and Michigan Ceded Territory Waters During Spring 2005

by

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Introduction

Skinless fillet samples from walleye (*Stizostedion vitreum*) captured during the spring of 2005 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). The samples were a part of two separate grants and are reported separately. The first group consisted of one hundred fourteen skinless walleye fillets from ten lakes in Michigan that were collected and analyzed as part of the U.S. Environmental Protection Agency's (EPA) Science to Achieve Results (STAR) Grant Number RD83104701-0. The second group of fish consisted of three hundred fifty four skinless walleye fillets from thirty-two lakes in Wisconsin and Michigan collected by tribal spearers and GLIFWC Inland Fisheries assessment crews 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 -18°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).

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). Mercury concentrations and quality assurance calculations were done in Microsoft Excel according to SOP SA/37. The biota method detection limit was 0.0113 µg Hg/g for a tissue mass of 0.2 g. The detection limit was determined using a tuna fish sample 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 38 percent of the walleye analyzed for mercury had moisture content determined.

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 (Appendices A and B).

Duplicate agreement values were acceptable when having a relative percent agreement >75.9%. The acceptable value was calculated as the mean ± 2 times the standard deviation of all duplicate analyses conducted from Spring Walleye 2004 sample analysis at the LSRI laboratory.

A commercial canned tuna fish (*Thunnus sp.*) sample was used as a measurement of laboratory bias on the grinding process for sample preparation. One aliquot from a can of tuna 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. Results were considered acceptable when the relative percent agreement was in the range of 63.8 – 100%. 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 2004 project.

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 2004 sample analysis (mean ± 2 times the standard deviation of all DORM-2 analyses). The calculated acceptable range was 3.27 to 5.31 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 69.1 to 123 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 2004 sample analysis.

A quality assurance audit was conducted by the LSRI quality assurance officer during the Spring Walleye 2005 project. That report is provided in Appendix C.

Results from fish analyzed for the Science to Achieve Results (STAR) Grant (Number RD83104701-0)

Quality Assurance – Mercury analysis of the canned tuna fish from two occasions coincident with the grinding of walleye for the STAR grant resulted in a mean of 90.3 ± 9.3 relative percent agreement (Table 1). Both percent agreement values were within the acceptable range for relative percent agreement.

Analysis of the dogfish shark tissue (DORM-2) standard reference material was conducted in duplicate with each set of walleye tissues analyzed (Table 2). The certified mercury concentration for the dogfish tissue was 4.64 ± 0.26 µg Hg/g. The grand mean and standard deviation was 94.9 ± 7.4 percent of the certified value. All analyses were within the acceptance range of 70.4 to 114% for DORM-2 samples.

Fish tissues were analyzed in duplicate 12 times. Two portions of the same tissue were digested and analyzed independently. Relative percent agreement between the two mercury analyses of the same tissue averaged 94.5 ± 5.5 percent (Table 3). All duplicates were above the minimum acceptable value.

Samples of tissue were spiked with known concentrations of mercury prior to digestion. Mean recovery for the 14 spiked samples was 82.3 ± 22.9 percent (Table 4). Two of the spiked samples had recoveries outside the acceptable range (69.1 – 123%) and were reanalyzed. The reanalysis resulted in recoveries within the acceptable range.

Mercury Analysis – Skinless fillets of 114 walleye from 10 lakes in Michigan were analyzed for total mercury concentration. Total mercury concentrations on a wet weight basis (Table 5) ranged from 0.083 to 1.78 µg Hg/g (parts per million).

Tissue Moisture Analysis – Percent moisture was measured in the muscle of 38 of the 114 (33.3%) ground fillets immediately following grinding (Table 6). Walleye muscle tissue contained an average of 79.0 ± 1.2 percent moisture.

Table 1. Relative Percent Agreement of Total Mercury for Procedural Blank Samples (Before and After Grinding) from the STAR Grant Fish Analysis.

Date of Analysis	Grinding Date	Before Grinding µg Hg/g	After Grinding µg Hg/g	Mean µg Hg/g	Relative* Percent Agreement
8/23/2005	7/18/2005	0.392	0.333	0.363	83.7
8/24/2005	6/22/2005	0.092	0.095	0.094	96.8
			Mean ± Std. Dev.		90.3 ± 9.3

* Relative percent agreement is calculated by the equation (1- |before – after|/mean)100

Table 2. Mercury Concentrations of Dogfish Tissue (Standard Reference Material DORM-2) Analyzed during the STAR Grant Fish Analysis. The Tissue has a Certified Mercury Concentration of 4.64 ± 0.26 µg 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
6/28/05	4.73	102	4.56	98.3
8/11/05	4.44	95.7	4.30	92.7
8/16/05	4.62	99.6	4.23	91.2
8/17/05	3.86	83.2	3.70	79.7
8/25/05	4.98	107	4.52	97.4
9/7/05	4.63	99.8	4.10	88.4
9/20/05	4.65	100	4.31	92.9
		Mean ± Std. Dev.	4.40±0.35	94.9±7.4

Table 3. Relative Percent Agreement for Duplicate Analysis of Total Mercury Content in Skinless Fillet Tissue of Walleye Coincident with STAR Grant Fish Analysis.

Date of Analysis	Lake	Tag Number	µg Hg/g	Duplicate µg Hg/g	Mean µg Hg/g	Relative Percent Agreement
6/28/05	Brule	168	0.200	0.188	0.194	93.8
8/11/05	Beatons	126	0.519	0.553	0.536	93.7
8/11/05	Cisco	7540	0.480	0.501	0.491	95.7
8/11/05	James	7521	0.302	0.346	0.324	86.4
8/16/05	James	7512	1.40	1.68	1.54	81.8
8/17/05	Indian	7593	0.306	0.310	0.308	98.7
8/17/05	Marion	7574	0.377	0.381	0.379	98.9
8/17/05	Ottawa	147	0.081	0.085	0.083	95.2
8/25/05	Tamarack	9184	0.396	0.425	0.411	92.9
8/25/05	Winslow	195	0.717	0.711	0.714	99.2
8/25/05	Winslow	9199	0.300	0.305	0.303	98.3
9/7/05	Ste. Kathryn	157	0.289	0.291	0.290	99.3
				Mean ± Std. Dev.		94.5 ± 5.5

Table 4. Percent of Mercury Recovered from Skinless Walleye Fillet Samples Spiked with Mercury Concurrent with the Analysis of Walleye from the STAR Grant.

Date of Analysis	Lake	Tag Number	Spike #1	Spike #2	Mean	Std. Dev.
6/28/05	Brule	168	121	117	119	2.83
8/11/05	Beatons	126	94.7	92.1	93.4	1.84
8/11/05	Cisco	7540	80.3	73.7	77.0	4.67
8/11/05	James	7512	27.6	10.7	19.2*	12.0
8/11/05	James	7521	95.7	97.0	96.4	0.92
8/16/05	James	7512	95.1	105	100 [R]	7.00
8/17/05	Indian	7593	86.5	68.1	77.3	13.0
8/17/05	Marion	7574	69.9	71.1	70.5	0.85
8/17/05	Ottawa	147	75.1	75.3	75.2	0.14
8/17/05	Ste .Kathryn	157	51.6	76.8	64.2*	17.8
8/25/05	Tamarack	9184	88.4	88.7	88.6	0.21
8/25/05	Winslow	195	85.6	99.0	92.3	9.48
8/25/05	Winslow	9199	86.4	86.5	86.5	0.07
9/7/05	Ste .Kathryn	157	90.3	94.1	92.2 [R]	2.69
				Mean ± Std. Dev.		82.3 ± 22.9

*Spike recoveries for the initial analysis of these samples were out of the acceptable range. Samples were reanalyzed in duplicate on a later date [(R)].

Table 5. Total Mercury Concentration (Wet Weight) in Walleye Fillets from Fish Captured in the Spring of 2005 for the STAR Grant.

Analysis Date	Lake	Tag Number	Fresh Length (in)	Sex	Age(Spine)	µg Hg/g
6/28/05	Brule	165	17.1	Female	6	0.812
6/28/05	Brule	166	13.1	Male	4	0.487
6/28/05	Brule	167	16.1	Male	7	0.424
6/28/05	Brule	168	12.0	Male	4	0.194
6/28/05	Brule	169	13.0	Male	6	0.431
6/28/05	Brule	170	14.6	Female	5	0.503
6/28/05	Brule	171	12.4	Male	5	0.440
6/28/05	Brule	175	15.8	Male	7	0.716
6/28/05	Brule	176	16.7	Male	9	0.893
6/28/05	Brule	177	15.2	Female	5	0.999
6/28/05	Brule	178	15.8	Male	7	0.544
6/28/05	Brule	179	17.6	Female	6	0.830
8/11/05	Beatons	119	20.5	Male	6	0.305
8/11/05	Beatons	120	22.8	Male	10	0.579
8/11/05	Beatons	121	21.8	Male	7	0.408
8/11/05	Beatons	122	20.7	Male	7	0.373
8/11/05	Beatons	125	22.8	Male	10	0.649
8/11/05	Beatons	126	21.0	Male	10	0.536
8/11/05	Beatons	127	21.0	Male	10	0.612
8/11/05	Beatons	128	21.2	Male	8	0.444
8/11/05	Beatons	129	20.2	Male	10	0.524
8/11/05	Beatons	130	22.9	Male	11	0.604
8/11/05	Beatons	131	20.3	Male	10	0.559
8/11/05	Beatons	133	20.7	Male	1	0.695
8/11/05	Cisco	7538	20.1	Male	9	0.436
8/11/05	Cisco	7539	16.0	Male	7	0.212
8/11/05	Cisco	7540	22.9	Female	8	0.490
8/11/05	Cisco	7541	14.0	Male	6	0.165
8/11/05	Cisco	7543	16.6	Male	7	0.371
8/11/05	Cisco	7546	19.7	Male	7	0.317
8/11/05	Cisco	7547	16.8	Male	5	0.206
8/11/05	Cisco	7548	13.6	Male	4	0.109
8/11/05	Cisco	7549	20.6	Female	8	0.276
8/11/05	Cisco	7550	17.2	Male	6	0.369
8/11/05	Cisco	7551	19.0	Male	10	0.498
8/11/05	Cisco	7552	27.3	Female	12	0.882
8/11/05	James	7508	22.0	Male	10	1.26
8/11/05	James	7509	21.2	Male	Unknown	1.67
8/11/05	James	7510	17.7	Male	4	0.415

