2009-2010 Walleye Total Mercury Analyses

by

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INTRODUCTION

Walleye (*Sander vitreus*) are targeted for harvest by Chippewa tribal members from many off-reservation inland lakes in Wisconsin each spring (Krueger 2010). Tribal representatives have expressed concern about the health risk that mercury in fish may pose to tribal members. As a result, the Great Lakes Indian Fish and Wildlife Commission (GLIFWC) has been collecting walleye annually since 1989 during the spring harvest from various lakes routinely harvested by tribal members. In some years, muskellunge (*Esox masquinongy*) and northern pike (*Esox lucius*) have also been included, but these species were not collected in 2009 or 2010. Mercury in the muscle tissue of top predator fish is known to exist primarily (>95%) in the organic form as methylmercury (Bloom, 1992; Lasorsa and Allen-Gil, 1995). Thus, total mercury concentration was measured in fish tissues and used as a surrogate for methylmercury concentration.

The resulting walleye data are used to prepare tribal and lake specific, color-coded GIS maps that include walleye consumption advice (Appendix 1). These maps provide lake specific meal-based consumption advice intended to assist tribal members in selecting lakes for harvest in which walleye contain lower mercury concentrations, reducing the risk of dietary methylmercury exposure. These maps were last updated in 2006 and have been made available to tribal members at offices where permits for off-reservation spearing are issued as well as at health service provider offices. Large, wall-sized maps have also been posted at these offices and in various public locations such as tribal administration buildings, grocery stores, school libraries, or community centers (DeWeese *et al.* 2009). The maps for the six Wisconsin Ojibwe tribes were updated in 2005 using the methodology described in Madsen *et al*. (2008). In 2006, the maps were expanded 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 the results of mercury testing of walleye collected from off-reservation lakes during the spring in 2009 and 2010. Funding for the collection and analysis of these samples was provided by the United States Environmental Protection Agency (U.S. EPA) Great Lakes National Program Office (GLNPO) as part of Grant #GL00E06501 and by the Bureau of Indian Affairs (BIA).

METHODS

Sample Collection

Walleye from inland lakes were collected during spring from tribal spearers and netters and by GLIFWC fishery assessment crews. According to the sampling plan, twelve walleye were collected from each lake with three fish taken from each of four size ranges (12.0-14.9, 15.0-17.9, 18.0-22.0, and >22.0 inches).

Upon collection, walleye total length and sex were determined and a metal identification tag with a unique number was attached to each fish. Whole fish were then placed on ice in a cooler and transferred to a freezer ($\leq -10^{o}$C) within 36 hours. A chain-of-custody form ("Field Chain-of-

Custody/Data Form") was filled out nightly for each lake to identify the fish collected and record who collected and transported the samples and when they were placed on ice or transferred to a freezer. A second chain-of-custody form ("Transfer Chain-of-Custody Form") was used when transferring fish samples to the Lake Superior Research Institute (LSRI) in Superior. Both chain-of-custody forms are included in 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 at the time of processing: a second length measurement (denoted as frozen length), sex, round weight, and fillet weight. 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. At the time of processing, the second or third dorsal spine was also removed for aging. The age of the fish was determined by counting the number of annuli (translucent zones) in the spine cross-section, as described by Schram (1989). Experienced GLIFWC Inland Fisheries technicians performed the spine preparation and subsequent walleye aging. All chain-of-custody forms and GLIFWC laboratory data sheets were filed in a three-ring binder and are kept at GLIFWC's main office.

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

Project: Spring Mercury Walleye	**Client:** GLIFWC
Species: Walleye	**Tag No.** 1121
Month/Day Collected: 3/31	**Year:** 2010
Lake Name: Sherman Lake (Vilas)	**Sample Processing:** Hg
Tissue Type: Fillet	**Processor:** LSRI

Total Mercury Analyses

Walleye fillets were received by LSRI from GLIFWC 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 (200-300 mg) of the ground tissue was digested and analyzed for total mercury using a Cold Vapor Atomic Absorption Spectroscopy (CVAAS; Perkin Elmer FIMS-100 Flow Injection Mercury Analysis System) method based on EPA Method 245.6.

Quality Control

Data quality at LSRI was assessed via four methods. This included analysis of: *1.)* certified reference materials (DORM-2 and DORM-3, dogfish shark tissue, *Squalus acanthias*) to determine accuracy, *2.)* spiked tissue samples to test for extraction efficiency and possible analytical interferences, *3.)* duplicate samples from a single fillet to measure analytical precision, and *4.)* procedural blanks (canned tuna, *Thunnus sp.*) before and after the tissue grinding process to measure laboratory bias.

A quality assurance reports from the audits of the laboratory processing and analysis are included in the LSRI Final Reports in Appendices 3 and 4.

RESULTS

Quality Control

Standard Reference Material

The DORM-2 and DORM-3 reference tissues have certified concentrations of 4.64 ± 0.26 and 0.382 ± 0.060µg Hg/g tissue, respectively. Both reference materials were included during the analysis of spring 2009 walleye samples. DORM-3, but not DORM-2, was included during the analysis of spring 2010 walleye samples. Acceptable ranges of mercury concentrations for the certified reference material samples were defined as the mean (± 2 standard deviations) of the values obtained for these materials during the previous three spring walleye assessments (i.e., spring 2006-2008 assessments for the samples analyzed in 2009 and spring 2007-2009 assessments for the samples analyzed in 2010). The acceptable range for DORM-2 was calculated to be 3.58-5.20µg Hg/g. The acceptable range for DORM-3 was calculated to be 0.294-0.428µg Hg/g in 2009 and 0.295-0.420µg Hg/g in 2010.

In 2009, DORM-2 was analyzed in duplicate with the first set of walleye tissues and DORM-3 was analyzed in triplicate with the remaining five sets of walleye tissues. Recovery values ranged from 81.2-97.7% with the grand mean and standard deviation of the recoveries being 89.2 ± 5.6% of the certified value. All values were within the acceptance range. In 2010, DORM-3 was analyzed in triplicate with the first three sets of walleye tissue samples and in duplicate for the fourth set of samples due to the small sample size in this final set. Recovery values ranged from 76.6-97.9% with the grand mean and standard deviation of the recoveries being 90.8 ± 6.7% of the certified value. One value (0.293µg Hg/g) fell slightly outside the acceptable range of 0.295-0.420µg Hg/g, but the set was deemed acceptable by LSRI since the mean of the triplicates was well within the acceptable range.

Spikes

As with certified reference materials, the acceptable spike recovery was calculated as the mean ± 2 times the standard deviation of all analyses of the spiked samples conducted during the previous 3 years of walleye sample analysis. In 2009, 26 spiked samples were analyzed (13% of

samples). Spike recovery was considered acceptable when it was in the range of 65.1 to 113% of the expected value. Mean recovery for the 26 spiked samples was 84.8 ± 19.9% with individual values ranging from 40.5-163.2%. One spike recovery value (Willow Flowage 11570) was below the acceptance range (57.1% mean recovery). This sample was reanalyzed and found to have an acceptable recovery upon reanalysis. In addition, one spike recovery value (Annabelle 11431) was above the acceptable range (161.6% mean recovery). This sample continued to exhibit unacceptable spike recovery upon reanalysis, suggesting a possible interference in this sample.

In 2010, 12 spiked samples were analyzed (10% of samples). Spike recovery was considered acceptable when it was in the range of 56.9 to 117% of the expected value. Mean recovery for the 12 spiked samples was 91.7 ± 8.2% with individual values ranging from 78.3-100.6%. All samples were within the acceptable spike recovery range.

Duplicates

Fish tissues were analyzed for mercury in duplicate 26 times (13% of total samples) in 2009 and 12 times (10% of total samples) in 2010. Two portions of the same tissue were digested and analyzed independently. Duplicate agreement values were acceptable when having a relative percent agreement >82.4% (2009) or >78.5% (2010). The acceptable value was calculated as the mean ± 2 times the standard deviations of all duplicate analyses conducted during the previous three spring walleye sample analyses at the LSRI laboratory. In 2009, relative percent agreement between the duplicate analyses of the same tissue ranged from 43.9-100% with the average and standard deviation of the agreements being 96.0 ± 10.8 percent. One relative percent agreement value (Minocqua 11579, 43.9%) was below the acceptance range of >82.4%. This sample was reanalyzed in duplicate on another date. The results for the reanalyzed sample fell within the acceptance range. In 2010, all duplicate analyses were within the acceptable range. Relative percent agreement between the duplicate analyses of the same tissue ranged from 78.6-99.3% with the average and standard deviation of the agreements being 94.2 ± 5.8 percent.

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 (i.e., homogenization). Results for the procedural blanks were considered acceptable when the relative percent agreement was >70.0% (2009) or >65.9% (2010). 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 previous three spring walleye projects. Four tuna procedural blanks in 2009 and three in 2010 were processed coincident with the grinding of walleye. One procedural blank was analyzed with each set of mercury samples for a total of six analyses in 2009 and four in 2010. In 2009, the mean and standard deviation of the four procedural blanks was 85.3 ± 9.94 relative percent agreement. Relative percent agreement values ranged from 67.6-96.7%, with all but one within the acceptable range of >70.0%. In 2010, the mean and standard deviation of the four procedural blanks was 80.8 ± 20.6 relative percent agreement. Relative percent agreement values ranged

from 52.1-96.4%, with all but one within the acceptable range of >65.9%. The very low concentrations of the procedural blanks increase the probability that QA/QC criteria will not be met. Thus, the single sample exceeding quality assurance criteria was considered an acceptable result.

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 75 quality control samples measured in 2009 and 39 in 2010. In 2009, three samples, or 4.0% of the total samples, exceeded the quality control parameters. In 2010, two samples, or 5.1% of the total samples, exceeded the quality control parameters. In both years, the percentage of samples exceeding quality control parameters met the goal of <10%. Overall, the sample data were in good agreement with the quality assurance parameters, so the data were determined to be precise and accurate.

Mercury in Walleye

During 2009, skinless fillets of 180 walleye from 15 lakes in Wisconsin and 19 walleye from two lakes in Minnesota were analyzed for total mercury concentration (Appendix 3). Overall, total mercury concentrations on a wet weight basis ranged from 0.048 to 1.59 µg Hg/g from Wisconsin lakes and from 0.098 to 0.787 µg Hg/g from the two Minnesota lakes. Walleye lengths ranged from 11.3 to 28.8 inches from Wisconsin lakes and 12.2 to 22.7 inches from the Minnesota lakes. Walleye length and mercury data from 2009 are summarized in Table 1.

Table 1. Summary statistics for wet weight mercury concentration (µg Hg/g fish tissue) and fish length (inches) for walleye collected from 15 Wisconsin lakes and 2 Minnesota lakes during spring 2009.

COUNTY	LAKE	# of Fish	Hg Concentration (µg/g)					Length (inches)	
			Mean	Std Dev	Median	Min	Max	Mean	Std Dev
VILAS	ANNABELLE L	12	0.725	0.342	0.662	0.426	1.59	18.0	5.0
WASHBURN	BASS-PATTERSON L	12	0.318	0.144	0.318	0.110	0.533	18.2	4.0
FOREST	BUTTERNUT L	12	0.162	0.115	0.106	0.048	0.390	18.3	4.0
SAWYER	L CHETAC	12	0.159	0.092	0.138	0.070	0.387	17.6	4.4
SAWYER	L CHIPPEWA	12	0.383	0.216	0.403	0.104	0.809	18.3	4.9
SAWYER	LAC COURTE OREILLES	12	0.264	0.150	0.211	0.126	0.621	18.6	4.3
VILAS	KENTUCK L	13	0.354	0.097	0.350	0.201	0.507	16.5	3.7
ONEIDA	MINOQUA L	12	0.351	0.247	0.253	0.122	0.863	19.0	5.0
BAYFIELD	NAMEKAGON L	12	0.375	0.344	0.262	0.126	1.35	18.1	4.1
VILAS	NORTH TWIN L	12	0.319	0.322	0.185	0.083	1.11	19.4	5.5
VILAS	SHERMAN L	10	0.348	0.095	0.333	0.239	0.482	16.9	3.5
BAYFIELD	SISKIWIT L	13	0.603	0.220	0.612	0.316	0.988	15.6	2.2
ONEIDA	SQUIRREL L	12	0.389	0.184	0.325	0.137	0.676	18.0	4.1
IRON	TURTLE-FLAMBEAU FL	12	0.620	0.243	0.585	0.318	1.14	17.4	4.0
ONEIDA	WILLOW FL	12	0.729	0.322	0.776	0.199	1.25	17.8	3.4
ONTONAGON (MI)	BOND FALLS FL	9	0.500	0.133	0.480	0.301	0.787	17.1	2.2
GOGEBIC (MI)	GOGEBIC L	10	0.283	0.174	0.205	0.098	0.600	16.6	3.3

During 2010, skinless fillets of 106 walleye from 9 lakes in Wisconsin and 12 walleye from one lake in Minnesota were analyzed for total mercury concentration (Appendix 4). Overall, total mercury concentrations on a wet weight basis ranged from 0.063 to 0.962 µg Hg/g from Wisconsin lakes and from 0.056 to 0.359 from the Minnesota lake. Walleye lengths ranged from 12.0 to 27.4 inches from Wisconsin lakes and 14.4 to 24.7 inches from the Minnesota lake. Walleye length and mercury data from 2010 are summarized in Table 2.

Table 2. Summary statistics for wet weight mercury concentration (µg Hg/g fish tissue) and fish length (inches) for walleye collected from 9 Wisconsin lakes and 1 Minnesota lake during spring 2010.

COUNTY	LAKE	# of Fish	Hg Concentration (µg/g)					Length (inches)	
			Mean	Std Dev	Median	Min	Max	Mean	Std Dev
ONEIDA	BEARSKIN L	12	0.156	0.074	0.147	0.063	0.303	18.5	4.9
SAWYER	CHIPPEWA L	12	0.489	0.265	0.414	0.132	0.962	18.7	5.2
VILAS	NORTH TWIN L	12	0.199	0.101	0.169	0.095	0.407	18.1	4.3
ONEIDA	PELICAN L	12	0.273	0.083	0.269	0.136	0.409	18.7	3.4
SAWYER	ROUND L	12	0.236	0.143	0.213	0.103	0.612	18.1	4.1
VILAS	SHERMAN L	10	0.302	0.068	0.302	0.220	0.411	16.1	2.8
VILAS	SQUAW L	12	0.385	0.097	0.349	0.226	0.513	16.8	3.2
SAWYER	TEAL L	12	0.295	0.182	0.225	0.122	0.738	18.7	4.7
IRON	TURTLE-FLAMBEAU FL	12	0.407	0.179	0.391	0.184	0.798	17.9	4.0
MILLE LACS (MI)	MILLE LACS	12	0.193	0.175	0.141	0.056	0.650	18.9	4.0

Percent Moisture

In 2009, percent moisture was measured in 51 of the 199 walleye tissues (25.6% of samples). Walleye muscle tissue had a mean moisture value of 79.6 ± 1.13% (Appendix 3). Of the 51 tissues analyzed for moisture, nine were analyzed in duplicate, all yielding relative percent agreements of ≥99.4%. Ten samples were also dried an additional 24 hours and reweighed to ensure dryness, all yielding agreements greater than 99%.

In 2010, percent moisture was measured in 30 of the 118 walleye tissues (25.4% of samples). Walleye muscle tissue had a mean moisture value of 79.0 ± 0.7% (Appendix 4). Of the 30 tissues analyzed for moisture, four were analyzed in duplicate, all yielding relative percent agreements of ≥98.2%. Seven samples were also dried an additional 24 hours and reweighed to ensure dryness, all yielding agreements greater than 99%.

SUMMARY

Walleye total mercury results from 2009 and 2010 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 (Madsen et al. 2008).

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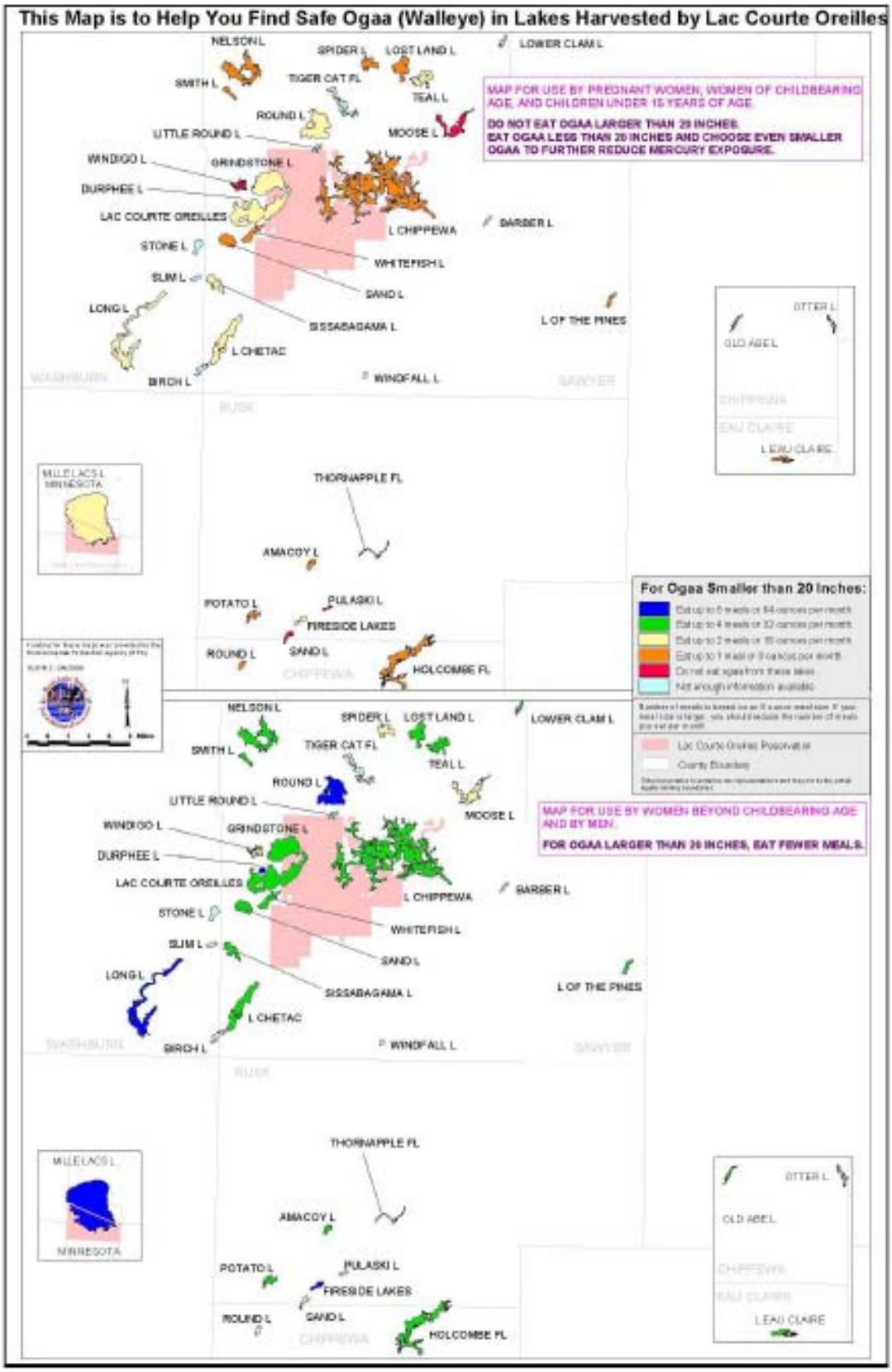
LIST OF APPENDICES

APPENDIX 1

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


This Map is to Help You Find Safe Ogaa (Walleye) in Lakes Harvested by Lac Courte Oreilles
NELSON L
SPIDER L
LOST LAND L
LOWER CLAM L
SMITH L
TIGER CAT FL
TEAL L
ROUND L
LITTLE ROUND L
MOOSE L
WINDIGO L
GRINDSTONE L
DURPHEE L
LAC COURTE OREILLES
L CHIPPEWA
BARBER L
STONE L
WHITEFISH L
SLIM L
SAND L
LONG L
SISSABAGAMA L
L OF THE PINES
L CHETAC
BIRCH L
WINDFALL L
WASHBURN
RUSK
SAWYER
CHIPPEWA
EAU CLAIRE
OTTER L
OLD ABE L
LEAU CLAIRE
MILLE LACS L
MINNESOTA
THORNAPPLE FL
AMACOY L
POTATO L
PULASKI L
FIRESIDE LAKES
HOLCOMBE FL
MAP FOR USE BY PREGNANT WOMEN, WOMEN OF CHILDBEARING AGE, AND CHILDREN UNDER 15 YEARS OF AGE.
DO NOT EAT OGAA LARGER THAN 20 INCHES.
EAT OGAA LESS THAN 20 INCHES AND CHOOSE EVEN SMALLER OGAA TO FURTHER REDUCE MERCURY EXPOSURE.
For Ogaa Smaller than 20 Inches:
Not enough information available
Lac Courte Oreilles Reservation
County Boundary
MAP FOR USE BY WOMEN BEYOND CHILDBEARING AGE AND BY MEN.
FOR OGAA LARGER THAN 20 INCHES, EAT FEWER MEALS.

