GREAT LAKES INDIAN FISH & WILDLIFE COMMISSION
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• MEMBER TRIBES •

MICHIGAN	WISCONSIN		MINNESOTA
Bay Mills Community	Bad River Band	Red Cliff Band	Fond du Lac Band
Keweenaw Bay Community	Lac Courte Oreilles Band	St. Croix Chippewa	Mille Lacs Band
Lac Vieux Desert Band	Lac du Flambeau Band	Sokaogon Chippewa	

To: Neil Kmiecik, Biological Services Director

From: Sara Moses, Environmental Biologist Sara K. Moses

Date: August 14, 2013

Re: Results of Mercury Testing of Walleye Collected During Spring 2012

GLIFWC has collected information on mercury in walleye every year since 1989. The data are used to provide walleye consumption advice to member tribes so that tribal members can reduce their exposure to mercury while continuing to exercise their treaty rights to harvest and enjoy the health benefits of eating this resource. In 2012 GLIFWC was funded through a U.S. EPA Great Lakes Restoration Initiative (GLRI) grant [GL00E00613-0] to collect and test for mercury 360 walleye from inland lakes within the ceded territories. The data will be used to update GLIFWC's mercury maps and provide safe walleye consumption advice to our member tribes. The maps were last updated in early 2012 with data through 2011. All walleye collection and analysis was conducted according to the Quality Assurance Project Plan (QAPP) "Great Lakes Indian Fish and Wildlife Commission Mercury Testing and Updating Tribal Walleye Consumption Advice" approved June 24, 2011.

A total of 387 walleye were collected from 35 inland lakes within the 1837 and 1842 ceded territories of Wisconsin, Mille Lacs in the 1837 ceded territory of Minnesota, Lake Gogebic in the 1842 ceded territory of Michigan, and the Kakagon Slough on the Bad River reservation. The number of walleye collected from each targeted lake, by size class, is shown in the attached Table 1. A total of 42 lakes were targeted for walleye collection to account for the inability to collect 12 fish from some lakes.

Skin-off walleye fillets were analyzed for total mercury content by the Lake Superior Research Institute (LSRI) at the University of Wisconsin, Superior. LSRI provided the final report detailing these analyses on October 12, 2012 together with results of the QA/QC audit for these analyses (Appendix 1). With the exception of only one sample spike, all QA/QC samples were within their respective acceptance ranges. The out of range sample spike was redigested and reanalyzed and fell well within the acceptable range. The QA audit found only one minor deviation:

> *"Deviation #2012-GLIFWC-01: The balance used to weigh processed tissue for digestion was calibrated using three ASTM Class 1 weights; however, the lowest verification weight used (i.e., 0.2 g) was greater than that of the Certified Reference Material for Trace Metals (i.e., DORM-3)*

being measured (i.e., DORM-3 weight was 0.1 g – 0.15 g). According to LSRI SOP GLM/12, v.5 – Procedure for Verification of Laboratory Balances, three ANSI/ASTM Class 1 weights must be selected that "bracket the weight being determined". This was discussed with the project staff during the audit, and it was suggested that a 0.1 g verification weight or lower mass be used as the lowest verification weight whenever the Certified Reference Material for Trace Metals is weighed."

Total mercury concentrations on a wet weight basis ranged from 0.045 to 1.37 µg/g (parts per million or ppm). Figure 1 shows the number of walleye falling into each of 5 mercury concentration ranges. Summary statistics for walleye mercury concentrations by lake can be found in the attached Table 2. The results of mercury analysis for each individual sample are included in Table 3.

cc: John Coleman, Environmental Section Leader

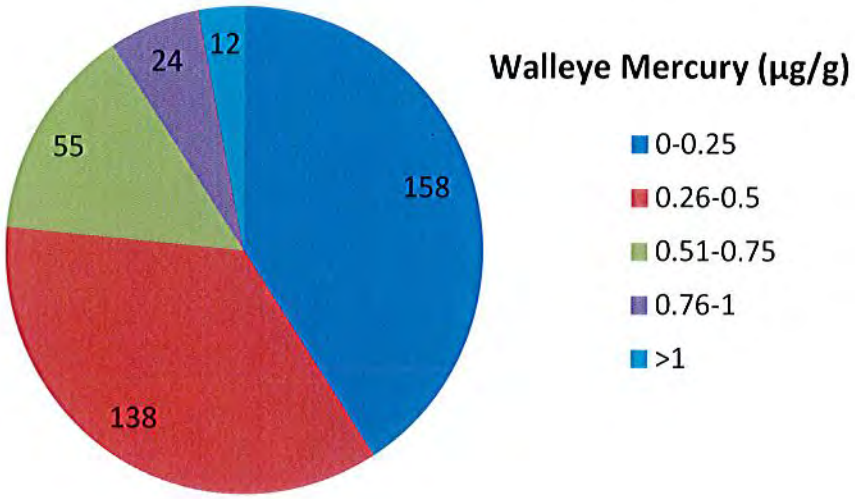

Figure 1: Number of walleye collected during Spring 2012 by mercury content.

Table 1: Number of Walleye Collected from Inland Lakes during Spring 2012

STATE	COUNTY	LAKE	Collected by: Warden/ Assessment Crew	12.0 to 14.9	15.0 to 17.9	18.0 to 22.0	> 22.0	Total Collected	% of Goal
MI	GOGEBIC	L GOGEBIC	North	4	8	0	0	12	100%
MI	ONTONAGON	BOND FALLS FL	North						0%
MN	MILLE LACS	MILLE LACS L	Arunagiri	3	3	3	3	12	100%
WI	ASHLAND	KAKAGON SLOUGH	Bad River DNR	3	3	3	3	12	100%
WI	BARRON	DUCK L	Assessment	3	3	3	1	10	83%
WI	BARRON	RED CEDAR L	Kacizak	2	3	0	0	5	42%
WI	BARRON	SILVER L	Kacizak						0%
WI	BAYFIELD	ATKINS L	J. Stone	1	3	2	1	7	58%
WI	BAYFIELD	MIDDLE EAU CLAIRE L	J. Stone	4	3	3	0	10	83%
WI	BAYFIELD	SISKIWIT L	Assessment	3	3	3	0	9	75%
WI	CHIPPEWA	L WISSOTA	V. Stone						0%
WI	DOUGLAS	WHITEFISH L	V.Stone	2	5	4	1	12	100%
WI	FOREST	BUTTERNUT L	Assessment	3	3	3	0	9	75%
WI	IRON	TURTLE-FLAMBEAU FL	Moermond	3	3	3	1	10	83%
WI	ONEIDA	BEARSKIN L	Assessment	3	3	2	3	11	92%
WI	ONEIDA	LONG L	McGeshick						0%
WI	ONEIDA	PELICAN L	McGeshick	3	3	3	3	12	100%
WI	ONEIDA	PLANTING GROUND L	McGeshick	3	3	0	4	10	83%
WI	ONEIDA	SQUIRREL L	Assessment	3	3	5	2	13	108%
WI	ONEIDA	THUNDER L	McGeshick						0%
WI	PRICE	BUTTERNUT L	Moermond	3	3	2	2	10	83%
WI	SAWYER	CONNORS L	V. Stone	3	3	3	1	10	83%
WI	SAWYER	L CHIPPEWA	Tuori	5	3	4	0	12	100%
WI	SAWYER	LAC COURTE OREILLES	Tuori	3	4	3	2	12	100%
WI	SAWYER	ROUND L	Tuori	3	3	3	3	12	100%
WI	ST CROIX	CEDAR L	Kacizak						0%
WI	VILAS	ANNABELLE L	V. Stone	3	3	1	0	7	58%
WI	VILAS	BIG ARBOR VITAE L	Moermond	3	3	3	3	12	100%
WI	VILAS	BIG GIBSON L	Moermond	3	3	3	0	9	75%
WI	VILAS	BIG L (MI BORDER)	Moermond	3	3	3	1	10	83%
WI	VILAS	BIG SAND L	McGeshick	3	3	3	1	10	83%
WI	VILAS	CRANBERRY L	McGeshick	3	3	3	1	10	83%
WI	VILAS	EAGLE L	Moermond	3	3	3	1	10	83%
WI	VILAS	MAMIE L	V. Stone	3	3	3	3	12	100%
WI	VILAS	OXBOW L	Moermond	3	3	3	2	11	92%
WI	VILAS	PRESQUE ISLE L CHAIN	V. Stone	3	2	4	3	12	100%
WI	VILAS	SCATTERING RICE L	Moermond	1	1	0	2	4	33%
WI	VILAS	SHERMAN L	Assessment						0%
WI	VILAS	SQUAW L	Assessment	3	3	1	3	10	83%
WI	VILAS	TENDERFOOT L	V. Stone	3	3	3	3	12	100%
WI	VILAS	TROUT L	Moermond	3	3	3	3	12	100%
WI	VILAS	TWIN L CHAIN	McGeshick	3	3	3	3	12	100%
WI	WASHBURN	DUNN L	Kacizak	0	1	2	1	4	33%
WI	WASHBURN	SHELL L	Kacizak	3	3	2	0	8	67%
WI	WASHBURN	STONE L	Tuori	3	3	3	3	12	100%
							TOTAL:	387	104%

Table 2: Summary Statistics by Lake for Length, Weight, and Mercury Concentration (µg/g wet weight) of Walleye Collected from Inland Lakes during Spring 2012

Lake	County	n	Length (Inches)		Weight (Pounds)		Mercury (µg/g ww)	
			Range	Mean	Range	Mean	Range	Mean
L Gogebic	Gogebic (MI)	12	13.1-17.5	15.9	0.69-1.71	1.29	0.09-0.27	0.17
Mille Lacs	Mille Lacs (MN)	12	14.2-23.8	18.6	0.87-4.92	2.44	0.05-0.21	0.12
Kakagon Slough	Ashland	12	13.2-23.6	17.9	0.84-4.37	2.23	0.11-0.79	0.24
Duck L	Barron	10	14.3-22.1	16.8	0.94-3.94	1.78	0.10-0.29	0.18
Red Cedar L	Barron	5	14.0-15.8	14.6	0.96-1.43	1.14	0.26-0.41	0.33
Atkins L	Bayfield	7	14.5-24.3	18.5	0.98-6.03	2.66	0.39-0.81	0.54
Middle Eau Claire L	Bayfield	10	13.4-18.2	16.0	0.74-1.92	1.34	0.16-0.59	0.36
Siskiwit L	Bayfield	9	12.4-19.9	16.0	0.64-2.70	1.44	0.46-1.11	0.76
Whitefish L	Douglas	12	14.6-23.4	17.9	0.90-3.92	1.91	0.12-0.72	0.36
Butternut L	Forest	9	13.8-19.6	16.5	0.75-2.58	1.50	0.05-0.30	0.12
Turtle-Flambeau FL	Iron	10	12.3-23.0	16.8	0.56-4.84	1.75	0.23-0.88	0.47
Bearskin L	Oneida	11	12.7-25.9	18.4	0.54-6.52	2.33	0.05-0.40	0.17
Pelican L	Oneida	12	13.0-24.2	17.8	0.66-5.31	2.15	0.10-0.52	0.26
Planting Ground L	Oneida	10	13.0-24.6	18.7	0.67-6.15	2.83	0.20-1.33	0.58
Squirrel L	Oneida	13	13.2-24.7	19.1	0.53-5.53	2.46	0.18-0.70	0.34
Butternut L	Price	10	12.2-26.5	17.6	0.60-6.65	2.44	0.49-1.37	0.83
Connors L	Sawyer	10	12.7-26.1	17.3	0.58-7.47	1.99	0.27-0.61	0.36
L Chippewa	Sawyer	12	13.5-19.3	16.2	0.81-2.56	1.51	0.20-0.58	0.38
Lac Courte Oreilles	Sawyer	12	12.5-24.0	17.5	0.59-5.26	2.14	0.09-0.51	0.26
Round L	Sawyer	12	14.4-22.8	18.4	0.83-3.78	2.15	0.11-0.48	0.23
Annabelle L	Vilas	7	13.2-18.2	15.1	0.66-2.14	1.13	0.53-0.90	0.69
Big Arbor Vitae L	Vilas	12	14.8-28.0	18.8	0.98-8.18	2.76	0.13-0.48	0.21
Big Gibson L	Vilas	9	13.3-20.0	16.5	0.67-2.74	1.67	0.31-0.64	0.41
Big L (MI Border)	Vilas	10	13.6-23.0	17.1	0.73-4.98	1.92	0.18-0.64	0.28
Big Sand L	Vilas	10	13.1-22.9	17.1	0.70-4.34	1.89	0.20-0.78	0.40
Cranberry L	Vilas	10	11.4-24.8	16.9	0.50-6.29	2.15	0.13-0.58	0.28
Eagle L	Vilas	10	12.3-22.1	16.8	0.59-3.66	1.78	0.17-0.99	0.44
Mamie L	Vilas	12	12.2-25.5	18.6	0.92-4.96	2.50	0.19-0.88	0.40
Oxbow L	Vilas	11	12.8-25.2	17.3	0.60-6.68	2.16	0.49-1.10	0.76
Presque Isle L Chain	Vilas	12	13.0-24.8	18.4	0.57-6.05	2.38	0.13-0.42	0.27
Scattering Rice L	Vilas	4	12.5-24.1	18.5	0.63-5.22	2.89	0.20-1.12	0.53
Squaw L	Vilas	10	13.4-27.2	18.6	0.62-7.75	2.93	0.49-1.03	0.70
Tenderfoot L	Vilas	12	14.0-26.3	18.5	0.86-7.63	2.66	0.25-0.60	0.45
Trout L	Vilas	12	13.3-28.0	18.9	0.70-6.22	2.61	0.13-0.74	0.26
Twin L Chain	Vilas	12	12.7-24.3	18.5	0.78-5.04	2.61	0.11-0.37	0.22
Dunn L	Washburn	4	17.0-24.2	21.0	1.81-6.16	3.91	0.21-0.97	0.48
Shell L	Washburn	8	12.8-21.1	16.0	0.66-3.21	1.40	0.24-0.70	0.40
Stone L	Washburn	12	13.0-24.3	18.2	0.58-4.37	2.14	0.20-0.91	0.43