8/11/05	James	7511	21.5	Male	9	1.05
8/16/05	James	7512	25.5	Female	13	1.54*
8/11/05	James	7513	25.4	Female	11	1.29
8/11/05	James	7514	19.5	Male	Unknown	1.36
8/11/05	James	7515	20.8	Male	10	1.48
8/11/05	James	7516	20.8	Male	9	1.34
8/11/05	James	7518	21.0	Male	10	1.78
8/11/05	James	7521	18.0	Male	5	0.324
8/11/05	James	7522	20.5	Male	9	1.61
8/17/05	Indian	7588	23.0	Male	8	0.324
8/17/05	Indian	7589	17.1	Female	3	0.141
8/17/05	Indian	7590	16.8	Male	5	0.258
8/17/05	Indian	7591	22.4	Male	14	0.815
8/17/05	Indian	7592	23.0	Male	9	0.346
8/17/05	Indian	7593	18.7	Male	6	0.308
8/17/05	Indian	7594	17.8	Male	5	0.175
8/17/05	Indian	7595	19.7	Male	5	0.276
8/17/05	Indian	7596	19.2	Male	4	0.199
8/17/05	Indian	7597	21.7	Male	13	0.771
8/17/05	Marion	1752	22.4	Male	13	0.789
8/17/05	Marion	1755	19.7	Male	9	0.925
8/17/05	Marion	7568	19.9	Male	11	1.00
8/17/05	Marion	7572	14.7	Male	3	0.372
8/17/05	Marion	7574	16.6	Male	3	0.379
8/17/05	Marion	7578	15.8	Male	5	0.370
8/17/05	Marion	7579	19.7	Male	10	0.736
8/17/05	Marion	7581	17.9	Male	4	0.421
8/17/05	Ottawa	134	25.0	Male	10	0.222
8/17/05	Ottawa	137	19.2	Male	9	0.358
8/17/05	Ottawa	139	16.2	Male	5	0.201
8/17/05	Ottawa	140	22.0	Male	9	0.329
8/17/05	Ottawa	141	19.0	Male	10	0.231
8/17/05	Ottawa	142	17.7	Male	10	0.179
8/17/05	Ottawa	143	15.1	Male	6	0.135
8/17/05	Ottawa	144	12.6	Unknown	5	0.107
8/17/05	Ottawa	145	19.0	Male	4	0.372
8/17/05	Ottawa	146	15.9	Male	11	0.177
8/17/05	Ottawa	147	12.4	Female	5	0.083
8/17/05	Ottawa	148	18.5	Male	4	0.489
8/17/05	Ste. Kathryn	149	16.3	Male	7	0.191
8/17/05	Ste. Kathryn	150	21.1	Female	9	0.344
8/17/05	Ste. Kathryn	152	19.3	Male	10	0.462
8/17/05	Ste. Kathryn	153	17.5	Male	8	0.250
8/17/05	Ste. Kathryn	154	15.2	Male	4	0.198

8/17/05	Ste. Kathryn	155	16.8	Male	8	0.292
8/17/05	Ste. Kathryn	156	22.0	Female	9	0.358
9/7/05	Ste. Kathryn	157	21.4	Female	7	0.290*
8/17/05	Ste. Kathryn	158	15.1	Female	5	0.193
8/17/05	Ste. Kathryn	160	19.6	Female	7	0.263
8/17/05	Ste. Kathryn	161	18.6	Male	10	0.660
8/17/05	Ste. Kathryn	162	17.7	Male	5	0.232
8/25/05	Tamarack	9176	17.5	Male	10	0.723
8/25/05	Tamarack	9177	16.0	Male	5	0.896
8/25/05	Tamarack	9178	13.8	Male	6	0.676
8/25/05	Tamarack	9182	25.5	Female	10	0.544
8/25/05	Tamarack	9183	21.5	Female	10	0.412
8/25/05	Tamarack	9184	16.5	Male	7	0.411
8/25/05	Tamarack	9185	18.4	Male	8	0.653
8/25/05	Tamarack	9186	12.8	Male	5	0.212
8/25/05	Tamarack	9187	23.0	Female	8	0.493
8/25/05	Tamarack	9188	19.7	Male	10	0.826
8/25/05	Tamarack	9189	14.6	Male	5	0.240
8/25/05	Tamarack	9190	22.5	Female	9	0.593
8/25/05	Winslow	159	12.3	Male	3	0.347
8/25/05	Winslow	163	19.5	Female	10	0.752
8/25/05	Winslow	195	21.1	Female	8	0.714
8/25/05	Winslow	9191	16.7	Male	7	0.264
8/25/05	Winslow	9192	18.7	Male	9	0.738
8/25/05	Winslow	9193	20.4	Female	10	0.721
8/25/05	Winslow	9194	17.0	Female	8	0.485
8/25/05	Winslow	9195	18.3	Female	7	0.612
8/25/05	Winslow	9196	13.6	Male	6	0.311
8/25/05	Winslow	9197	15.2	Male	7	0.245
8/25/05	Winslow	9198	14.9	Male	6	0.477
8/25/05	Winslow	9199	12.7	Male	3	0.303

*Spike recoveries for the initial analysis of these samples were out of the acceptable range. Samples were reanalyzed in duplicate. Reported results are the mean of those duplicates.

Table 6. Percent Moisture in Walleye Fillets (Measured Immediately After Grinding) from the STAR Grant.

Lake	Tag ID	% moisture	Relative Percent Agreement
Beatons	122	78.1	
Beatons	127	77.8	
Beatons	129	76.6	
Beatons	129 Dup	76.7	99.9
Beatons	131	77.2	
Brule	165	81.3	
Brule	167	79.3	
Brule	170	81.3	
Brule	175	79.3	
Cisco	7540	78.1	
Cisco	7541	78.6	
Cisco	7541 Dup	78.6	100
Cisco	7543	79.1	
Cisco	7546	78.2	
Cisco	7550	79.0	
Indian	7593	80.3	
Indian	7594	78.9	
Indian	7595	79.0	
Indian	7596	79.9	
James	7510	77.9	
James	7514	79.7	
James	7515	79.4	
James	7516	80.5	
James	7518	80.4	
James	7518 Dup	78.9	98.1
Marion	1752	76.8	
Marion	7568	78.8	
Marion	7574	78.5	
Marion	7581	78.4	
Ottawa	134	80.1	
Ottawa	145	77.9	
Ottawa	146	78.5	
Ottawa	148	78.3	
Ste. Kathryn	152	80.7	
Ste. Kathryn	153	79.3	
Ste. Kathryn	156	79.5	
Ste. Kathryn	157	79.3	

Tamarack	9177	79.8	
Tamarack	9177 Dup	79.8	100
Tamarack	9178	79.7	
Tamarack	9184	80.2	
Tamarack	9185	80.0	

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

Quality Assurance – Mercury analysis of the canned tuna fish from 5 occasions coincident with the grinding of walleye collected for the GLIFWC EPA Mercury/Mapping Grant resulted in a mean of 87.7 ± 7.7 relative percent agreement (Table 7). All percent agreement values were within the acceptable range.

Analysis of the dogfish shark tissue (DORM-2) standard reference material was conducted in duplicate with all 14 sets of walleye tissues analyzed (Table 8). The certified mercury concentration for the dogfish tissue was 4.64 ± 0.26 µg Hg/g. The grand mean and standard deviation was 95.1 ± 9.0 percent of the certified value. All results were within the acceptable range of 70.4 – 114%.

Fish tissues were analyzed in duplicate 40 times. Two portions of the same tissue were digested and analyzed independently. Relative percent agreement between the two mercury analyses of the same tissue averaged 94.1 ± 6.3 percent (Table 9). Two of the relative percent agreement values were below the acceptance range and were analyzed a second time and the results of the second analysis were within the acceptance range.

Samples of tissue were spiked with known concentrations of mercury prior to digestion. Mean recovery for the 43 spiked samples was 87.3 ± 12.8 percent (Table 10). Five spike recovery values were outside of the acceptance range (69.1 – 123%). The sample spiking was repeated and the results of the second analysis were within the acceptance range.

Mercury Analysis – Skinless fillets of 354 walleye from 32 lakes in Wisconsin and Michigan were analyzed for total mercury concentration. Total mercury concentrations on a wet weight basis (Table 11) ranged from 0.061 to 1.36 µg Hg/g (parts per million).

Tissue Moisture Analysis – Percent moisture was measured in 140 fish of the 354 fish immediately following grinding (Table 12). Walleye muscle tissue had a mean moisture value of 79.0 ± 0.9 percent moisture.

Table 7. Relative Percent Agreement of Total Mercury in Procedural Blank Samples (Before and After Grinding) from the EPA Mercury/Mapping Grant.

Date of Analysis	Grinding Date	Before Grinding µg Hg/g	After Grinding µg Hg/g	Mean µg Hg/g	Relative* Percent Agreement
8/9/05	7/11/05	0.229	0.181	0.205	76.6
8/11/05	6/27/05	0.076	0.069	0.073	90.4
8/23/05	7/18/05	0.392	0.333	0.363	83.7
8/23/05	6/1/05	0.091	0.083	0.087	90.8
8/24/05	6/22/05	0.092	0.095	0.094	96.8
				Mean ± Std. Dev.	87.7 ± 7.7

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

Table 8. Mercury Concentrations of Dogfish Tissue (Standard Reference Material DORM-2) Analyzed during the GLIFWC EPA Mercury/Mapping Grant Fish Analysis. The Tissue has a Certified Mercury Concentration of 4.64 ± 0.26 µg 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
6/9/05	3.82	82.5	4.04	87.1
6/28/05	4.73	102	4.56	98.3
8/9/05	3.56	76.7	3.75	80.8
8/10/05	4.41	95.0	3.88	83.6
8/16/05	4.62	99.6	4.23	91.2
8/18/05	4.40	94.8	4.33	93.3
8/23/05	5.07	109	3.88	83.6
8/23/05	4.81	104	4.52	97.4
8/23/05	5.07	109	3.88	83.6
8/23/05	4.81	104	4.52	97.4
8/24/05	4.93	106	4.58	98.7
8/25/05	4.98	107	4.52	97.4
9/7/05	4.63	99.8	4.10	88.4
9/20/05	4.65	100	4.31	92.9
		Mean ± Std. Dev.	4.41 ± 0.42	95.1 ± 9.0

Table 9. Relative Percent Agreement for Duplicate Analysis of Total Mercury Content in Skinless Fillet Tissue of Walleye Coincident with the GLIFWC EPA Mercury/Mapping Grant Fish Analysis.