Recommended Maximum Number of Ogaa Meals per Month for Lakes Harvested by Lac Courte Oreilles

SORTING AND LABELING *OGAA* PRIOR TO FREEZING

When Cleaning *Ogaa*:

- Put ogaa under 20 inches in bags labeled "under 20 inches."
- Put ogaa over 20 inches in bags labeled "over 20 inches."
- Label bags with the lake name.
- Follow the advice below for maximum number of meals per month.

USING THIS CHART TO FIND SAFER *GITGOONH*

MAXIMUM NUMBER OF MEALS PER MONTH

Advice is for all lakes combined. For example, if you eat four meals in a month from green lakes you should not eat any other meals of ogaa in that month.

MEAL SIZE

Meal size is based on 8 ounces. An average 19 inch ogaa will have 8 ounces of meat. If your meal size is larger you should eat fewer meals of ogaa. If it is smaller you can eat more meals of ogaa.

OTHER *GITGOONH*

Gitgoonh such as muskellunge, largemouth bass, smallmouth bass, and northern pike will have more mercury than gitgoonh such as lake whitefish, herring, bluegill, sunfish, crappie or perch. Try to choose safer gitgoonh.

LAKE	COUNTY	Women of childbearing age and children less than 15: Maximum number of meals per month	Women beyond childbearing years and men 15 and older: Maximum number of meals per month
AMACOY L	RUSK	1	4
BARBER L	SAWYER	Not Enough Information	
BIRCH L	WASHBURN	Not Enough Information	
DURPHEE L	SAWYER	2	8
FIRESIDE LAKES	RUSK	2	8
GRINDSTONE L	SAWYER	2	4
HOLCOMBE FL	CHIPPEWA	1	4
L CHETAC	SAWYER	2	4
L CHIPPEWA	SAWYER	1	4
L EAU CLAIRE	EAU CLAIRE	1	4
L OF THE PINES	SAWYER	1	4
LAC COURTE OREILLES	SAWYER	2	8
LITTLE ROUND L	SAWYER	Not Enough Information	
LONG L	WASHBURN	2	8
LOST LAND L	SAWYER	1	4
LOWER CLAM L	SAWYER	2	4
MILLE LACS L	MILLE LACS	2	8
MOOSE L	SAWYER	0	2
NELSON L	SAWYER	1	4
OLD ABE L	CHIPPEWA	1	4
OTTER L	CHIPPEWA	Not Enough Information	
POTATO L	RUSK	1	4
PULASKI L	RUSK	0	2
ROUND L	SAWYER	2	8
ROUND L	CHIPPEWA	1	2
SAND L	RUSK	0	2
SAND L	SAWYER	1	4
SISSABAGAMA L	SAWYER	2	4
SLIM	WASHBURN	Not Enough Information	
SMITH L	SAWYER	1	4
SPIDER L	SAWYER	1	2
STONE L	WASHBURN	Not Enough Information	
TEAL L	SAWYER	2	4
THORNAPPLE FL	RUSK	Not Enough Information	
TIGER CAT FL	SAWYER	Not Enough Information	
WHITEFISH L	SAWYER	1	4
WINDFALL L	SAWYER	Not Enough Information	
WINDIGO L	SAWYER	0	2

For many native people, *gitgoonh* are part of a traditional and healthy diet. If you rely on *gitgoonh*, choose safer *gitgoonh* with lower levels of mercury by following the advice on this map.

RISKS AND BENEFITS

Risk: Mercury can damage the nervous system, especially the brain. Fetuses and babies are the most at risk because their nervous systems are rapidly developing. Children exposed to unsafe levels while in the womb have been found to experience delayed development in walking and talking, even though the mother was not affected. Mercury cannot be removed by trimming or cooking.

Benefit: Eating even as few as two to three meals of *gitgoonh* a month may reduce your risk of death due to heart disease.

If you have questions about finding safer ogaa, call GLIFWC at 1-800-258-7574.
To learn more about mercury in ogaa, visit GLIFWC's website at www.glifwc.org/bio/mercury.htm

Appendix 2

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

FIELD CHAIN-OF-CUSTODY/DATA FORM

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

Name of Lake:_____ County_____ Area _____

SECTION A: SAMPLE COLLECTION

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

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

SECTION B: SAMPLE STORAGE AND CUSTODY

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

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

Comments:_____
_____
_____

OFFICE USE ONLY– DO NOT WRITE BELOW THIS LINE

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

TRANSFER CHAIN-OF-CUSTODY FORM

Study Title: Spring Walleye Sampling For Mercury **Year:**
Purpose: Transfer Filets to UW-Superior, LSRI

PAGE 1 of 2

SECTION A: SAMPLE STORAGE

Container Type Enter: 1 = Cooler + Ice 2 = Freezer (≤-10°C)		Placed INTO Container				Taken OUT of Container			
		Date	Time	Initials	^{0}C	Date	Time	Initials	^{0}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. _____ _____
1. _____ _____ 12. _____ _____
2. _____ _____ 13. _____ _____
3. _____ _____ 14. _____ _____
4. _____ _____ 15. _____ _____
5. _____ _____ 16. _____ _____
6. _____ _____ 17. _____ _____
7. _____ _____ 18. _____ _____
8. _____ _____ 19. _____ _____
9. _____ _____ 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 during the Spring 2009 in Wisconsin Ceded Territory Waters

Total Mercury Concentrations in Muscle Tissue from Walleye Captured during the Spring 2009 in Wisconsin and Michigan Ceded Territory Waters

by

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Introduction

Skinless fillet samples from walleye (*Sander vitreus*) captured during the spring of 2009 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). One hundred ninety nine skinless walleye fillets, from a total of seventeen lakes in Wisconsin and Michigan, collected by tribal spearers and GLIFWC Inland Fisheries assessment crews were analyzed.

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

Before processing the fish tissues, all glassware, utensils, and grinders were cleaned according to the appropriate methods (LSRI SOP SA/8 v.5). 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 passed through a grinder three times. A small amount of the initial tissue that passed through the grinder was collected and discarded (LSRI SOP SA/10 v.4). A sub-sample of the ground tissue was placed into a certified 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 v.5).

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

Fish tissues were weighed for mercury analysis following standard laboratory procedure (SOP SA/11 v.4). Mercury solutions for making tissue spikes and preparing analytical standards were prepared following 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/49). Sample analysis yielded triplicate absorbance readings whose mean value was used to calculate the concentration of each sample. If the relative standard deviation (RSD) of the three measurements was greater than 5%, additional aliquots of the sample were analyzed in an attempt to obtain an RSD of less than 5%. If an RSD of < 5% was not able to be achieved, the sample was redigested and reanalyzed. Mercury concentrations and quality assurance calculations were done in Microsoft Excel according to SOP SA/37. The biota method detection limit was 0.0066 µg Hg/g for a tissue mass of 0.2 g (Appendix A). This limit of detection was determined using a whole fish composite of rainbow trout containing a low concentration of mercury (SOP SA/35).

Moisture content of tissue was calculated using the wet and dried tissue weights (SOP NT/15 v.2). 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 25 percent of the walleye analyzed for mercury had moisture content determined. In general, three fish per lake were randomly selected for determination of percent moisture.

Data Quality Assessment

Data quality was assessed using four data quality indicators: 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 (*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 possible analytical interferences. Analytical standards with known amounts of mercury were analyzed with each group (maximum of 40 samples plus QA samples) of tissue samples. On the initial analysis date, July 29, 2009, the analytical standards contained 0, 50, 100, 500, 1000 and 6000 ng Hg/L. To avoid the necessity of diluting high concentration samples or spikes a decision was made to switch to a set of analytical standards containing 0, 100, 500, 1000, 6000, and 10,000 ng Hg/L. This set of standards was used for the remaining five analysis dates. Standards were prepared from a purchased 1000 ± 10 ppm mercury (prepared from mercuric nitrate) reference standard solution (Fisher Scientific, Pittsburgh, PA). Summary tables of the mercury calibration curve data are provided (Appendix B).

Results for the quality assurance samples were considered acceptable when the value determined for a quality assurance sample fell within the mean ± 2 times the standard deviation of the values obtained from the Spring Walleye 2006 through 2008 projects (previous three years) for the respective quality assurance parameter. Results for the procedural blanks were considered acceptable when the relative percent agreement was > 70.0%. Duplicate agreement values were acceptable when having a relative percent agreement > 82.4%. The calculated acceptable range for the DORM standard reference material was 77.1 to 112% of certified value. 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 65.1 to 113 percent of the expected value.

Two tissue samples had an initial RSD of >5% for the triplicate measurements made on the digested sample. The digestate of those samples was reanalyzed on the same date and each resulted in an RSD of <5%. Several QA/QC samples (blanks or lowest concentration standard) also failed the 5% RSD check for their initial analysis. Calibration blanks and the lowest concentration mercury standard were normally not reanalyzed if they failed the RSD requirement because they have low absorbance values and thus are more likely to fail the RSD limit.

A quality assurance audit was conducted by the LSRI quality assurance manager during the Spring Walleye 2009 project. That report is provided in Appendix C.

Results of Fish Tissue Analyses

Quality Assurance – Four tuna procedural blanks were processed coincident with the grinding of walleye collected for the project. One of the four procedural blanks was analyzed with each set of mercury samples for a total of six analyses resulting in a mean of 85.3 ± 9.94 relative percent agreement (Table 1). The relative percent agreement values ranged from 67.6 to 96.7%, all but one of which was within the acceptable range of > 70.0%.

Analysis of the dogfish shark tissue DORM-2 standard reference material was conducted in duplicate with the first set of walleye tissues analyzed. Analysis of dogfish shark tissue DORM-3 was conducted in triplicate on the remaining five sets of walleye tissues analyzed because the supply of DORM-2 reference had run out (Table 2). The certified mercury concentration for the dogfish tissue was 4.64 ± 0.26 µg Hg/g for DORM-2 and 0.382 ± 0.060 µg Hg/g for DORM-3. The recovery values ranged from 81.2 to 97.7% with the grand mean and standard deviation of the recoveries being 89.2 ± 5.64 percent of the certified value. All of the values were within the acceptable QC range of 77.1 – 112% of the certified mercury concentration in the standard reference samples.

Fish tissues were analyzed for mercury in duplicate 26 times. Two portions of the same tissue were digested and analyzed independently. Relative percent agreement between the duplicate analyses of the same tissue ranged from 43.9 to 100% with the average and standard deviation of the agreements being 96.0 ± 10.8 percent (Table 3). One relative percent agreement value (Minocqua 11579) was below the acceptance range of > 82.4% and that sample was reanalyzed in duplicate on another date. The results for the reanalyzed samples fell within the acceptance range.

Samples of tissue were spiked with known concentrations of mercury prior to digestion. Mean recovery for the 26 spiked samples was 84.8 ± 19.9 percent with the individual values ranging from 40.5 to 163.2% (Table 4). Two initial spike recovery values (Willow Flowage 11570 and Annabelle 11431) were outside of the spike recovery acceptance range (65.1 to 113%). These samples were reanalyzed. The Willow Flowage 11570 sample was reanalyzed on August 25, 2009 and was found to have an acceptable recovery on that date. The Annabelle 11431 sample was reanalyzed on August 18, 2009. The spike recovery was unacceptable on the second analysis date as well, suggesting that there is possibly some interference in the sample precluding

an acceptable spike recovery.

Mercury Analysis – Skinless fillets of 199 walleye collected from a total of 17 lakes in Wisconsin and Michigan were analyzed for total mercury concentration. Total mercury concentrations on a wet weight basis (Table 5) ranged from 0.048 to 1.59 µg Hg/g (parts per million).

Tissue Moisture Analysis – Percent moisture was measured in 51 of the 199 walleye tissues. Moisture analysis took place immediately following grinding of the fillets. Walleye muscle tissue had a mean moisture value of 79.6 ± 1.13 percent (Table 6). Of the 51 tissues analyzed for moisture, nine were analyzed in duplicate, all yielding relative percent agreements of 99.4 percent or greater. Ten samples were also dried an additional 24 hours and reweighed to ensure dryness, all yielding agreements greater than 99 percent.

Table 1. Relative Percent Agreement of Total Mercury for Procedural Blank Samples (Before and After Grinding). Data quality indicator for laboratory bias was >70.0% relative percent agreement.

Analysis Date	Grinding Date	Before Grinding µg Hg/g	After Grinding µg Hg/g	Mean µg Hg/g	Relative Percent Agreement*
7/29/2009	6/30/2009	0.063	0.053	0.058	82.2
8/6/2009	7/8/2009	0.045	0.049	0.047	90.5
8/11/2009	7/14/2009	0.092	0.079	0.085	85.5
8/13/2009	6/2/2009	0.050	0.036	0.043	67.6
8/18/2009	7/8/2009	0.049	0.050	0.050	96.7
8/25/2009	7/14/2009	0.078	0.070	0.074	89.1
			Mean ± Std. Dev.		85.3 ± 9.94 %

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

Table 2. Mercury Concentrations of Dogfish Shark Tissue (Standard Reference Material DORM-2 and DORM-3) Analyzed during Fish Analysis. The Standard Reference has a Certified Mercury Concentration of 4.64 ± 0.26µg Hg/g Tissue for DORM-2 and 0.382 ± 0.060µg Hg/g Tissue for DORM-3. Data quality indicator for accuracy was 77.1 to 112% agreement between the nominal and measured reference standard values.

	DORM 2-1		DORM 2-2			
Date of Analysis	µg Hg/g	% of Certified Value	µg Hg/g	% of Certified Value		
7/29/2009	4.50	97.0	4.31	92.9		
	DORM 3-1		DORM 3-2		DORM 3-3	
Date of Analysis	µg Hg/g	% of Certified Value	µg Hg/g	% of Certified Value	µg Hg/g	% of Certified Value
8/6/2009	0.344	90.2	0.349	91.3	0.343	89.9
8/11/2009	0.344	90.1	0.317	83.1	0.328	86.0
8/13/2009	0.314	82.4	0.310	81.4	0.317	82.9
8/18/2009	0.361	94.6	0.373	97.7	0.361	94.8
8/25/2009	0.360	94.4	0.310	81.2	0.329	86.2
			Mean ± Std. Dev.		89.2 ± 5.64 %	

Table 3. Relative Percent Agreement for Duplicate Analysis of Total Mercury Content in Skinless Walleye Fillet Tissue. Data quality indicator for precision was >82.4% relative percent agreement.

Date of Analysis	Lake and Tag Number	µg Hg/g	Duplicate µg Hg/g	Mean µg Hg/g	Relative Percent Agreement
7/29/2009	Lac Courte Oreille 11539	0.238	0.238	0.238	100
7/29/2009	Chetac 11517	0.142	0.141	0.142	99.3
7/29/2009	Chetac 11529	0.080	0.082	0.081	97.5
7/29/2009	Willow Flowage 11570	0.769	0.821	0.795	93.5
8/6/2009	Squirrel Lake 11406	0.303	0.304	0.304	99.7
8/6/2009	Gogebic 11159	0.110	0.112	0.111	98.2
8/6/2009	Namekagon 11646	0.151	0.145	0.148	95.9
8/6/2009	Namekagon 11658	0.541	0.560	0.551	96.5
8/11/2009	Bond Falls Flowage 11606	0.464	0.471	0.468	98.5
8/11/2009	Siskiwit 11465	0.317	0.314	0.316	99.0
8/11/2009	Kentuck 11453	0.423	0.423	0.423	100
8/11/2009	Kentuck 11460	0.360	0.339	0.350	94.0
8/13/2009	Sherman 11668	0.327	0.335	0.331	97.6
8/13/2009	Minocqua 11579	0.219	0.123	0.171	43.9
8/13/2009	Annabelle 11431	1.1	1.09	1.095	98.2
8/13/2009	Annabelle 11445	0.425	0.427	0.426	99.5
8/18/2009	North Twin Lake 11636	0.093	0.090	0.092	96.7
8/18/2009	Chippewa Flowage 11505	0.280	0.284	0.282	98.6
8/18/2009	Chippewa Flowage 11514	0.588	0.598	0.593	98.3
8/18/2009	Turtle Flambeau Flowage 11558	0.518	0.515	0.517	99.4
8/18/2009	Annabelle 11431	1.19	1.19	1.19	100
8/25/2009	Bass-Patterson 11684	0.474	0.481	0.478	98.5
8/25/2009	Butternut 11417	0.228	0.230	0.229	99.1
8/25/2009	Butternut 11426	0.136	0.143	0.140	95.0
8/25/2009	Willow Flowage 11570	0.707	0.714	0.711	99.0
8/25/2009	Minocqua 11579	0.220	0.219	0.220	99.5
			Mean ± Std. Dev.		96.0 ± 10.8 %

Table 4. Percent of Mercury Recovered from Skinless Walleye Fillet Samples Spiked with a Known Concentration of Mercury. Data quality indicator for accuracy was 65.1 to 113% spike-recovery.

Date of Analysis	Lake and Tag Number	Spike #1	Spike #2	Mean Spike Recovery (%)	Std. Dev.
7/29/2009	Lac Courte Oreille 11539	83.7	83.9	83.8	0.11
7/29/2009	Chetac 11517	92.0	95.4	93.7	2.45
7/29/2009	Chetac 11529	94.0	94.2	94.1	0.10
7/29/2009	Willow Flowage 11570	63.4	50.7	57.1	8.97
8/6/2009	Squirrel Lake 11406	86.7	82.9	84.8	2.64
8/6/2009	Gogebic 11159	94.5	93.6	94.0	0.63
8/6/2009	Namekagon 11646	91.5	89.8	90.7	1.17
8/6/2009	Namekagon 11658	75.2	75.3	75.2	0.03
8/11/2009	Bond Falls Flowage 11606	74.9	70.0	72.4	3.41
8/11/2009	Siskiwit 11465	80.9	81.1	81.0	0.1
8/11/2009	Kentuck 11453	77.1	72.1	74.6	3.57
8/11/2009	Kentuck 11460	78.0	84.2	81.1	4.34
8/13/2009	Sherman 11668	85.1	88.8	86.9	2.63
8/13/2009	Minocqua 11579	100.2	99.5	99.9	0.49
8/13/2009	Annabelle 11431	40.5	52.3	46.4	8.3
8/13/2009	Annabelle 11445	76.2	83.6	79.9	5.27
8/18/2009	North Twin Lake 11636	96.3	96.6	96.4	0.17
8/18/2009	Chippewa Flowage 11505	87.1	86.9	87.0	0.10
8/18/2009	Chippewa Flowage 11514	69.4	76.3	72.9	4.93
8/18/2009	Turtle Flambeau Flowage 11558	83.1	77.2	80.2	4.2
8/18/2009	Annabelle 11431	163.2	159.9	161.6	2.29
8/25/2009	Bass-Patterson 11684	74.7	76.4	75.6	1.19
8/25/2009	Butternut 11417	90.0	87.7	88.8	1.63
8/25/2009	Butternut 11426	92.1	91.0	91.6	0.77
8/25/2009	Willow Flowage 11570	66.9	66.3	66.6	0.37
8/25/2009	Minocqua 11579	85.6	93.5	89.6	5.54
		Mean ± Std. Dev.		84.8 ± 19.9 %	

Table 5. Total Mercury Concentration (Wet Weight) in Walleye Fillets from Fish Captured during the Spring of 2009.