Table 3: Mercury Concentration (µg/g wet weight), Length (inches), Sex, and Weight (Pounds) of Individual Walleye Collected from Inland Lakes during the Spring 2012 Spearing Season

Lake	County	Length (Inches)	Mercury (µg/g ww)	Sample Number	Sex	Age	Date	Weight (Pounds)
L GOGEBIC	GOGEBIC (MI)	13.1	0.088	11745	M	3	4/12/2012	0.69
L GOGEBIC	GOGEBIC (MI)	14.9	0.120	11746	M	4	4/12/2012	1.01
L GOGEBIC	GOGEBIC (MI)	17.3	0.265	11747	F	7	4/12/2012	1.71
L GOGEBIC	GOGEBIC (MI)	16.7	0.166	11748	M	7	4/12/2012	1.48
L GOGEBIC	GOGEBIC (MI)	16.7	0.180	11749	M	8	4/12/2012	1.68
L GOGEBIC	GOGEBIC (MI)	17.0	0.165	11750	M	7	4/12/2012	1.55
L GOGEBIC	GOGEBIC (MI)	16.9	0.244	11751	M	5	4/12/2012	1.38
L GOGEBIC	GOGEBIC (MI)	17.5	0.215	11752	M	8	4/12/2012	1.68
L GOGEBIC	GOGEBIC (MI)	13.4	0.192	11753	M	2	4/12/2012	0.80
L GOGEBIC	GOGEBIC (MI)	16.5	0.157	11754	M	6	4/12/2012	1.27
L GOGEBIC	GOGEBIC (MI)	14.0	0.127	11755	M	3	4/12/2012	0.85
L GOGEBIC	GOGEBIC (MI)	16.2	0.126	11756	M	5	4/12/2012	1.36
MILLE LACS	MILLE LACS (MN)	14.9	0.072	12746	M	5	4/42012	1.14
MILLE LACS	MILLE LACS (MN)	19.9	0.203	12747	M	8	4/42012	2.39
MILLE LACS	MILLE LACS (MN)	23.0	0.211	12748	F	11	4/42012	4.48
MILLE LACS	MILLE LACS (MN)	23.8	0.19	12749	F	9	4/42012	4.84
MILLE LACS	MILLE LACS (MN)	23.8	0.179	12750	F	9	4/42012	4.92
MILLE LACS	MILLE LACS (MN)	21.3	0.126	12751	F	7	4/42012	3.13
MILLE LACS	MILLE LACS (MN)	18.8	0.07	12752	F	5	4/42012	2.05
MILLE LACS	MILLE LACS (MN)	16.6	0.08	12753	M	4	4/42012	1.51
MILLE LACS	MILLE LACS (MN)	14.5	0.057	12754	M	5	4/42012	1.15
MILLE LACS	MILLE LACS (MN)	16.5	0.096	12755	M	4	4/42012	1.43
MILLE LACS	MILLE LACS (MN)	14.2	0.068	12756	M	4	4/42012	0.87
MILLE LACS	MILLE LACS (MN)	15.9	0.05	12757	M	7	4/42012	1.33
VILAS	ANNABELLE L	18.2	0.695	12191	F	9	4/12/2012	2.14
VILAS	ANNABELLE L	16.0	0.529	12192	F	5	4/12/2012	1.4
VILAS	ANNABELLE L	15.1	0.903	12193	M	5	4/12/2012	1.1
VILAS	ANNABELLE L	15.6	0.811	12194	M	5	4/12/2012	1.11
VILAS	ANNABELLE L	13.4	0.583	12371	M	5	4/12/2012	0.68
VILAS	ANNABELLE L	14.3	0.698	12391	M	6	4/12/2012	0.82
VILAS	ANNABELLE L	13.2	0.581	12392	M	4	4/12/2012	0.66
BAYFIELD	ATKINS L	16.1	0.387	12232	M	2	3/29/2012	1.4
BAYFIELD	ATKINS L	21.5	0.592	12262	F	8	3/29/2012	4.27
BAYFIELD	ATKINS L	20.3	0.622	12320	F	4	3/29/2012	3.1
BAYFIELD	ATKINS L	24.3	0.807	12533	F	6	3/29/2012	6.03
BAYFIELD	ATKINS L	16.4	0.47	12539	M	3	3/29/2012	1.42
BAYFIELD	ATKINS L	14.5	0.438	12556	M	4	3/29/2012	0.98
BAYFIELD	ATKINS L	16.1	0.485	12586	M	3	3/29/2012	1.39
ONEIDA	BEARSKIN L	18.7	0.14	12089	F	5	3/26/2012	2.03
ONEIDA	BEARSKIN L	21.9	0.212	12547	F	8	3/26/2012	4.03
ONEIDA	BEARSKIN L	13.0	0.048	12762	M	NA	3/26/2012	0.69
ONEIDA	BEARSKIN L	14.6	0.081	12763	M	5	3/26/2012	0.95
ONEIDA	BEARSKIN L	12.7	0.076	12764	M	4	3/26/2012	0.65
ONEIDA	BEARSKIN L	17.9	0.144	12765	M	9	3/26/2012	1.86
ONEIDA	BEARSKIN L	25.9	0.401	12766	F	13	3/26/2012	6.52
ONEIDA	BEARSKIN L	15.5	0.125	12767	M	5	3/26/2012	1.13
ONEIDA	BEARSKIN L	16.1	0.118	12768	M	7	3/26/2012	1.46
ONEIDA	BEARSKIN L	22.8	0.272	12769	F	11	3/26/2012	0.54
ONEIDA	BEARSKIN L	23.3	0.275	12770	F	12	3/26/2012	5.82
VILAS	BIG ARBOR VITAE L	18.0	0.188	11901	M	7	4/4/2012	2.02
VILAS	BIG ARBOR VITAE L	18.6	0.129	11902	F	6	4/4/2012	2.36
VILAS	BIG ARBOR VITAE L	18.6	0.185	11933	F	8	4/4/2012	2.2
VILAS	BIG ARBOR VITAE L	28.0	0.478	11988	F	11	4/4/2012	8.18
VILAS	BIG ARBOR VITAE L	14.8	0.134	12901	M	6	4/4/2012	1.05
VILAS	BIG ARBOR VITAE L	15.8	0.141	12902	M	6	4/4/2012	1.25
VILAS	BIG ARBOR VITAE L	16.7	0.181	12903	M	6	4/4/2012	1.41
VILAS	BIG ARBOR VITAE L	14.8	0.158	12904	M	4	4/4/2012	0.98
VILAS	BIG ARBOR VITAE L	14.9	0.161	12905	M	4	4/4/2012	1.12
VILAS	BIG ARBOR VITAE L	17.1	0.176	12906	M	6	4/4/2012	1.72
VILAS	BIG ARBOR VITAE L	25.4	0.409	12983	F	13	4/4/2012	7.06
VILAS	BIG ARBOR VITAE L	22.3	0.214	12984	F	11	4/4/2012	3.81
VILAS	BIG GIBSON L	17.3	0.425	11903	F	7	4/2/2012	1.86
VILAS	BIG GIBSON L	14.9	0.368	11904	M	6	4/2/2012	1.06