Date of Analysis	Lake	Tag Number	µg Hg/g	Duplicate µg Hg/g	Mean µg Hg/g	Relative Percent Agreement
6/9/2005	Razorback	9281	0.226	0.244	0.235	92.3
6/9/2005	Upper Turtle	9169	0.305	0.319	0.312	95.5
6/28/2005	Bond Falls	9145	0.750	0.700	0.725	93.1
6/28/2005	Enterprise	9097	0.576	0.595	0.586	96.8
6/28/2005	Enterprise	9099	0.261	0.343	0.302	72.8*
8/9/2005	Chetac	1790	0.152	0.160	0.156	94.9
8/9/2005	Kawaguesaga	1864	0.561	0.591	0.576	94.8
8/9/2005	Kawaguesaga	1873	0.061	0.061	0.061	100
8/9/2005	Namekagon	1765	0.271	0.252	0.262	92.7
8/10/2005	Bass Patterson	1967	0.304	0.318	0.311	95.5
8/10/2005	Big	9213	0.330	0.305	0.318	92.1
8/10/2005	Big	9219	0.320	0.321	0.321	99.7
8/10/2005	Big Fork	5060	1.07	0.959	1.02	89.1
8/16/2005	Big Muskellunge	1592	0.177	0.172	0.175	97.1
8/16/2005	Butternut	6480	0.188	0.190	0.189	98.9
8/16/2005	Dam	1894	0.679	0.700	0.690	97.0
8/16/2005	Dam	5078	0.583	0.597	0.590	97.6
8/18/2005	Squash	9107	0.288	0.304	0.296	94.6
8/18/2005	Star	10186	0.358	0.345	0.352	96.3
8/18/2005	Teal	6571	0.256	0.316	0.286	79.0
8/18/2005	Twin Lake Chain	6470	0.178	0.184	0.181	96.7
8/23/2005	Pike	10148	0.133	0.133	0.133	100
8/23/2005	Pike	10160	0.474	0.500	0.487	94.7
8/23/2005	Plum	9076	0.353	0.329	0.341	93.0
8/23/2005	Red Cedar	10199	0.259	0.254	0.257	98.1
8/23/2005	Sherman	1985	0.398	0.382	0.390	95.9
8/23/2005	Squirrel	6567	0.176	0.134	0.155	72.9*
8/24/2005	Chippewa	1952	0.477	0.519	0.498	91.6
8/24/2005	Chippewa	1960	0.264	0.252	0.258	95.3
8/24/2005	Long	10163	0.437	0.453	0.445	96.4
8/24/2005	Minocqua	10106	0.167	0.171	0.169	97.6
9/7/2005	Annabelle	10130	0.529	0.478	0.504	89.9

9/7/2005	Anvil	9277	0.613	0.644	0.629	95.1
9/7/2005	Enterprise	9099	0.212	0.210	0.211	99.1 [R]
9/7/2005	Island	9148	0.210	0.206	0.208	98.1
9/7/2005	Kentuck	1992	0.259	0.260	0.260	99.6
9/7/2005	Presque Isle	10136	0.517	0.517	0.517	100
9/7/2005	Squirrel	6558	0.291	0.298	0.295	97.6
9/7/2005	Squirrel	6567	0.172	0.192	0.182	89.0[R]
9/7/2005	Willow	1782	0.763	0.702	0.733	91.7
				Mean ± Std. Dev.		94.1 ± 6.3

* Duplicate relative percent agreements for the initial analysis of these samples were out of the acceptable range. Samples were reanalyzed in duplicate on 9/7/05 [(R)].

Table 10. Percent of Mercury Recovered from Skinless Walleye Fillet Samples Spiked with Mercury Concurrent with the Analysis of Walleye from the GLIFWC EPA Mercury/Mapping Grant.

Date of Analysis	Lake	Tag Number	Spike #1	Spike #2	Mean	STD
6/9/05	Annabelle	10130	67.1	63.3	65.2*	2.69
6/9/05	Anvil	9277	56.8	61.8	59.3*	3.54
6/9/05	Razorback	9281	101	98.5	99.8	1.77
6/9/05	Upper Turtle	9169	88.8	85.2	87.0	2.55
6/28/05	Bond Falls Flowage	9145	110	103	107	4.95
6/28/05	Enterprise	9097	78.6	86.9	82.8	5.87
6/28/05	Enterprise	9099	122	94.7	108	19.3
8/9/05	Kawaguesaga	1864	80.4	80.8	80.6	0.28
8/9/05	Kawaguesaga	1873	87.0	87.9	87.5	0.64
8/9/05	Chetac	1790	88.7	88.9	88.8	0.14
8/9/05	Namekagon	1765	84.2	80.5	82.4	2.62
8/10/05	Bass Patterson	1967	91.4	88.9	90.2	1.77
8/10/05	Big	9213	93.3	81.1	87.2	8.63
8/10/05	Big	9219	85.1	88.4	86.8	2.33
8/10/05	Big Fork	5060	66.5	73.9	70.2	5.23
8/16/05	Big Muskellunge	1592	99.8	96.8	98.3	2.12
8/16/05	Butternut	6480	97.2	97.6	97.4	0.28
8/16/05	Dam	1894	71.6	73.0	72.3	0.99
8/16/05	Dam	5078	76.5	77.0	76.8	0.35
8/18/05	Squash	9107	92.6	96.1	94.4	2.47
8/18/05	Star	10186	83.7	85.6	84.7	1.34

8/18/05	Teal	6571	101	104	103	2.12
8/18/05	Twin Lake Chain	6470	98.6	95.6	97.1	2.12
8/23/05	Pike	10148	88.3	88.3	88.3	0.00
8/23/05	Pike	10160	82.0	81.1	81.6	0.64
8/23/05	Plum	9076	86.5	82.6	84.6	2.76
8/23/05	Presque Isle	10136	41.6	82.1	61.9*	28.6
8/23/05	Red Cedar	10199	78.0	86.5	82.3	6.01
8/23/05	Sherman	1985	60.5	88.0	74.3	19.4
8/23/05	Squirrel	6558	105	145	125*	28.3
8/23/05	Squirrel	6567	89.7	94.8	92.3	3.61
8/24/05	Chippewa	1952	91.6	88.5	90.1	2.19
8/24/05	Chippewa	1960	89.2	90.6	89.9	0.99
8/24/05	Long	10163	83.3	88.4	85.9	3.61
8/24/05	Minocqua	10106	93.7	95.5	94.6	1.27
8/25/05	Willow	1782	63.8	65.7	64.8 *	1.34
9/7/05	Annabelle	10130	93.2	86.5	89.9[R]	4.74
9/7/05	Anvil	9277	87.8	80.2	84.0[R]	5.37
9/7/05	Island	9148	91.1	90.4	90.8	0.49
9/7/05	Kentuck	1992	102	101	102	0.71
9/7/05	Presque Isle	10136	85.2	89.4	87.3[R]	2.97
9/7/05	Squirrel	6558	98.8	95.0	96.9[R]	2.69
9/7/05	Willow	1782	85.4	77.0	81.2[R]	5.94
			Mean ± Std. Dev.		87.3 ± 12.8	

* Spike Recoveries for the initial analysis of these samples were out of the acceptable range. Samples were reanalyzed in duplicate on a later date [(R)].

Table 11. Total Mercury Concentration (Wet Weight) in Walleye Fillets from Fish Captured in the Spring of 2005 for the GLIFWC EPA Mercury/Mapping Grant.

Analysis Date	Lake	Tag Number	Fresh Length (in)	Sex	Age (Spine)	µg Hg/g
6/9/05	Annabelle	10116	12.0	Male	4	0.383
6/9/05	Annabelle	10117	16.2	Female	6	0.569
6/9/05	Annabelle	10118	12.3	Male	4	0.420
6/9/05	Annabelle	10119	13.0	Male	5	0.406

6/9/05	Annabelle	10120	13.1	Male	7	0.674
6/9/05	Annabelle	10121	12.2	Male	5	0.493
6/9/05	Annabelle	10124	12.7	Male	4	0.474
6/9/05	Annabelle	10125	21.0	Female	11	0.944
6/9/05	Annabelle	10126	16.5	Female	5	0.821
6/9/05	Annabelle	10128	15.4	Female	7	0.531
6/9/05	Annabelle	10129	13.0	Male	6	0.483
6/9/05	Annabelle	10130	12.6	Male	5	0.503*
6/9/05	Anvil	9265	16.5	Male	5	0.244
6/9/05	Anvil	9266	23.8	Female	10	0.261
6/9/05	Anvil	9267	18.6	Male	8	0.402
6/9/05	Anvil	9268	17.1	Male	8	0.428
6/9/05	Anvil	9269	14.4	Male	9	0.617
6/9/05	Anvil	9270	13.1	Male	4	0.220
6/9/05	Anvil	9271	14.9	Male	6	0.293
6/9/05	Anvil	9273	15.4	Male	6	0.299
6/9/05	Anvil	9275	18.1	Male	6	0.285
9/7/05	Anvil	9277	16.8	Male	7	0.628*
8/10/05	Bass Patterson	1961	13.8	Male	4	0.360
8/10/05	Bass Patterson	1962	24.5	Female	8	0.488
8/10/05	Bass Patterson	1963	14.2	Male	4	0.271
8/10/05	Bass Patterson	1965	23.6	Female	8	0.554
8/10/05	Bass Patterson	1966	19.0	Female	6	0.399
8/10/05	Bass Patterson	1967	12.7	Male	4	0.311
8/10/05	Bass Patterson	1968	21.5	Female	6	0.433
8/10/05	Bass Patterson	1970	20.6	Male	8	0.625
8/10/05	Bass Patterson	1971	15.5	Male	5	0.326
8/10/05	Bass Patterson	1972	26.5	Female	11	0.720
8/10/05	Bass Patterson	1973	16.0	Male	5	0.297
8/10/05	Bass Patterson	1974	15.2	Male	6	0.327
8/10/05	Big	9205	16.0	Female	6	0.203
8/10/05	Big	9207	14.9	Female	6	0.202
8/10/05	Big	9208	19.6	Male	8	0.323
8/10/05	Big	9210	15.6	Female	6	0.166
8/10/05	Big	9212	17.0	Female	6	0.175
8/10/05	Big	9213	18.0	Female	8	0.317
8/10/05	Big	9214	14.8	Male	5	0.148