Analysis Date	Lake	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g tissue
8/18/2009	North Twin	11631	Vilas	14.1	M	0.083
8/18/2009	North Twin	11632	Vilas	14.9	M	0.184
8/18/2009	North Twin	11633	Vilas	16.2	M	0.185
8/18/2009	North Twin	11634	Vilas	15.3	M	0.128
8/18/2009	North Twin	11635	Vilas	16.7	M	0.168
8/18/2009	North Twin	11636	Vilas	13.1	M	0.092
8/18/2009	North Twin	11637	Vilas	20.4	F	0.290
8/18/2009	North Twin	11638	Vilas	28.5	F	0.850
8/18/2009	North Twin	11642	Vilas	19.9	F	0.302
8/18/2009	North Twin	11643	Vilas	28.8	F	1.11
8/18/2009	North Twin	11644	Vilas	25.8	F	0.278
8/18/2009	North Twin	11645	Vilas	18.9	F	0.156
8/18/2009	Chippewa Flowage	11504	Sawyer	20.3	F	0.521
8/18/2009	Chippewa Flowage	11505	Sawyer	19.2	F	0.282
8/18/2009	Chippewa Flowage	11506	Sawyer	15.5	M	0.406
8/18/2009	Chippewa Flowage	11507	Sawyer	15.0	M	0.210
8/18/2009	Chippewa Flowage	11508	Sawyer	22.0	F	0.399
8/18/2009	Chippewa Flowage	11509	Sawyer	13.5	M	0.163
8/18/2009	Chippewa Flowage	11510	Sawyer	11.5	M	0.104
8/18/2009	Chippewa Flowage	11511	Sawyer	12.8	M	0.115
8/18/2009	Chippewa Flowage	11512	Sawyer	25.7	F	0.554
8/18/2009	Chippewa Flowage	11513	Sawyer	16.9	M	0.434
8/18/2009	Chippewa Flowage	11514	Sawyer	20.7	F	0.593
8/18/2009	Chippewa Flowage	11515	Sawyer	26.4	F	0.809
8/18/2009	Turtle-Flambeau Flowage	11546	Iron	15.2	M	0.566
8/18/2009	Turtle-Flambeau Flowage	11547	Iron	13.4	M	0.318
8/18/2009	Turtle-Flambeau Flowage	11548	Iron	18.1	M	0.867
8/18/2009	Turtle-Flambeau Flowage	11549	Iron	18.6	M	0.571
8/18/2009	Turtle-Flambeau Flowage	11551	Iron	20.1	F	0.614
8/18/2009	Turtle-Flambeau Flowage	11552	Iron	24.0	F	1.14
8/18/2009	Turtle-Flambeau Flowage	11554	Iron	24.4	F	0.857
8/18/2009	Turtle-Flambeau Flowage	11556	Iron	18.1	F	0.687
8/18/2009	Turtle-Flambeau Flowage	11557	Iron	15.8	M	0.599
8/18/2009	Turtle-Flambeau Flowage	11558	Iron	15.8	F	0.517

8/18/2009	Turtle-Flambeau Flowage	11559	Iron	12.5	M	0.368
8/18/2009	Turtle-Flambeau Flowage	11560	Iron	12.5	M	0.330
8/6/2009	Squirrel	11401	Oneida	24.5	F	0.669
8/6/2009	Squirrel	11402	Oneida	18.8	M	0.676
8/6/2009	Squirrel	11403	Oneida	24.1	F	0.619
8/6/2009	Squirrel	11404	Oneida	16.2	F	0.330
8/6/2009	Squirrel	11405	Oneida	24.1	F	0.521
8/6/2009	Squirrel	11406	Oneida	15.1	M	0.304
8/6/2009	Squirrel	11407	Oneida	15.1	M	0.260
8/6/2009	Squirrel	11408	Oneida	18.2	F	0.345
8/6/2009	Squirrel	11409	Oneida	14.0	M	0.226
8/6/2009	Squirrel	11410	Oneida	14.0	M	0.261
8/6/2009	Squirrel	11411	Oneida	13.6	M	0.137
8/6/2009	Squirrel	11412	Oneida	18.4	F	0.319
8/6/2009	Gogebic	11157	Gogebic	14.6	M	0.157
8/6/2009	Gogebic	11159	Gogebic	12.2	M	0.111
8/6/2009	Gogebic	11160	Gogebic	12.2	M	0.098
8/6/2009	Gogebic	11161	Gogebic	18.2	M	0.432
8/6/2009	Gogebic	11616	Gogebic	18.4	M	0.432
8/6/2009	Gogebic	11619	Gogebic	22.7	F	0.600
8/6/2009	Gogebic	12359	Gogebic	15.2	M	0.166
8/6/2009	Gogebic	12360	Gogebic	19.2	F	0.427
8/6/2009	Gogebic	12361	Gogebic	17.6	F	0.223
8/6/2009	Gogebic	12365	Gogebic	15.4	M	0.187
8/11/2009	Bond Falls Flowage	11601	Ontonagon	15.3	M	0.441
8/11/2009	Bond Falls Flowage	11602	Ontonagon	16.5	M	0.505
8/11/2009	Bond Falls Flowage	11603	Ontonagon	21.0	F	0.787
8/11/2009	Bond Falls Flowage	11604	Ontonagon	18.9	F	0.520
8/11/2009	Bond Falls Flowage	11605	Ontonagon	18.3	M	0.581
8/11/2009	Bond Falls Flowage	11606	Ontonagon	17.8	F	0.468
8/11/2009	Bond Falls Flowage	11607	Ontonagon	17.2	F	0.480
8/11/2009	Bond Falls Flowage	11608	Ontonagon	14.4	M	0.301
8/11/2009	Bond Falls Flowage	11609	Ontonagon	14.2	M	0.414
8/6/2009	Namekagon	11646	Bayfield	14.5	M	0.148
8/6/2009	Namekagon	11647	Bayfield	13.2	M	0.146
8/6/2009	Namekagon	11648	Bayfield	22.2	M	1.35
8/6/2009	Namekagon	11649	Bayfield	16.0	M	0.222
8/6/2009	Namekagon	11650	Bayfield	15.5	M	0.184
8/6/2009	Namekagon	11651	Bayfield	11.9	M	0.126

8/6/2009	Namekagon	11652	Bayfield	17.4	M	0.327
8/6/2009	Namekagon	11653	Bayfield	18.5	F	0.156
8/6/2009	Namekagon	11654	Bayfield	24.8	F	0.569
8/6/2009	Namekagon	11655	Bayfield	22.5	F	0.425
8/6/2009	Namekagon	11656	Bayfield	18.5	F	0.301
7/29/2009	Namekagon	11658	Bayfield	21.8	F	0.551
7/29/2009	Lac Courte Oreilles	11531	Sawyer	24.8	F	0.357
7/29/2009	Lac Courte Oreilles	11534	Sawyer	12.8	M	0.143
7/29/2009	Lac Courte Oreilles	11535	Sawyer	25.7	F	0.621
7/29/2009	Lac Courte Oreilles	11537	Sawyer	14.3	M	0.166
7/29/2009	Lac Courte Oreilles	11538	Sawyer	13.4	M	0.153
7/29/2009	Lac Courte Oreilles	11539	Sawyer	18.6	M	0.238
7/29/2009	Lac Courte Oreilles	11540	Sawyer	16.2	M	0.126
7/29/2009	Lac Courte Oreilles	11541	Sawyer	20.2	M	0.223
7/29/2009	Lac Courte Oreilles	11542	Sawyer	17.1	M	0.144
7/29/2009	Lac Courte Oreilles	11543	Sawyer	17.6	M	0.198
7/29/2009	Lac Courte Oreilles	11544	Sawyer	23.5	F	0.380
7/29/2009	Lac Courte Oreilles	11545	Sawyer	19.7	M	0.413
7/29/2009	Chetac	11516	Sawyer	15.1	M	0.117
7/29/2009	Chetac	11517	Sawyer	18.5	M	0.142
7/29/2009	Chetac	11518	Sawyer	15.5	M	0.147
7/29/2009	Chetac	11519	Sawyer	12.9	M	0.084
7/29/2009	Chetac	11520	Sawyer	17.2	M	0.133
7/29/2009	Chetac	11521	Sawyer	18.2	M	0.172
7/29/2009	Chetac	11522	Sawyer	23.2	F	0.195
7/29/2009	Chetac	11526	Sawyer	15.5	M	0.106
7/29/2009	Chetac	11527	Sawyer	27.1	F	0.387
7/29/2009	Chetac	11528	Sawyer	13.7	M	0.070
7/29/2009	Chetac	11529	Sawyer	13.0	M	0.081
7/29/2009	Chetac	11530	Sawyer	21.5	M	0.278
8/25/2009	Bass-Patterson	11676	Washburn	17.8	M	0.384
8/25/2009	Bass-Patterson	11677	Washburn	24.2	F	0.533
8/25/2009	Bass-Patterson	11679	Washburn	16.4	M	0.345
8/25/2009	Bass-Patterson	11681	Washburn	18.5	M	0.290
8/25/2009	Bass-Patterson	11682	Washburn	19.4	M	0.438
8/25/2009	Bass-Patterson	11684	Washburn	23.7	F	0.478
8/25/2009	Bass-Patterson	11685	Washburn	17.0	M	0.249
8/25/2009	Bass-Patterson	11686	Washburn	13.3	M	0.110
8/25/2009	Bass-Patterson	11687	Washburn	19.2	F	0.261

8/25/2009	Bass-Patterson	11688	Washburn	13.1	M	0.136
8/25/2009	Bass-Patterson	11689	Washburn	12.5	M	0.137
8/25/2009	Bass-Patterson	11690	Washburn	23.1	F	0.453
8/11/2009	Siskiwit	11461	Bayfield	15.5	M	0.612
8/11/2009	Siskiwit	11462	Bayfield	13.1	M	0.371
8/11/2009	Siskiwit	11463	Bayfield	15.6	M	0.603
8/11/2009	Siskiwit	11464	Bayfield	15.3	M	0.586
8/11/2009	Siskiwit	11465	Bayfield	13.2	M	0.316
8/11/2009	Siskiwit	11466	Bayfield	18.7	F	0.868
8/11/2009	Siskiwit	11467	Bayfield	18.1	M	0.784
8/11/2009	Siskiwit	11468	Bayfield	18.2	F	0.632
8/11/2009	Siskiwit	11469	Bayfield	14.2	M	0.318
8/11/2009	Siskiwit	11470	Bayfield	14.8	M	0.763
8/11/2009	Siskiwit	11471	Bayfield	16.5	M	0.675
8/11/2009	Siskiwit	11472	Bayfield	17.9	M	0.988
8/11/2009	Siskiwit		Bayfield	12.0*	M	0.323
8/11/2009	Kentuck	11453	Vilas	14.6	M	0.423
8/11/2009	Kentuck	11446	Vilas	12.8	M	0.294
8/11/2009	Kentuck	11447	Vilas	17.9	F	0.386
8/11/2009	Kentuck	11448	Vilas	14.5	M	0.276
8/11/2009	Kentuck	11449	Vilas	18.4	F	0.527
8/11/2009	Kentuck	11451	Vilas	13.5	F	0.350
8/11/2009	Kentuck	11455	Vilas	25.8	F	0.492
8/11/2009	Kentuck	11456	Vilas	17.2	M	0.391
8/11/2009	Kentuck	11457	Vilas	15.3	M	0.230
8/11/2009	Kentuck	11458	Vilas	20.0	F	0.402
8/11/2009	Kentuck	11459	Vilas	17.9	M	0.281
8/11/2009	Kentuck	11460	Vilas	15.2	M	0.350
8/11/2009	Kentuck		Vilas	11.3*	M	0.201
8/25/2009	Butternut	11416	Forest	24.1	F	0.390
8/25/2009	Butternut	11417	Forest	18.6	M	0.229
8/25/2009	Butternut	11418	Forest	14.9	M	0.090
8/25/2009	Butternut	11419	Forest	15.6	M	0.091
8/25/2009	Butternut	11420	Forest	24.0	F	0.299
8/25/2009	Butternut	11421	Forest	24.1	F	0.310
8/25/2009	Butternut	11422	Forest	14.8	M	0.065
8/25/2009	Butternut	11423	Forest	16.0	M	0.075
8/25/2009	Butternut	11424	Forest	12.1	M	0.048
8/25/2009	Butternut	11425	Forest	17.0	M	0.117

8/25/2009	Butternut	11426	Forest	19.3	M	0.140
8/25/2009	Butternut	11427	Forest	19.0	F	0.094
8/13/2009	Annabelle	11431	Vilas	25.7	F	1.10
8/13/2009	Annabelle	11432	Vilas	18.0	F	0.489
8/13/2009	Annabelle	11436	Vilas	22.1	F	0.738
8/13/2009	Annabelle	11437	Vilas	27.8	F	1.59
8/13/2009	Annabelle	11438	Vilas	18.5	F	0.861
8/13/2009	Annabelle	11439	Vilas	18.2	F	0.799
8/13/2009	Annabelle	11440	Vilas	12.4	F	0.462
8/13/2009	Annabelle	11441	Vilas	12.4	M	0.458
8/13/2009	Annabelle	11442	Vilas	15.3	M	0.721
8/13/2009	Annabelle	11443	Vilas	17.4	F	0.603
8/13/2009	Annabelle	11444	Vilas	12.4	M	0.448
8/13/2009	Annabelle	11445	Vilas	15.9	F	0.426
7/29/2009	Willow Flowage	11561	Oneida	15.9	M	0.744
7/29/2009	Willow Flowage	11562	Oneida	17.5	M	0.757
7/29/2009	Willow Flowage	11563	Oneida	14.5	M	0.390
7/29/2009	Willow Flowage	11564	Oneida	14.2	M	0.271
7/29/2009	Willow Flowage	11565	Oneida	17.8	M	0.907
7/29/2009	Willow Flowage	11566	Oneida	17.8	M	1.04
7/29/2009	Willow Flowage	11567	Oneida	11.9	M	0.199
7/29/2009	Willow Flowage	11568	Oneida	23.8	F	1.08
7/29/2009	Willow Flowage	11569	Oneida	19.4	F	0.474
7/29/2009	Willow Flowage	11570	Oneida	19.3	M	0.795
7/29/2009	Willow Flowage	11571	Oneida	18.5	M	0.845
7/29/2009	Willow Flowage	11574	Oneida	23.0	F	1.25
8/13/2009	Sherman	11661	Vilas	12.2	M	0.239
8/13/2009	Sherman	11662	Vilas	12.0	M	0.334
8/13/2009	Sherman	11663	Vilas	14.5	F	0.266
8/13/2009	Sherman	11664	Vilas	22.6	F	0.477
8/13/2009	Sherman	11667	Vilas	15.3	M	0.252
8/13/2009	Sherman	11668	Vilas	18.2	F	0.331
8/13/2009	Sherman	11669	Vilas	20.8	F	0.482
8/13/2009	Sherman	11672	Vilas	17.1	M	0.266
8/13/2009	Sherman	11673	Vilas	19.1	F	0.398
8/13/2009	Sherman	11674	Vilas	16.7	F	0.439
8/13/2009	Minoqua	11576	Oneida	28.0	F	0.776
8/13/2009	Minoqua	11577	Oneida	27.3	F	0.863
8/13/2009	Minoqua	11578	Oneida	19.8	M	0.524

8/13/2009	Minoqua	11579	Oneida	15.4	M	0.220
8/13/2009	Minoqua	11580	Oneida	16.6	M	0.218
8/13/2009	Minoqua	11581	Oneida	13.5	M	0.145
8/13/2009	Minoqua	11582	Oneida	14.2	M	0.149
8/13/2009	Minoqua	11583	Oneida	18.6	M	0.285
8/13/2009	Minoqua	11584	Oneida	14.3	M	0.122
8/13/2009	Minoqua	11585	Oneida	19.5	F	0.394
8/13/2009	Minoqua	11586	Oneida	23.8	F	0.299
8/13/2009	Minoqua	11590	Oneida	16.6	M	0.214

*The reported length is the frozen length as no fresh length was recorded. There were a total of 26 other walleye with a "frozen" length from 11.0-12.9 inches and a "fresh" length recorded. The difference between the "fresh" and "frozen" lengths for these 26 fish ranged from 0.2-1.2 inches and averaged 0.6 inches, with the "fresh" length being longer than the "frozen".

Table 6. Percent Moisture in Walleye Fillets (Measured Immediately after Grinding).

Lake and Tag Number		Percent Moisture	Relative Percent Agreement
Chippewa Flowage 11509*		78.7	
Chippewa Flowage 11513*		78.5	99.8
Chippewa Flowage 11513 *	Dup	78.6	
Chippewa Flowage 11512*		79.0	
Bond Falls 11602*		78.6	
Bond Falls 11605*		77.6	
Bond Falls 11608*		79.5	
Gogebic 12365*		79.8	
Gogebic 12360*		80.7	
Gogebic 11616*		79.5	
North Twin Lake 11632		77.3	
North Twin Lake 11634		79.2	99.8
North Twin Lake 11634	Dup	79.0	
North Twin Lake 11637		80.7	
Namekagon 11646		79.7	
Namekagon 11648		79.0	
Namekagon 11654		80.1	
Turtle Flambeau Flowage 11548		80.6	
Turtle Flambeau Flowage 11552		80.7	99.9
Turtle Flambeau Flowage 11552	Dup	80.6	

Turtle Flambeau Flowage 11556		81.7	
Kentuck 11448		79.9	
Kentuck 11447		79.6	99.5
Kentuck 11447	Dup	79.2	
Kentuck 11453		80.7	
Butternut 11418		79.3	
Butternut 11419		79.0	
Butternut 11424		80.1	
Annabelle 11443		82.0	
Annabelle 11442		80.3	
Annabelle 11439		82.5	99.9
Annabelle 11439	Dup	82.6	
Minocqua 11580		78.4	
Minocqua 11582		79.3	
Minocqua 11583		78.3	99.8
Minocqua 11583	Dup	78.5	
Sherman 11663		79.8	
Sherman 11667		79.2	
Sherman 11664		79.3	
Squirrel 11410		80.4	
Squirrel 11407		79.1	99.9
Squirrel 11407	Dup	79.1	
Squirrel 11404		80.7	
Bass-Patterson 11686		79.8	
Bass-Patterson 11688		79.7	
Bass-Patterson 11676		78.2	
Siskiwit 11467		80.6	
Siskiwit 11464		79.3	
Siskiwit 11468		81.0	
Chetac 11517		78.7	99.9
Chetac 11517	Dup	78.7	
Chetac 11519		79.4	
Chetac 11526		79.3	
Lac Courte Oreilles 11534		78.0	
Lac Courte Oreilles 11537		77.4	99.7
Lac Courte Oreilles 11537	Dup	77.7	
Lac Courte Oreilles 11542		78.9	
Willow Flowage 11561		80.4	
Willow Flowage 11564		80.3	
Willow Flowage 11562		79.2	
Mean ± Std. Dev.		79.3 ± 1.13 %	99.8 ± 0.1 %

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

Appendix A

Determination of 2009 Limit of Detection (LOD) and Limit of Quantitation (LOQ) using GP-RT-HRC-3 sample from 2006

Sample	Tissue Type	ng/L	ng Hg	g sample	µg Hg/g
GP-RT-HRC-3 #1	whole fish composite	59.6	2.98	0.206	0.014
GP-RT-HRC-3 #2	whole fish composite	96.1	4.81	0.253	0.019
GP-RT-HRC-3 #3	whole fish composite	55.1	2.75	0.207	0.013
GP-RT-HRC-3 #4	whole fish composite	82.4	4.12	0.243	0.017
GP-RT-HRC-3 #5	whole fish composite	73.3	3.67	0.219	0.017
GP-RT-HRC-3 #6	whole fish composite	100.7	5.03	0.292	0.017
GP-RT-HRC-3 #7	whole fish composite	114.4	5.72	0.288	0.020
GP-RT-HRC-3 #8	whole fish composite	105.2	5.26	0.284	0.019
				Mean	0.0170
				Std. Dev.	0.00222

LOD = Std. Dev. x t = 0.00222 x 2.998 = 0.0066

LOQ = 10/3 x LOD = 0.0220

2009 Hg LOD = 0.0066 µg/g LOQ = 0.0220 µg/g

2008 Hg LOD = 0.0126 µg/g LOQ = 0.0421 µg/g
2007 Hg LOD = 0.0047 µg/g LOQ = 0.0157 µg/g
2006 Hg LOD = 0.0042 µg/g LOQ = 0.0141 µg/g
2005 Hg LOD = 0.0113 µg/g LOQ = 0.0368 µg/g
2004 Hg LOD = 0.0013 µg/g LOQ = 0.0042 µg/g

Appendix B

Calibration Curve Data Generated During the Analysis of GLIFWC's 2009 Walleye Fillets

Analysis Date	Standard Conc. ng Hg/L	Blank Corrected Abs 1*	Blank Corrected Abs 2*	Blank Corrected Mean	Std. Dev.	Slope	Y-Intercept	Correlation
7/29/09	0	0.0000	0.0000	0.0000	0.0001			
7/29/09	50	0.0013	0.0017	0.0015	0.0003			
7/29/09	100	0.0025	0.0026	0.0026	0.0001			
7/29/09	500	0.0139	0.0141	0.0140	0.0001			
7/29/09	1000	0.0277	0.0278	0.0278	0.0001			
7/29/09	6000	0.1611	0.1593	0.1602	0.0013	2.667E-05	3.30E-04	0.99997
8/6/09	0	0.0000	0.0000	0.0000	0.0001			
8/6/09	100	0.0024	0.0025	0.0025	0.0001			
8/6/09	500	0.0129	0.0123	0.0126	0.0004			
8/6/09	1000	0.0253	0.0241	0.0247	0.0008			
8/6/09	6000	0.1455	0.1351	0.1403	0.0074			
8/6/09	10000	0.2377	0.2233	0.2305	0.0102	2.304E-05	8.15E-04	0.99996
8/11/09	0	0.0000	0.0000	0.0000	0.0000			
8/11/09	100	0.0025	0.0024	0.0025	0.0001			
8/11/09	500	0.0129	0.0125	0.0127	0.0003			
8/11/09	1000	0.0252	0.0244	0.0248	0.0006			
8/11/09	6000	0.1489	0.1415	0.1452	0.0052			
8/11/09	10000	0.2401	0.2284	0.2343	0.0083	2.353E-05	8.82E-04	0.99984
8/13/09	0	0.0000	0.0000	0.0000	0.0001			
8/13/09	100	0.0025	0.0024	0.0025	0.0001			
8/13/09	500	0.0126	0.0125	0.0126	0.0001			
8/13/09	1000	0.0248	0.0236	0.0242	0.0008			
8/13/09	6000	0.1424	0.1373	0.1399	0.0036			
8/13/09	10000	0.2301	0.2193	0.2247	0.0076	2.257E-05	1.09E-03	0.99980
8/18/09	0	0.0000	0.0000	0.0000	0.0001			
8/18/09	100	0.0022	0.0024	0.0023	0.0001			
8/18/09	500	0.0113	0.0111	0.0112	0.0001			
8/18/09	1000	0.0223	0.0218	0.0221	0.0004			
8/18/09	6000	0.1312	0.1274	0.1293	0.0027			
8/18/09	10000	0.2126	0.2082	0.2104	0.0031	2.110E-05	6.45E-04	0.99991
8/25/09	0	0.0000	0.0000	0.0000	0.0002			
8/25/09	100	0.0021	0.0022	0.0022	0.0001			
8/25/09	500	0.012	0.0113	0.0117	0.0005			
8/25/09	1000	0.024	0.0221	0.0231	0.0013			
8/25/09	6000	0.1415	0.1303	0.1359	0.0079			
8/25/09	10000	0.2261	0.2097	0.2179	0.0116	2.193E-05	7.92E-04	0.99977

Appendix C

Quality Assurance Audit Report: 2009 Technical Systems Audit of Great Lakes Indian Fish and Wildlife Commission (GLIFWC) Testing of Fish for Mercury

Auditee: Lake Superior Research Institute (LSRI) staff assigned to GLIFWC Project (i.e., Thomas Markee, Christine Polkinghorne, Heidi Saillard, and Kimberly Beesley)

Auditor: Kelsey Prihoda, LSRI Quality Assurance Manager (QAM)

Audit Date: Tuesday, July 14, 2009

Closing Meeting with LSRI-GLIFWC Staff: Tuesday, August 4, 2009

Description and Scope of Audit

A technical systems audit (TSA) of the 2009 GLIFWC Testing of Fish for Mercury Project, hereafter referred to as the 2009 GLIFWC Project, was conducted on Tuesday, July 14, 2009. The TSA included an observation of tissue grinding procedures during processing of 12 walleye samples collected from Lac Courte Oreilles Lake (LCO, Sawyer County). The GLIFWC Project Manager at LSRI is Thomas Markee, and project staff members include Christine Polkinghorne and Heidi Saillard. Kimberly Beesley is the student researcher assisting with the project. Christine Polkinghorne and Kimberly Beesley were present during this TSA, and were observed during walleye tissue grinding and weighing. The objectives of this audit were to review the project quality system documentation, compliance with standard operating procedures (SOPs), training and safety, and equipment/analytical instrumentation calibration and maintenance. The 2009 GLIFWC project laboratory notebook was reviewed to determine whether correct documentation procedures were being followed in accordance with LSRI's Quality Management Plan (QMP). The sample processing and tissue moisture content determination procedures were observed to verify that these procedures are done in accordance with the appropriate LSRI SOPs.