VILAS	BIG GIBSON L	15.0	0.346	11905	M	5	4/2/2012	0.99
VILAS	BIG GIBSON L	15.1	0.316	11906	M	5	4/2/2012	1.16
VILAS	BIG GIBSON L	14.6	0.42	11907	M	6	4/2/2012	1.14
VILAS	BIG GIBSON L	13.3	0.321	11908	M	4	4/2/2012	0.67
VILAS	BIG GIBSON L	19.0	0.635	11909	F	8	4/2/2012	2.73
VILAS	BIG GIBSON L	20.0	0.481	11910	F	7	4/2/2012	2.74
VILAS	BIG GIBSON L	19.1	0.393	11911	U	5	4/2/2012	2.71
VILAS	BIG L (MI BORDER)	15.5	0.317	11918	F	6	4/11/2012	1.18
VILAS	BIG L (MI BORDER)	13.6	0.237	11919	M	7	4/11/2012	0.8
VILAS	BIG L (MI BORDER)	13.8	0.176	11920	M	5	4/11/2012	0.73
VILAS	BIG L (MI BORDER)	14.7	0.215	11921	F	8	4/11/2012	1.06
VILAS	BIG L (MI BORDER)	19.2	0.29	11922	F	9	4/11/2012	2.48
VILAS	BIG L (MI BORDER)	23.0	0.638	11923	F	10	4/11/2012	4.98
VILAS	BIG L (MI BORDER)	19.2	0.215	11924	F	7	4/11/2012	2.49
VILAS	BIG L (MI BORDER)	16.1	0.219	11925	M	9	4/11/2012	1.25
VILAS	BIG L (MI BORDER)	17.6	0.3	11926	F	7	4/11/2012	2.06
VILAS	BIG L (MI BORDER)	18.0	0.179	11927	M	6	4/11/2012	2.12
VILAS	BIG SAND L	21.6	0.432	11276	U	7	4/12/2012	3.73
VILAS	BIG SAND L	15.3	0.274	11277	M	3	4/12/2012	1.08
VILAS	BIG SAND L	13.1	0.204	11283	U	2	4/12/2012	0.7
VILAS	BIG SAND L	13.4	0.205	11284	M	2	4/12/2012	0.76
VILAS	BIG SAND L	13.5	0.219	11285	U	2	4/12/2012	0.82
VILAS	BIG SAND L	16.6	0.26	11286	U	4	4/12/2012	1.46
VILAS	BIG SAND L	17.2	0.394	11287	M	8	4/12/2012	1.57
VILAS	BIG SAND L	18.2	0.784	11288	U	7	4/12/2012	1.85
VILAS	BIG SAND L	19.5	0.487	11289	U	9	4/12/2012	2.61
VILAS	BIG SAND L	22.9	0.741	11290	U	8	4/12/2012	4.34
FOREST	BUTTERNUT L	19.6	0.303	12601	M	9	3/28/2012	2.58
FOREST	BUTTERNUT L	16.2	0.087	12602	M	6	3/28/2012	1.3
FOREST	BUTTERNUT L	14.8	0.115	12603	M	5	3/28/2012	0.95
FOREST	BUTTERNUT L	14.6	0.047	12604	M	3	3/28/2012	0.95
FOREST	BUTTERNUT L	15.1	0.045	12605	M	6	3/28/2012	1.02
FOREST	BUTTERNUT L	13.8	0.094	12606	M	3	3/28/2012	0.75
FOREST	BUTTERNUT L	16.7	0.106	12607	M	6	3/28/2012	1.49
FOREST	BUTTERNUT L	19.4	0.137	12608	F	6	3/28/2012	2.41
FOREST	BUTTERNUT L	18.6	0.13	12609	F	6	3/28/2012	2.05
PRICE	BUTTERNUT L	12.2	0.497	11934	M	4	3/25/2012	0.6
PRICE	BUTTERNUT L	14.8	0.805	11935	M	8	3/25/2012	1.03
PRICE	BUTTERNUT L	12.4	0.489	11936	M	3	3/25/2012	0.56
PRICE	BUTTERNUT L	15.4	0.596	11937	F	3	3/25/2012	1.14
PRICE	BUTTERNUT L	15.3	0.641	11938	M	4	3/25/2012	1.09
PRICE	BUTTERNUT L	26.5	1.37	11939	F	13	3/25/2012	6.65
PRICE	BUTTERNUT L	18.1	0.804	11940	F	6	3/25/2012	2.27
PRICE	BUTTERNUT L	16.0	0.852	11941	F	6	3/25/2012	1.63
PRICE	BUTTERNUT L	21.0	1.04	11942	F	10	3/27/2012	3.83
PRICE	BUTTERNUT L	24.5	1.24	11943	F	10	3/27/2012	5.62
SAWYER	CONNORS L	19.2	0.607	12198	M	8	4/7/2012	2.13
SAWYER	CONNORS L	12.7	0.367	12199	M	2	4/7/2012	0.58
SAWYER	CONNORS L	13.3	0.368	12200	M	3	4/7/2012	0.68
SAWYER	CONNORS L	18.4	0.359	12292	M	6	4/7/2012	2.02
SAWYER	CONNORS L	17.6	0.276	12293	M	6	4/7/2012	1.53
SAWYER	CONNORS L	16.4	0.273	12294	M	6	4/7/2012	0.95
SAWYER	CONNORS L	13.0	0.295	12295	M	4	4/7/2012	0.69
SAWYER	CONNORS L	26.1	0.472	12296	F	13	4/7/2012	7.47
SAWYER	CONNORS L	17.6	0.311	12297	M	7	4/7/2012	1.88
SAWYER	CONNORS L	18.5	0.274	12298	M	7	4/7/2012	2
VILAS	CRANBERRY L	18.1	0.236	11596	F	6	4/5/2012	2.24
VILAS	CRANBERRY L	21.6	0.583	11597	F	8	4/5/2012	3.78
VILAS	CRANBERRY L	24.8	0.298	11598	F	7	4/5/2012	6.29
VILAS	CRANBERRY L	20.8	0.453	11599	F	7	4/5/2012	3.53
VILAS	CRANBERRY L	15.4	0.193	12291	F	7	4/2/2012	1.31
VILAS	CRANBERRY L	12.6	0.178	12796	M	5	4/2/2012	0.66
VILAS	CRANBERRY L	11.4	0.134	12797	M	4	4/2/2012	0.5
VILAS	CRANBERRY L	14.0	0.142	12798	M	5	4/2/2012	0.86
VILAS	CRANBERRY L	15.3	0.24	12799	M	7	4/2/2012	1.19
VILAS	CRANBERRY L	15.3	0.337	12800	M	8	4/2/2012	1.15
BARRON	DUCK L	15.4	0.21	12188	M	2	3/22/2012	1.24
BARRON	DUCK L	18.5	0.208	12189	F	5	3/22/2012	2.26
BARRON	DUCK L	18.5	0.26	12490	F	6	3/22/2012	2.56
BARRON	DUCK L	16.1	0.162	12512	M	4	3/22/2012	1.39
BARRON	DUCK L	14.3	0.13	12537	M	4	3/22/2012	0.94

BARRON	DUCK L	14.8	0.117	12540	M	3	3/22/2012	1.13
BARRON	DUCK L	14.7	0.113	12546	M	4	3/22/2012	1.03
BARRON	DUCK L	15.7	0.104	12551	M	3	3/22/2012	1.17
BARRON	DUCK L	18.0	0.293	12555	F	8	3/22/2012	2.1
BARRON	DUCK L	22.1	0.229	12725	F	8	3/22/2012	3.94
WASHBURN	DUNN L	21.5	0.347	12261	F	7	3/24/2012	4.06
WASHBURN	DUNN L	24.2	0.971	12265	F	13	3/24/2012	6.16
WASHBURN	DUNN L	17.0	0.205	12305	U	3	3/24/2012	1.81
WASHBURN	DUNN L	21.1	0.39	12307	F	7	3/24/2012	3.61
VILAS	EAGLE L	13.2	0.171	12937	M	5	4/11/2012	0.68
VILAS	EAGLE L	16.3	0.674	12938	M	11	4/11/2012	1.38
VILAS	EAGLE L	18.2	0.264	12939	F	10	4/11/2012	2.48
VILAS	EAGLE L	22.1	0.685	12941	F	9	4/11/2012	3.66
VILAS	EAGLE L	21.1	0.985	12946	M	12	4/11/2012	3.16
VILAS	EAGLE L	19.0	0.572	12947	M	10	4/11/2012	2.54
VILAS	EAGLE L	12.3	0.242	12948	M	3	4/11/2012	0.59
VILAS	EAGLE L	12.9	0.273	12949	M	7	4/11/2012	0.67
VILAS	EAGLE L	15.6	0.286	12950	M	6	4/11/2012	1.17
VILAS	EAGLE L	16.8	0.267	12951	M	6	4/11/2012	1.45
ASHLAND	KAKAGON R	15.4	0.112	12061	M	4	4/5/2012	1.34
ASHLAND	KAKAGON R	16.1	0.108	12062	M	4	4/5/2012	1.51
ASHLAND	KAKAGON R	15.1	0.165	12063	M	3	4/5/2012	1.11
ASHLAND	KAKAGON R	14.2	0.157	12064	M	3	4/5/2012	1.01
ASHLAND	KAKAGON R	13.2	0.153	12065	M	3	4/5/2012	0.84
ASHLAND	KAKAGON R	23.6	0.218	12066	F	9	4/5/2012	4.25
ASHLAND	KAKAGON R	23.2	0.794	12067	M	16	4/5/2012	4.37
ASHLAND	KAKAGON R	23.0	0.453	12068	M	11	4/5/2012	4.31
ASHLAND	KAKAGON R	18.1	0.161	12069	M	5	4/5/2012	2.21
ASHLAND	KAKAGON R	19.0	0.201	12070	M	5	4/5/2012	2.27
ASHLAND	KAKAGON R	14.0	0.122	12071	M	3	4/5/2012	0.86
ASHLAND	KAKAGON R	20.1	0.205	12072	M	5	4/5/2012	2.64
SAWYER	LAC COURTE OREILLES	13.4	0.106	12968	M	3	4/8/2012	0.76
SAWYER	LAC COURTE OREILLES	12.5	0.089	12969	M	3	4/8/2012	0.59
SAWYER	LAC COURTE OREILLES	20.5	0.368	12970	M	9	4/8/2012	2.65
SAWYER	LAC COURTE OREILLES	18.1	0.416	12971	M	9	4/8/2012	2.14
SAWYER	LAC COURTE OREILLES	23.3	0.449	12972	F	10	4/8/2012	4.5
SAWYER	LAC COURTE OREILLES	24.0	0.509	12973	F	10	4/8/2012	5.26
SAWYER	LAC COURTE OREILLES	20.2	0.355	12977	M	8	4/8/2012	3.05
SAWYER	LAC COURTE OREILLES	15.8	0.116	12978	M	4	4/8/2012	1.26
SAWYER	LAC COURTE OREILLES	15.3	0.152	12979	M	4	4/8/2012	1.21
SAWYER	LAC COURTE OREILLES	15.0	0.103	12980	M	4	4/8/2012	1.21
SAWYER	LAC COURTE OREILLES	14.6	0.122	12981	M	4	4/8/2012	1.06
SAWYER	LAC COURTE OREILLES	17.7	0.352	12982	M	8	4/8/2012	1.97
SAWYER	L CHIPPEWA	16.0	0.445	11787	M	7	3/26/2012	1.26
SAWYER	L CHIPPEWA	18.1	0.392	11788	M	8	3/26/2012	1.99
SAWYER	L CHIPPEWA	15.8	0.198	11790	M	3	3/26/2012	0.81
SAWYER	L CHIPPEWA	19.3	0.436	11791	F	9	3/26/2012	2.32
SAWYER	L CHIPPEWA	13.5	0.353	11792	M	7	3/26/2012	1.31
SAWYER	L CHIPPEWA	19.3	0.473	11793	F	7	3/26/2012	1.21
SAWYER	L CHIPPEWA	18.7	0.333	11794	F	6	3/26/2012	2.37
SAWYER	L CHIPPEWA	15.8	0.534	11795	M	8	3/26/2012	2.56
SAWYER	L CHIPPEWA	14.2	0.313	11796	M	5	3/28/2012	1.06
SAWYER	L CHIPPEWA	14.7	0.235	11797	M	4	3/28/2012	1.14
SAWYER	L CHIPPEWA	14.9	0.584	11798	M	4	3/28/2012	0.96
SAWYER	L CHIPPEWA	14.5	0.304	11800	M	5	3/28/2012	1.1
VILAS	MAMIE L	16.8	0.318	11491	F	7	4/6/2012	0.93
VILAS	MAMIE L	25.5	0.876	11494	F	12	4/6/2012	4.96
VILAS	MAMIE L	13.8	0.295	12091	M	5	4/6/2012	1
VILAS	MAMIE L	15.8	0.387	12092	M	7	4/6/2012	1.12
VILAS	MAMIE L	16.2	0.212	12093	U	6	4/6/2012	1.16
VILAS	MAMIE L	23.5	0.46	12094	F	8	4/6/2012	4.36
VILAS	MAMIE L	14.2	0.339	12095	M	6	4/6/2012	1.12
VILAS	MAMIE L	12.2	0.193	12096	M	6	4/6/2012	0.92
VILAS	MAMIE L	21.6	0.62	12097	F	8	4/6/2012	4.46
VILAS	MAMIE L	21.5	0.338	12098	F	11	4/6/2012	4.38
VILAS	MAMIE L	18.0	0.292	12099	F	6	4/6/2012	1.16
VILAS	MAMIE L	23.6	0.467	12100	F	8	4/6/2012	4.39
BAYFIELD	MIDDLE EAU CLAIRE L	15.9	0.258	12317	M	5	3/31/2012	1.19
BAYFIELD	MIDDLE EAU CLAIRE L	18.0	0.46	12318	M	7	3/31/2012	1.9
BAYFIELD	MIDDLE EAU CLAIRE L	14.6	0.198	12321	M	5	3/31/2012	1.05
BAYFIELD	MIDDLE EAU CLAIRE L	17.0	0.511	12322	M	9	3/31/2012	1.61