8/10/05	Big	9215	22.1	Female	9	0.354
8/10/05	Big	9216	19.1	Female	7	0.317
8/10/05	Big	9217	26.0	Unknown	12	0.405
8/10/05	Big	9218	12.1	Male	4	0.071
8/10/05	Big	9219	24.5	Unknown	11	0.320
8/10/05	Big Fork	5058	16.3	Female	7	0.838
8/10/05	Big Fork	5059	14.9	Female	6	0.586
8/10/05	Big Fork	5060	23.1	Female	14	1.02
8/10/05	Big Fork	5061	17.9	Female	8	0.714
8/10/05	Big Fork	5064	23.4	Female	14	0.968
8/10/05	Big Fork	5065	18.9	Female	12	1.08
8/10/05	Big Fork	5066	12.9	Female	7	0.630
8/10/05	Big Fork	5069	13.2	Male	7	0.760
8/10/05	Big Fork	5070	15.3	Male	6	0.578
8/10/05	Big Fork	5071	20.4	Female	11	0.818
8/10/05	Big Fork	5072	19.7	Female	8	0.617
8/16/05	Big Muskellunge	1586	15.2	Male	6	0.253
8/16/05	Big Muskellunge	1587	25.0	Female	11	0.927
8/16/05	Big Muskellunge	1588	17.7	Male	9	0.454
8/16/05	Big Muskellunge	1589	17.6	Male	10	0.482
8/16/05	Big Muskellunge	1591	16.1	Male	8	0.435
8/16/05	Big Muskellunge	1592	12.3	Male	3	0.174
8/16/05	Big Muskellunge	1594	24.8	Female	12	0.613
8/16/05	Big Muskellunge	1595	16.6	Female	5	0.311
8/16/05	Big Muskellunge	1596	16.6	Male	8	0.288
8/16/05	Big Muskellunge	1597	14.6	Male	5	0.381
8/16/05	Big Muskellunge	1599	13.4	Male	6	0.286
8/16/05	Big Muskellunge	1600	14.2	Male	6	0.218
6/28/05	Bond Falls Flowage	9131	12.1	Male	4	0.578
6/28/05	Bond Falls Flowage	9132	20.0	Male	9	0.566
6/28/05	Bond Falls Flowage	9134	15.2	Male	5	0.643
6/28/05	Bond Falls Flowage	9135	17.4	Male	6	0.572
6/28/05	Bond Falls Flowage	9136	23.1	Male	7	0.353
6/28/05	Bond Falls Flowage	9137	15.8	Female	5	1.10
6/28/05	Bond Falls Flowage	9138	23.9	Female	8	0.530
6/28/05	Bond Falls Flowage	9139	14.1	Male	6	0.607
6/28/05	Bond Falls Flowage	9141	18.3	Female	7	0.286

6/28/05	Bond Falls Flowage	9142	23.0	Female	8	0.556
6/28/05	Bond Falls Flowage	9144	18.8	Female	9	0.371
6/28/05	Bond Falls Flowage	9145	13.6	Male	5	0.725
8/16/05	Butternut	6478	21.2	Female	12	0.352
8/16/05	Butternut	6479	17.5	Male	7	0.317
8/16/05	Butternut	6480	17.4	Male	7	0.189
8/16/05	Butternut	6481	13.7	Male	5	0.133
8/16/05	Butternut	6482	17.6	Male	9	0.229
8/16/05	Butternut	6483	14.4	Male	5	0.115
8/16/05	Butternut	6484	18.4	Male	12	0.288
8/16/05	Butternut	6487	22.9	Unknown	12	0.392
8/16/05	Butternut	6488	18.5	Male	9	0.265
8/16/05	Butternut	6490	18.8	Male	9	0.279
8/16/05	Butternut	6491	14.5	Male	5	0.141
8/16/05	Butternut	6492	22.7	Female	8	0.568
8/16/05	Dam	1890	15.3	Male	7	0.456
8/16/05	Dam	1891	18.3	Female	7	0.494
8/16/05	Dam	1892	14.9	Male	6	0.340
8/16/05	Dam	1893	19.3	Female	10	0.604
8/16/05	Dam	1894	23.9	Female	9	0.690
8/16/05	Dam	1895	18.7	Female	6	0.407
8/16/05	Dam	1896	13.9	Male	6	0.467
8/16/05	Dam	5073	12.8	Male	7	0.278
8/16/05	Dam	5074	17.6	Male	8	0.616
8/16/05	Dam	5075	19.5	Male	10	0.900
8/16/05	Dam	5077	24.8	Female	13	0.946
8/16/05	Dam	5078	22.8	Female	10	0.590
6/28/05	Enterprise	6495	14.9	Male	7	0.400
6/28/05	Enterprise	6496	16.8	Male	7	0.213
6/28/05	Enterprise	9091	17.7	Female	8	0.375
6/28/05	Enterprise	9093	16.0	Male	8	0.363
6/28/05	Enterprise	9095	13.0	Male	5	0.263
6/28/05	Enterprise	9096	21.8	Female	10	0.318
6/28/05	Enterprise	9097	21.2	Female	11	0.586
6/28/05	Enterprise	9098	18.1	Male	10	0.602
9/7/05	Enterprise	9099	13.4	Male	5	0.211*
9/7/05	Island	9146	16.5	Male	7	0.148

9/7/05	Island	9147	15.4	Female	4	0.100
9/7/05	Island	9148	22.0	Female	6	0.208
9/7/05	Island	9149	14.4	Male	6	0.175
9/7/05	Island	9150	19.1	Female	6	0.181
9/7/05	Island	9151	14.4	Male	5	0.149
9/7/05	Island	9152	14.4	Male	4	0.099
9/7/05	Island	9153	15.8	Male	6	0.212
9/7/05	Island	9154	18.6	Male	9	0.513
9/7/05	Island	9155	21.0	Female	6	0.142
8/9/05	Kawaguesaga	1860	13.1	Male	5	0.098
8/9/05	Kawaguesaga	1861	16.1	Male	7	0.216
8/9/05	Kawaguesaga	1862	15.1	Male	5	0.102
8/9/05	Kawaguesaga	1863	24.5	Female	8	0.288
8/9/05	Kawaguesaga	1864	24.9	Female	11	0.576
8/9/05	Kawaguesaga	1865	22.4	Female	11	0.323
8/9/05	Kawaguesaga	1866	19.4	Female	11	0.125
8/9/05	Kawaguesaga	1867	13.2	Male	5	0.073
8/9/05	Kawaguesaga	1868	19.6	Male	12	0.427
8/9/05	Kawaguesaga	1870	16.4	Male	5	0.135
8/9/05	Kawaguesaga	1871	18.9	Male	10	0.268
8/9/05	Kawaguesaga	1873	12.9	Male	5	0.061
9/7/05	Kentuck	1986	18.0	Female	5	0.225
9/7/05	Kentuck	1987	15.6	Male	6	0.250
9/7/05	Kentuck	1988	16.6	Male	6	0.288
9/7/05	Kentuck	1990	28.2	Female	16	1.19
9/7/05	Kentuck	1991	16.5	Male	6	0.219
9/7/05	Kentuck	1992	18.0	Male	6	0.260
9/7/05	Kentuck	1993	22.5	Female	7	0.266
9/7/05	Kentuck	1995	28.9	Female	16	1.11
9/7/05	Kentuck	1997	12.5	Male	4	0.173
9/7/05	Kentuck	1998	19.5	Female	6	0.218
9/7/05	Kentuck	1999	12.9	Male	4	0.137
9/7/05	Kentuck	2000	12.7	Male	4	0.176
8/9/05	Chetac	1788	14.2	Male	4	0.104
8/9/05	Chetac	1789	17.4	Male	6	0.226
8/9/05	Chetac	1790	13.5	Male	4	0.156
8/9/05	Chetac	1791	12.3	Male	3	0.066

8/9/05	Chetac	1792	19.9	Male	7	0.281
8/9/05	Chetac	1794	19.2	Male	8	0.236
8/9/05	Chetac	1795	22.5	Female	8	0.233
8/9/05	Chetac	1798	20.0	Male	8	0.190
8/9/05	Chetac	1799	18.2	Male	7	0.230
8/9/05	Chetac	1800	21.5	Male	9	0.393
8/9/05	Chetac	6599	16.3	Female	4	0.099
8/9/05	Chetac	6600	15.5	Male	5	0.090
8/24/05	Chippewa	1946	19.1	Female	6	0.382
8/24/05	Chippewa	1947	23.3	Female	10	0.703
8/24/05	Chippewa	1948	13.2	Male	5	0.304
8/24/05	Chippewa	1950	22.0	Male	13	0.871
8/24/05	Chippewa	1951	18.1	Male	10	0.940
8/24/05	Chippewa	1952	18.0	Female	7	0.498
8/24/05	Chippewa	1953	15.0	Male	7	0.547
8/24/05	Chippewa	1954	16.0	Male	7	0.513
8/24/05	Chippewa	1957	22.9	Female	11	1.36
8/24/05	Chippewa	1958	16.0	Male	7	0.765
8/24/05	Chippewa	1959	14.0	Male	8	0.545
8/24/05	Chippewa	1960	12.0	Male	5	0.258
8/24/05	Long	10161	22.8	Female	11	0.418
8/24/05	Long	10162	22.6	Female	9	0.357
8/24/05	Long	10163	20.2	Female	8	0.445
8/24/05	Long	10164	18.2	Male	8	0.379
8/24/05	Long	10165	16.3	Male	8	0.434
8/24/05	Long	10166	17.3	Male	9	0.292
8/24/05	Long	10167	19.7	Male	10	0.317
8/24/05	Long	10168	16.6	Male	7	0.295
8/24/05	Long	10169	13.5	Male	6	0.170
8/24/05	Long	10170	14.6	Male	6	0.132
8/24/05	Long	10171	12.9	Male	5	0.163
8/24/05	Minocqua	10101	15.6	Male	6	0.165
8/24/05	Minocqua	10102	27.4	Female	12	0.560
8/24/05	Minocqua	10103	22.7	Female	8	0.291
8/24/05	Minocqua	10104	17.2	Male	6	0.232
8/24/05	Minocqua	10105	22.0	Male	10	0.424
8/24/05	Minocqua	10106	14.0	Male	5	0.169