A draft audit report was distributed to LSRI-GLIFWC staff on July 31, 2009 and a closing meeting to discuss the TSA findings was held on Tuesday, August 4, 2009. This final audit report includes the findings from the TSA (detailed in the first section of this report), as well as, a summary of the conclusions, recommendations, and follow-up comments made during the closing meeting (detailed in the second section of this report).

Summary of Findings

Quality System Documentation

- The current GLIFWC Quality Assurance Project Plan (QAPP) for Testing of Fish for Mercury was approved in November 16, 2004 and was added to the 2009 GLIFWC Project three-ring binder. The QAPP should always be included with the study records (i.e., in a three-ring binder with the data sheets).
- The QAPP was followed during glassware preparation, weighing of tissue for moisture determination, and tissue grinding with one deviation observed. The QAPP specifies that "moisture content will be determined randomly on 4 fish from each water body", however, only 3 fish from LCO were used to determine tissue moisture. There is no expected affect on data quality as a result of this deviation. The appropriate amount of tissue was weighed according to the LSRI SOP NT/15 v.2.

    - Scintillation vials used to store ground tissue were certified clean from the vendor. Vials were labeled with sample number, lake, year, and project. The sample processing date and processor's initials were not included on the label.
    - The balance was verified at the beginning of the day (prior to tissue grinding) according to LSRI SOP GLM/12 v.3, and data was recorded on data sheets in binder 05-9-26-BAL. A signature page was added to 05-9-26-BAL to identify the initials of individuals responsible for balance calibration.
    - Fish fillet grinding was conducted according to LSRI SOP SA/10 v.4. During sample processing, LSRI SOP SA/8 v.5 was followed for labware cleaning between homogenized samples. One deviation from LSRI SOP SA/10 v.4 was observed, the student researcher did not mix the ground tissue with a spatula before the second and third pass through the grinder, however, the tissue was mixed thoroughly with a spatula after the third pass though the grinder. There is no expected effect on the 2009 GLIFWC Project as a result of this deviation.
- One tissue matrix/procedural blank was included in the batch of samples to be processed for the day. As specified by the QAPP, the procedural blank (canned tuna fish in water) consisted of an unground sample of canned tuna and a ground canned tuna sample.
- Chain of Custody (COC) forms are included with the project records (in the 2009 GLIFWC binder and laboratory notebook 06-07-10-CNP). The COC from June 5, 2009 indicated that the GLIFWC freezer was at -9°C, which is a deviation from the <-10°C that is specified in the QAPP. In addition the COC from June 5, 2009 did not indicate the date/time that the fish were put into the freezer at LSRI and no LSRI freezer temperature was recorded.
- All SOPs relevant to the GLIFWC Project were located in the laboratory (Barstow 9) where the sample grinding/tissue weighing procedures were carried out. The binder did not contain the most recent SOP Master List of active LSRI SOPs and there was one out of date SOP (REC/9 – Laboratory Notebook Preparation); the most recent version of the LSRI Active SOP Master List was added to the SOP binder and the old version of REC/9 was replaced. It is the responsibility of the LSRI QAM to ensure the most recent SOP Master List and SOP versions are located in the laboratory SOP binders.
- Laboratory notebook 06-07-10-CNP was reviewed. The table of contents was properly completed according to project year. All initials were identified on the inside front cover of the notebook with the individual's full name except the initials "EO". An exact copy of the subcontract for 2009 was also included in the laboratory notebook (original in 2009 GLIFWC binder). Sample numbers of fish that were used for tissue moisture content analysis were recorded; however, sample numbers were not recorded from fish that were ground for mercury analysis. These numbers should also be recorded in the laboratory notebook to provide a record of the samples that are processed each day.

    - Pan dry time (time in/out of drying oven) should be recorded in the laboratory notebook.
- It appears that 06-07-10-CNP has been inspected by LSRI QA Staff annually during past TSAs, however, this has not been indicated in the laboratory notebook. The QAM signed the bottom of the laboratory notebook pages that were inspected during this TSA, and this will be the practice in the future.
- The lakes that are sampled for the 2009 GLIFWC Project are referred to by 3-letter codes after the initial complete lake name is written once in the laboratory notebook. If designated codes can be assigned to each lake to provide a consistent format of identification that can be added to the study records, the complete lake name need not be written out at all.

Organization and Responsibilities

- There are adequate LSRI personnel dedicated to the GLIFWC Project to maintain the level of quality required by the QAPP.
- The QAPP describes the project organization and responsibilities of the LSRI personnel dedicated to the GLIFWC Project. However, the QAPP should be revised to specify Thomas Markee as the Laboratory Manager rather than Larry Brooke, and Kelsey Prihoda as the Quality Assurance Director rather than Dianne Brooke. Project staff includes Christine Polkinghorne and Heidi Saillard. LSRI maintains position descriptions for all of the LSRI personnel involved in the QLIFWC Project.

Training and Safety

- The LSRI QA Staff have the most current resumes on file for Christine Polkinghorne and Kimberly Beesley.
- Kimberly completed the LSRI Introduction to Good Laboratory Practices (GLPs) workshop in March 2009. Christine completed the Introduction to GLPs workshop in 1997, and completed refresher GLP training in June 2009.
- LSRI maintains records documenting that Christine and Kimberly have read all LSRI SOPs that are applicable to the GLIFWC Project.
- Kimberly completed the UWS Laboratory Health and Safety Training course in June 2009, and Christine has also completed the UWS Laboratory Health and Safety Training course.
- There was sufficient personal protective equipment (PPE) present in the laboratory. Christine and Kim were appropriately outfitted with safety glasses, gloves, and lab coats.

Equipment and Analytical Instrumentation

- The analytical balance used to weigh ground tissue for moisture determination was a Mettler PB303-S. This balance reads to 0.001 g and has a maximum capacity of 310 g. The

balance was calibrated according to LSRI SOP GLM/12 v.3 using Class 1 Weights from Denver Instrument Company (serial number: 95-J066802). Data was recorded in 05-9-26-BAL, which is kept next to the balance. The balance was received in September 2002, has not been professionally serviced/calibrated since purchase, but has not fallen outside the quality control acceptance limits during any verification on record.

- The 0.1 M HCl solution used to clean labware was labeled "0.1 M HCl", but the label did not include the complete name (i.e., hydrochloric acid), date of preparation, prepared by initials, expiration date, or any other relevant information.
- Drying oven temperature was 62.5°C, which is +2.5°C above temperature specified in LSRI SOP NT/15 v.2. SOP should be revised to read "oven set at 60°C", and should ideally provide a range of acceptability for the temperature of the drying oven.
- The sample freezer, which is kept locked, was at -25.0°C when it was opened for the first time. Minimum temperature was -26.1°C, and maximum temperature was -22.7°C. The storage temperature of the ground tissue samples is not listed in any LSRI SOP nor in the QAPP. The LSRI SOP SA/10 v.4 should be revised to read "ground tissue samples will be stored at ≤-20°C".

Other

- The sample archival procedure needs clarification. The samples are currently being stored frozen at LSRI indefinitely. The QAPP specifies that the data and study records be stored at GLIFWC permanently, however, it does not include any information about sample archival procedures. Since the data/study records will be permanently stored and the data has been collected according to the QAPP and reviewed by project personnel, is it necessary to keep the samples after the final report has been signed? This is especially important to resolve given that LSRI laboratory and storage space will be reduced with the move to the basement of Barstow.

Conclusions, Recommendations, and Suggested Corrective Actions (Bullets Indicate Follow-Up Comments from Closing Meeting)

Quality System Documentation

Summary of Conclusions: Overall project documentation using laboratory notebook 06-07-10-CNP and the 2009 GLIFWC Project 3-ring binder was good, and provided sufficient documentation to follow the samples from receipt at LSRI though tissue grinding and weighing for tissue moisture determination. The COC forms are difficult to interpret, and reformatting should be considered.

Recommendations/Suggested Corrective Actions:

1. It is recommended that the time in and out of the oven be recorded for the pans that are used for tissue moisture determination. LSRI SOP NT/15 v.2 specifies that the pans be

dried at 60°C for a minimum of two hours, and there is no way to verify that this procedure is followed unless time in/out of the oven is recorded.

- Following the closing meeting, the data sheets for tissue moisture determination were revised to include the date/time aluminum pans are put into and taken out of the drying oven.

2. The QAPP is unclear regarding the number of samples that should be placed into the drying oven for an additional 24 hours, stating, "Five percent of the total number of samples chosen as the first five percent of the samples processed for moisture will be placed back in the oven for a minimum of an additional 24 hr." Based on this, 6-7 samples from the first day of tissue grinding are placed back into the drying oven for an additional 24 hours, but it cannot be determined if this number of samples is sufficient according to the QAPP.
   - It is recommended that if the GLIFWC QAPP is revised the second, 24-hour tissue drying procedure be conducted on the first set of moisture data that is collected within a sampling year.
3. Tissue sample vials were labeled with sample code, lake, year, and project. It is also recommended that the date of tissue grinding and initials of responsible individual be added to the vial label. This allows for cross-referencing from the sample vial to the laboratory notebook.
4. One less fish was chosen from each lake than is specified in the QAPP, and this deviation should be written into the final report.
   - There is a discrepancy between the QAPP and the LSRI-GLIFWC contract. The contract states that up to 51 fillets should be tested for moisture, since there were 17 lakes sampled in 2009, moisture content was determined on three fish per lake. Therefore, the contract was followed for tissue moisture determination based on funding. It is recommended that if the QAPP is revised the number of fish samples per lake to be tested for moisture content be changed from 4 to 3 in order to be consistent with the contract terms.
5. The COC forms should always include the date/time fish samples left the GLIFWC freezer, temperature of the sample freezer at departure, date/time fish samples were transferred to LSRI personnel, date/time fish samples were added to sample freezer at LSRI, and temperature of sample freezer upon addition. The current COC for this project may need to be reformatted so all of the needed information can be more easily recorded and verified.
6. Lakes sampled for the 2009 GLIFWC Project are designated by three-letter codes in the laboratory notebook. It is recommended that these codes be written down for consistency, and added to the laboratory notebook so that they can be referenced. This

will eliminate the need to write out the complete lake name the first time that it is referred to in the notebook.

Organization and Responsibilities
Summary of Conclusions: There are adequate LSRI personnel dedicated to the 2009 GLIFWC Project to maintain the level of quality required by the QAPP.

Recommendations/Suggested Corrective Actions:

1. It is recommended for future project years that the QAPP be revised to include the LSRI personnel that are currently working on the 2009 GLIFWC Project.

Training and Safety
Summary of Conclusions: Resumes are on file for LSRI staff working on the 2009 GLIFWC Project. Christine Polkinghorne and Kimberly Beesley have read all relevant SOPs and have completed the LSRI Introduction to GLPs workshop and the UWS Laboratory Health and Safety Training course. All laboratory safety procedures were followed during the TSA and Christine and Kim were appropriately outfitted with PPE.

Equipment and Analytical Instrumentation
Summary of Conclusions: The LSRI SOP GLM/12 v.3 was followed during tissue weighing for moisture determination. The Mettler PB303-S balance was verified using Class 1 Weights, and verification information was appropriately recorded in 05-9-26-BAL. The balance has not fallen outside the quality control acceptance limits during any verification on record.

Recommendations/Suggested Corrective Actions:

1. It is recommended that the 0.1 M HCl solution be labeled "0.1 M Hydrochloric Acid (HCl)", so it can be easily identified by anyone entering the laboratory (particularly non-scientists). It is also recommended that the preparation date and the preparer's initials be added on future labels. Although the 0.1 M HCl may not have an expiration date per se, if there is a recommended frequency with which this solution should be remade then this information should also be added to future labels.
   - The storage container that the 0.1 M HCl solution is prepared in is properly labeled according to the LSRI QMP (with the information above); however, the 0.1 M HCl rinse basin should be relabeled to "0.1 M Hydrochloric Acid (HCl)" and health/safety information should be placed on the basin (e.g., corrosive label).
2. It is recommended that LSRI SOP NT/15 v.2 be revised to read "oven set at 60°C", and should ideally provide a range of acceptability for the temperature of the drying oven. As it is written now, a deviation from the SOP constitutes any temperature outside 60°C.
3. It is recommended that LSRI SOP SA/10 v.4 be revised to read "ground tissue samples will be stored at ≤-20°C".

Other
Summary of Conclusions: Samples are currently being stored at LSRI indefinitely. It is not known how long the samples can be stored before they begin to degrade and will no longer be useful for reanalysis. GLIFWC stores the

data and study records permanently, therefore, there may not be a need to permanently store the samples after the final report is signed.

Recommendations/Suggested Corrective Actions:

1. It is recommended that clarification be received from GLIFWC regarding sample archival procedures. This information should be added to the QAPP if this project continues beyond 2009. It is suggested that samples be stored until the final report is signed and data/study records be stored permanently at GLIFWC.

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., Mettler AG245, PB303, AB204, H34, H72 and H80)
- Aluminum Weighing Pans
- Drying Oven (60^{o} C)
- Desiccation Container
- Spatula
- Laboratory Notebook

PROCEDURE

1. Label the aluminum weighing pans and dry at 60^{o} C for a minimum of two hours. Record the date and time the pans were placed in the oven in the appropriate lab notebook.

2. Place dried weighing pans in desiccator until cool.

3. Check balance calibration using Class 1 weights (SOP GLM/12). Weigh the dried and cooled weighing pans on balance to 0.001 g.

4. Weigh 1.0- 5.0 g of 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^{o} C for a minimum of 16 hours or until constant dry weight is achieved. Record the date and time that the pans were placed in the oven in the appropriate lab notebook.

7. Remove dried pans and tissue from the oven and place in desiccator until cool. Record the date and time that the pans were placed in the oven in the appropriate lab notebook.

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. Record the date and

time that the pans were weighed a second time.

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
- Spatula (Stainless Steel)
- Hydrochloric Acid, Concentrated
- 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. Replace soapy water as needed during washing process when the water becomes contaminated with fish tissue.
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 acid comes in contact with all surfaces of equipment).
6. Rinse equipment three times with deionized water.
7. Upon drying, cover equipment with aluminum foil to store until used. Equipment should be processed through this entire cleaning procedure before the initial use each day, as well as, after each use.

PROCEDURE: LABWARE CLEANING

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.

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.

PROCEDURES FOR DETERMINING DETECTION LIMITS

INTRODUCTION

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

EQUIPMENT

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

PROCEDURE

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

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

Student's t:

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

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

PROCEDURES FOR CALCULATING MERCURY CONCENTRATIONS USING COLD VAPOR MERCURY ANALYSIS

INTRODUCTION

The following equations are used in calculating mercury concentrations.

PROCEDURE

Concentration of Mercury Stock Solution:

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

Concentration of Mercury Sub-Stocks:

$C_1V_1 = C_2V_2$

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

Amount of Hg in Each Standard:

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

Calibration Curve:

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

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

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

Calculation of µg Hg/g Tissue:

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

FIMS MERCURY ANALYSIS - STOCK, STANDARD AND SPIKE PREPARATION

INTRODUCTION

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

EQUIPMENT LIST

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

PROCEDURE

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

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

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

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

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

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

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

COLD VAPOR MERCURY DETERMINATION IN BIOTA USING THE FMS-100

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 AND REAGENT LIST

- Stannous Chloride, Analytical Reagent
- 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
- Hot Block (Environmental Express)
- FIMS- 100 (Perkin Elmer) Mercury Analyzer
- Lab industries Repipet II Dispenser, 3 - 10 mL and 1 - 5 mL
- Wheaton Instruments Socorex Dispenser Model 511, 10 mL
- Polypropylene Digestion Cups and Covers (Environmental Express)
- Pipets/Pipettors
- Beakers
- Spatulas
- Kimwipes
- 5% (w/v) Potassium Permanganate
- 5% (w/v) Potassium Persulfate
- 3% Hydrochloric Acid
- 10% (w/v) Hydroxylamine Hydrochloride- 1 O%(w/v) Sodium Chloride
- 5% Stannous Chloride in 3% Hydrochloric Acid
- 1000 ug/mL Mercuric Nitrate Stock
- 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

PROCEDURE

Digestion

1. **The addition of acids and digestion of samples must be conducted in a fume hood.** 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 a setting of 110°C. Place the Plexiglas fume cover over the Hot Block with tubing connected to all Erlenmeyer flasks and vacuum pump. Turn on vacuum pump. Allow samples to digest for approximately 15 minutes or until all the fish tissue is dissolved.
3. Disconnect and remove Plexiglas fume cover. **Be careful in handling the fume cover as it will contain acid vapors!** Turn off the Hot Block. Remove the digestion cups from the Hot Block and allow to cool to room temperature in the fume hood.
4. Add 5.0 mL of 5% potassium permanganate to each digestion cup, swirling the digestion cups after each addition.
5. Add an additional 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 cups 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

1. Prepare the following:
   Carrier Solution (3% HCI)
   Reductant Solution (5% SnC12, 1% Silicon Defoaming Agent, in 3% HC1)
   Weigh 50g $SnCl_2$ and add to 990 mL 3% HC1.
   Add 10 mL Silicon Defoaming Agent using 5 mL micropipettor. Note: The Silicon Defoaming Agent is optional, needed only if the samples appear to be producing foam during analysis.
2. Turn on computer and printer.
3. Turn on Nitrogen (400 kPa or 60 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, choose drive D: AAUSER SW2007 and enter a new data set name (DateProject). Be sure that the save data box is checked.
13. Turn pump magazine pressure adjustment levers so that they fit into the notch on the back of the pump magazine.
14. Check gas/liquid separator cover to see that it has been tightened.
15. Attach tubing from gad/liquid separator to the FIMS-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 and mix thoroughly.
19. Rinse the sample aspiration 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 sample aspiration 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 the 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. Prior to analyzing samples check the following parameters:
    a. The slope of the line should fall between $2.0x10^{-5}$ – $3.0x10^{-5}$ b. Review peak shape
    c. The 6000 ng/L standard should give a response between 0.12 and 0.18.
    d. If these checks do not fall in the acceptable range, check carrier and reductant flows and/or perform other maintenance as needed.
22. Rinse the collection tube with deionized water and place in appropriate sample. Enter sample ID code into the appropriate field. Rinse the sample aspiration tube with deionized water and place in appropriate sample. Click on “analyze sample” and allow the 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.
23. 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) (~1 3 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:

24. Place sample aspiration tube, and lines from reductant and carrier solutions into beaker of deionized water.
25. Flush/clean tubing with deionized water by running FIAS two times.
26. 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.
27. Raise waste lines out of liquid in waste container so liquid does not back up.
28. Release the pump magazine pressure adjustment levers so that tubing is not compressed.
29. Detach line from FIMS-cell.
30. Unscrew the gas/liquid separator cover and, using forceps to handle filter, dry filter with a Kimwipe.
31. Print report. Choose “file” > “utilities” > “reporter”. “Open Design” Choose “WRO1 Mussel” (double-click), then double-click on the number l under result name and choose the data set for that day. Click “OK” > “Print Report” and close the reporter window.
32. Save Excel file to floppy disk.
33. Turn off FIMS instrument, computer, nitrogen gas, and printer.
34. Record the date, project, analyst, number of injections, and time run in FIMS-100 usage record book located in the drawer below the instrument.