BAYFIELD	MIDDLE EAU CLAIRE L	14.5	0.282	12323	M	4	3/31/2012	0.94
BAYFIELD	MIDDLE EAU CLAIRE L	13.4	0.16	12324	M	4	3/31/2012	0.74
BAYFIELD	MIDDLE EAU CLAIRE L	18.0	0.542	12325	M	8	3/31/2012	1.78
BAYFIELD	MIDDLE EAU CLAIRE L	14.6	0.306	12326	M	5	3/31/2012	1.11
BAYFIELD	MIDDLE EAU CLAIRE L	18.2	0.588	12488	M	10	3/31/2012	1.92
BAYFIELD	MIDDLE EAU CLAIRE L	15.5	0.27	12990	M	5	3/31/2012	1.16
VILAS	OXBOW L	18.2	1.05	12907	F	9	4/7/2012	2.25
VILAS	OXBOW L	23.8	1.06	12912	F	10	4/7/2012	1.1
VILAS	OXBOW L	18.6	0.616	12913	M	9	4/7/2012	5.32
VILAS	OXBOW L	15.3	0.698	12914	M	7	4/7/2012	2.28
VILAS	OXBOW L	15.5	0.782	12915	M	6	4/7/2012	1.12
VILAS	OXBOW L	15.3	0.777	12916	M	6	4/7/2012	1.02
VILAS	OXBOW L	25.2	1.1	12917	F	13	4/7/2012	6.68
VILAS	OXBOW L	18.4	0.734	12918	M	7	4/7/2012	1.94
VILAS	OXBOW L	13.9	0.565	12919	M	5	4/7/2012	0.76
VILAS	OXBOW L	12.8	0.522	12920	M	5	4/7/2012	0.6
VILAS	OXBOW L	13.4	0.492	12921	M	4	4/7/2012	0.7
ONEIDA	PELICAN L	13.0	0.098	12776	M	2	3/23/2012	0.66
ONEIDA	PELICAN L	17.0	0.246	12777	M	6	3/23/2012	1.55
ONEIDA	PELICAN L	15.3	0.165	12778	M	5	3/23/2012	1.14
ONEIDA	PELICAN L	18.6	0.292	12779	M	8	3/23/2012	2.06
ONEIDA	PELICAN L	15.4	0.147	12780	M	4	3/23/2012	1.23
ONEIDA	PELICAN L	18.8	0.379	12781	M	9	3/23/2012	2.46
ONEIDA	PELICAN L	16.6	0.199	12782	M	5	3/23/2012	1.41
ONEIDA	PELICAN L	14.7	0.117	12783	M	5	3/25/2012	1.15
ONEIDA	PELICAN L	14.3	0.16	12784	M	3	3/25/2012	0.78
ONEIDA	PELICAN L	22.2	0.371	12785	F	10	3/25/2012	3.95
ONEIDA	PELICAN L	24.2	0.524	12786	U	12	3/25/2012	5.31
ONEIDA	PELICAN L	23.7	0.408	12787	F	8	3/25/2012	4.14
ONEIDA	PLANTING GROUND L	13.5	0.472	11979	M	5	3/24/2012	0.78
ONEIDA	PLANTING GROUND L	14.2	0.304	11980	M	6	3/24/2012	0.89
ONEIDA	PLANTING GROUND L	13.0	0.249	11981	M	5	3/24/2012	0.67
ONEIDA	PLANTING GROUND L	16.2	0.392	11982	F	6	3/24/2012	1.49
ONEIDA	PLANTING GROUND L	17.3	0.343	11983	U	7	3/24/2012	1.58
ONEIDA	PLANTING GROUND L	16.0	0.204	11984	F	6	3/24/2012	1.53
ONEIDA	PLANTING GROUND L	24.6	1.14	11985	F	13	3/24/2012	6.15
ONEIDA	PLANTING GROUND L	23.5	1.33	11986	F	11	3/24/2012	4.89
ONEIDA	PLANTING GROUND L	23.8	0.591	11987	F	9	3/25/2012	5.04
ONEIDA	PLANTING GROUND L	24.6	0.822	11988	F	10	3/25/2012	5.27
VILAS	PRESQUE ISLE L	19.5	0.418	12922	M	11	3/31/2012	2.08
VILAS	PRESQUE ISLE L	22.4	0.286	12923	F	9	3/31/2012	4.25
VILAS	PRESQUE ISLE L	14.0	0.214	12924	M	6	3/31/2012	0.88
VILAS	PRESQUE ISLE L	19.5	0.415	12925	F	7	4/7/2012	2.24
VILAS	PRESQUE ISLE L	19.3	0.29	12926	F	5	4/7/2012	2.8
VILAS	PRESQUE ISLE L	24.8	0.386	12930	F	8	4/7/2012	6.05
VILAS	PRESQUE ISLE L	13.0	0.172	12932	M	3	3/31/2012	0.57
VILAS	PRESQUE ISLE L	19.0	0.203	12933	F	7	3/31/2012	2.11
VILAS	PRESQUE ISLE L	16.3	0.191	12934	M	6	3/31/2012	1.36
VILAS	PRESQUE ISLE L	16.2	0.125	12935	F	4	3/31/2012	1.23
VILAS	PRESQUE ISLE L	14.3	0.154	12936	M	4	3/31/2012	0.76
VILAS	PRESQUE ISLE L	22.5	0.417	12951	F	9	4/7/2012	4.19
BARRON	RED CEDAR L	14.0	0.374	12277	M	3	4/6/2012	0.96
BARRON	RED CEDAR L	14.1	0.328	12290	M	5	4/6/2012	1.23
BARRON	RED CEDAR L	14.4	0.26	12356	M	4	4/6/2012	1.04
BARRON	RED CEDAR L	14.6	0.292	12358	M	5	4/6/2012	1.06
BARRON	RED CEDAR L	15.8	0.408	12372	M	6	4/6/2012	1.43
SAWYER	ROUND L	14.7	0.13	11772	M	4	4/10/2012	0.88
SAWYER	ROUND L	17.5	0.21	11773	M	6	4/10/2012	1.46
SAWYER	ROUND L	14.4	0.114	11774	M	4	4/10/2012	0.83
SAWYER	ROUND L	16.7	0.198	11775	M	7	4/10/2012	1.27
SAWYER	ROUND L	14.7	0.164	11776	M	5	4/10/2012	0.83
SAWYER	ROUND L	15.2	0.12	11777	M	4	4/10/2012	1.01
SAWYER	ROUND L	19.2	0.205	11778	M	7	4/10/2012	2.16
SAWYER	ROUND L	20.6	0.203	11779	F	7	4/10/2012	2.94
SAWYER	ROUND L	22.1	0.268	11780	F	7	4/10/2012	3.66
SAWYER	ROUND L	21.1	0.418	11784	M	14	4/10/2012	2.84
SAWYER	ROUND L	22.8	0.305	11785	M	12	4/10/2012	4.11
SAWYER	ROUND L	22.0	0.477	11786	M	10	4/10/2012	3.78
VILAS	SCATTERING RICE L	24.1	1.12	12940	F	10	4/12/2012	5.22
VILAS	SCATTERING RICE L	14.7	0.208	12943	M	4	4/12/2012	1.15
VILAS	SCATTERING RICE L	12.5	0.233	12944	M	4	4/12/2012	0.63

VILAS	SCATTERING RICE L	22.7	0.575	12945	F	15	4/12/2012	4.55
WASHBURN	SHELL L	15.3	0.382	12266	M	7	4/11/2012	1.18
WASHBURN	SHELL L	18.2	0.544	12268	F	8	4/11/2012	1.85
WASHBURN	SHELL L	12.8	0.244	12289	M	4	4/11/2012	0.66
WASHBURN	SHELL L	16.4	0.306	12316	M	5	4/11/2012	1.33
WASHBURN	SHELL L	14.3	0.259	12348	M	6	4/11/2012	0.83
WASHBURN	SHELL L	13.8	0.457	12351	M	6	4/11/2012	0.81
WASHBURN	SHELL L	16.0	0.32	12369	M	6	4/11/2012	1.29
WASHBURN	SHELL L	21.1	0.703	12670	F	8	4/11/2012	3.21
BAYFIELD	SISKIWIT L	15.2	0.706	12459	M	6	3/22/2012	1.08
BAYFIELD	SISKIWIT L	16.4	0.872	12513	M	8	3/22/2012	1.34
BAYFIELD	SISKIWIT L	12.4	0.527	12514	M	3	3/22/2012	0.64
BAYFIELD	SISKIWIT L	18.3	1.11	12549	F	10	3/22/2012	2.09
BAYFIELD	SISKIWIT L	13.5	0.464	12552	M	4	3/22/2012	0.78
BAYFIELD	SISKIWIT L	13.8	0.541	12554	M	5	3/22/2012	0.81
BAYFIELD	SISKIWIT L	15.6	0.692	12559	M	7	3/22/2012	1.17
BAYFIELD	SISKIWIT L	19.0	0.923	12560	F	9	3/22/2012	2.35
BAYFIELD	SISKIWIT L	19.9	1.04	12561	F	11	3/22/2012	2.7
VILAS	SQUAW L	13.4	0.486	12059	M	5	4/24/2012	0.82
VILAS	SQUAW L	23.7	1.03	12231	F	11	4/24/2012	5.66
VILAS	SQUAW L	27.2	0.924	12548	F	12	4/24/2012	7.75
VILAS	SQUAW L	21.9	0.653	12557	F	7	4/24/2012	3.55
VILAS	SQUAW L	17.8	0.882	12591	F	5	3/23/2012	2.19
VILAS	SQUAW L	12.7	0.574	12592	M	6	3/23/2012	0.62
VILAS	SQUAW L	13.5	0.512	12593	M	5	3/23/2012	0.87
VILAS	SQUAW L	15.2	0.594	12594	M	8	3/23/2012	1.31
VILAS	SQUAW L	15.7	0.581	12595	M	7	3/23/2012	1.04
VILAS	SQUAW L	24.4	0.786	12596	F	9	4/24/2012	5.44
ONEIDA	SQUIRREL L	24.4	0.213	12132	M	3	3/26/2012	0.71
ONEIDA	SQUIRREL L	13.2	0.281	12133	M	5	3/26/2012	0.81
ONEIDA	SQUIRREL L	13.9	0.178	12134	M	5	3/26/2012	1.07
ONEIDA	SQUIRREL L	15.1	0.242	12135	M	6	3/26/2012	1.27
ONEIDA	SQUIRREL L	15.7	0.24	12136	M	5	3/26/2012	1.17
ONEIDA	SQUIRREL L	15.0	0.273	12137	F	7	3/26/2012	2.72
ONEIDA	SQUIRREL L	19.5	0.324	12138	F	8	3/26/2012	2.53
ONEIDA	SQUIRREL L	18.9	0.661	12139	F	10	3/26/2012	5.48
ONEIDA	SQUIRREL L	23.5	0.448	12140	F	8	3/26/2012	3.75
ONEIDA	SQUIRREL L	21.9	0.319	12141	M	8	3/26/2012	2.94
ONEIDA	SQUIRREL L	20.4	0.365	12142	F	8	3/26/2012	3.47
ONEIDA	SQUIRREL L	21.6	0.696	12143	F	11	3/26/2012	5.53
ONEIDA	SQUIRREL L	24.7	0.204	12458	M	3	3/26/2012	0.53
WASHBURN	STONE L	13.9	0.201	11757	M	3	4/4/2012	0.78
WASHBURN	STONE L	23.4	0.909	11758	F	7	4/4/2012	4.07
WASHBURN	STONE L	19.9	0.402	11759	F	6	4/4/2012	2.42
WASHBURN	STONE L	15.8	0.242	11760	M	3	4/4/2012	1.09
WASHBURN	STONE L	19.8	0.412	11761	F	5	4/7/2012	2.4
WASHBURN	STONE L	13.0	0.241	11762	M	4	4/4/2012	0.58
WASHBURN	STONE L	19.3	0.495	11763	F	7	4/7/2012	2.85
WASHBURN	STONE L	16.4	0.251	11767	M	5	4/4/2012	1.18
WASHBURN	STONE L	16.4	0.442	11768	M	5	4/4/2012	1.29
WASHBURN	STONE L	22.6	0.521	11769	F	6	4/4/2012	3.77
WASHBURN	STONE L	24.3	0.846	11770	F	10	4/4/2012	4.37
WASHBURN	STONE L	14.1	0.226	11771	M	3	4/4/2012	0.93
VILAS	TENDERFOOT L	23.2	0.546	11479	F	8	4/6/2012	4.47
VILAS	TENDERFOOT L	18.2	0.551	11480	M	10	4/6/2012	2.31
VILAS	TENDERFOOT L	18.2	0.537	11481	M	7	4/6/2012	2.15
VILAS	TENDERFOOT L	14.0	0.318	11482	M	7	4/6/2012	0.87
VILAS	TENDERFOOT L	16.6	0.426	11483	M	7	4/6/2012	1.46
VILAS	TENDERFOOT L	14.7	0.429	11484	M	6	4/6/2012	1.01
VILAS	TENDERFOOT L	17.5	0.416	11485	F	6	4/6/2012	1.88
VILAS	TENDERFOOT L	14.0	0.254	11486	M	6	4/6/2012	0.86
VILAS	TENDERFOOT L	23.9	0.601	11487	F	11	4/6/2012	5.59
VILAS	TENDERFOOT L	26.3	0.485	11488	F	9	4/6/2012	7.63
VILAS	TENDERFOOT L	16.9	0.399	11489	F	7	4/6/2012	1.48
VILAS	TENDERFOOT L	18.5	0.399	11490	F	7	4/6/2012	2.19
VILAS	TROUT L	28.0	0.737	11914	U	14	4/11/2012	6.22
VILAS	TROUT L	18.0	0.154	12953	M	6	4/10/2012	1.8
VILAS	TROUT L	13.3	0.137	12954	M	4	4/10/2012	0.7
VILAS	TROUT L	16.5	0.172	12956	M	5	4/10/2012	1.31
VILAS	TROUT L	25.4	0.349	12958	F	10	4/10/2012	6.22
VILAS	TROUT L	23.9	0.362	12959	F	11	4/10/2012	5.14