8/24/05	Minocqua	10108	18.3	Female	6	0.187
8/24/05	Minocqua	10109	14.6	Male	5	0.170
8/24/05	Minocqua	10111	15.0	Male	8	0.321
8/24/05	Minocqua	10112	18.2	Male	9	0.295
8/24/05	Minocqua	10114	25.1	Female	14	0.712
8/24/05	Minocqua	10115	14.0	Male	5	0.172
8/9/05	Namekagon	1758	14.2	Male	6	0.290
8/9/05	Namekagon	1760	15.7	Male	7	0.340
8/9/05	Namekagon	1761	18.3	Male	12	0.630
8/9/05	Namekagon	1762	23.0	Female	11	0.446
8/9/05	Namekagon	1764	14.7	Male	5	0.260
8/9/05	Namekagon	1765	14.6	Male	4	0.262
8/9/05	Namekagon	1767	16.5	Male	9	0.438
8/9/05	Namekagon	1768	22.0	Female	7	0.438
8/9/05	Namekagon	1769	16.0	Male	10	0.477
8/9/05	Namekagon	1770	20.4	Female	9	0.484
8/9/05	Namekagon	1771	23.1	Female	8	0.312
8/9/05	Namekagon	1772	19.3	Male	12	0.605
8/23/05	Pike	10146	15.5	Male	6	0.183
8/23/05	Pike	10147	14.5	Male	8	0.192
8/23/05	Pike	10148	13.0	Male	4	0.133
8/23/05	Pike	10149	13.0	Male	5	0.135
8/23/05	Pike	10150	24.0	Female	10	0.795
8/23/05	Pike	10152	18.2	Female	6	0.153
8/23/05	Pike	10153	17.8	Male	8	0.221
8/23/05	Pike	10155	20.0	Female	7	0.277
8/23/05	Pike	10156	18.5	Male	8	0.423
8/23/05	Pike	10157	15.6	Male	7	0.206
8/23/05	Pike	10159	24.9	Female	8	0.511
8/23/05	Pike	10160	22.7	Female	7	0.487
8/23/05	Plum	9076	19.0	Male	10	0.341
8/23/05	Plum	9078	17.9	Male	9	0.295
8/23/05	Plum	9079	17.9	Male	11	0.454
8/23/05	Plum	9080	14.6	Male	7	0.270
8/23/05	Plum	9081	15.3	Male	6	0.319
8/23/05	Plum	9082	13.2	Male	6	0.180
8/23/05	Plum	9083	18.6	Female	8	0.355

8/23/05	Plum	9084	24.5	Unknown	12	0.472
8/23/05	Plum	9085	24.4	Female	12	0.522
8/23/05	Plum	9086	22.2	Female	12	0.415
8/23/05	Plum	9087	18.5	Female	8	0.422
8/23/05	Plum	9089	16.4	Male	9	0.358
8/23/05	Presque Isle	10132	16.5	Male	9	0.358
8/23/05	Presque Isle	10133	14.0	Male	5	0.249
8/23/05	Presque Isle	10134	19.5	Female	8	0.215
9/7/05	Presque Isle	10136	19.8	Male	12	0.517*
8/23/05	Presque Isle	10137	13.5	Male	4	0.174
8/23/05	Presque Isle	10138	21.7	Female	8	0.331
8/23/05	Presque Isle	10139	15.3	Male	5	0.132
8/23/05	Presque Isle	10140	15.7	Male	9	0.399
8/23/05	Presque Isle	10141	12.8	Male	4	0.177
8/23/05	Presque Isle	10143	22.1	Female	10	0.298
8/23/05	Presque Isle	10144	27.4**	Female	19	0.877
8/23/05	Presque Isle	10145	25.7	Female	12	0.477
6/9/05	Razorback	9280	14.6	Male	5	0.279
6/9/05	Razorback	9281	14.5	Male	7	0.235
6/9/05	Razorback	9282	15.9	Female	8	0.437
6/9/05	Razorback	9283	12.2	Male	4	0.105
6/9/05	Razorback	9284	15.2	Female	6	0.321
6/9/05	Razorback	9285	18.1	Female	8	0.204
6/9/05	Razorback	9287	15.8	Male	8	0.432
6/9/05	Razorback	9290	21.6	Female	11	0.426
8/23/05	Red Cedar	10191	15.1	Male	4	0.260
8/23/05	Red Cedar	10192	16.1	Male	5	0.286
8/23/05	Red Cedar	10193	19.3	Male	11	0.617
8/23/05	Red Cedar	10194	14.5	Male	5	0.238
8/23/05	Red Cedar	10195	13.9	Male	4	0.217
8/23/05	Red Cedar	10196	20.4	Male	9	0.519
8/23/05	Red Cedar	10197	14.5	Male	3	0.175
8/23/05	Red Cedar	10198	19.1	Male	8	0.507
8/23/05	Red Cedar	10199	16.2	Male	4	0.257
8/23/05	Sherman	1753	14.9	Male	4	0.229
8/23/05	Sherman	1757	21.8	Female	6	0.440
8/23/05	Sherman	1976	14.3	Male	4	0.192

8/23/05	Sherman	1977	18.8	Male	7	0.402
8/23/05	Sherman	1978	22.1	Female	7	0.426
8/23/05	Sherman	1979	17.3	Male	4	0.224
8/23/05	Sherman	1980	22.7	Female	7	0.543
8/23/05	Sherman	1981	22.1	Female	8	0.378
8/23/05	Sherman	1982	16.6	Unknown	5	0.239
8/23/05	Sherman	1983	13.6	Male	5	0.261
8/23/05	Sherman	1984	18.6	Female	5	0.213
8/23/05	Sherman	1985	17.8	Male	6	0.390
6/28/05	Siskiwit	10276	14.9	Male	7	0.564
6/28/05	Siskiwit	10277	13.7	Male	5	0.284
6/28/05	Siskiwit	10278	18.5	Female	8	0.877
6/28/05	Siskiwit	10282	16.4	Male	8	0.867
6/28/05	Siskiwit	10283	15.4	Male	7	0.650
6/28/05	Siskiwit	10285	15.3	Female	5	0.235
6/28/05	Siskiwit	10287	13.4	Male	4	0.368
8/18/05	Squash	9102	17.1	Male	8	0.336
8/18/05	Squash	9103	22.0	Female	11	0.558
8/18/05	Squash	9105	15.8	Male	4	0.363
8/18/05	Squash	9106	19.7	Female	9	0.469
8/18/05	Squash	9107	14.6	Male	6	0.296
8/18/05	Squash	9113	16.6	Male	5	0.326
8/18/05	Squash	9115	14.6	Male	6	0.278
8/23/05	Squirrel	6554	15.0	Male	7	0.302
8/23/05	Squirrel	6555	15.7	Male	9	0.287
8/23/05	Squirrel	6557	17.2	Female	7	0.295
9/7/05	Squirrel	6558	23.8	Female	11	0.295*
8/23/05	Squirrel	6559	23.0	Female	10	0.856
8/23/05	Squirrel	6560	21.0	Female	11	0.388
8/23/05	Squirrel	6561	24.2	Female	12	0.702
8/23/05	Squirrel	6562	20.3	Female	10	0.327
8/23/05	Squirrel	6563	12.3	Male	5	0.137
8/23/05	Squirrel	6564	15.6	Male	8	0.345
8/23/05	Squirrel	6565	18.6	Female	7	0.285
8/23/05	Squirrel	6566	20.5	Female	10	0.277
9/7/05	Squirrel	6567	14.6	Male	7	0.182*
8/18/05	Star	10177	19.7	Female	8	0.330

8/18/05	Star	10178	19.8	Female	8	0.317
8/18/05	Star	10180	16.6	Male	7	0.259
8/18/05	Star	10181	17.1	Male	8	0.261
8/18/05	Star	10182	22.8	Female	10	0.471
8/18/05	Star	10183	12.8	Male	4	0.102
8/18/05	Star	10184	13.8	Male	4	0.134
8/18/05	Star	10186	18.4	Male	10	0.351
8/18/05	Star	10187	12.0	Male	4	0.131
8/18/05	Star	10188	15.8	Male	8	0.245
8/18/05	Star	10189	26.3	Female	14	0.680
8/18/05	Star	10190	23.0	Female	10	0.495
8/18/05	Teal	6569	21.0	Female	8	0.283
8/18/05	Teal	6570	12.1	Male	5	0.158
8/18/05	Teal	6571	16.3	Male	9	0.286
8/18/05	Teal	6572	13.1	Male	4	0.198
8/23/05	Teal	6574	19.5	Female	9	0.290
8/23/05	Teal	6575	16.7	Male	7	0.225
8/18/05	Teal	6576	20.7	Female	11	0.467
8/18/05	Teal	6577	13.4	Male	6	0.284
8/18/05	Teal	6580	15.1	Male	9	0.409
8/18/05	Twin Lake Chain	6463	20.5	Male	8	0.291
8/18/05	Twin Lake Chain	6464	24.2	Female	11	0.431
8/18/05	Twin Lake Chain	6465	24.4	Female	8	0.434
8/18/05	Twin Lake Chain	6466	19.1	Female	7	0.224
8/18/05	Twin Lake Chain	6469	26.5	Female	14	0.893
8/18/05	Twin Lake Chain	6470	15.9	Male	5	0.181
8/18/05	Twin Lake Chain	6471	25.5	Female	11	0.526
8/18/05	Twin Lake Chain	6473	13.2	Male	6	0.173
8/18/05	Twin Lake Chain	6474	17.0	Male	8	0.175
8/18/05	Twin Lake Chain	6475	12.8	Male	7	0.168
8/18/05	Twin Lake Chain	6476	15.4	Male	5	0.196
8/18/05	Twin Lake Chain	6477	18.7	Male	12	0.385
6/9/05	Upper Turtle	9161	14.6	Male	3	0.134
6/9/05	Upper Turtle	9162	16.3	Male	5	0.202
6/9/05	Upper Turtle	9164	16.9	Male	5	0.179
6/9/05	Upper Turtle	9166	18.9	Male	8	0.210
6/9/05	Upper Turtle	9168	12.0	Male	3	0.123

6/9/05	Upper Turtle	9169	20.0**	Male	8	0.312
6/9/05	Upper Turtle	9171	17.8	Male	7	0.244
6/9/05	Upper Turtle	9173	18.5	Male	10	0.311
6/9/05	Upper Turtle	9174	23.5	Female	12	0.497
6/9/05	Upper Turtle	9175	14.2	Male	6	0.092
8/25/05	Willow	1773	14.1	Male	4	0.424
8/25/05	Willow	1774	20.8	Female	10	1.25
8/25/05	Willow	1775	13.5	Male	4	0.337
8/25/05	Willow	1777	14.6	Male	4	0.336
8/25/05	Willow	1778	18.9	Male	11	0.925
8/25/05	Willow	1779	22.0	Female	11	1.09
8/25/05	Willow	1780	15.5	Male	5	0.472
8/25/05	Willow	1781	18.0	Female	6	0.611
9/7/05	Willow	1782	16.2	Male	10	0.733*
8/25/05	Willow	1783	22.2	Female	9	1.04
8/25/05	Willow	1784	16.8	Male	8	0.751
8/25/05	Willow	1785	22.0	Female	11	0.718

*Spike recoveries or duplicate relative percent agreements for the initial analysis of these samples were out of the acceptable range. Samples were reanalyzed in duplicate. Reported results are the mean of those duplicates.