APPENDIX 4

Lake Superior Research Institute Final Report: Total Mercury Concentrations in Muscle Tissue from Walleye Captured during the Spring 2010 in Wisconsin Ceded Territory Water

Total Mercury Concentrations in Muscle Tissue from Walleye Captured during Spring 2010 in Wisconsin and Minnesota Ceded Territory Waters

by

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Introduction

Skinless fillet samples from walleye (*Sander vitreus*) captured during the spring of 2010 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). One hundred eighteen skinless walleye fillets, from a total of ten lakes in Wisconsin and Minnesota, collected by tribal spearers and GLIFWC Inland Fisheries assessment crews were analyzed.

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

Before processing the fish tissues, all glassware, utensils, and grinders were cleaned according to the appropriate methods (LSRI SOP SA/8 v.6). 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 passed through a grinder three times. A small amount of the initial tissue that passed through the grinder was collected and discarded (LSRI SOP SA/10 v.5). A sub-sample of the ground tissue was placed into a certified 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 v.6).

Commercial canned tuna fish (*Thunnus sp.*) were used as procedural blanks for this project. These procedural blanks consisted of one aliquot from a can of tuna that was transferred directly into a sample bottle after the packing liquid was removed from the tuna. 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. Three procedural blanks were prepared during this project. The initial procedural blank was prepared on the first day fish were ground for the project and the last procedural blank was generated on the last day fish were processed. The other one was prepared on an intermediate date when fish were being ground.

Fish tissues were weighed for mercury analysis following standard laboratory procedure (SOP

SA/11 v.5). Mercury solutions for making tissue spikes and preparing analytical standards were prepared following the procedures in SOP SA/42 v.1. Mercury analyses were performed using cold vapor mercury analysis techniques on a Perkin Elmer FIMS 100 mercury analysis system (SOP SA/49 v.1). Sample analysis yielded triplicate absorbance readings whose mean value was used to calculate the concentration of each sample. If the relative standard deviation (RSD) of the three measurements was greater than 5%, additional aliquots of the sample were analyzed in an attempt to obtain an RSD of less than 5%. If an RSD of < 5% was not able to be achieved, the sample was redigested and reanalyzed. Mercury concentrations and quality assurance calculations were done in Microsoft Excel according to SOP SA/37 v.1. The biota method detection limit was 0.0046 µg Hg/g for a tissue mass of 0.2 g (Appendix A). This limit of detection was determined using a whole fish composite of rainbow trout containing a low concentration of mercury (SOP SA/35 v.1).

Moisture content of tissue was calculated using the wet and dried tissue weights (SOP SA/51 v.3). 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 25 percent of the walleye analyzed for mercury had moisture content determined. Three fish per lake were randomly selected for determination of percent moisture.

Data Quality Assessment

Data quality was assessed using four data quality indicators: 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 (*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 possible analytical interferences. Analytical standards with known amounts of mercury were analyzed with each group (maximum of 40 samples plus QA samples) of tissue samples. A set of analytical standards containing 0, 100, 500, 1000, 6000, and 10,000 ng Hg/L was used on all analysis dates. Standards were prepared from a purchased 1000 ± 10 ppm mercury (prepared from mercuric nitrate) reference standard solution (Fisher Scientific, Pittsburgh, PA). Summary tables of the mercury calibration curve data are provided (Appendix B).

Results for the quality assurance samples were considered acceptable when the value determined for a quality assurance sample fell within the mean ± 2 times the standard deviation of the values obtained from the Spring Walleye 2007 through 2009 projects (previous three years) for the respective quality assurance parameter. Results for the procedural blanks were considered acceptable when the relative percent agreement was > 65.9%. Duplicate agreement values were acceptable when having a relative percent agreement > 78.5%. The calculated acceptable range for the DORM standard reference material was 77.2 to 110% of certified value. 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 56.9 to 117 percent of the expected value.

During the first day (6/22/10) of tissue analysis for mercury content, a problem occurred with the instrument resulting in the final twenty tissue samples having an RSD of >5% for the triplicate measurements made on the digested sample. No results are reported for the samples, standards, or QA/QC samples that were analyzed after the problem occurred on that date. Prior to another analysis being initiated, the problem was corrected, resulting in improved RSD values. The twenty samples were redigested and analyzed on 7/29/10 and each resulted in an RSD of <5%. Several QA/QC samples (blanks or lowest concentration standard) also failed the 5% RSD check for their initial analysis. These calibration blanks and the lowest concentration mercury standard were normally not reanalyzed if they failed the RSD requirement because they have low absorbance values and thus are more likely to fail the RSD limit.

A quality assurance audit was conducted by the LSRI quality assurance manager during the Spring Walleye 2010 project. That report is provided in Appendix C.

Results of Fish Tissue Analyses

Quality Assurance – Three tuna procedural blanks were processed coincident with the grinding of walleye collected for the project. One of the three procedural blanks was analyzed with each set of mercury samples for a total of four analyses resulting in a mean of 80.8 ± 20.6 relative percent agreement (Table 1). A procedural blank was analyzed on 6/22/10 but the data was not reported due to the RSD being greater than 5%. The relative percent agreement values ranged from 52.1 to 96.4%, all but one of which was within the acceptable range of > 65.9%. All of the tuna samples were found to have very low mercury concentrations. These low concentrations increase the probability that the QA/QC requirement for relative percent agreement will not be met.

Analysis of dogfish shark tissue DORM-3 was conducted concurrently with walleye tissue analysis (Table 2). The certified mercury concentration for the dogfish tissue was 0.382 ± 0.060 µg Hg/g. The recovery values ranged from 76.6 to 97.9% with the grand mean and standard deviation of the recoveries being 90.8 ± 6.7 percent of the certified value. One of the values from analysis on 7/14/10 was outside the acceptable range of 77.2 – 110% of the certified mercury concentration in the standard reference samples, however, the average recovery for the analysis date was 87.1 so the set was considered acceptable.

Fish tissues were analyzed for mercury in duplicate twelve times. Two portions of the same tissue were digested and analyzed independently. Relative percent agreement between the duplicate analyses of the same tissue ranged from 78.6 to 99.3% with the average and standard deviation of the agreements being 94.2 ± 5.8 percent (Table 3).

Samples of tissue were spiked with known concentrations of mercury prior to digestion. Mean recovery for the 12 spiked samples was 91.7 ± 8.2 percent with the individual values ranging from 78.3 to 100.6% (Table 4).

Mercury Analysis – Skinless fillets of 118 walleye collected from a total of 10 lakes in Wisconsin and Minnesota were analyzed for total mercury concentration. Total mercury concentrations on a wet weight basis (Table 5) ranged from 0.056 to 0.962 µg Hg/g (parts per million).

Tissue Moisture Analysis – Percent moisture was measured in 30 of the 118 walleye tissues. Moisture analysis took place immediately following grinding of the fillets. Walleye muscle tissue had a mean moisture value of 79.0 ± 0.7 percent (Table 6). Of the 30 tissues analyzed for moisture, four were analyzed in duplicate, all yielding relative percent agreements of 98.2 percent or greater. Seven samples were also dried an additional 24 hours and reweighed to ensure dryness, all yielding agreements greater than 99 percent.

Table 1. Relative Percent Agreement of Total Mercury for Procedural Blank Samples (Before and After Grinding). Data quality indicator for laboratory bias was >65.9% relative percent agreement.

Analysis Date	Grinding Date	Before Grinding µg Hg/g	After Grinding µg Hg/g	Mean µg Hg/g	Relative Percent Agreement *
6/30/2010	6/14/2010	0.021	0.022	0.022	95.3
7/14/2010	6/15/2010	0.044	0.027	0.036	52.1
7/29/2010	6/1/2010	0.028	0.027	0.028	96.4
7/29/2010	6/15/2010	0.043	0.035	0.039	79.5
			Mean ± Std. Dev.		80.8 ± 20.6

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

Table 2. Mercury Concentrations of Dogfish Shark Tissue (Standard Reference Material DORM-3) Analyzed during Fish Analysis. The Standard Reference has a Certified Mercury Concentration of 0.382 ± 0.060µg Hg/g Tissue for DORM-3. Data quality indicator for accuracy was 77.2 to 110% agreement between the nominal and measured reference standard values.

	DORM 3-1		DORM 3-2		DORM 3-3		
Date of Analysis	µg Hg/g	% of Certified Value	µg Hg/g	% of Certified Value	µg Hg/g	% of Certified Value	Mean
6/22/2010	0.374	97.9	0.327	85.5	NR	NR	91.7
6/30/2010	0.354	92.7	0.363	95.0	0.370	96.7	94.8
7/14/2010	0.351	91.9	0.355	92.9	0.293	76.6	87.1
7/29/2010	NA	NA	0.361	94.5	0.320	83.8	89.2
				Mean ± Std. Dev.		90.8 ± 6.7	

NR – Not reported because none of samples from the end of this set were reported due to high % RSD and shift in standard curve.

NA – Not applicable because only two DORM samples were processed and analyzed on 7/29/10 due to small number of samples.

Table 3. Relative Percent Agreement for Duplicate Analysis of Total Mercury Content in Skinless Walleye Fillet Tissue. Data quality indicator for precision was >78.5% relative percent agreement.

Date of Analysis	Lake and Tag Number	µg Hg/g	Duplicate µg Hg/g	Mean µg Hg/g	Relative Percent Agreement
6/22/2010	Teal 1038	0.243	0.196	0.220	78.6
6/22/2010	North Twin 1047	0.130	0.137	0.134	94.8
6/30/2010	Chippewa 1029	0.131	0.133	0.132	98.5
6/30/2010	Pelican 1062	0.305	0.323	0.314	94.3
6/30/2010	Sherman 1123	0.250	0.227	0.239	90.4
6/30/2010	Turtle Flambeau Flowage 1005	0.265	0.294	0.280	89.6
7/14/2010	Mille Lacs 1115	0.348	0.369	0.359	94.1
7/14/2010	Round 1093	0.304	0.302	0.303	99.3
7/14/2010	Sherman 1129	0.407	0.415	0.411	98.1
7/14/2010	Squaw 1157	0.231	0.221	0.226	95.6
7/29/2010	North Twin 1059	0.161	0.164	0.163	98.2
7/29/2010	Bearskin 1147	0.159	0.157	0.158	98.7
			Mean ± Std. Dev.		94.2 ± 5.8

Table 4. Percent of Mercury Recovered from Skinless Walleye Fillet Samples Spiked with a Known Concentration of Mercury. Data quality indicator for accuracy was 56.9 to 117% spike-recovery.

Date of Analysis	Lake and Tag Number	Spike #1	Spike #2	Mean Spike Recovery	Std. Dev.
6/22/2010	Teal 1038	91.0	88.6	89.8	1.70
6/22/2010	North Twin 1047	89.6	88.9	89.2	0.54
6/30/2010	Chippewa 1029	101.5	97.1	99.3	3.14
6/30/2010	Pelican 1062	88.6	94.6	91.6	4.25
6/30/2010	Sherman 1123	96.0	105.1	100.6	6.47
6/30/2010	Turtle Flambeau Flowage 1005	99.7	100.6	100.2	0.62
7/14/2010	Mille Lacs 1115	80.3	76.4	78.3	2.71
7/14/2010	Round 1093	83.6	81.9	82.8	1.19
7/14/2010	Sherman 1129	84.9	90.7	87.8	4.07
7/14/2010	Squaw 1157	80.4	84.0	82.2	2.54
7/29/2010	North Twin 1059	90.1	88.3	89.2	1.23
7/29/2010	Bearskin 1147	87.6	89.0	88.3	0.99
		Mean ± Std. Dev.		91.7 ± 8.2	

Table 5. Total Mercury Concentration (Wet Weight) in Walleye Fillets from Fish Captured during the Spring of 2010.

Analysis Date	Lake	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
6/30/2010	Sherman	1121	Vilas	14.0	M	0.321
6/30/2010	Sherman	1122	Vilas	13.3	M	0.223
6/30/2010	Sherman	1123	Vilas	12.7	M	0.239
6/30/2010	Sherman	1124	Vilas	13.3	M	0.220
6/30/2010	Sherman	1125	Vilas	18.2	F	0.355
7/14/2010	Sherman	1126	Vilas	17.5	M	0.282
7/14/2010	Sherman	1127	Vilas	17.2	M	0.376
7/14/2010	Sherman	1128	Vilas	15.2	M	0.256
7/14/2010	Sherman	1129	Vilas	20.9	F	0.411
7/14/2010	Sherman	1130	Vilas	18.4	F	0.341
6/30/2010	Turtle-Flambeau Flowage	1001	Iron	14.5	M	0.243
6/30/2010	Turtle-Flambeau Flowage	1002	Iron	15.0	M	0.236
6/30/2010	Turtle-Flambeau Flowage	1003	Iron	12.0	M	0.279
6/30/2010	Turtle-Flambeau Flowage	1004	Iron	15.2	M	0.184
6/30/2010	Turtle-Flambeau Flowage	1005	Iron	16.4	F	0.280
6/30/2010	Turtle-Flambeau Flowage	1006	Iron	15.5	M	0.464
6/30/2010	Turtle-Flambeau Flowage	1007	Iron	25.7	F	0.551
6/30/2010	Turtle-Flambeau Flowage	1008	Iron	18.3	F	0.350
6/30/2010	Turtle-Flambeau Flowage	1009	Iron	18.4	F	0.431
6/30/2010	Turtle-Flambeau Flowage	1010	Iron	18.8	F	0.570
6/30/2010	Turtle-Flambeau Flowage	1011	Iron	22.2	F	0.493
6/30/2010	Turtle-Flambeau Flowage	1012	Iron	22.5	F	0.798
6/30/2010	Chippewa	1016	Sawyer	17.5	M	0.373
6/30/2010	Chippewa	1017	Sawyer	25.4	F	0.962
6/30/2010	Chippewa	1018	Sawyer	27.4	F	0.650
6/30/2010	Chippewa	1019	Sawyer	18.3	M	0.772
6/30/2010	Chippewa	1020	Sawyer	25.5	F	0.738
6/30/2010	Chippewa	1021	Sawyer	21.2	F	0.251
6/30/2010	Chippewa	1025	Sawyer	19.6	F	0.269
6/30/2010	Chippewa	1026	Sawyer	16.0	M	0.709
6/30/2010	Chippewa	1027	Sawyer	12.9	M	0.455
6/30/2010	Chippewa	1028	Sawyer	13.0	M	0.307
6/30/2010	Chippewa	1029	Sawyer	15.6	M	0.132
6/30/2010	Chippewa	1030	Sawyer	12.2	M	0.255

6/22/2010	Teal	1031	Sawyer	23.2	F	0.470
6/22/2010	Teal	1033	Sawyer	26.5	F	0.738
6/22/2010	Teal	1035	Sawyer	21.5	M	0.427
6/22/2010	Teal	1036	Sawyer	17.8	M	0.238
6/22/2010	Teal	1037	Sawyer	18.3	M	0.212
7/14/2010	Teal	1038	Sawyer	19.4	M	0.252
6/22/2010	Teal	1039	Sawyer	16.3	M	0.183
6/22/2010	Teal	1041	Sawyer	15.9	M	0.167
6/22/2010	Teal	1042	Sawyer	25.9	F	0.408
6/22/2010	Teal	1043	Sawyer	12.2	M	0.134
6/22/2010	Teal	1044	Sawyer	14.3	M	0.188
6/22/2010	Teal	1045	Sawyer	13.6	M	0.122
7/29/2010	North Twin	1046	Vilas	13.7	M	0.095
6/22/2010	North Twin	1047	Vilas	14.0	M	0.134
6/22/2010	North Twin	1048	Vilas	13.8	M	0.165
6/22/2010	North Twin	1049	Vilas	15.0	M	0.121
6/22/2010	North Twin	1050	Vilas	16.3	M	0.172
6/22/2010	North Twin	1051	Vilas	18.6	F	0.208
7/29/2010	North Twin	1052	Vilas	26.7	F	0.404
7/29/2010	North Twin	1053	Vilas	15.3	M	0.190
6/22/2010	North Twin	1054	Vilas	19.9	M	0.177
6/22/2010	North Twin	1055	Vilas	18.0	F	0.154
7/29/2010	North Twin	1059	Vilas	22.1	F	0.163
6/22/2010	North Twin	1060	Vilas	24.2	F	0.407
7/29/2010	Bearskin	1136	Oneida	27.3	F	0.303
6/22/2010	Bearskin	1137	Oneida	13.9	M	0.063
7/29/2010	Bearskin	1138	Oneida	20.4	M	0.181
7/29/2010	Bearskin	1139	Oneida	16.4	M	0.146
7/29/2010	Bearskin	1140	Oneida	16.0	M	0.141
7/29/2010	Bearskin	1141	Oneida	15.3	M	0.147
7/29/2010	Bearskin	1144	Oneida	12.4	M	0.066
7/29/2010	Bearskin	1145	Oneida	25.2	F	0.203
7/29/2010	Bearskin	1146	Oneida	17.3	M	0.106
7/29/2010	Bearskin	1147	Oneida	18.6	M	0.158
7/29/2010	Bearskin	1148	Oneida	14.8	M	0.090
7/29/2010	Bearskin	1150	Oneida	24.8	F	0.266
6/30/2010	Pelican	1061	Oneida	17.8	M	0.409
6/30/2010	Pelican	1062	Oneida	23.4	F	0.314
6/30/2010	Pelican	1063	Oneida	14.3	M	0.136
6/30/2010	Pelican	1064	Oneida	17.5	M	0.233

6/30/2010	Pelican	1068	Oneida	23.0	F	0.268
6/30/2010	Pelican	1069	Oneida	22.5	F	0.269
6/30/2010	Pelican	1070	Oneida	16.9	M	0.215
6/30/2010	Pelican	1071	Oneida	21.9	F	0.317
6/30/2010	Pelican	1072	Oneida	14.9	M	0.179
6/30/2010	Pelican	1073	Oneida	19.8	M	0.377
6/30/2010	Pelican	1074	Oneida	18.3	M	0.349
6/30/2010	Pelican	1075	Oneida	14.5	M	0.213
7/14/2010	Squaw	1151	Vilas	14.5	F	0.327
7/14/2010	Squaw	1152	Vilas	13.2	M	0.289
7/14/2010	Squaw	1153	Vilas	15.0	F	0.346
7/14/2010	Squaw	1154	Vilas	14.5	M	0.470
7/14/2010	Squaw	1155	Vilas	15.0	M	0.332
7/14/2010	Squaw	1156	Vilas	23.3	F	0.503
7/14/2010	Squaw	1157	Vilas	16.0	F	0.226
7/14/2010	Squaw	1158	Vilas	18.8	F	0.352
7/14/2010	Squaw	1159	Vilas	16.0	F	0.451
7/14/2010	Squaw	1160	Vilas	17.0	F	0.314
7/14/2010	Squaw	1161	Vilas	22.7	F	0.513
7/14/2010	Squaw	1162	Vilas	15.2	F	0.499
7/14/2010	Mille Lacs	1106	Mille Lacs	17.0	M	0.074
7/14/2010	Mille Lacs	1107	Mille Lacs	14.4	M	0.065
7/14/2010	Mille Lacs	1108	Mille Lacs	15.4	M	0.058
7/14/2010	Mille Lacs	1109	Mille Lacs	15.7	M	0.068
7/14/2010	Mille Lacs	1110	Mille Lacs	24.7	F	0.301
7/14/2010	Mille Lacs	1111	Mille Lacs	21.9	F	0.141
7/14/2010	Mille Lacs	1112	Mille Lacs	21.7	F	0.152
7/14/2010	Mille Lacs	1113	Mille Lacs	19.9	F	0.141
7/14/2010	Mille Lacs	1114	Mille Lacs	23.9	F	0.237
7/14/2010	Mille Lacs	1115	Mille Lacs	22.8	F	0.359
7/14/2010	Mille Lacs	1116	Mille Lacs	14.7	M	0.056
7/14/2010	Mille Lacs	1117	Mille Lacs	14.9	M	0.081
7/14/2010	Round	1091	Sawyer	13.0	M	0.107
7/14/2010	Round	1092	Sawyer	15.3	M	0.144
7/14/2010	Round	1093	Sawyer	17.5	M	0.303
7/14/2010	Round	1094	Sawyer	14.5	M	0.103
7/14/2010	Round	1095	Sawyer	13.5	M	0.124
7/14/2010	Round	1096	Sawyer	15.4	M	0.151
7/14/2010	Round	1097	Sawyer	19.5	F	0.246
7/14/2010	Round	1098	Sawyer	20.5	M	0.245

7/14/2010	Round	1099	Sawyer	26.7	F	0.612
7/14/2010	Round	1100	Sawyer	18.0	F	0.181
7/14/2010	Round	1101	Sawyer	21.0	F	0.341
7/14/2010	Round	1102	Sawyer	22.0	F	0.279

Table 6. Percent Moisture in Walleye Fillets (Measured Immediately after Grinding).