VILAS	TROUT L	17.2	0.28	12960	M	10	4/10/2012	1.53
VILAS	TROUT L	14.6	0.127	12961	M	4	4/10/2012	0.97
VILAS	TROUT L	18.3	0.175	12963	F	6	4/10/2012	2.06
VILAS	TROUT L	14.2	0.125	12964	M	4	4/10/2012	0.85
VILAS	TROUT L	21.2	0.318	12965	F	12	4/10/2012	3.25
VILAS	TROUT L	16.0	0.158	12966	M	7	4/10/2012	1.23
IRON	TURTLE-FLAMBEAU FL	23.0	0.884	11912	F	12	4/9/2012	4.84
IRON	TURTLE-FLAMBEAU FL	14.0	0.378	11964	M	4	4/8/2012	0.93
IRON	TURTLE-FLAMBEAU FL	14.9	0.227	11965	M	4	4/8/2012	1.1
IRON	TURTLE-FLAMBEAU FL	12.3	0.381	11966	M	4	4/8/2012	0.56
IRON	TURTLE-FLAMBEAU FL	15.1	0.247	11967	M	4	4/8/2012	1.16
IRON	TURTLE-FLAMBEAU FL	15.2	0.448	11968	M	6	4/8/2012	0.95
IRON	TURTLE-FLAMBEAU FL	16.1	0.405	11969	M	6	4/8/2012	1.31
IRON	TURTLE-FLAMBEAU FL	18.8	0.575	11970	F	9	4/8/2012	1.82
IRON	TURTLE-FLAMBEAU FL	20.6	0.757	11971	F	9	4/8/2012	2.98
IRON	TURTLE-FLAMBEAU FL	18.3	0.42	11972	M	9	4/8/2012	1.87
VILAS	TWIN L CHAIN	16.5	0.374	11496	M	12	3/29/2012	1.25
VILAS	TWIN L CHAIN	12.7	0.123	11498	M	5	3/29/2012	0.78
VILAS	TWIN L CHAIN	16.9	0.137	11499	M	3	3/29/2012	1.83
VILAS	TWIN L CHAIN	19.7	0.256	11692	F	9	3/29/2012	2.48
VILAS	TWIN L CHAIN	24.3	0.218	11694	F	10	3/29/2012	5.04
VILAS	TWIN L CHAIN	21.1	0.271	11695	M	10	3/29/2012	3.83
VILAS	TWIN L CHAIN	18.3	0.232	11696	M	10	3/29/2012	2.12
VILAS	TWIN L CHAIN	22.4	0.176	11697	F	7	3/29/2012	4.98
VILAS	TWIN L CHAIN	14.9	0.108	11698	M	5	3/29/2012	1.01
VILAS	TWIN L CHAIN	17.0	0.198	11699	M	8	3/29/2012	2.16
VILAS	TWIN L CHAIN	14.7	0.231	11700	M	10	3/29/2012	1.03
VILAS	TWIN L CHAIN	23.1	0.324	11788	F	10	4/4/2012	4.75
DOUGLAS	WHITEFISH L	14.9	0.134	12014	M	3	4/3/2012	1
DOUGLAS	WHITEFISH L	16.8	0.452	12187	M	9	4/3/2012	1.64
DOUGLAS	WHITEFISH L	19.8	0.505	12263	M	10	4/3/2012	2.76
DOUGLAS	WHITEFISH L	20.8	0.724	12339	M	12	4/10/2012	2.85
DOUGLAS	WHITEFISH L	17.2	0.311	12340	M	5	4/10/2012	1.48
DOUGLAS	WHITEFISH L	21.2	0.525	12341	M	8	4/10/2012	2.75
DOUGLAS	WHITEFISH L	16.5	0.184	12342	M	6	4/10/2012	1.42
DOUGLAS	WHITEFISH L	18.2	0.341	12489	M	6	4/3/2012	1.83
DOUGLAS	WHITEFISH L	16.1	0.115	12535	M	4	4/3/2012	1.29
DOUGLAS	WHITEFISH L	23.4	0.603	12558	F	9	4/10/2012	3.92
DOUGLAS	WHITEFISH L	14.6	0.165	12625	M	3	4/3/2012	0.9
DOUGLAS	WHITEFISH L	15.6	0.207	12626	M	4	4/3/2012	1.11

APPENDIX 1

LSRI Analytical and QA/QC Reports on Mercury Analysis of Walleye Collected and During Spring 2011

Total Mercury Concentrations in Muscle Tissue from Walleye Collected from Inland Lakes and the Kakagon Slough during Spring 2012 in Wisconsin, Michigan and Minnesota Ceded Territory Waters

by

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Introduction

Skinless fillet samples from walleye (*Sander vitreus*) captured during the spring of 2012 from the 1837 and 1842 Treaty ceded territories were analyzed for total mercury (Hg) content at the University of Wisconsin-Superior's Lake Superior Research Institute (LSRI). Three hundred eighty seven skinless walleye fillets, from a total of thirty seven inland lakes (thirty five in Wisconsin, one in Michigan and one in Minnesota) and the Kakagon Slough (Wisconsin) collected by tribal spearers and GLIFWC Inland Fisheries assessment crews, were analyzed.

Methods

At the time fish were captured, a tribal warden or creel clerk 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 in the field via extrusion. If it could not be determined in the field, sex was determined via direct examination of the gonads at the time the fillet was removed in the GLIFWC lab. A dorsal fin spine was removed from each fish to determine its age. At the LSRI laboratories, the walleye fillets 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.7). 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.6). 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.7).

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. Five 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 three 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.6). Mercury solutions for making tissue spikes and preparing analytical standards were prepared following the procedures in SOP SA/42 v.2. 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 re-digested and re-analyzed. Mercury concentrations and quality assurance calculations were done in Microsoft Excel according to SOP SA/37 v.1. The biota method detection limit was 0.0030 µg Hg/g for an average sample mass of 0.21 g (Appendix A). This limit of detection was determined using a ground tuna sample (6-2-11) 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.4). A portion (1.0 to 5.0 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 17 to 120 hours. After the initial drying and weighing, the samples were returned to the oven for a minimum of an additional 24 hours and then reweighed to confirm that the tissue samples were dry. Approximately 30 percent of the walleye analyzed for mercury had moisture content determined. Three fish per lake were randomly selected for determination of percent moisture. Ten percent of these fish were analyzed for moisture in duplicate.

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. Several sets of analytical standards with known amounts of mercury were analyzed with each group (maximum of 40 samples plus QA samples) of tissue samples. The concentrations of the mercury standards analyzed with each set of samples were 0, 100, 500, 1000, 5000, and 10,000 ng Hg/L. 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 limits established in the Quality Assurance Project Plan (QAPP) for this project approved in June 2011. Results for the procedural blanks were considered acceptable when the relative percent difference was < 50%. Duplicate agreement values were acceptable when having a relative percent difference < 25%. The acceptable range for the DORM standard reference material was 75 to 125% 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 the calculated mean recovery was 70 to 130% of the spike. If a spike recovery did not fall within the acceptable range, the sample was spiked and analyzed again during a succeeding analysis set.

A quality assurance audit was conducted by the LSRI quality assurance manager during the Spring Walleye 2012 project on July 31 and August 2. That report is provided in Appendix C.

Results of Fish Tissue Analyses

Quality Assurance – Five tuna procedural blanks were processed coincident with the grinding of walleye collected for the project. One of the five procedural blanks was digested with each set of mercury samples for a total of twelve analyses resulting in a mean of 17.6 ± 9.7 relative percent difference (Table 1). The relative percent difference values ranged from 2.5 to 37.3%, all were within the acceptable range of <50%. On September 5, an instrument malfunction occurred prior to the end of the analysis. The tuna sample which was digested with that set was not analyzed. All of the tuna samples were found to have very low mercury concentrations.

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 individual recovery values ranged from 74.0 to 111.8% with the grand mean and standard deviation of the recoveries being 92.3 ± 5.7 percent of the certified value. All of the DORM-3 reference sample daily mean values were within the acceptance range. One individual value (DORM 3-3) from 6-15-12 was outside the acceptance range but the daily mean was within the acceptance range. Due to larger than usual changes in the calibration standards analyzed on 6-15-12 and the one DORM-3 Reference Standard falling outside the acceptance range the entire set was digested and reanalyzed in two sets analyzed on 9-7-12 and 9-11-12. No sample data is reported from the analysis done on 6-15-12.

Fish tissues were analyzed for mercury in duplicate forty four times. Two portions of the same tissue were digested and analyzed independently. The relative percent difference between duplicate analyses of the same tissue ranged from 0 to 24.8% with the average and standard deviation of the differences being 5.7 ± 5.5% (Table 3).

Samples of tissue were spiked in duplicate with known concentrations of mercury prior to digestion. Mean recovery for the forty six spiked samples was 92.9 ± 8.2 percent with the reported individual average recovery values ranging from 59.4 to 104.2% (Table 4). The Butternut Lake (Price County) 11939 sample was spiked on three separate occasions. The resulting average recoveries for the first two times were below the 70% acceptance limit. When this sample was spiked for a third time (9-11-12), only about 0.12 grams was used (half the mass that was used for the previous two spikes) and this gave us an acceptable spike recovery of 85.1%. The value reported for the Butternut Lake 11939 sample is the value obtained from the 9-11-12 analysis. The percent difference between the highest and lowest values obtained for the sample on the three analysis dates was 3.4%.

Mercury Analysis – Skinless fillets of 387 walleye collected from a total of 37 inland lakes in Wisconsin, Michigan and Minnesota (thirty-five in Wisconsin, one in Michigan and one in Minnesota) and the Kakagon Slough (Wisconsin) were analyzed for total mercury concentration. Total mercury concentrations on a wet weight basis (Table 5) ranged from 0.045 to 1.37 µg Hg/g (parts per million).

Tissue Moisture Analysis – Percent moisture was measured in 116 of the 387 walleye tissues. Moisture analysis took place immediately following grinding of the fillets. The data obtained

drying and weighing the samples twice indicates that drying for 17 hours was sufficient to remove the moisture from the samples used for moisture determination. The longer drying time reported was due to samples that were placed in the oven and not removed for 5 days because of a long weekend.

Walleye muscle tissue had a mean moisture value of 78.8 ± 1.0 percent (Table 6). Of the 116 tissues analyzed for moisture, twelve were analyzed in duplicate, all yielding relative differences of 0.0 to 1.0 percent. All samples were dried a minimum of an additional 24 hours and reweighed to ensure dryness, all yielding relative percent differences of <0.1 percent.

Table 1. Relative Percent Difference of Total Mercury for Procedural Blank Samples (Before and After Grinding). Data quality indicator for laboratory bias is <50% relative percent difference.