** No fresh length available due to recording error so frozen length was used.

Table 12. Percent Moisture in Walleye Fillets (Measured Immediately After Grinding) from the GLIFWC EPA Mercury/Mapping Grant.

Lake	Tag Number	% moisture	Relative Percent Agreement
Annabelle	10117 *	81.4	
Annabelle	10120 *	80.2	
Annabelle	10120 Dup*	80.2	100
Annabelle	10121 *	79.2	
Annabelle	10125 *	80.1	
Anvil	9267	78.2	
Anvil	9268	80.1	
Anvil	9269	78.4	
Anvil	9270	78.5	
Bass Patterson	1967	79.6	
Bass Patterson	1970	78.9	
Bass Patterson	1971	78.6	
Bass Patterson	1971 Dup	78.6	100
Bass Patterson	1974	80.8	
Big Fork	5058	80.0	
Big Fork	5059	79.9	
Big Fork	5061	80.6	
Big Fork	5070	78.5	
Big	9205	79.2	
Big	9215	78.3	
Big	9216	77.2	
Big	9219	79.4	
Big Muskellunge	1589	78.4	
Big Muskellunge	1591	78.4	
Big Muskellunge	1591 Dup	79.0	99.2
Big Muskellunge	1596	78.8	
Big Muskellunge	1597	78.9	
Big Muskellunge	1600	79.0	
Bond Falls Flowage	9134	79.7	
Bond Falls Flowage	9134 Dup	79.0	99.1

Bond Falls Flowage	9135	77.6	
Bond Falls Flowage	9141	78.3	
Bond Falls Flowage	9144	80.2	
Butternut	6480	79.0	
Butternut	6482	78.4	
Butternut	6484	78.2	
Butternut	6487	79.5	
Chetac	1789	79.1	
Chetac	1790	77.4	
Chetac	1790 Dup	79.2	97.7
Chetac	6599	79.1	
Chetac	6600	78.8	
Chippewa	1946	78.2	
Chippewa	1948	79.3	
Chippewa	1953	78.3	
Chippewa	1960	79.0	
Dam	1890	78.8	
Dam	1892	78.5	
Dam	1896	79.0	
Dam	5073	78.6	
Dam	5077	77.8	
Dam	5077 Dup	78.2	99.5
Enterprise	9091	78.4	
Enterprise	9093	79.9	
Enterprise	9099	78.8	
Enterprise	96060	79.3	
Island	9146	79.7	
Island	9147	78.4	
Island	9148	79.2	
Island	9150	79.1	
Island	9152	78.1	
Island	9152 Dup	78.6	99.4
Kawaguesaga	1864	80.0	
Kawaguesaga	1866	77.9	
Kawaguesaga	1868	79.1	

Kawaguesaga	1871	78.9	
Kentuck	1986	78.9	
Kentuck	1990	80.3	
Kentuck	1995	80.2	
Kentuck	1998	79.9	
Long	10164	78.3	
Long	10166	79.1	
Long	10168	78.7	
Long	10169	81.3	
Minocqua	10106	80.4	
Minocqua	10106 Dup	80.4	100
Minocqua	10109	79.3	
Minocqua	10111	79.0	
Minocqua	10115	78.7	
Namekagon	1760	79.1	
Namekagon	1764	79.1	
Namekagon	1765	78.5	
Namekagon	1769	78.7	
Namekagon	1769 Dup	78.8	99.9
Pike Lake Chain	10146	77.2	
Pike Lake Chain	10147	79.3	
Pike Lake Chain	10152	77.2	
Pike Lake Chain	10159	80.6	
Plum	9078	78.4	
Plum	9079	78.1	
Plum	9080	77.4	
Plum	9082	77.7	
Presque Isle	10131	80.0	
Presque Isle	10136	79.0	
Presque Isle	10136 Dup	78.5	99.4
Presque Isle	10139	78.9	
Presque Isle	10143	80.1	
Presque Isle	10145	79.1	
Razorback	9281	79.5	
Razorback	9284	79.8	

Razorback	9285	79.9	
Razorback	9287	78.6	
Red Cedar	10191	78.2	
Red Cedar	10191 Dup	77.7	99.4
Red Cedar	10192	77.6	
Red Cedar	10193	78.2	
Red Cedar	10196	78.3	
Red Cedar	10198	78.9	
Sherman	1977	79.0	
Sherman	1980	80.7	
Sherman	1982	78.7	
Sherman	1985	79.4	
Siskiwit	10276	78.4	
Siskiwit	10277	77.6	
Siskiwit	10282	78.6	
Siskiwit	10285	79.7	
Squash	9103	78.2	
Squash	9107	79.3	
Squash	9113	77.8	
Squash	9115	77.1	
Squash	9115 Dup	78.0	98.8
Squirrel	6554	79.6	
Squirrel	6554 Dup	79.3	99.6
Squirrel	6555	79.4	
Squirrel	6564	80.0	
Squirrel	6567	79.8	
Star	10181	79.8	
Star	10182	79.8	
Star	10189	80.8	
Star	10190	79.4	
Teal	6570	78.1	
Teal	6571	78.6	
Teal	6575	78.8	
Teal	6577	80.3	
Teal	6577 Dup	80.6	99.6

Teal	6580	79.1	
Twin Lake Chain	6464	77.7	
Twin Lake Chain	6464 Dup	78.8	98.6
Twin Lake Chain	6467	76.8	
Twin Lake Chain	6473	79.3	
Twin Lake Chain	6476	78.2	
Twin Lake Chain	6477	78.6	
Upper Turtle	9164	78.3	
Upper Turtle	9166	77.9	
Upper Turtle	9169	78.7	
Upper Turtle	9169 Dup	77.9	99.0
Upper Turtle	9173	78.4	
Willow	1774	80.0	
Willow	1780	79.6	
Willow	1781	79.8	
Willow	1783	80.0	
Winslow	9191	78.8	
Winslow	9192	80.3	
Winslow	9195	79.9	
Winslow	9197	79.8	
Winslow	9197 Dup	80.8	98.8
Winslow	9198	79.2	

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

Appendix A

Standard Curve Data Run Coincident with the Star Grant Fish Analysis.

Analysis Date	Standard Conc. ngHg/L	Blank Corrected Abs 1	Blank Corrected Abs 2	Blank Corrected Mean	Slope	Intercept	Correlation
6/28/05	0	0.0002*	0.0001*	0.000			
6/28/05	50	0.0012	0.0015	0.0014			
6/28/05	100	0.0020	0.0025	0.0023			
6/28/05	500	0.0127	0.0122	0.0125			
6/28/05	1000	0.0254	0.0239	0.0247			
6/28/05	6000	0.1476	0.1071	0.1274	2.12E-05	0.0011	0.9996
8/11/05	0	0.0001*	0.0002*	0.000			
8/11/05	50	0.0013	0.0013	0.0013			
8/11/05	100	0.0025	0.0029	0.0027			
8/11/05	500	0.0123	0.0143	0.0133			
8/11/05	1000	0.0275	0.0288	0.0282			
8/11/05	6000	0.1682	0.1633	0.1658	2.76E-05	-4.16E-05	0.9999
8/16/05	0	0.0008*	0.0009*	0.000			
8/16/05	50	0.0013	0.0012	0.0013			
8/16/05	100	0.0025	0.0024	0.0025			
8/16/05	500	0.0132	0.0127	0.0130			
8/16/05	1000	0.0264	0.0244	0.0254			
8/16/05	6000	0.1514	0.1424	0.1469	2.45E-05	0.0003	0.9999
8/17/05	0	0.0009*	0.0007*	0.000			
8/17/05	50	0.0016	0.0013	0.0015			
8/17/05	100	0.0032	0.0025	0.0029			
8/17/05	500	0.0175	0.0115	0.0145			
8/17/05	1000	0.0314	0.0227	0.0271			
8/17/05	6000	0.1829	0.1822	0.1826	3.05E-05	-0.0008	0.9998
8/25/05	0	0.0014*	0.0013*	0.000			
8/25/05	50	0.0010	0.0013	0.0012			
8/25/05	100	0.0023	0.0022	0.0023			
8/25/05	500	0.0119	0.0106	0.0113			
8/25/05	1000	0.0214	0.0212	0.0213			
8/25/05	6000	0.1371	0.1335	0.1353	2.26E-05	-0.0002	0.9999
9/7/05	0	0.0014*	0.0013*	0.000			
9/7/05	50	0.0011	0.0007	0.0009			

9/7/05	100	0.0021	0.0018	0.0020			
9/7/05	500	0.0109	0.0092	0.0101			
9/7/05	1000	0.0215	0.0190	0.0190			
9/7/05	6000	0.1289	0.1102	0.1196	1.99E-05	-0.0002	0.9999
9/20/05	0	0.0010*	0.0012*	0.000			
9/20/05	50	0.0011	0.0010	0.0011			
9/20/05	100	0.0022	0.0021	0.0022			
9/20/05	500	0.0111	0.0109	0.0110			
9/20/05	1000	0.0224	0.0217	0.0221			
9/20/05	6000	0.1301	0.1246	0.1274	2.12E-05	0.0002	0.9999

* 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

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

Set	Analysis Date	Standard Conc. ngHg/L	Blank Corrected Abs 1	Blank Corrected Abs 2	Blank Corrected Mean	Slope	Intercept	Correlation
1	6/9/05	0	0.0001*	0.0002*	0.0000			
1	6/9/05	50	0.0013	0.0013	0.0013			
1	6/9/05	100	0.0025	0.0029	0.0027			
1	6/9/05	500	0.0123	0.0143	0.0133			
1	6/9/05	1000	0.0275	0.0288	0.0282			
1	6/9/05	6000	0.1682	0.1633	0.1658	2.76E-05	-4.16-05	0.9999
1	6/28/05	0	0.0002*	0.0001*	0.0000			
1	6/28/05	50	0.0012	0.0015	0.0014			
1	6/28/05	100	0.0020	0.0025	0.0023			
1	6/28/05	500	0.0127	0.0122	0.0125			
1	6/28/05	1000	0.0254	0.0239	0.0247			
1	6/28/05	6000	0.1476	0.1071	0.1274	2.11E-05	0.0011	0.9996
1	8/9/05	0	0.0004*	0.0003*	0.0000			
1	8/9/05	50	0.0010	0.0015	0.0013			
1	8/9/05	100	0.0022	0.0028	0.0025			
1	8/9/05	500	0.0120	0.0137	0.0129			
1	8/9/05	1000	0.0294	0.0279	0.0287			
1	8/9/05	6000	0.1953	0.1590	0.1772	2.96E-05	-0.0007	0.9999
1	8/10/05	0	0.0010*	0.0009*	0.0000			
1	8/10/05	50	0.0012	0.0013	0.0013			
1	8/10/05	100	0.0027	0.0024	0.0026			
1	8/10/05	500	0.0112	0.0114	0.0113			
1	8/10/05	1000	0.0232	0.0230	0.0231			
1	8/10/05	6000	0.1379	0.1348	0.1364	2.27E-05	0.0001	0.9999
1	8/16/05	0	0.0008*	0.0009*	0.0000			
1	8/16/05	50	0.0013	0.0012	0.0013			
1	8/16/05	100	0.0025	0.0024	0.0025			
1	8/16/05	500	0.0132	0.0127	0.0130			
1	8/16/05	1000	0.0264	0.0244	0.0254			
1	8/16/05	6000	0.1514	0.1424	0.1469	2.44E-05	0.0003	0.9999
1	8/18/05	0	0.0004*	0.0006*	0.0000			