Sample ID		Percent Moisture	Relative Percent Agreement
Round Lake 1092*		78.6	
Round Lake 1096*		79.4	99.7
Round Lake 1096*	dup	79.6	
Round Lake 1102*		79.3	
Bearskin Lake 1139*		78.2	
Bearskin Lake 1147*		78.7	
Bearskin Lake 1148*		79.7	
Turtle Flambeau Flowage 1006		80.1	98.2
Turtle Flambeau Flowage 1006	dup	78.7	
Turtle Flambeau Flowage 1001		78.0	
Turtle Flambeau Flowage 1005		79.4	
Sherman Lake 1125		80.5	
Sherman Lake 1128		79.3	
Sherman Lake 1129		79.2	
North Twin Lake 1050		78.7	
North Twin Lake 1052		79.3	
North Twin Lake 1055		78.8	
Teal Lake 1044		79.3	
Teal Lake 1039		79.1	
Teal Lake 1037		78.9	
Mille Lacs 1108		79.6	
Mille Lacs 1113		78.8	
Mille Lacs 1115		79.5	99.9
Mille Lacs 1115	dup	79.4	
Pelican 1061		78.2	
Pelican 1063		76.7	
Pelican 1064		78.5	
Chippewa Flowage 1016		78.6	
Chippewa Flowage 1019		79.5	
Chippewa Flowage 1020		78.5	
Squaw 1152		79.0	

Squaw 1153		79.7	
Squaw 1154		79.6	99.7
Squaw 1154	dup	79.3	
Mean ± Std. Dev.		79.0 ± 0.7	

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

Appendix A

Determination of 2010 Limit of Detection (LOD) and Limit of Quantitation (LOQ) using GP-RT-HRC-3 sample from 2006

Sample	Tissue Type	ng/L	ng Hg	g sample	ug Hg/g
GP-RT-HRC-3 #1	whole fish composite	131.7	6.58	0.227	0.029
GP-RT-HRC-3 #2	whole fish composite	123.5	6.17	0.220	0.028
GP-RT-HRC-3 #3	whole fish composite	131.7	6.58	0.224	0.029
GP-RT-HRC-3 #4	whole fish composite	123.5	6.17	0.212	0.029
GP-RT-HRC-3 #5	whole fish composite	107.0	5.35	0.211	0.025
GP-RT-HRC-3 #6	whole fish composite	123.5	6.17	0.230	0.027
GP-RT-HRC-3 #7	whole fish composite	115.2	5.76	0.208	0.028
GP-RT-HRC-3 #8	whole fish composite	111.1	5.56	0.215	0.026
				Mean	0.0277
				Std. Dev.	0.00153

2010 LOD = Std. Dev. x t = 0.00153 x 2.998 = 0.00459

2010 LOQ = 10/3 x LOD = 0.0153

2009 **Hg LOD = 0.0066 µg/g LOQ = 0.0220 µg/g**
2008 **Hg LOD = 0.0126 µg/g LOQ = 0.0421 µg/g**
2007 **Hg LOD = 0.0047 µg/g LOQ = 0.0157 µg/g**
2006 **Hg LOD = 0.0042 µg/g LOQ = 0.0141 µg/g**
2005 **Hg LOD = 0.0113 µg/g LOQ = 0.0368 µg/g**
2004 **Hg LOD = 0.0013 µg/g LOQ = 0.0042 µg/g**

Appendix B

Calibration Curve Data Generated During the Analysis of GLIFWC's 2010 Walleye Fillets

Analysis Date	Standard Conc. ng Hg/L	Blank Corrected Abs 1	Blank Corrected Abs 2	Blank Corrected Mean	Std. Dev.	Slope	Y-Intercept	Correlation
6/22/2010	0	0.0000	*	0.0000				
6/22/2010	100	0.0064	*	0.0064				
6/22/2010	500	0.0150	*	0.0150				
6/22/2010	1000	0.0314	*	0.0314				
6/22/2010	6000	0.1884	*	0.1884				
6/22/2010	10000	0.3064	*	0.3064		3.068 E-05	1.26E-03	0.99987
6/30/2010	0	0.0000	0.0000	0.0000	0.0000			
6/30/2010	100	0.0022	0.0026	0.0024	0.0003			
6/30/2010	500	0.0112	0.0121	0.0117	0.0006			
6/30/2010	1000	0.0230	0.0243	0.0237	0.0009			
6/30/2010	6000	0.1372	0.1447	0.1410	0.0053			
6/30/2010	10000	0.2155	0.2272	0.2214	0.0083	2.237E-05	1.05E-03	0.99946
7/14/2010	0	0.0000	0.0000	0.0000	0.0000			
7/14/2010	100	0.0025	0.0027	0.0026	0.0001			
7/14/2010	500	0.0127	0.0120	0.0124	0.0005			
7/14/2010	1000	0.0255	0.0260	0.0258	0.0004			
7/14/2010	6000	0.1488	0.1520	0.1504	0.0023			
7/14/2010	10000	0.2425	0.2371	0.2398	0.0038	2.416E-05	9.57E-04	0.99970
7/29/2010	0	0.0000	0.0000	0.0000	0.0000			
7/29/2010	100	0.0026	0.0025	0.0026	0.0001			
7/29/2010	500	0.0126	0.0121	0.0124	0.0004			
7/29/2010	1000	0.0250	0.0245	0.0248	0.0004			
7/29/2010	6000	0.1498	0.1488	0.1493	0.0007			
7/29/2010	10000	0.2434	0.2400	0.2417	0.0024	2.430E-05	5.00E-04	0.99987

*No data reported due to problem encountered with instrument during 6/22/10 analysis,

Appendix C

Quality Assurance Audit Report: 2010 Technical Systems Audit of Great Lakes Indian Fish and Wildlife Commission (GLIFWC) Testing of Fish for Mercury

Auditee: Lake Superior Research Institute (LSRI) staff assigned to GLIFWC Project (i.e., Thomas Markee, Christine Polkinghorne, Heidi Saillard, Thomas Johnson, and Kimberly Beesley)
Auditors: Kelsey Prihoda, LSRI Quality Assurance Manager (QAM)
Audit Date: June 29-30, 2010 and August 23-24, 2010
Closing Meeting with LSRI-GLIFWC Staff: September 20, 2010

Description and Scope of Audit

A technical systems audit (TSA) of the 2010 GLIFWC Testing of Fish for Mercury Project, hereafter referred to as the 2010 GLIFWC Project, was conducted June 29-30, 2010 and August 23-24, 2010. The objectives of this audit were to review the project quality system documentation, training and safety, and equipment/analytical instrumentation calibration and maintenance. The TSA included a procedural audit of digestion and mercury analysis of walleye collected from Sherman Lake, Turtle-Flambeau Flowage, Pelican Lake, and Lake Chippewa. The sample digestion and analysis procedures were observed to verify that these procedures were conducted in accordance with LSRI standard operating procedures (SOPs). The digestion was conducted on June 29, 2010 and the mercury analysis was conducted on June 30, 2010. In addition, the project documentation (GLIFWC Project Laboratory Notebook and 2010 GLIFWC Project Three-Ring Binder) was thoroughly reviewed on June 29-30, 2010 and August 23-24, 2010 in order to verify compliance with LSRI's Quality Management Plan and the GLIFWC Quality Assurance Project Plan (QAPP). A draft quality assurance report was sent to LSRI staff on September 7, 2010 and staff members met with the LSRI Quality Assurance Manager on September 20, 2010 to discuss the TSA findings. This final quality assurance report is based on findings from the 2010 GLIFWC Project TSA, as well as, follow-up discussion during the closing meeting.

The GLIFWC Project Manager at LSRI is Thomas Markee, and project staff members include Christine Polkinghorne and Heidi Saillard. Kimberly Beesley and Thomas Johnson are the student researchers assisting with the project.

Technical Systems Audit Findings

Quality System Documentation

1. The current GLIFWC QAPP was approved on November 16, 2004 and is past the five-year review date, as per US Environmental Protection Agency requirements. However, this GLIFWC QAPP was still used to audit the 2010 GLIFWC Project, as it is the most recently approved version that has been reviewed by all appropriate personnel.
2. The implementation of the 2010 GLIFWC Project was done in accordance with the QAPP (issued: November 16, 2004). However, it should be noted that the acceptance

ranges for all data quality objectives (DQO) are based on historical data from this project, and may differ from the acceptance ranges in Section 3 of the GLIFWC QAPP.

3. According to the GLIFWC Project QAPP, §5.1.2. , "*The custody of the samples will be transferred to the EHL laboratory manager and the date of transfer will be recorded on a separate chain-of-custody…The temperature inside the freezer, as well as the date and time of the transfer into the freezer, will be recorded on the chain-of-custody.*" The Chain of Custody (COC) form is included in the 2010 GLIFWC Project binder, and the date that samples were transferred to GLIFWC Project staff at LSRI (and to the freezer) was recorded on the COC. The freezer temperature is recorded on LSRI's "Temperature Monitoring Data Record", rather than on the GLIFWC COC form. On June 15, 2010 the temperature of the freezer was -23°C, with a minimum temperature of -24°C and a maximum temperature of -8°C.
4. All SOPs relevant to the GLIFWC Project were located in the laboratory (Barstow 9) where the sample digestion and analysis procedures were carried out.
    a. The mercury analysis SOP (LSRI SOP SA/49 *v*.1 – *Cold Vapor Mercury Determination in Biological Tissues using the FIMS-100*) was in draft form and did not contain up-to-date procedural information regarding the current version of the WinLab32 for AA™ analysis software that is new for the 2010 GLIFWC Project.
5. The laboratory notebook for the GLIFWC Project (06-07-10-CNP) was reviewed. Initials of all individuals responsible for the data were identified on the signature page on the inside front cover of the notebook, with the exception of the initials "HJS". Heidi Saillard added her initials to the front cover of 06-07-10-CNP on September 17, 2010.
    a. Page 52 of 06-07-10-CNP refers to a specific fish that contained two filets in the storage bag; however, the fish ID number was never recorded and it cannot be determined which walleye sample this notebook entry refers to.
6. Several samples were reanalyzed due to lower-than-expected reproducibility, as measured by relative percent agreement. On the original sample analysis date, the project staff member conducting the analysis observed lower-than-expected reproducibility and contacted the LSRI QAM. The current version of the GLIFWC QAPP (issued November 16, 2004) states that the relative percent agreement of one sample analyzed in triplicate must be >75%, and based on this information the analysis continued as planned. In actuality, the GLIFWC Project requirement for a sample analyzed in triplicate is >95% agreement. Therefore, all samples that did not meet the reproducibility criteria of >95% were later re-digested and analyzed.
    a. All samples that were re-digested and analyzed were noted in the 2010 GLIFWC Project Binder (10-06-02-GLIFWC), however, the staff collecting the data did not note the anomalous event at the time that the event occurred as it appeared that the reproducibility was within the acceptable range. The documentation

regarding the low reproducibility occurred after internal review of the data by another project staff member.

a. The relative percent agreement requirement is based on historical GLIFWC Project data, and is not in any LSRI SOPs or in the GLIFWC Project QAPP. Therefore, a deviation form was not filled out and the documentation regarding this event is in the 2010 GLIFWC Project Binder.

Organization and Responsibilities

1. There are adequate LSRI personnel dedicated to the GLIFWC Project to maintain the level of quality required by the QAPP.
2. The QAPP describes the project organization and responsibilities of the LSRI personnel dedicated to the GLIFWC Project. However, the QAPP should be revised to specify Thomas Markee as the Laboratory Manager rather than Larry Brooke, and Kelsey Prihoda as the Quality Assurance Director rather than Dianne Brooke. Project staff includes Christine Polkinghorne and Heidi Saillard. LSRI maintains position descriptions for all of the LSRI personnel involved in the GLIFWC Project.

Training and Safety

1. GLIFWC Project staff members have read the appropriate SOPs, and have adequate training/expertise to perform these routine procedures.
2. The LSRI QAM has the most current resumes on file for all GLIFWC Project staff.
3. There was sufficient personal protective equipment (PPE) present in the laboratory.

Equipment and Analytical Instrumentation

1. The analytical balance used to weigh homogenized tissue for digestion was a Mettler PB303-S. This balance reads to 0.001 g and has a maximum capacity of 310 g. The balance was verified according to LSRI SOP GLM/12 *v*.4 – *Procedure for Verification of Laboratory Balances* using Class 1 Weights from Denver Instrument Company (serial number: 95-J066802). Data was recorded in 05-9-26-BAL. Homogenized tissue was weighed prior to digestion according to LSRI SOP SA/11v.5 – *Sample Weighing for Metals Analysis*.
2. The mercury analysis was done using the Perkin-Elmer FIMS 100 Mercury Analysis System. The FIMS 100 is controlled by a personal computer using WinLab32 for AA™ software. A new version of the WinLab32 software was installed prior to the beginning of the 2010 GLIFWC Project.
    a. The maintenance log (02-03-06-CNP) for the FIMS 100 was located adjacent to the instrument. Maintenance was performed on the FIMS 100 on June 21, 2010, which was prior to sample analysis for the 2010 GLIFWC Project. The carrier and reductant tubing were changed, the air filter was replaced and the screen was cleaned, the manifold was cleaned, and the rollers were lubricated.

b. On June 28, 2010 the fill and read time for the FIMS 100 was adjusted by five seconds to decrease the relative standard deviation because a portion of the peak for each sample was being missed prior to this change.
    i. Most of the samples that were analyzed prior to this adjustment (i.e., samples analyzed on June 22, 2010) were re-digested and analyzed due to failure to meet the reproducibility standards.
    ii. The new version of the WinLab32 software was not validated prior to use.
        1. Validation: *Establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes* (FDA 1995).
            a. Specificity, accuracy, limit of detection, limit of quantification, linearity, and precision should be validated on all new instruments and on instruments with new analysis software.

3. Limit of Detection and Quantification for 2010 GLIFWC Project was determined 29 July 2010 (after the fill and read time adjustment was made): LOD = 0.00459 μg Hg/g and LOQ = 0.0153 μg/g.
4. Preparation of calibration standards were not documented in the laboratory notebook.
5. The number of samples analyzed for DQO's was in accordance to the GLIFWC QAPP.

Other

1. A new version of WinLab32 for AA™ software was installed on April 27, 2010 prior to the start of the 2010 GLIFWC Project. Heidi Saillard and Thomas Markee had a training session with the technician on the date of installation. The software was not validated prior to sample analysis for the 2010 GLIFWC Project. A validation should always be conducted upon receipt of new software (including new versions of software already in use), and this validation must be documented to provide proof that the software is working as intended.

Conclusions, Recommendations, and Suggested Corrective Actions

Quality System Documentation

Summary of Conclusions: Overall project documentation using laboratory notebook 06-07-10-CNP and the 2010 GLIFWC Project Binder (10-06-02-GLIFWC) was good, and provided sufficient documentation to follow the samples from receipt at LSRI though mercury analysis. Although the QAPP is out-of-date (as defined by US EPA requirements), it should always be located where it can be easily referenced, preferably with the study data and in or near the laboratory. The mercury analysis SOP (LSRI SOP SA/49) was in draft form and did not contain any information regarding the new WinLab32 for AA™ software.

Recommendations/Suggested Corrective Actions:

- It is recommended that the next revision of the GLIFWC QAPP contain language to clarify that DQO acceptance ranges are based on historical project data. For example, the current version of the GLIFWC QAPP (issued November 16, 2004) states that the relative percent agreement of triplicate sample analysis (i.e., one sample measured three times) is >75%, however, based on historical project data the requirement for the 2010 GLIFWC Project is >95% agreement.
- It is suggested that the >95% agreement requirement for sample reproducibility be incorporated into the Quality Assurance/Quality Control section of the mercury analysis SOP (LSRI SOP SA/49).
- It is recommended that GLIFWC Project staff review all applicable SOPs prior to receipt of samples for a project year and ensure that the SOPs are up-to-date. SOPs that are revised as part of this review process should be finalized prior to sample processing and analysis. As part of this review process, any information regarding new equipment, instrumentation, hardware, and software should be incorporated into the appropriate SOP(s). When applicable, staff should also be trained on the use and implementation of new instrumentation, hardware, and/or software.
- It is recommended that GLIFWC Project staff review the current version of the GLIFWC QAPP prior to receipt of samples for a project year and understand the quality assurance requirements outlined in the QAPP.
- It is suggested that reanalysis of samples be documented very thoroughly, utilizing the project laboratory notebook and binder to detail any anomalous events/unforeseen circumstances/SOP deviations, as well as, the outcome of any corrective actions. Details regarding the nature of any anomalous event, the reason the event occurred (if known), the corrective action (i.e., reanalysis), and the outcome of the reanalysis should be documented.
- It is recommended that the "Temperature Monitoring Data Record" be referenced on the GLIFWC COC, as this is where the freezer temperature is recorded. The LSRI QAM will assign an identification code to the three-ring binder containing the temperature monitoring data so that it may be more easily referenced. The current COC for this project may need to be revised/reformatted so all of the needed information (i.e., date/time fish samples left the GLIFWC freezer, temperature of the sample freezer at departure, date/time fish samples were transferred to LSRI personnel, date/time fish samples were added to sample freezer at LSRI, and temperature of sample freezer upon addition) can be more easily recorded and verified.

Organization and Responsibilities

Summary of Conclusions: There are adequate LSRI personnel dedicated to the 2010 GLIFWC Project to maintain the level of quality required by the QAPP.

Recommendations/Suggested Corrective Actions:

- It is recommended for future project years that the QAPP be revised to include the LSRI personnel that are currently working on the GLIFWC Project, in addition to several other revisions mentioned in this report.

Training and Safety

Summary of Conclusions: Resumes are on file for LSRI staff working on the 2010 GLIFWC Project. GLIFWC Project staff have read all relevant SOPs and have completed the LSRI Introduction to GLPs workshop and the UWS Laboratory Health and Safety Training course. All laboratory safety procedures were followed during the TSA.

Recommendations/Suggested Corrective Actions:

- No recommendations other than a continued dedication to training and safety.

Equipment and Analytical Instrumentation

Summary of Conclusions: The analytical balance used to weigh homogenized tissue for digestion was a Mettler PB303-S. The balance was verified for accuracy (according to LSRI SOP GLM/12 *v.*4), and samples were weighed according to LSRI SOP SA/11 *v.*5. The mercury analysis was conducted using the Perkin-Elmer FIMS 100 Mercury Analysis System, with a newly-installed version of WinLab32 for AA™ software. Routine maintenance was performed on the FIMS 100 on June 21, 2010, prior to sample analysis for the 2010 GLIFWC Project. On June 28, 2010 the fill and read time for the FIMS 100 was adjusted by five seconds to decrease the relative standard deviation, and most of the samples that had been analyzed prior to this change were reanalyzed after failing to meet the reproducibility data quality objective. The number of samples analyzed for data quality objectives determination was in accordance with the GLIFWC QAPP, and were within the respective acceptance ranges at the conclusion of sample analysis for the 2010 GLIFWC Project.

Recommendations/Suggested Corrective Actions:

- It is recommended that the existing LSRI SOP for routine maintenance of the FIMS 100 (LSRI SOP SA/50 *v.*1) be revised to include additional procedural information on the use of the Perkin-Elmer FIMS 100 Mercury Analysis System with WinLab32 for AA™ software. This SOP should also detail the methods, materials, and schedules to be used in the routine inspection, cleaning, maintenance, testing, calibration, and use of this instrument. This SOP should be used in conjunction with the existing LSRI SOP for mercury analysis (LSRI SOP SA/49 *v.*1).
- It is suggested that preparation of all solutions used during analysis, especially preparation of calibration standards, be documented in the projection notebook.

- A blank on the datasheet for the GLIFWC Project (datasheet made using MS Excel) will be added to document the preparation of standards. The preparation of reagents will be documented in the project laboratory notebook.

- Data that falls outside the acceptance ranges for any data quality objective should be noted in the project documentation. In addition, a corrective action for this invalid data should be given (e.g., reanalysis of the sample). This should be noted at the time that the data is collected, whenever possible.
- It is suggested that quality control samples having mercury concentrations that fall below or within a certain percentage of the LOQ not be used to determine compliance with project DQO's. It is the responsibility of LSRI-GLIFWC staff to determine the appropriate concentration above which data can be used for DQO calculations.

Other

Summary of Conclusions: New WinLab32 for AA™ software was installed prior to the start of the 2010 GLIFWC Project. The software was not validated prior to sample analysis for the 2010 GLIFWC Project, in order to ensure that the software was working correctly and fit the needs of the GLIFWC Project. However, there have been several sets of quality control standards/samples and tissue samples that have been analyzed for the 2010 GLIFWC Project and these data will serve as the validation for the current version of WinLab32 for AA™ software.

Recommendations/Suggested Corrective Actions:

- As part of LSRI's quality assurance training program, a training course/workshop on equipment/analytical instrument validation should be offered and this represents a current gap in LSRI's training program. This is the responsibility of LSRI's QAM, and should be developed as soon as possible.
- A general LSRI SOP for equipment/instrument/software validation will be developed by the LSRI QAM and LSRI staff working on the GLIFWC Project.