Analysis Date	Grinding Date	Before Grinding µg Hg/g	After Grinding µg Hg/g	Mean µg Hg/g	Relative Percent Difference
6/12/2012	6/1/2012	0.049	0.039	0.044	22.7
6/15/2012	6/1/2012	0.012	0.009	0.011	27.5
6/19/2012	6/1/2012	0.041	0.034	0.038	18.7
6/28/2012	6/1/2012	0.039	0.040	0.040	2.5
7/12/2012	7/2/2012	0.046	0.036	0.041	24.4
7/13/2012	7/2/2012	0.035	0.024	0.030	37.3
7/20/2012	7/17/2012	0.059	0.070	0.065	17.1
8/1/2012	7/17/2012	0.072	0.066	0.069	8.7
8/17/2012	8/2/2002	0.062	0.053	0.058	15.7
8/21/2012	8/15/2012	0.025	0.023	0.024	8.3
*9/5/2012	8/15/2012	-	-	-	-
9/7/2012	8/15/2012	0.02	0.022	0.021	9.5
9/11/2012	6/1/2012	0.04	0.033	0.037	19.2
			Mean ± Std. Dev.		**17.6 ± 9.7**

* An instrument problem occurred after the sample for Trout Lake 12963 was run so no other samples were analyzed on that date. The tuna procedural blank was not analyzed on 9/5/2012.

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 ± 0.060 µg Hg/g Tissue. Data quality indicator for accuracy is 75.0 to 125% agreement between the certified concentration and the measured value for the reference standard.

Date of Analysis	DORM 3-1		DORM 3-2		DORM 3-3		Mean
	µg Hg/g	% of Certified Value	µg Hg/g	% of Certified Value	µg Hg/g	% of Certified Value	
6/12/2012	0.350	91.5	0.353	92.5	0.324	84.9	89.6
6/15/2012	0.377	98.8	0.324	84.7	0.282	74.0	85.8
6/19/2012	0.367	96.0	0.340	89.1	0.369	96.6	93.9
6/28/2012	0.375	98.1	0.390	102.1	0.379	99.2	99.8
7/12/2012	0.393	102.8	0.406	106.2	0.389	101.9	103.6
7/13/2012	0.345	90.4	0.339	88.7	0.309	80.9	86.7
7/20/2012	0.343	89.8	0.361	94.6	0.366	95.9	93.4
8/1/2012	0.357	93.5	0.329	86.2	0.327	85.7	88.5
8/17/2012	0.352	92.1	0.344	90.1	0.427	111.8	98.0
8/21/2012	0.359	93.9	0.363	95.0	0.364	95.2	94.7
*9/5/2012	0.338	88.5	0.339	88.7	-	-	88.6
9/7/2012	0.361	94.5	0.354	92.8	0.344	90.0	92.4
9/11/2012	0.330	86.5	0.329	86.0	0.317	83.1	85.2
						Mean ± Std. Dev.	**92.3 ± 5.7**

* An instrument problem occurred after the sample for Trout Lake 12963 was run so no other samples were analyzed on that date. Dorm 3-3 was not analyzed on 9/5/2012.

Table 3. Relative Percent Difference for Duplicate Analysis of Total Mercury Content in Skinless Walleye Fillet Tissue. Data quality indicator for precision is <25% relative percent difference.

Date of Analysis	Lake and Tag Number	µg Hg/g	Duplicate µg Hg/g	Mean µg Hg/g	Relative Percent Difference
6/12/2012	Cranberry Lake 12796	0.176	0.179	0.178	1.7
6/12/2012	Dunn Lake 12307	0.392	0.387	0.390	1.3
6/12/2012	Lac Courte Oreilles 12979	0.153	0.151	0.152	1.3
6/12/2012	Middle Eau Claire Lake 12326	0.296	0.315	0.306	6.2
6/19/2012	Atkins Lake 12556	0.441	0.434	0.438	1.6
6/19/2012	Lake Chippewa 11794	0.313	0.353	0.333	12.0
6/19/2012	Planting Ground Lake 11982	0.381	0.402	0.392	5.4
6/19/2012	Shell Lake 12348	0.260	0.258	0.259	0.8
6/28/2012	Presque Isle Lake 12930	0.394	0.377	0.386	4.4
6/28/2012	Squirrel Lake 12133	0.273	0.289	0.281	5.7
6/28/2012	Squirrel Lake 12142	0.369	0.360	0.365	2.5
6/28/2012	Tenderfoot Lake 11488	0.479	0.490	0.485	2.3

Date of Analysis	Lake and Tag Number	µg Hg/g	Duplicate µg Hg/g	Mean µg Hg/g	Relative Percent Difference
7/12/2012	Kakagon River 12066	0.219	0.217	0.218	0.9
7/12/2012	Red Cedar Lake 12290	0.306	0.350	0.328	13.4
7/12/2012	Siskiwit Lake 12554	0.529	0.553	0.541	4.4
7/12/2012	Squaw Lake 12594	0.608	0.580	0.594	4.7
7/13/2012	Big Sand Lake 11286	0.239	0.281	0.260	16.2
7/13/2012	Butternut Lake 12604	0.047	0.058	0.053	21.0
7/13/2012	Connors Lake 12292	0.363	0.355	0.359	2.2
7/13/2012	Turtle-Flambeau Flowage 11968	0.437	0.458	0.448	4.7
7/20/2012	Bearskin Lake 12765	0.141	0.146	0.144	3.5
7/20/2012	Whitefish Lake 12263	0.567	0.442	0.505	24.8
7/20/2012	Whitefish Lake 12626	0.199	0.215	0.207	7.7
7/20/2012	Mamie Lake 12099	0.292	0.292	0.292	0.0
8/1/2012	Duck Lake 12512	0.158	0.166	0.162	4.9
8/1/2012	Scattering Rice Lake 12944	0.227	0.239	0.233	5.2
8/1/2012	Twin Lake Chain 11698	0.110	0.106	0.108	3.7
8/17/2012	Annabelle Lake 12391	0.651	0.744	0.698	13.3
8/17/2012	Big Gibson Lake 11909	0.633	0.636	0.635	0.5
8/17/2012	Eagle Lake 12948	0.239	0.245	0.242	2.5
8/17/2012	Oxbow Lake 12918	0.742	0.725	0.734	2.3
8/21/2012	Big Arbor Vitae Lake 12902	0.135	0.147	0.141	8.5
8/21/2012	Big Lake 11919	0.243	0.230	0.237	5.5
8/21/2012	Pelican Lake 12786	0.538	0.510	0.524	5.3
9/5/2012	Stone Lake 11762	0.247	0.234	0.241	5.4
9/5/2012	Stone Lake 11770	0.869	0.823	0.846	5.4
9/5/2012	Trout Lake 12953	0.158	0.149	0.154	5.9
*9/5/2012	Trout Lake 12965	-	-	-	-
*9/5/2012	Butternut Lake (Price) 11936	-	-	-	-
9/7/2012	Trout Lake 12965	0.321	0.314	0.318	2.2
9/7/2012	Butternut Lake(Price) 11936	0.509	0.468	0.489	8.4
9/7/2012	Round Lake 11784	0.413	0.423	0.418	2.4
9/11/2012	Lake Gogebic 11750	0.164	0.166	0.165	1.2
9/11/2012	Mille Lacs 12747	0.202	0.204	0.203	1.0
9/11/2012	Mille Lacs 12756	0.063	0.073	0.068	14.7
9/11/2012	Butternut Lake(Price) 11939	1.37	1.34	1.36	2.2
			Mean ± Std. Dev.		**5.7 ± 5.5**

* An instrument problem occurred after the sample for Trout Lake 12963 was run so no other samples were analyzed on that date. Trout Lake 12965 and Butternut Lake (Price) 11936 duplicate samples were not analyzed on 9/5/2012 but were re-digested and analyzed on 9/7/2012.

Table 4. Percent of Mercury Recovered from Skinless Walleye Fillet Samples Spiked with a Known Concentration of Mercury. Data quality indicator for accuracy is a spike recovery of 70 to 130%.

Date of Analysis	Lake and Tag Number	Spike #1	Spike #2	Mean Spike Recovery	Std. Dev.
6/12/2012	Cranberry Lake 12796	100.5	98.3	99.4	1.53
6/12/2012	Dunn Lake 12307	98.4	96.7	97.6	1.16
6/12/2012	Lac Courte Oreilles 12979	96.7	98.1	97.4	0.98
6/12/2012	Middle Eau Claire Lake 12326	97.1	95.4	96.3	1.18
6/19/2012	Atkins Lake 12556	93.4	91.0	92.2	1.71
6/19/2012	Lake Chippewa 11794	93.0	89.1	91.0	2.73
6/19/2012	Planting Ground Lake 11982	91.4	90.2	90.8	0.79
6/19/2012	Shell Lake 12348	88.8	88.7	88.8	0.13
6/28/2012	Presque Isle Lake 12930	102.8	98.6	100.7	2.96
6/28/2012	Squirrel Lake 12133	99.2	100.8	100.0	1.08
6/28/2012	Squirrel Lake 12142	97.8	96.3	97.0	1.06
6/28/2012	Tenderfoot Lake 11488	90.0	89.0	89.5	0.72
7/12/2012	Kakagon Slough 12066	96.2	101.5	98.9	3.75
7/12/2012	Red Cedar Lake 12290	101.2	107.3	104.2	4.32
7/12/2012	Siskiwit lake 12554	97.0	94.8	95.9	1.58
7/12/2012	Squaw Lake 12594	92.6	86.0	89.3	4.65
7/13/2012	Big Sand Lake 11286	101.8	99.1	100.5	1.87
7/13/2012	Butternut Lake 12604	104.1	102.8	103.4	0.92
7/13/2012	Connors Lake 12292	96.2	96.5	96.3	0.25
7/13/2012	Turtle-Flambeau Flowage 11968	91.8	87.3	89.6	3.16
7/20/2012	Bearskin Lake 12765	98.8	96.8	97.8	1.41
7/20/2012	Whitefish Lake 12263	96.2	91.2	93.7	3.54
7/20/2012	Whitefish Lake 12626	97.7	95.3	96.5	1.72
7/20/2012	Mamie Lake 12099	94.3	95.1	94.7	0.52
8/1/2012	Butternut Lake (Price) 11939	56.4	62.5	59.4	4.30
8/1/2012	Duck Lake 12512	97.9	94.7	96.3	2.25
8/1/2012	Scattering Rice Lake 12944	95.3	93.2	94.3	1.50
8/1/2012	Twin Lake Chain 11698	94.1	95.4	94.7	0.93
8/17/2012	Annabelle Lake 12391	87.5	81.3	84.4	4.38
8/17/2012	Big Gibson Lake 11909	85.8	81.0	83.4	3.39
8/17/2012	Eagle Lake 12948	98.2	102.4	100.3	3.01
8/17/2012	Oxbow Lake 12918	91.5	95.8	93.7	3.04
8/21/2012	Big Arbor Vitae Lake 12902	96.7	96.3	96.5	0.30
8/21/2012	Big Lake 11919	91.2	96.5	93.9	3.74
8/21/2012	Butternut Lake (Price) 11939	68.0	71.5	69.7	2.49
8/21/2012	Pelican Lake 12786	89.4	96.2	92.8	4.80

Date of Analysis	Lake and Tag Number	Spike #1	Spike #2	Mean Spike Recovery	Std. Dev.
9/5/2012	Stone Lake 11762	95.8	91.7	93.8	2.94
9/5/2012	Stone Lake 11770	77.6	76.5	77.1	0.78
9/5/2012	Trout Lake 12953	95.0	96.0	95.5	0.70
*9/5/2012	Trout Lake 12965	-	-	-	-
*9/5/2012	Butternut Lake (Price) 11936	-	-	-	-
9/7/2012	Trout Lake 12965	88.4	86.4	87.4	1.45
9/7/2012	Butternut Lake (Price) 11936	88.4	87.9	88.1	0.32
9/7/2012	Round Lake 11784	94.7	92.0	93.4	1.90
9/11/2012	Lake Gogebic 11750	100.0	100.3	100.2	0.19
9/11/2012	Mille Lacs 12747	93.6	97.6	95.6	2.82
9/11/2012	Mille Lacs 12756	97.1	99.6	98.3	1.76
9/11/2012	Butternut Lake(Price) 11939	83.7	86.4	85.1	1.92
		Mean ± Std. Dev.		**92.9 ± 8.2**	

* An instrument problem occurred after the sample for Trout Lake 12963 was run so no other samples were analyzed on that date. Trout Lake 12965 and Butternut Lake (Price) 11936 spike samples were not analyzed on 9/5/2012 but were re-digested and analyzed on 9/7/2012.

Table 5. Total Mercury Concentration (Wet Weight) in Walleye Fillets from Fish Captured during the Spring of 2012.