1	8/18/05	50	0.0021	0.0013	0.0017			
1	8/18/05	100	0.0036	0.0024	0.0030			
1	8/18/05	500	0.0153	0.0121	0.0137			
1	8/18/05	1000	0.0293	0.0225	0.0259			
1	8/18/05	6000	0.1394	0.1338	0.1366	2.26E-05	0.0013	0.9997
1	8/23/05	0	0.0007*	0.0008*	0.0000			
1	8/23/05	50	0.0013	0.0008	0.0011			
1	8/23/05	100	0.0025	0.0019	0.0022			
1	8/23/05	500	0.0118	0.0100	0.0109			
1	8/23/05	1000	0.0229	0.0197	0.0213			
1	8/23/05	6000	0.1369	0.1111	0.1240	2.06E-05	0.0003	0.9999
2	8/23/05	0	0.0015*	0.0016*	0.0000			
2	8/23/05	50	0.0019	0.0017	0.0018			
2	8/23/05	100	0.0036	0.0035	0.0036			
2	8/23/05	500	0.0174	0.0169	0.0172			
2	8/23/05	1000	0.0356	0.0338	0.0347			
2	8/23/05	6000	0.2027	0.1949	0.1988	3.31E-05	0.0005	0.9999
1	8/24/05	0	0.0013*	0.0013*	0.0000			
1	8/24/05	50	0.0012	0.0012	0.0012			
1	8/24/05	100	0.0023	0.0023	0.0023			
1	8/24/05	500	0.0112	0.0114	0.0113			
1	8/24/05	1000	0.0216	0.0230	0.0223			
1	8/24/05	6000	0.1363	0.1373	0.1368	2.28E-05	-0.0001	0.9999
1	8/25/05	0	0.0014*	0.0013*	0.0000			
1	8/25/05	50	0.0010	0.0013	0.0012			
1	8/25/05	100	0.0023	0.0022	0.0023			
1	8/25/05	500	0.0119	0.0106	0.0113			
1	8/25/05	1000	0.0214	0.0212	0.0213			
1	8/25/05	6000	0.1371	0.1335	0.1353	2.26E-05	-0.0002	0.9999
1	9/7/05	0	0.0014*	0.0013*	0.0000			
1	9/7/05	50	0.0011	0.0007	0.0009			
1	9/7/05	100	0.0021	0.0018	0.0020			
1	9/7/05	500	0.0109	0.0092	0.0101			
1	9/7/05	1000	0.0215	0.0190	0.0190			
1	9/7/05	6000	0.1289	0.1102	0.1196	1.99E-05	-0.0002	0.9999
1	9/20/05	0	0.0010*	0.0012*	0.0000			
1	9/20/05	50	0.0011	0.0010	0.0011			
1	9/20/05	100	0.0022	0.0021	0.0022			
1	9/20/05	500	0.0111	0.0109	0.0110			

1	9/20/05	1000	0.0224	0.0217	0.0221			
1	9/20/05	6000	0.1301	0.1246	0.1274	2.12E-05	0.0002	0.9999

* 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 C

Quality Assurance Audit Report on the Spring 2005 Walleye Project

Audit Date: September 2005
Report Date: October 25, 2005

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 analyses for mercury and adherence to LSRI SOPs SA/13 (*Cold Vapor Mercury Determination in Biota*), and SA/42 (*FIMS Mercury Analysis - Stock, Standard and Spike Preparation*). This audit outlines the QA/QC observations for the analyses conducted with the Kentuck and Island Lakes samples, as well as a review of the analyses bench sheets and mercury analyzer computer output. The findings are listed in the subsequent section.

2. Major Findings

Spring 2005 Walleye Project - Preparation of Solutions, SOP Review, Labeling

On September 6 - 7, 2005 Dianne Brooke (LSRI QA Manager) observed one staff member preparing sub-stock solutions, and preparing the samples for the digestion process. The following observations were made and discussed with the project staff.

3. Staff member was properly attired in lab coat, gloves, and safety glasses when performing the procedures listed in SOPs SA/13 and SA/42.
4. The staff member prepared the Hg sub-stock solutions (10 mg/L and 100 µg/L) according to SOP SA/42. The standards were prepared properly by using a micropipette to deliver aliquots of the 100 µg/L Hg sub-stock solution into the digestion cups. The type of digestion cup (i.e., SC475) will be added to SOP SA/42 for further clarification when ordering supplies for the mercury analyses.
5. All SOPs for the project need to be reviewed and updated, if changes are needed. The revision dates on the SOPs ranged from 1997 - 2000. The following SOPs should be reviewed: SA/8, SA/10, SA/11, SA/13, SA/14, SA/35, SA/37, SA/38, and SA/42.
6. In reviewing the September 2002 report "Total Mercury in Walleye Muscle Tissue Captured in the Ceded Territories During the Spring of 2002 and in Some Commercial Fish Products", two SOPs were listed in the appendices that are not on the master LSRI SOP list. An SOP for cleaning the meat grinder and an SOP for determining the percent moisture in tissue samples should be incorporated into the current project's SOP list. They should also be reviewed since there have been no changes since 2002.

7. The staff member had received training in Good Laboratory Practices. A training certificate was on file.
8. The staff member also prepared a new hazardous waste container as the stock solutions were being prepared. The waste container was labeled with the date and element. An entry into the waste log was recorded, listing the amount of material, date, initials of researcher, and description of the waste process.
9. The dorm samples (dogfish muscle and liver), a set of standards for the beginning of analyses and one interspersed throughout the analyses, calibration blanks, duplicate samples, and spike recovery samples were prepared for QA/QC purposes.
10. The glass vials containing the tissue samples were well labeled, as were the digestion cups. Project personnel recently developed a computerized system for printing labels that are color-coded and contain the following information: lake code/name, year, project designation, and sample ID. This information is cross-referenced to the bench sheet to check for accuracy when processing the samples.

Spring 2005 Walleye Project - Sample Digestion Process, Mercury Analyses, Data Output

Observed the various processing steps used to digest the samples for mercury analyses. This also included a review of the instrument analytical output and data bench sheets.

- Approximately 0.2 - 0.3 g of fish tissue was removed from each glass vial and placed into a pre-labeled digestion cup. The SOP SA/ll should be revised and list "50 mL digestion cup" rather than "glass bottle" in the Procedure section of the SOP.
- When the staff member was removing tissue from the vial labeled "1986 Kentuck L", the balance had first been tared with the certified clean digestion cup before the tissue was added. The balance was calibrated prior to use. All balance calibration information should now be recorded in the "Balance Calibration Check Log" notebook (next to the balance) and referenced in the specific project lab book. This change was made to SOP GLM/12 on August 31, 2005.
- The staff member performed steps 5 - 8 of SOP SA/11 in the proper manner. The tissue from "1986 Kentuck L" was removed with a spatula that had been rinsed in 10% nitric acid, then rinsed with deionized water, and wiped with a Kimwipe. As an added precaution for preventing contamination, several spatulas were soaking in the beaker containing nitric acid and a different one was used for each tissue sample. Another precaution used is the tissue samples are weighed in sequential fashion (i.e., from low to high according to the sample numbers). This method of weighing follows the sample numbers on the bench sheet.
- Observed the procedure of adding the sulfuric and nitric acids to each sample before being placed in the "Hot Block". The temperature for the "Hot Block" was set at 110° C, whereas the temperature listed in SA/13 procedure number 2 for the water bath was 80 - 90° C. This change should be reflected in the SOP. A timer was used to allow the samples to digest for 15 minutes. The samples were then allowed to cool (an approximate time for cooling should be added to SA/13).
- The potassium permanganate was added to the samples in the prescribed

increments. The digestion cups were swirled between additions. In talking with the staff member, it was noted that the potassium persulfate bottle had vapor locked in the past when adding the solutions. Perhaps the dispensing device should be changed or at least check the calibration on it before adding solution to the sample. More potassium permanganate is added if the samples do not remain purple for at least 15 minutes (this occurs very infrequently and the additional amounts are recorded and added to the standards as well). *(Corrective Action: In a discussion with a project staff member on October 19, 2005, I was informed that the dispensing device had been changed. However, the potassium persulfate bottle vapor locked as it had before. Because the solution is supersaturated, and settles out at room temperature, crystals are apparently forming in the bottle and block the flow of solution. The staff member is working on different methods to remedy the problem.)*

- Diary information for sample processing is recorded in the lab notebook "04-10-14-HS: GLIFWC". All analysts' names and initials were recorded in the front cover of the notebook. The Table of Contents has been completed and the study ID number had been placed on each page of the notebook. A plastic label tab should be attached to the notebook to separate the 2004 from the 2005 data. All sample processing steps had been dated and initialed on the Excel spreadsheet summary, and in the notebook.
- In reviewing the mercury analyzer output data sheets, it was noted that each sample for the Kentuck and Island Lakes was analyzed in triplicate. A mean, standard deviation, and percent relative standard deviation were calculated for each triplicate sample set. If the percent relative standard deviation is above 5.0, the samples are rerun. For the Kentuck and Island Lakes samples, the %RSD values were not above 5.0. However the sample "Dorm 2-2 #2" had an initial %RSD = 98.55. When the sample was rerun, the %RSD = 4.52. *(Corrective Action: In a discussion with a project staff member on October 19, 2005 it was noted that the "Dorm 2-2#2" sample %RSD = 98.55 may have been due to a clogged tube or the tubing may have been inadvertently pulled out of the sample. Thus, the sample was rerun and a new %RSD recalculated.)*
- According to the mercury analyzer output, a set of standards were run at the beginning of the analyses (i.e, calibration blank, 50 ng/L, 100 ng/L, 500 ng/L, 1000 ng/L, and 6000 ng/L). The other set of standards was interspersed throughout the analyses.
- If possible, the study ID and page numbers should be included on the mercury analyzer measurement output. The initials of the analyst would also be helpful.
- The one page Excel spreadsheet QA/QC summary for each analyses set is excellent. In addition to listing the measurement values, it also lists the sample processing steps, when each was completed, and the initials of the analyst performing the procedure. Copies of the QA/QC summary and project bench sheets were glued in the notebook "04-10-14-HS: GLIFWC" for previous analyses.