Appendix D

Standard Operating Procedures (SOPs) Used During Project

Standard Operating Procedure SA/8 v.6

ROUTINE LABWARE CLEANING FOR METALS ANALYSIS

INTRODUCTION

This standard operating procedure (SOP) describes the process used for the routine cleaning of labware and equipment used for metals analysis. Labware consists of all glassware or plasticware used in the preparation of samples, analytical standards, and spikes; as well as, all equipment used for weighing tissue samples (e.g., spatulas). Labware is typically in contact with higher metal concentrations than the equipment used for tissue grinding (e.g., meat grinder, blender, bowls, fillet knife, etc.) and, therefore, must be cleaned using a different procedure than the tissue grinding equipment. The proper personal protective equipment must be worn during the entire cleaning procedure. This includes gloves, safety glasses or goggles, and lab coat.

EQUIPMENT LIST

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

PROCEDURE

1. **Cleaning Equipment used for Tissue Grinding** (e.g., Grinder, Blender, Stainless Steel Bowls, Fillet Knife, Spatula)

    1.1. Dismantle the meat grinder or blender before washing.
    1.2. Scrub all grinding equipment in hot water containing Liquinox® detergent. Replace soapy water as needed during washing process when the water becomes contaminated with fish tissue.
    1.3. Rinse equipment with tap water until there is no presence of soap.

1.4. Rinse equipment once with deionized water.
1.5. Soak equipment in 0.1 M HCl for 30 seconds (be sure acid comes in contact with all surfaces of equipment).
1.6. Rinse equipment three times with deionized water.
1.7. Upon drying, cover equipment with aluminum foil to store until used. Equipment should be processed through this entire cleaning procedure before the initial use if it has been unused for more than one week, as well as, after each use.

2. **Labware Cleaning** (e.g., Volumetric Flasks, Beakers, Spatulas used for Weighing)

2.1. Scrub the labware thoroughly in hot water containing Liquinox® detergent.
2.2. Rinse the labware with hot water until there is no presence of soap.
2.3. Rinse the labware once with deionized water.
2.4. Place the labware in a plastic tank containing 10% nitric acid or fill the container with acid. Be sure the labware is completely filled with acid. Allow the labware to soak for a minimum of 60 minutes.
2.5. Remove the labware from the tank, emptying the acid back into the tank or empty the acid from the container back into the acid storage carboy.
2.6. Rinse the labware a minimum of three times with deionized water.
2.7. 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.

3. **Preparing 0.1 M Hydrochloric Acid (HCl) for Cleaning Tissue Grinding Equipment**

3.1. 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 grinding equipment (i.e., for 30 seconds). Used acid should not be returned to the 2½ gallon carboy. Remake the 0.1 M hydrochloric solution every six months or when the supply has been depleted.
3.2. Used acid should be neutralized and diluted prior to disposing in a laboratory sink. Neutralize the acid with ammonium hydroxide or sodium bicarbonate until a pH of between 6 and 9 is achieved. Measure the pH with pH indicator strips.
3.3. Pour the neutralized acid down the drain with running cold water.

4. **Preparing 10% (v/v) Nitric Acid (HNO_3) for Labware Cleaning**

Note: This procedure should only be used to clean glassware or plastic labware and to clean spatulas used to weigh tissue samples. It should not be used to clean tissue grinding equipment because the stronger acid concentration would cause damage to the grinding equipment. If an acid bath is used, be sure that the bath is neutralized and emptied as soon as possible after being used.

4.1. The acid is made by adding concentrated nitric acid to deionized water in the ratio of 1 volume of acid per 9 volumes of deionized water. The acid solution can be made in a carboy or soaking tank
4.2. If a soaking tank is used, neutralize the acid in the tank immediately after use. Acid can be neutralized with ammonium hydroxide until a pH of between 6 and 9 is achieved. Measure the pH in the tank with pH indicator strips.
4.3. Pour the neutralized acid down the drain with running cold water. Run the cold water for an additional 10 minutes.
4.4. Rinse the tank with warm tap water and then with deionized water. Fill the tank with 10% nitric acid as in step 1.

Standard Operating Procedure SA/10 v.5

SAMPLE GRINDING FOR METALS ANALYSIS

INTRODUCTION

This standard operating procedure (SOP) describes the method used for grinding of biological tissues into homogeneous samples. The grinder and labware used to grind the tissue are cleaned using the Lake Superior Research Institute (LSRI) SOP, *Routine Labware Cleaning for Metals Analysis* (LSRI/SOP/SA/8, issued 1992). The proper safety equipment must be worn during the entire grinding procedure, including gloves, safety glasses, and lab coat.

EQUIPMENT LIST

- Aluminum Foil
- Beaker or Stainless Steel Bowls
- Certified-Clean Sample Containers
- Fillet Knife
- Food Processor with Grinding Attachments
- Gloves
- Grinder
- Lab Coat
- Procedural Blank (i.e., Canned Tuna Fish)
- Safety Glasses
- Samples
- Spatula
- Tissue Samples to be Ground

PROCEDURE

1. Grinding Tissue Samples

1.1. Prior to grinding tissue samples on each processing day, label certified-clean sample containers with the

appropriate sample number, collection site, project, and year of collection. The processing date and initials of individuals responsible for sample processing should be recorded on pre-printed data sheets.

1.2. Remove the samples to be ground from the storage freezer and allow to partially thaw prior to grinding.

1.3. If necessary, cut the sample into small pieces that will fit through the grinder feed tube or food processor with grinding attachments.

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

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

1.6. Mix the tissue to ensure homogeneity.

1.7. Place the ground tissue in a labeled, certified-clean sample container. Seal the vial securely with the screw top lid. Store ground tissue samples in a freezer set at <-10°C.

1.8. Wash the grinder (or food processor) and labware by following the procedure in *LSRI/SOP/SA/8- Routine Labware Cleaning for Metals Analysis* prior to grinding the next sample.

1.9. Continue to grind each sample by repeating steps 1.3. to 1.8.

2. Preparing the Procedural Blank

2.1. Prepare an appropriate procedural blank based on the type of tissue being ground, e.g., canned tuna fish from a commercial supplier can be used as a procedural blank when grinding fish tissue samples. When using tuna, drain the liquid from the can. Grind half the tuna as a procedural blank following the procedure outlined in 1.4. to 1.8. Label this procedural blank as "Tuna after Grinding" and include the date of processing. The ground blank is included with the analysis set.

2.2. The other half of the tuna is not ground, but is mixed with a spatula and placed in a certified-clean sample container following steps 1.6. to 1.7. Label this procedural blank as "Tuna before Grinding" and include the date of processing. The unground blank is included with the analysis set.

Standard Operating Procedure SA/11 v.5

SAMPLE WEIGHING FOR METALS ANALYSIS

INTRODUCTION

This standard operating procedure (SOP) describes the method used to weigh biological tissue samples for metals analysis. The tissue samples should be processed according to *LSRI/SOP/SA/10 - Sample Grinding for Metals Analysis* (issued 1992) or *LSRI/SOP/SA/38 - Preparation of Tissues for Analytical Determinations Using Liquid Nitrogen* (issued 1999). All labware used in this procedure should be cleaned according to *LSRI/SOP/SA/8 - Routine Labware Cleaning for Metals Analysis* (issued 1992). The proper personal protective equipment must be worn during this entire procedure. This includes gloves, safety glasses, and lab coat.

EQUIPMENT LIST

- Deionized Water
- Gloves
- Ground/Processed Samples
- Kimwipes
- Lab Coat
- Nitric Acid (10%)
- Polypropylene Digestion Vessels (from a commercial supplier, such as Environmental Express)
- Safety Glasses
- Spatula
- Top-Loading or Analytical Balance (must be capable of reading to at least 0.001 g)

PROCEDURE

1. Remove the sample(s) to be analyzed from the freezer and allow the sample(s) to thaw until able to be mixed with a spatula.

2. Check the level of the balance and adjust if necessary. Clean the balance pan by removing any foreign materials with a soft brush.

3. Zero the balance with the zero adjustment. If balance calibration check has not been previously performed on the day of sample weighing, the balance calibration must be verified following *LSRI/SOP/GLM/12 - Procedure for Verifying Calibration of Laboratory Balances* (issued 1995).

4. Place a clean, labeled sample digestion vessel on the pan of the balance and tare the balance.

5. With a spatula, stir the sample to ensure homogeneity. Weigh the appropriate quantity (i.e., approximately 0.2-0.3 g for mercury analyses and 1.0 g for other metals analyses) of tissue into the sample container. Be sure that none of the tissue adheres to the upper sides of the sample container.

6. Record the weight of the sample on the appropriate datasheet or in a study-specific laboratory notebook.

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

8. Repeat steps 4 to7 for all tissue samples to be weighed.

Standard Operating Procedure SA/35 v.1

PROCEDURE FOR DETERMINATION OF METHOD DETECTION LIMIT AND LIMIT OF QUANTIFICATION

INTRODUCTION

Method detection limits (MDL) and limit of quantification (LOQ) should be determined using the following procedure for each analyte and analytical method of interest, for those 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 spectrophotometer, and specific ion electrodes.

DEFINITIONS

Method Detection Limit (MDL): The constituent concentration that, when processed through the complete method, produces a signal with a 99% probability that is different from the blank (Eaton et al. 2005)

Limit of Quantification (LOQ): The constituent concentration that produces a signal sufficiently greater than the blank that it can be detected within specified levels during routine conditions (Eaton et al. 2005). Typically, it is the concentration that produces a signal 10/3 that of the method detection limit.

EQUIPMENT

- Calculator capable of doing standard deviations (or MS Excel spreadsheet)
- Standard or sample estimated to be within 5-10 times the expected detection limit
- Student's *t*-distribution chart

PROCEDURE

1. Select a low-level standard or sample that is estimated to be within 5-10 times the method detection limit for the analyte and analytical method.

2. If the analysis method involves sample preparation before analysis, the standard or sample should be carried through the entire preparation method before instrumental analysis is conducted. A minimum of seven aliquots/replicates of the standard or sample are carried through the entire preparation and analysis.

3. Determine a mean and standard deviation, $SD_{(n-1),}$ for the calculated concentration of each of the seven or more replicates.

4. Calculate the method detection limit by multiplying the standard deviation of the

   concentrations by the Student's *t* value (Appendix 1) for the number of replicates (*n*-1):

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

5. Compare the detection limit to the mean concentration. If the mean concentration is greater than 5-10 times the calculated detection limit, repeat steps 1-4 using a lower concentration for the replicates.

6. Once the MDL has been determined, the limit of quantification is calculated by multiplying the MDL by 10/3.

$$LOQ = MDL \times \frac{10}{3}$$

REFERENCES

Eaton, AD, Clesceri, LS, Rice, EW, and AE Greenberg, Eds. (2005). Standard Methods for the Examination of Water and Wastewater, 21st Edition. American Public Health Association, Washington, DC.

US Environmental Protection Agency, Electronic Code of Federal Regulations. Definition and Procedure for the Determination of the Method Detection Limit (revision 1.11). Title 40, Part 136, Appendix B. Accessed from: http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&tpl=/ecfrbrowse/Title40/40cfr136_main_02.tpl November 2009.

Standard Operating Procedure SA/37 v.1

PROCEDURES FOR CALCULATING MERCURY CONCENTRATIONS USING COLD VAPOR MERCURY ANALYSIS

INTRODUCTION

This standard operating procedure (SOP) describes the process used to calculate mercury concentrations at various stages during the analysis of mercury using the cold-vapor atomic absorption method. The following equations are used in calculating mercury concentrations in stock solutions, sub-stock solutions, and in biological tissue samples.

EQUIPMENT

- Calculator (or MS Excel Spreadsheet)
- Certified Mercury Standard Solution (i.e., to be used as a stock)
- Study-Specific Laboratory Notebook/Three-Ring Binder

PROCEDURE

1. Use a purchased a mercury stock solution with a certified concentration of mercury
   Note: µg/mL = mg/L = ppm.

Conversion from µg/mL to ng/mL

$$\frac{\mu g}{mL} \times 10^3 \frac{ng}{\mu g} = \frac{ng}{mL}$$

Concentration of Mercury Sub-Stocks

$$C_1 \times V_1 = C_2 \times V_2$$

Where, C_1 = Concentration of Mercury Stock Solution (see above)
C_2 = Desired Concentration of Mercury Sub-Stock/Diluted Solution
V_1 = Volume of Stock Solution Needed
V_2 = Desired Volume of Mercury Sub-Stock/Diluted Solution

Amount of Mercury in each Standard Solution

$$ng\ of\ Hg = Concentration\ of\ Hg\ Sub\ Stock\ \left(\frac{ng}{mL}\right) \times Volume\ of\ Sub\ Stock\ Used\ (mL)$$

2. Determine the concentration of mercury in each prepared sample using the calibration curve generated from the mercury standard solutions prepared in step 1. Plot the amount of mercury in each standard solution (x) vs. the mean blank-corrected peak height for each sample of interest (y), and use the resulting linear regression line's slope and intercept to calculate sample mercury concentration:

Amount of Mercury in each Sample

$$y = mx + b$$

Where, m = Slope of Linear Regression Line
b = Intercept of Linear Regression Line

y = Mean Blank-Corrected Peak Height for Sample of Interest
x = Amount (ng) of Mercury in Sample of Interest

3. Multiply the resulting amount of mercury in each sample by "1 µg/1000 ng" to convert to amount

of mercury in µg.

4. Calculate the concentration of mercury in each tissue sample by diving the amount of mercury in each sample by the mass of the tissue analyzed:

Concentration of Mercury in each Biological Tissue Sample

$$\frac{Amount\ of\ Hg\ in\ Sample\ (\mu g)}{Mass\ of\ Tissue\ Sample\ (g)}$$

Standard Operating Procedure SA/42 v.1

STOCK, STANDARD, AND SPIKE PREPARATION FOR MERCURY ANALYSIS

INTRODUCTION

This standard operating procedure (SOP) is used for the preparation of the stock, analytical standards, blanks, and spikes for mercury analysis. The fish/tissue used for the spikes should be weighed according to *LSRI/SOP/SA/11 - Sample Weighing for Metals Analysis* (issued 1992). The labware used in this procedure should be cleaned following the method described in *LSRI/SOP/SA/8 - Routine Labware Cleaning for Metals Analysis* (issued 1992).

EQUIPMENT LIST

- Adjustable-Volume Micropipetters (10-100 µL and 100-1000 µL) and Tips
- Adjustable-Volume Pipetters (ranging from 1-5 mL) and Tips
- Concentrated Hydrochloric Acid (Trace Metal Grade)
- Deionized Water
- Ground Tissue Samples for Spikes
- Mercury Stock/Reference Solution, (i.e. 1000 mg/L from mercuric nitrate)
- Mercury Waste Container
- Polypropylene Digestion Vessels (from commercial supplier, such as Environmental Express)
- Potassium Permanganate ($KMnO_4$), 5% (w/v)
- Volumetric Flasks (100 mL)

PROCEDURE

1. **Mercury (Hg) Sub-Stock Preparation: 10.0 mg/L Hg Sub-Stock**
   1.1. Add ~60 mL deionized (DI) water to a 100-mL volumetric flask.
   1.2. Into the flask, add (i.e., using an adjustable pipetter) the following:
   - 1.00 mL of a 1000 mg/L mercury stock solution
   - 1 mL trace metal grade concentrated HCl
   - 100 µL 5% KMnO4

   1.3. Dilute to 100 mL with deionized water to prepare the 10.0 mg/L Hg sub-stock.
   1.4. Label this solution with the concentration, date and initials as it must be remade **once a month**.

2. **Mercury Sub-Stock Preparation: 100 µg/L Hg Sub-Stock**
   2.1. Add ~60 mL of deionized water to a 100-mL volumetric flask.
   2.2. Into the flask, add (i.e., using an adjustable pipette) the following:
   - 1.00 mL of the 10.0 mg/L Hg substock solution prepared in step 1
   - 0.5 mL trace metal grade concentrated HCl
   - 100 µL 5% KMnO4

   2.3. Dilute to 100 mL with deionzed water to prepare a 100 µg/L Hg sub-stock.
   2.4. Label this solution with the concentration, date and initials as it must be remade **once a week**.

3. **Mercury Standards Preparation**

3.1. Label digestion cups with the appropriate Hg concentrations (concentrations are listed in Table 1).

3.2. Pipet the volumes of deionized water and 100 µg/L Hg sub-stock into digestion vessels according to the tables below (Table 1). Mercury concentrations in standards are based on the final volume (50 mL) of standard at time of analysis.

3.3. Use a micropipetter to deliver all water volumes and stock Hg volumes less than 1 mL. Use a pipetter to deliver the 3.0 and 5.0 mL volumes of 100 µg Hg/L sub-stock.

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

Table 1. Mercury (Hg) Standard Preparation Volumes for Standards Ranging from 0 ng/L to 10,000 ng/L Hg.

Hg Standard Concentration (ng/L)	Volume of 100 µg/L Sub-Stock	Volume of DI Water
Blank	0	5.00 mL
100	50 µL	4950 µL
500	250 µL	4750 µL
1000	500 µL	4500 µL
6000	3.00 mL	2000 µL
10,000	5.00 mL	0 mL

4. **Mercury Spike Preparation**

4.1. A total of 10% of samples analyzed for mercury should be spiked in duplicate.

4.2. Spiking is accomplished by pipetting a known volume of the 100 µg/L Hg sub-stock into a digestion vessel containing a known weight of fish tissue. A pipetter is used to deliver 1.50 mL of 100 µg/L Hg sub-stock onto pre-weighed tissue.

5. 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.

Standard Operating Procedure SA/49v.1

COLD VAPOR MERCURY DETERMINATION IN BIOLOGICAL TISSUES USING THE FIMS-100

INTRODUCTION

This standard operating procedure (SOP) describes the operation of the FIMS-100 (PerkinElmer Life and Analytical Sciences, Shelton, CT) to determine total mercury (organic and inorganic) concentrations in fish, hair, and other biological tissue samples. Do not use this procedure for analyzing human blood.
In this method, pre-weighed tissue samples are digested with sulfuric acid and nitric acid and oxidized overnight with potassium permanganate and potassium persulfate. Mercury in the digested samples is reduced with stannous chloride to elemental mercury and measured using flow-injection technique with atomic absorption detection (Lobring and Potter 1991).

REFERENCES

Lobring, L.B. and Potter, B.B. 1991. Method 245.6, Revision 2.3: *Determination of Mercury in Tissues by Cold Vapor Atomic Absorption Spectrometry*. Method from US Environmental Protection Agency, Office of Research and Development, Environmental Monitoring Systems Laboratory.

EQUIPMENT AND REAGENT LIST

- 10 mg/L Mercuric Nitrate Sub-Stock for FIMS-100 Analysis
- 10% (w/v) Hydroxylamine Hydrochloride with 10% (w/v) Sodium Chloride
- 100 µg/L Mercuric Nitrate Sub-Stock for FIMS-100 Analysis
- 1000 µg/mL Mercuric Nitrate Stock
- 3% (v/v) Hydrochloric Acid
- 5% (w/v) Potassium Permanganate
- 5% (w/v) Potassium Persulfate
- 5% Stannous Chloride in 3% (v/v) Hydrochloric Acid
- Beakers
- Deionized Water
- FIMS-100 (PerkinElmer) Mercury Analyzer
- HotBlock™ (Environmental Express)
- Hydrochloric Acid, Trace Metals Grade
- Hydroxylamine Hydrochloride, Reagent Suitable for Mercury Determination
- KimWipes®
- Lab Coat
- Nitric Acid, Fisher, Trace Metals Grade
- Pipets/Pipettors
- Polypropylene Digestion Cups and Covers
- Potassium Permanganate, Certified ACS
- Potassium Persulfate, Reagent Suitable for Mercury Determination
- Repipet Dispensers, 10 mL and 1-5 mL
- Safety Glasses or Goggles
- Silicon Defoaming Agent
- Sodium Chloride, Certified ACS
- Spatulas
- Stannous Chloride, Analytical Reagent
- Sulfuric Acid, Certified ACS, Reagent Suitable for Mercury Determination
- WinLab32™ for AA Software (PerkinElmer)

PROCEDURE

1. Prepare samples for mercury digestion and analysis following the appropriate SOP (e.g., *LSRI SOP SA/10 – Sample Grinding for Metals Analysis*; *LSRI SOP SA/46 – Processing Several Large Fish into one Homogenous Fish Composite*; or *LSRI SOP SA/38 – Preparation of Tissues for Analytical Determinations using Liquid Nitrogen*).

2. Weigh samples using the procedure outlined in *LSRI SOP SA/11 – Sample Weighing for Metals Analysis.*

3. Prepare standards and spikes for mercury digestion and analysis following *LSRI SOP SA/42 – Stock, Standard, and Spike Preparation for Mercury Analysis*.

4. **Sample Digestion**

Note: The addition of acids and digestion of samples must be conducted in a fume hood. Proper personal protective clothing must be worn.