Analysis Date	Lake	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
8/17/2012	Annabelle Lake	12191	Vilas	18.2	F	0.695
8/17/2012	Annabelle Lake	12192	Vilas	16.0	F	0.529
8/17/2012	Annabelle Lake	12193	Vilas	15.1	M	0.903
8/17/2012	Annabelle Lake	12194	Vilas	15.6	M	0.811
8/17/2012	Annabelle Lake	12371	Vilas	13.4	M	0.583
8/17/2012	Annabelle Lake	12391	Vilas	14.3	M	0.698
8/17/2012	Annabelle Lake	12392	Vilas	13.2	M	0.581
6/19/2012	Atkins Lake	12232	Bayfield	16.1	M	0.387
6/19/2012	Atkins Lake	12262	Bayfield	21.5	F	0.592
6/19/2012	Atkins Lake	12320	Bayfield	20.3	F	0.622
6/19/2012	Atkins Lake	12533	Bayfield	24.3	F	0.807
6/19/2012	Atkins Lake	12539	Bayfield	16.4	M	0.470
6/19/2012	Atkins Lake	12556	Bayfield	14.5	M	0.438
6/19/2012	Atkins Lake	12586	Bayfield	16.1	M	0.485
7/20/2012	Bearskin Lake	12089	Oneida	18.7	F	0.140
7/20/2012	Bearskin Lake	12547	Oneida	21.9	F	0.212
7/20/2012	Bearskin Lake	12762	Oneida	13.0	M	0.048

Analysis Date	Lake	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
7/20/2012	Bearskin Lake	12763	Oneida	14.6	M	0.081
7/20/2012	Bearskin Lake	12764	Oneida	12.7	M	0.076
7/20/2012	Bearskin Lake	12765	Oneida	17.9	M	0.144
7/20/2012	Bearskin Lake	12766	Oneida	25.9	F	0.401
7/20/2012	Bearskin Lake	12767	Oneida	15.5	M	0.125
7/20/2012	Bearskin Lake	12768	Oneida	16.1	M	0.118
7/20/2012	Bearskin Lake	12769	Oneida	22.8	F	0.272
7/20/2012	Bearskin Lake	12770	Oneida	23.3	F	0.275
8/21/2012	Big Arbor Vitae Lake	11901	Vilas	18.0	M	0.188
8/21/2012	Big Arbor Vitae Lake	11902	Vilas	18.6	F	0.129
8/21/2012	Big Arbor Vitae Lake	11933	Vilas	18.6	F	0.185
8/21/2012	Big Arbor Vitae Lake	11988	Vilas	28.0	F	0.478
8/21/2012	Big Arbor Vitae Lake	12901	Vilas	14.8	M	0.134
8/21/2012	Big Arbor Vitae Lake	12902	Vilas	15.8	M	0.141
8/21/2012	Big Arbor Vitae Lake	12903	Vilas	16.7	M	0.181
8/21/2012	Big Arbor Vitae Lake	12904	Vilas	14.8	M	0.158
8/21/2012	Big Arbor Vitae Lake	12905	Vilas	14.9	M	0.161
8/21/2012	Big Arbor Vitae Lake	12906	Vilas	17.1	M	0.176
8/21/2012	Big Arbor Vitae Lake	12983	Vilas	25.4	F	0.409
8/21/2012	Big Arbor Vitae Lake	12984	Vilas	22.3	F	0.214
8/17/2012	Big Gibson Lake	11903	Vilas	17.3	F	0.425
8/17/2012	Big Gibson Lake	11904	Vilas	14.9	M	0.368
8/17/2012	Big Gibson Lake	11905	Vilas	15.0	M	0.346
8/17/2012	Big Gibson Lake	11906	Vilas	15.1	M	0.316
8/17/2012	Big Gibson Lake	11907	Vilas	14.6	M	0.420
8/17/2012	Big Gibson Lake	11908	Vilas	13.3	M	0.321
8/17/2012	Big Gibson Lake	11909	Vilas	19.0	F	0.635
8/17/2012	Big Gibson Lake	11910	Vilas	20.0	F	0.481
8/17/2012	Big Gibson Lake	11911	Vilas	19.1	U	0.393
8/21/2012	Big Lake (MI Border)	11918	Vilas	15.5	F	0.317
8/21/2012	Big Lake (MI Border)	11919	Vilas	13.6	M	0.237
8/21/2012	Big Lake (MI Border)	11920	Vilas	13.8	M	0.176
8/21/2012	Big Lake (MI Border)	11921	Vilas	14.7	F	0.215
8/21/2012	Big Lake (MI Border)	11922	Vilas	19.2	F	0.290
8/21/2012	Big Lake (MI Border)	11923	Vilas	23.0	F	0.638
8/21/2012	Big Lake (MI Border)	11924	Vilas	19.2	F	0.215
8/21/2012	Big Lake (MI Border)	11925	Vilas	16.1	M	0.219
8/21/2012	Big Lake (MI Border)	11926	Vilas	17.6	F	0.300
8/21/2012	Big Lake (MI Border)	11927	Vilas	18.0	M	0.179

Analysis Date	Lake	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
7/13/2012	Big Sand Lake	11276	Vilas	21.6	U	0.432
7/13/2012	Big Sand Lake	11277	Vilas	15.3	M	0.274
7/13/2012	Big Sand Lake	11283	Vilas	13.1	U	0.204
7/13/2012	Big Sand Lake	11284	Vilas	13.4	M	0.205
7/13/2012	Big Sand Lake	11285	Vilas	13.5	U	0.219
7/13/2012	Big Sand Lake	11286	Vilas	16.6	U	0.260
7/13/2012	Big Sand Lake	11287	Vilas	17.2	M	0.394
7/13/2012	Big Sand Lake	11288	Vilas	18.2	U	0.784
7/13/2012	Big Sand Lake	11289	Vilas	19.5	U	0.487
7/13/2012	Big Sand Lake	11290	Vilas	22.9	U	0.741
7/13/2012	Butternut Lake	12601	Forest	19.6	M	0.303
7/13/2012	Butternut Lake	12602	Forest	16.2	M	0.087
7/13/2012	Butternut Lake	12603	Forest	14.8	M	0.115
7/13/2012	Butternut Lake	12604	Forest	14.6	M	0.047
7/13/2012	Butternut Lake	12605	Forest	15.1	M	0.045
7/13/2012	Butternut Lake	12606	Forest	13.8	M	0.094
7/13/2012	Butternut Lake	12607	Forest	16.7	M	0.106
7/13/2012	Butternut Lake	12608	Forest	19.4	F	0.137
7/13/2012	Butternut Lake	12609	Forest	18.6	F	0.130
8/1/2012	Butternut Lake	11934	Price	12.2	M	0.497
8/1/2012	Butternut Lake	11935	Price	14.8	M	0.805
8/1/2012	Butternut Lake	11936	Price	12.4	M	0.489
8/1/2012	Butternut Lake	11937	Price	15.4	F	0.596
8/1/2012	Butternut Lake	11938	Price	15.3	M	0.641
9/11/2012	Butternut Lake	11939	Price	26.5	F	1.37
8/1/2012	Butternut Lake	11940	Price	18.1	F	0.804
8/1/2012	Butternut Lake	11941	Price	16.0	F	0.852
8/1/2012	Butternut Lake	11942	Price	21.0	F	1.04
8/1/2012	Butternut Lake	11943	Price	24.5	F	1.24
7/13/2012	Connors Lake	12198	Sawyer	19.2	M	0.607
7/13/2012	Connors Lake	12199	Sawyer	12.7	M	0.367
7/13/2012	Connors Lake	12200	Sawyer	13.3	M	0.368
7/13/2012	Connors Lake	12292	Sawyer	18.4	M	0.359
7/13/2012	Connors Lake	12293	Sawyer	17.6	M	0.276
7/13/2012	Connors Lake	12294	Sawyer	16.4	M	0.273
7/13/2012	Connors Lake	12295	Sawyer	13.0	M	0.295
7/13/2012	Connors Lake	12296	Sawyer	26.1	F	0.472
7/13/2012	Connors Lake	12297	Sawyer	17.6	M	0.311
7/13/2012	Connors Lake	12298	Sawyer	18.5	M	0.274

Analysis Date	Lake	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
6/12/2012	Cranberry Lake	11596	Vilas	18.1	F	0.236
6/12/2012	Cranberry Lake	11597	Vilas	21.6	F	0.583
6/12/2012	Cranberry Lake	11598	Vilas	24.8	F	0.298
6/12/2012	Cranberry Lake	11599	Vilas	20.8	F	0.453
6/12/2012	Cranberry Lake	12291	Vilas	15.4	F	0.193
6/12/2012	Cranberry Lake	12796	Vilas	12.6	M	0.178
6/12/2012	Cranberry Lake	12797	Vilas	11.4	M	0.134
6/12/2012	Cranberry Lake	12798	Vilas	14.0	M	0.142
6/12/2012	Cranberry Lake	12799	Vilas	15.3	M	0.240
6/12/2012	Cranberry Lake	12800	Vilas	15.3	M	0.337
8/1/2012	Duck Lake	12188	Barron	15.4	M	0.210
8/1/2012	Duck Lake	12189	Barron	18.5	F	0.208
8/1/2012	Duck Lake	12490	Barron	18.5	F	0.260
8/1/2012	Duck Lake	12512	Barron	16.1	M	0.162
8/1/2012	Duck Lake	12537	Barron	14.3	M	0.130
8/1/2012	Duck Lake	12540	Barron	14.8	M	0.117
8/1/2012	Duck Lake	12546	Barron	14.7	M	0.113
8/1/2012	Duck Lake	12551	Barron	15.7	M	0.104
8/1/2012	Duck Lake	12555	Barron	18.0	F	0.293
8/1/2012	Duck Lake	12725	Barron	22.1	F	0.229
6/12/2012	Dunn Lake	12261	Washburn	21.5	F	0.347
6/12/2012	Dunn Lake	12265	Washburn	24.2	F	0.971
6/12/2012	Dunn Lake	12305	Washburn	17.0	U	0.205
6/12/2012	Dunn Lake	12307	Washburn	21.1	F	0.390
8/17/2012	Eagle Lake	12937	Vilas	13.2	M	0.171
8/17/2012	Eagle Lake	12938	Vilas	16.3	M	0.674
8/17/2012	Eagle Lake	12939	Vilas	18.2	F	0.264
8/17/2012	Eagle Lake	12941	Vilas	22.1	F	0.685
8/17/2012	Eagle Lake	12946	Vilas	21.1	M	0.985
8/17/2012	Eagle Lake	12947	Vilas	19.0	M	0.572
8/17/2012	Eagle Lake	12948	Vilas	12.3	M	0.242
8/17/2012	Eagle Lake	12949	Vilas	12.9	M	0.273
8/17/2012	Eagle Lake	12950	Vilas	15.6	M	0.286
8/17/2012	Eagle Lake	12951	Vilas	16.8	M	0.267
7/12/2012	Kakagon Slough	12061	Ashland	15.4	M	0.112
7/12/2012	Kakagon Slough	12062	Ashland	16.1	M	0.108
7/12/2012	Kakagon Slough	12063	Ashland	15.1	M	0.165
7/12/2012	Kakagon Slough	12064	Ashland	14.2	M	0.157
7/12/2012	Kakagon Slough	12065	Ashland	13.2	M	0.153