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 SOPs for the project are continually being revised and new ones are being written when a need arises. More complete documentation for SOP training needs should be coordinated between the principal investigator and the LSRI QA Manager. A brief description of the study should be written in the lab notebook at the onset of analyses (it would include the number of fish, sample lakes, personnel involved, contract number, project dates, sample collection methodology, and the list of SOPs needed to complete the project). The chain of custody forms received from the sponsor could be Xeroxed and glued into the study notebook to describe the number of fish and sample lakes. The contract memo received from the sponsor could be Xeroxed and portions of it reduced for inclusion in the study notebook. This would eliminate the need to write the study description in the notebook.

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:

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

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: 2005 Field Data Collection for EPA Grant # 96540801-0

Quality Assurance Report: 2005 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 39 lakes were selected for sampling and a total of 354 walleye samples from 32 lakes were collected (Table 1).

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 - One deviation form was completed (Appendix 4B). The deviation did not affect the quality of the data or the data collection process, so no corrective action was necessary.

4. Significant Quality Assurance Problems and Recommended Solutions - No significant quality assurance problems were noted during the 2005 field sample and data collection process.

Table 1. Summary of completeness of mercury walleye collections during spring 2005 as part of EPA Grant # 96540801-0.

Lake Name	State	County	Size Group				Collection Goal	Total Collected	Percent of Goal
			12.0 to 14.9	15.0 to 17.9	18.0 to 22.0	>22.0			
BOND FALLS FL	MI	ONTONAGON	3	3	3	3	12	12	100%
WINDFALL L	WI	SAWYER	0	0	0	0	12	0	0%
TRUDE L	WI	IRON	0	0	0	0	12	0	0%
TURTLE-FLAMBEAU FL	WI	IRON	0	0	0	0	12	0	0%
LOST LAND L	WI	SAWYER	0	0	0	0	12	0	0%
SAND L	WI	SAWYER	0	0	0	0	12	0	0%
GILE FL	WI	IRON	0	0	0	0	12	0	0%
CATFISH L	WI	VILAS	0	0	0	0	12	0	0%
SISKIWIT L	WI	BAYFIELD	3	3	1	0	12	7	58%
RAZORBACK L	WI	VILAS	3	3	2	0	12	8	67%
SQUASH L	WI	ONEIDA	2	3	2	0	12	7	58%
RED CEDAR L	WI	BARRON	3	3	3	0	12	9	75%
UPPER TURTLE L	WI	BARRON	4	3	2	1	12	10	83%
ISLAND L	WI	RUSK	3	3	4	0	12	10	83%
ANVIL L	WI	VILAS	3	4	2	1	12	10	83%
BIG FORK L	WI	ONEIDA	3	3	3	2	12	11	92%
SHERMAN L	WI	VILAS	3	3	3	3	12	12	100%
LONG L	WI	CHIPPEWA	3	3	3	2	12	11	92%
PIKE L CHAIN	WI	BAYFIELD	3	3	3	3	12	12	100%
BASS-PATTERSON L	WI	WASHBURN	3	3	3	3	12	12	100%
L CHETAC	WI	SAWYER	3	3	5	1	12	12	100%
BUTTERNUT L	WI	FOREST	3	3	4	2	12	12	100%
ANNABELLE L	WI	VILAS	8	3	1	0	12	12	100%
KENTUCK L	WI	VILAS	3	3	3	3	12	12	100%
PRESQUE ISLE L CHAIN	WI	VILAS	3	3	3	3	12	12	100%
BIG MUSKELLUNGE L	WI	VILAS	4	6	0	2	12	12	100%
DAM L	WI	ONEIDA	3	2	4	3	12	12	100%
TEAL L	WI	SAWYER	3	3	3	0	12	9	75%
L CHIPPEWA	WI	SAWYER	3	3	4	2	12	12	100%
WILLOW FL	WI	ONEIDA	3	3	5	1	12	12	100%
MINOCQUA L	WI	ONEIDA	3	3	3	3	12	12	100%
STAR L	WI	VILAS	3	3	3	3	12	12	100%
PLUM L	WI	VILAS	2	4	3	3	12	12	100%
KAWAGUESAGA L	WI	ONEIDA	3	3	3	3	12	12	100%
BIG L (MI BORDER)	WI	VILAS	3	3	3	3	12	12	100%
TWIN L CHAIN	WI	VILAS	2	3	3	4	12	12	100%
ENTERPRISE L	WI	LANGLADE	3	3	3	0	12	9	75%
NAMEKAGON L	WI	BAYFIELD	3	3	4	2	12	12	100%
SQUIRREL L	WI	ONEIDA	2	4	4	3	12	13	108%
		Total Collected	**99**	**101**	**95**	**59**	**468**	**354**	**76%**

Appendix 4A

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

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

Title: Field Audit of Biota Collection for Chemical Contaminant Samples

Introduction:
This procedure describes the auditing process for the collection of fish to be analyzed for chemical contaminants. The project manager or an appointed and properly trained GLIFWC staff member not involved in the fish collection will perform this audit.

Equipment:

Audit Form (see attachment)
Black indelible ink pen

Procedures:

1. All aspects of the biota sampling involving data collection, sample storage, sample processing, and transport should be audited.

2. At a minimum, audits will occur once during a sampling season less than or equal to 6 weeks in length. Two audits should occur for longer sampling seasons. Single audits should be conducted during the initial part or the sampling season, with second audits occurring after 6 weeks of sampling. If non-compliance to procedures is observed, further audits may be scheduled as deemed necessary by the project manager.

3. All types of field data collection should be observed such as the following possible parameters:
   a. Length
   b. Weight
   c. Sex
   d. Age

   Collection methods of this data will be according to the quality assurance protocol plan or work plan for which the data is being collected.

4. Tissue collection, packaging, storage, custody and transport procedures should be observed and documented for compliance to the quality assurance protocol plan or work plan for which the data is being collected.

5. The attached form should be completed and returned to the project manager for review and archiving.

Field Audit Form

Section 1: Data Collection

Data Type	(+/-)[a]	Comments	Date Observed
Length (field)	+	Consistent measurements	4/18/05
Sex (field)	+	followed protocol	4/18/05
Tag ID (field)	+	" "	4/18/05
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
Fish Descriptive Data	+		5/4/05 & 4/25/05
Spine Collection	+		5/4/05 & 4/25/05

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

General Comments:
Processor has a good system of calibrating the balance, weighing each fish, checking the length, collecting the spine, weighing the fillet and cleaning up between samples. No major problems noted although he occasionally needs to be reminded of the importance of QA/QC procedures

Section 3: Sample Packaging

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

Bagging fillet	+	Excellent method-very neat + organized	4/25/05 - 5/4/05
Storing fillets	+	Well packaged and frozen immediately after processing	4/25/05 - 5/4/05

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

General Comments:

Section 4: Storage

Data Type	(+/-)[a]	Temp (°C)[b]	Comments	Date Observed
Freezer, fish storage	+	< 70°C	Monitoring thermometer was not placed on freezer	4/18/05

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

General Comments: The monitoring thermometer was placed on the freezer by the field manager. The freezer temp was still below -10°C and although the freezer was in use for several days without the thermometer, this is not believed to be an issue. All fish were frozen.

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

Data Type	(+/-)[a]	Comments	Date Observed
CoC form	+	Excellent - followed established protocols	4/18/05

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

General Comments: In general, the crew leaders and wardens do a good job of properly filling out the COC forms

Section 6: Transport

Data Type	(+/-)[a]	Comments	Date Observed
Fish transport in field	+	Crew leaders did not have ice for cooler see comments below.	4/18/05

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

General Comments: After fish collection by the electrofishing crews, the fish were kept alive & fresh in water, rather than on ice. Fish were then placed directly into field freezer after collection. No problems seen as long As crew leader is aware of ice need and adapted well to situation without ice.

Auditor Name: Matt Hudson

Field Audit Auditor Signature: Matt Hudson Date Signed: 4/19/05

Law Audit (fillet processing) Matt Hudson 5/4/05

Appendix 4B

Deviation forms for EPA Grant # 96540801-0

DEVIATION FORM - GREAT LAKES INDIAN FISH AND WILDLIFE COMMISSION

Project Title: Testing of fish for mercury using EPA supplemental funding **Date/Time:** 8/2005

Explanation of Deviation: Section 2.8, "Project Organization", explains that the Lab Manager for the project will be Larry Brooke. Larry retired and has been replaced by Tom Markee. Thus, Tom Markee is now the Lab Manager for the project.

Corrective Procedure: None needed

Signature: Matt Hudson Date: 4/13/06

Route to Project Manager for Evaluation.

Impact on this Study:

NONE

Signature: [signature] Date: 7/18/06

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- 2005								
# 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 Sept. 20, 2005								
Sample	Tissue Type	ng/l	ng Hg	g sample	ug/g			
Tuna 6-27-05 #1	tuna	353.6875	17.68437	0.272	0.065016		0.00126	
Tuna 6-27-05 #2	tuna	292.3816	14.61908	0.22	0.06645			
Tuna 6-27-05 #3	tuna	264.0866	13.20433	0.223	0.059212			
Tuna 6-27-05 #4	tuna	254.655	12.73275	0.211	0.060345			
Tuna 6-27-05 #5	tuna	278.2341	13.91171	0.218	0.063815			
Tuna 6-27-05 #6	tuna	268.8025	13.44012	0.241	0.055768	**STDS**	**DL (ug/g)**	**LOQ**
Tuna 6-27-05 #7	tuna	249.9391	12.49696	0.207	0.060372	0.003763	0.011281	0.037602
Tuna 6-27-05 #8	tuna	240.5075	12.02537	0.21	0.057264			
		2005	**Hg LOD =0.011281ug/g LOQ=0.037602ug/g**					
		2004	**Hg LOD =0.00126ug/g LOQ=0.004194ug/g**					