4.1. Add 4.0 mL of concentrated sulfuric acid and 1.0 mL of concentrated nitric acid to each sample, standard, spike, duplicate, and blank to be analyzed.
4.2. Place the digestion cups in the HotBlock™ at a setting of 110°C. Allow samples to digest for approximately 15 minutes or until all the tissue is dissolved.
4.3. Turn off the HotBlock™, remove the digestion cups from the HotBlock™, and allow contents to cool to room temperature in the fume hood.
4.4. Add 15.0 mL of 5% (w/v) potassium permanganate to each digestion cup in 5.0 mL increments, swirling the digestion cups after each addition.
4.5. Ensure that the samples remain purple in color for at least 15 minutes. If not, add additional 5% potassium permanganate solution (maximum of 5 mL) or solid potassium permanganate to the samples. If additional potassium permanganate is added to a sample, an equal amount should be added to one set of standards and a blank.
4.6. Add 8.0 mL of 5% (w/v) potassium persulfate to each digestion cup, place a threaded cap loosely on top of each digestion cup to cover samples, and gently swirl to mix.
4.7. Allow the digestion cups to react overnight at room temperature to oxidize organic mercury compounds to inorganic mercury ions.
4.8. The samples can be stored covered in the fume hood, and will remain stable for up to several days before analysis. However, samples are typically analyzed the day following the digestion process.

5. **Sample Analysis Preparation**

5.1. Prepare the following:
- Carrier Solution: 3% (v/v) hydrochloric acid.
- Reductant Solution: 5% (w/v) stannous chloride in 3% hydrochloric acid.
  - For example, weigh 50 g stannous chloride. Dissolve and dilute to 1000 mL with 3% (v/v) hydrochloric acid. The volume of 5% stannous chloride prepared will depend on the number of samples to be analyzed.
  - If the samples appear to be producing excessive foam during analysis, 10 mL of Silicon Defoaming Agent may be added per liter reductant solution.

5.2. Turn on computer and printer.
5.3. Turn on Nitrogen (400 kPa or 60 psi).
5.4. Turn on FIMS-100 Mercury Analyzer and allow it to warm up for a minimum of 10 minutes.
5.5. Press Ctrl+Alt+Del on computer keyboard and enter your "uwsuper" username and password.
5.6. Open appropriate project MS Excel file prepared from the template MS Excel file "Hg Calculations-Master" and minimize the Excel window.
5.7. Double click on the " WinLab32 for AA" icon

5.8. Click on "Wrkspc" icon (Figure 1) and choose the Hg Analysis workspace. This should automatically bring up the correct method for analysis in the "Manual Analysis Control" window.

5.9. Enter a new results data set name (e.g., DateProject, see Figure 1). Click "open" and enter a new name or choose a file in the list. Be sure that the "save data" box is checked.

5.10. Choose or prepare the Sample Information File (SIF, Figure 1).

- 5.10.1. If a sample set is to be run again, a previous SIF may be chosen by clicking on the "open" button near the information file field in the "Manual Analysis Control" window.
- 5.10.2. To prepare an MS Excel file with the same format as SIF (Figure 2):
  - 5.10.2.1. Highlight the rows in the Excel file to be added to the SIF, and copy (Ctrl+C).
  - 5.10.2.2. In WinLab32™ for AA software, click on "SamInfo" button on top toolbar (Figure 1) and highlight the number of rows to be inserted and paste the rows from the Excel file (Ctrl+V).
  - 5.10.2.3. Close the Sample Information Editor window.
  - 5.10.2.4. In the "Manual Analysis Control" window click on the open button near the information file field a window will pop up prompting you to save changes in sample information file. Click yes and save your new SIF under an appropriate name. You will then be prompted to choose a file to open.

5.11. On the FIMS-100, turn pump magazine pressure adjustment levers so that they fit into the notch on the back of the pump magazine (Figure 3).

5.12. Check gas/liquid separator cover to see that it has been tightened (Figure 3).

5.13. Attach tubing from gas/liquid separator to the FIMS-absorbance [Quartz] cell (Figure 3).

5.14. With all three collection tubes (sample, carrier and reductant) in clean deionized water, run FIAS once by clicking on the "FIAS on/off" button in the FIAS control window (Figure 1).

5.15. Check the carrier and reductant flows. Place the carrier and reductant collection tubes into their appropriately labeled graduated cylinders with deionized water. Click on the valve fill/inject button to put pump in the fill position. Click on the "Pump 1" button to start the pump. Observe the volume withdrawn from each graduated cylinder over 1 minute. Carrier volume should be at between **9 and 11 mL/min** and reductant should be at about half the carrier flow (**5 to 6 mL/min**). If needed, flow rates may be adjusted by turning the top knobs (clockwise to increase flow) on the pump magazine pressure adjustment levers. The bottom knobs should not need any adjustment.

5.16. Place collection tubes into appropriate solution bottles (Red = Reductant solution, Yellow = Carrier Solution) and run FIAS one more time. Periodically check carrier and reductant volumes, so they do not deplete while running a sample set.

5.17. Just prior to analysis of blanks, standards, and samples (step 6), add 10 mL of 10% (w/v) Hydroxylamine Hydrochloride with 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 and mix thoroughly.

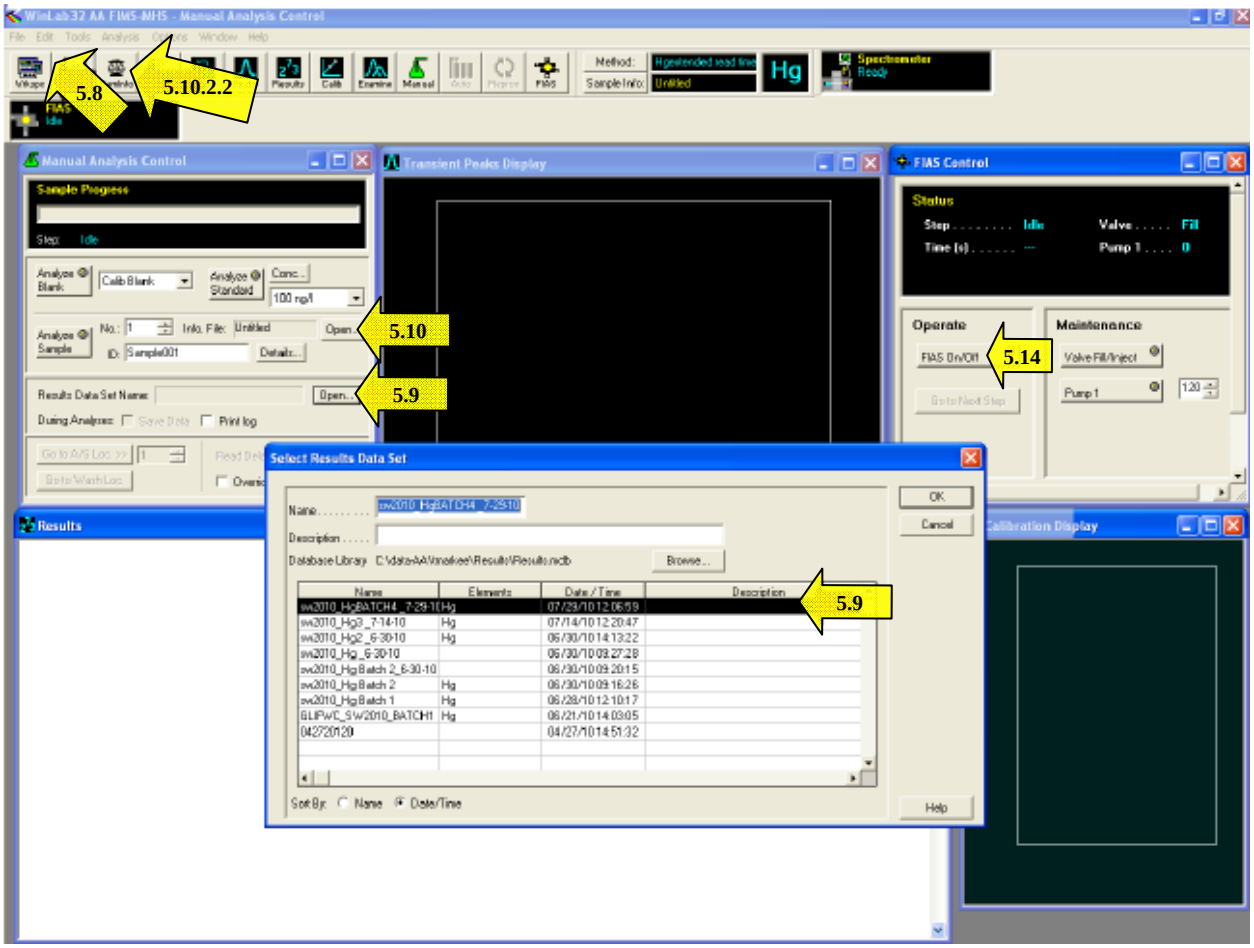

Figure 1. Screen shot of the control window in the WinLab32 for AA software. Yellow arrows indicate areas of importance and the corresponding steps referenced within this standard operating procedure.

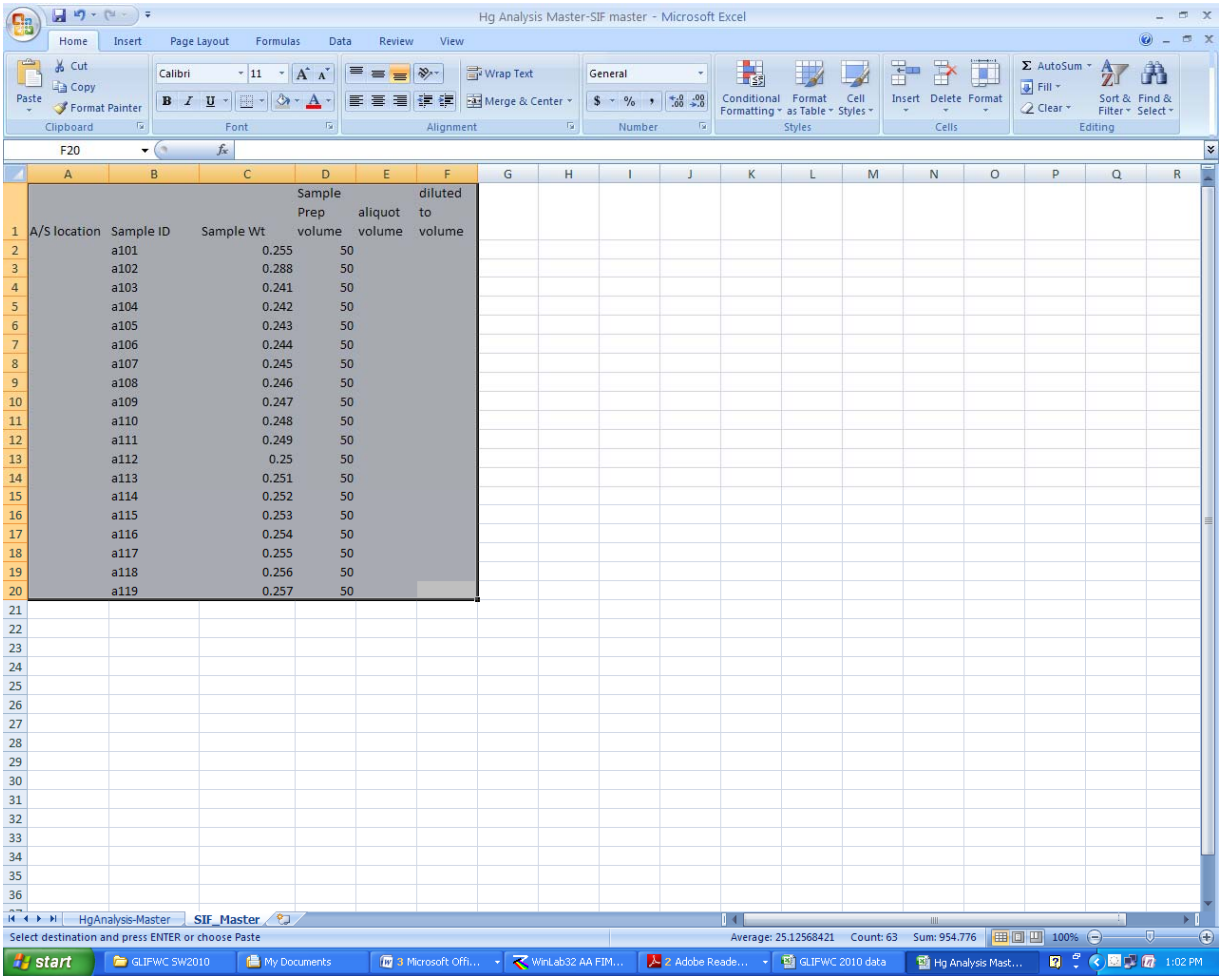

Figure 2. Preparation of a Sample Information File (SIF, in WinLab32™ for AA software) from an MS Excel file. Using MS Excel to create the SIF is ideal if a project MS Excel file has been previously prepared.

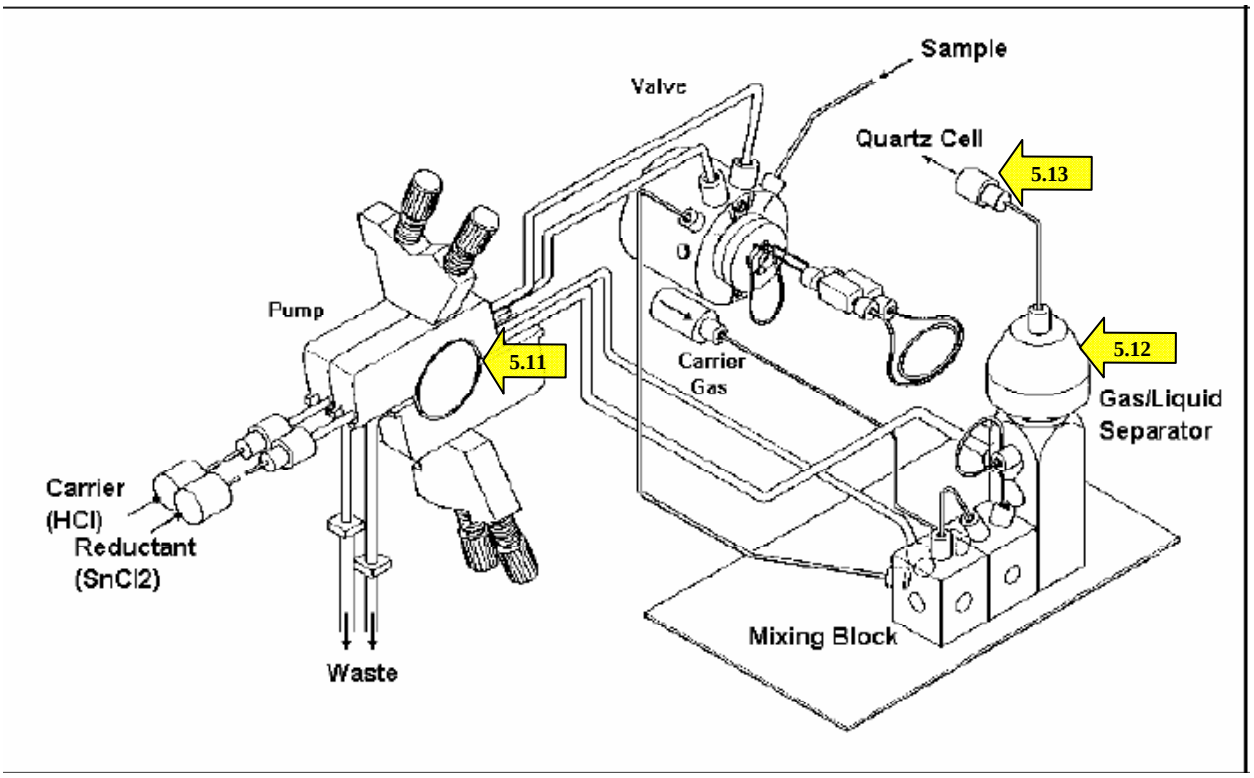

Figure 3. Diagram of the PerkinElmer FIMS-100. The yellow arrows indicate important areas of the instrument that need attention according to the referenced sections of this standard operating procedure.

6. **Sample Analysis**

6.1. Rinse the sample aspiration tube with deionized water and place in the blank solution. Click on "analyze blank" and allow instrument time to complete triplicate analysis. The pump will turn off in order to allow time to move the sample tube to the next sample/standard.

6.2. Rinse the sample aspiration 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 the 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.

6.3. Prior to analyzing samples check the following parameters:

- The slope of the line should fall between **2.0×10^{-5} to 3.0×10^{-5}**.
- Review peak shape.
- The 6000 ng/L standard should give a response between **0.12 and 0.18**.
- **If these checks do not fall in the acceptable range, check carrier and reductant flows and/or perform other maintenance as needed (see *LSRI SOP SA/50 – Routine Maintenance for FIMS-100*).**

6.4. Rinse the sample aspiration tube with deionized water and place in appropriate sample. Check that the sample ID in the ID field is correct. 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.

6.5. The second Blank, second set of standards, and reference standard (e.g., Dogfish Shark Muscle Tissue, DORM) 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, the lowest standard (100 ng/L), the reference standard, ~13 samples, the next two standards (500 ng/L and 1000 ng/L), the reference standard, ~13 samples, the 6000 ng/L standard, the reference standard, ~13 samples, and finally the 10,000 ng/L standard. 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 so that the samples can be kept together and in order.

6.6. As standards and samples are being analyzed, check to see that the relative standard deviation (RSD) of the three measurements is less than 5%. If not, reanalyze the sample in an attempt to obtain an RSD of less than 5%. If an RSD of less than 5% is not obtainable, the sample should be redigested and reanalyzed. The less than 5% RSD will not apply to standards or samples that have blank corrected absorbances of less than 0.005.

7. Completion of Analysis

7.1. Place sample aspiration tube, and lines from reductant and carrier solutions into beaker of deionized water.

7.2. Flush/clean tubing with deionized water by running FIAS two times.

7.3. 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.

7.4. Raise waste lines out of liquid in waste container so liquid does not back up.

7.5. Release the pump magazine pressure adjustment levers so that tubing is not compressed.

7.6. Detach line from FIMS-absorbance cell.

7.7. Unscrew the gas/liquid separator cover and, using forceps to handle filter, dry filter with a Kimwipe®.

7.8. Print report. Choose “file” > “utilities” > Choose the data set for that day > Click “Report” > “Use Existing Design” > Choose “hg.rep” > “next” > “open” > Select all the samples for that date > Choose “Preview” > If acceptable, print the report. If additional information or different settings are desired, “next” may be chosen and the design may be modified.

7.9. Save the Excel file to the appropriate project folder.

7.10. Turn off FIMS instrument, computer, nitrogen gas and printer.

7.11. Record the date, project, analyst, number of injections, and run time in FIMS-100 Record Notebook located in the laboratory with the instrument.

Standard Operating Procedure SA/51v.3

PROCEDURE FOR DETERMINING PERCENT MOISTURE IN TISSUE SAMPLES

INTRODUCTION

This standard operating procedure (SOP) describes the method used in determining the percent moisture content in biological tissue samples. This is a gravimetric method that requires careful weighing techniques. Once the aluminum weigh pans have been dried, they must only be handled with forceps to avoid addition of oils from the researchers' hands. The addition of oils will cause an error in the pan weight.

EQUIPMENT LIST

- Analytical Balance (i.e., capable of weighing to 0.001 g)
- Aluminum Weigh Pans
- Drying Oven (60°C ± 10°C)
- Desiccation Container with Dry Desiccant
- Spatula
- Laboratory Notebook
- Forceps

PROCEDURE

1. Label the aluminum weigh pans and dry at 60°C (±10°C) for a minimum of two hours. Record the date and time that the pans were placed into and removed from the oven in the appropriate laboratory notebook or on the Tissue Moisture Determination Datasheet (Appendix 1).

2. Using forceps, place dried weighing pans in desiccator until cool (i.e., to approximately room temperature).

3. Check analytical balance calibration using Class 1 weights according to *LSRI/SOP/GLM/12 – Procedure for Verifying Calibration of Laboratory Balances* (issued 1995). Weigh the dried and cooled weighing pans on balance to the 0.001 g and record weight in the appropriate laboratory notebook or datasheet (Appendix 1).

4. Add tissue (i.e., 1.0 g – 5.0 g) to the labeled weighing pan.

5. Weigh the pan and the tissue on balance to the nearest 0.001 g and record weight in the appropriate laboratory notebook or datasheet (Appendix 1).

6. Dry pan and tissue in drying oven at 60°C (±10°C) for a minimum of 16 hours or until constant dry weight is achieved. Record the date and time that the pans were placed in the oven in the appropriate laboratory notebook or datasheet (Appendix 1).

7. Remove dried pans and tissue from the oven and place in a desiccator until cool. Record the date and time that the pans were removed from the oven in the appropriate laboratory notebook or datasheet (Appendix 1).

8. Weigh the pan with the dried tissue on balance to the nearest 0.001 g and record weight in the appropriate laboratory notebook or datasheet (Appendix 1). 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 constant dry weight (i.e., the weight change is less than 50 mg from the previous weight) has been achieved. Record the date and time that the pans were weighed a second time, as well as, the second dry weight in the appropriate laboratory notebook or datasheet (Appendix 1).

9. Calculations:

$$\boldsymbol{Wet\ Weight\ of\ Tissue}\ (\boldsymbol{g}) = (Weight\ of\ Pan + Wet\ Tissue) - (Weight\ Dry\ Pan)$$

$$\boldsymbol{Percent\ Moisture\ of\ Tissue} = \left(\frac{(Weight\ Pan + Wet\ Tissue) - (Weight\ Pan + Dry\ Tissue)}{Wet\ Tissue\ Weight}\right) x\ 100\%$$