Analysis Date	Lake	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
7/12/2012	Kakagon Slough	12066	Ashland	23.6	F	0.218
7/12/2012	Kakagon Slough	12067	Ashland	23.2	M	0.794
7/12/2012	Kakagon Slough	12068	Ashland	23.0	M	0.453
7/12/2012	Kakagon Slough	12069	Ashland	18.1	M	0.161
7/12/2012	Kakagon Slough	12070	Ashland	19.0	M	0.201
7/12/2012	Kakagon Slough	12071	Ashland	14.0	M	0.122
7/12/2012	Kakagon Slough	12072	Ashland	20.1	M	0.205
6/12/2012	Lac Courte Oreilles	12968	Sawyer	13.4	M	0.106
6/12/2012	Lac Courte Oreilles	12969	Sawyer	12.5	M	0.089
6/12/2012	Lac Courte Oreilles	12970	Sawyer	20.5	M	0.368
6/12/2012	Lac Courte Oreilles	12971	Sawyer	18.1	M	0.416
6/12/2012	Lac Courte Oreilles	12972	Sawyer	23.3	F	0.449
6/12/2012	Lac Courte Oreilles	12973	Sawyer	24.0	F	0.509
6/12/2012	Lac Courte Oreilles	12977	Sawyer	20.2	M	0.355
6/12/2012	Lac Courte Oreilles	12978	Sawyer	15.8	M	0.116
6/12/2012	Lac Courte Oreilles	12979	Sawyer	15.3	M	0.152
6/12/2012	Lac Courte Oreilles	12980	Sawyer	15.0	M	0.103
6/12/2012	Lac Courte Oreilles	12981	Sawyer	14.6	M	0.122
6/12/2012	Lac Courte Oreilles	12982	Sawyer	17.7	M	0.352
6/19/2012	Lake Chippewa	11787	Sawyer	16.0	M	0.445
6/19/2012	Lake Chippewa	11788	Sawyer	18.1	M	0.392
6/19/2012	Lake Chippewa	11790	Sawyer	15.8	M	0.198
6/19/2012	Lake Chippewa	11791	Sawyer	19.3	F	0.436
6/19/2012	Lake Chippewa	11792	Sawyer	13.5	M	0.353
6/19/2012	Lake Chippewa	11793	Sawyer	19.3	F	0.473
6/19/2012	Lake Chippewa	11794	Sawyer	18.7	F	0.333
6/19/2012	Lake Chippewa	11795	Sawyer	15.8	M	0.534
6/19/2012	Lake Chippewa	11796	Sawyer	14.2	M	0.313
6/19/2012	Lake Chippewa	11797	Sawyer	14.7	M	0.235
6/19/2012	Lake Chippewa	11798	Sawyer	14.9	M	0.584
6/19/2012	Lake Chippewa	11800	Sawyer	14.5	M	0.304
9/11/2012	Lake Gogebic	11745	Gogebic	13.1	M	0.088
9/11/2012	Lake Gogebic	11746	Gogebic	14.9	M	0.120
9/11/2012	Lake Gogebic	11747	Gogebic	17.3	F	0.265
9/11/2012	Lake Gogebic	11748	Gogebic	16.7	M	0.166
9/11/2012	Lake Gogebic	11749	Gogebic	16.7	M	0.180
9/11/2012	Lake Gogebic	11750	Gogebic	17.0	M	0.165
9/11/2012	Lake Gogebic	11751	Gogebic	16.9	M	0.244
9/11/2012	Lake Gogebic	11752	Gogebic	17.5	M	0.215

Analysis Date	Lake	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
9/11/2012	Lake Gogebic	11753	Gogebic	13.4	M	0.192
9/11/2012	Lake Gogebic	11754	Gogebic	16.5	M	0.157
9/11/2012	Lake Gogebic	11755	Gogebic	14.0	M	0.127
9/11/2012	Lake Gogebic	11756	Gogebic	16.2	M	0.126
7/20/2012	Mamie Lake	11491	Vilas	16.8	F	0.318
7/20/2012	Mamie Lake	11494	Vilas	25.5	F	0.876
7/20/2012	Mamie Lake	12091	Vilas	13.8	M	0.295
7/20/2012	Mamie Lake	12092	Vilas	15.8	M	0.387
7/20/2012	Mamie Lake	12093	Vilas	16.2	U	0.212
7/20/2012	Mamie Lake	12094	Vilas	23.5	F	0.460
7/20/2012	Mamie Lake	12095	Vilas	14.2	M	0.339
7/20/2012	Mamie Lake	12096	Vilas	12.2	M	0.193
7/20/2012	Mamie Lake	12097	Vilas	21.6	F	0.620
7/20/2012	Mamie Lake	12098	Vilas	21.5	F	0.338
7/20/2012	Mamie Lake	12099	Vilas	18.0	F	0.292
7/20/2012	Mamie Lake	12100	Vilas	23.6	F	0.467
6/12/2012	Middle Eau Claire Lake	12317	Bayfield	15.9	M	0.258
6/12/2012	Middle Eau Claire Lake	12318	Bayfield	18.0	M	0.460
6/12/2012	Middle Eau Claire Lake	12321	Bayfield	14.6	M	0.198
6/12/2012	Middle Eau Claire Lake	12322	Bayfield	17.0	M	0.511
6/12/2012	Middle Eau Claire Lake	12323	Bayfield	14.5	M	0.282
6/12/2012	Middle Eau Claire Lake	12324	Bayfield	13.4	M	0.160
6/12/2012	Middle Eau Claire Lake	12325	Bayfield	18.0	M	0.542
6/12/2012	Middle Eau Claire Lake	12326	Bayfield	14.6	M	0.306
6/12/2012	Middle Eau Claire Lake	12488	Bayfield	18.2	M	0.588
6/12/2012	Middle Eau Claire Lake	12990	Bayfield	15.5	M	0.270
9/11/2012	Mille Lacs	12746	Mille Lacs	14.9	M	0.072
9/11/2012	Mille Lacs	12747	Mille Lacs	19.9	M	0.203
9/11/2012	Mille Lacs	12748	Mille Lacs	23.0	F	0.211
9/11/2012	Mille Lacs	12749	Mille Lacs	23.8	F	0.190
9/11/2012	Mille Lacs	12750	Mille Lacs	23.8	F	0.179
9/11/2012	Mille Lacs	12751	Mille Lacs	21.3	F	0.126
9/11/2012	Mille Lacs	12752	Mille Lacs	18.8	F	0.070
9/11/2012	Mille Lacs	12753	Mille Lacs	16.6	M	0.080
9/11/2012	Mille Lacs	12754	Mille Lacs	14.5	M	0.057
9/11/2012	Mille Lacs	12755	Mille Lacs	16.5	M	0.096
9/11/2012	Mille Lacs	12756	Mille Lacs	14.2	M	0.068
9/11/2012	Mille Lacs	12757	Mille Lacs	15.9	M	0.050
8/17/2012	Oxbow Lake	12907	Vilas	18.2	F	1.05

Analysis Date	Lake	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
8/17/2012	Oxbow Lake	12912	Vilas	23.8	F	1.06
8/17/2012	Oxbow Lake	12913	Vilas	18.6	M	0.616
8/17/2012	Oxbow Lake	12914	Vilas	15.3	M	0.698
8/17/2012	Oxbow Lake	12915	Vilas	15.5	M	0.782
8/17/2012	Oxbow Lake	12916	Vilas	15.3	M	0.777
8/17/2012	Oxbow Lake	12917	Vilas	25.2	F	1.10
8/17/2012	Oxbow Lake	12918	Vilas	18.4	M	0.734
8/17/2012	Oxbow Lake	12919	Vilas	13.9	M	0.565
8/17/2012	Oxbow Lake	12920	Vilas	12.8	M	0.522
8/17/2012	Oxbow Lake	12921	Vilas	13.4	M	0.492
8/21/2012	Pelican Lake	12776	Oneida	13.0	M	0.098
8/21/2012	Pelican Lake	12777	Oneida	17.0	M	0.246
8/21/2012	Pelican Lake	12778	Oneida	15.3	M	0.165
8/21/2012	Pelican Lake	12779	Oneida	18.6	M	0.292
8/21/2012	Pelican Lake	12780	Oneida	15.4	M	0.147
8/21/2012	Pelican Lake	12781	Oneida	18.8	M	0.379
8/21/2012	Pelican Lake	12782	Oneida	16.6	M	0.199
8/21/2012	Pelican Lake	12783	Oneida	14.7	M	0.117
8/21/2012	Pelican Lake	12784	Oneida	14.3	M	0.160
8/21/2012	Pelican Lake	12785	Oneida	22.2	F	0.371
8/21/2012	Pelican Lake	12786	Oneida	24.2	U	0.524
8/21/2012	Pelican Lake	12787	Oneida	23.7	F	0.408
6/19/2012	Planting Ground Lake	11979	Oneida	13.5	M	0.472
6/19/2012	Planting Ground Lake	11980	Oneida	14.2	M	0.304
6/19/2012	Planting Ground Lake	11981	Oneida	13.0	M	0.249
6/19/2012	Planting Ground Lake	11982	Oneida	16.2	F	0.392
6/19/2012	Planting Ground Lake	11983	Oneida	17.3	U	0.343
6/19/2012	Planting Ground Lake	11984	Oneida	16.0	F	0.204
6/19/2012	Planting Ground Lake	11985	Oneida	24.6	F	1.14
6/19/2012	Planting Ground Lake	11986	Oneida	23.5	F	1.33
6/19/2012	Planting Ground Lake	11987	Oneida	23.8	F	0.591
6/19/2012	Planting Ground Lake	11988	Oneida	24.6	F	0.822
6/28/2012	Presque Isle Lake	12922	Vilas	19.5	M	0.418
6/28/2012	Presque Isle Lake	12923	Vilas	22.4	F	0.286
6/28/2012	Presque Isle Lake	12924	Vilas	14.0	M	0.214
6/28/2012	Presque Isle Lake	12925	Vilas	19.5	F	0.415
6/28/2012	Presque Isle Lake	12926	Vilas	19.3	F	0.290
6/28/2012	Presque Isle Lake	12930	Vilas	24.8	F	0.386
6/28/2012	Presque Isle Lake	12932	Vilas	13.0	M	0.172

Analysis Date	Lake	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
6/28/2012	Presque Isle Lake	12933	Vilas	19.0	F	0.203
6/28/2012	Presque Isle Lake	12934	Vilas	16.3	M	0.191
6/28/2012	Presque Isle Lake	12935	Vilas	16.2	F	0.125
6/28/2012	Presque Isle Lake	12936	Vilas	14.3	M	0.154
6/28/2012	Presque Isle Lake	12951	Vilas	22.5	F	0.417
7/12/2012	Red Cedar Lake	12277	Barron	14.0	M	0.374
7/12/2012	Red Cedar Lake	12290	Barron	14.1	M	0.328
7/12/2012	Red Cedar Lake	12356	Barron	14.4	M	0.260
7/12/2012	Red Cedar Lake	12358	Barron	14.6	M	0.292
7/12/2012	Red Cedar Lake	12372	Barron	15.8	M	0.408
9/7/2012	Round Lake	11772	Sawyer	14.7	M	0.130
9/7/2012	Round Lake	11773	Sawyer	17.5	M	0.210
9/7/2012	Round Lake	11774	Sawyer	14.4	M	0.114
9/7/2012	Round Lake	11775	Sawyer	16.7	M	0.198
9/7/2012	Round Lake	11776	Sawyer	14.7	M	0.164
9/7/2012	Round Lake	11777	Sawyer	15.2	M	0.120
9/7/2012	Round Lake	11778	Sawyer	19.2	M	0.205
9/7/2012	Round Lake	11779	Sawyer	20.6	F	0.203
9/7/2012	Round Lake	11780	Sawyer	22.1	F	0.268
9/7/2012	Round Lake	11784	Sawyer	21.1	M	0.418
9/7/2012	Round Lake	11785	Sawyer	22.8	M	0.305
9/7/2012	Round Lake	11786	Sawyer	22.0	M	0.477
8/1/2012	Scattering Rice Lake	12940	Vilas	24.1	F	1.12
8/1/2012	Scattering Rice Lake	12943	Vilas	14.7	M	0.208
8/1/2012	Scattering Rice Lake	12944	Vilas	12.5	M	0.233
8/1/2012	Scattering Rice Lake	12945	Vilas	22.7	F	0.575
6/19/2012	Shell Lake	12266	Washburn	15.3	M	0.382
6/19/2012	Shell Lake	12268	Washburn	18.2	F	0.544
6/19/2012	Shell Lake	12289	Washburn	12.8	M	0.244
6/19/2012	Shell Lake	12316	Washburn	16.4	M	0.306
6/19/2012	Shell Lake	12348	Washburn	14.3	M	0.259
6/19/2012	Shell Lake	12351	Washburn	13.8	M	0.457
6/19/2012	Shell Lake	12369	Washburn	16.0	M	0.320
6/19/2012	Shell Lake	12670	Washburn	21.1	F	0.703
7/12/2012	Siskiwit Lake	12459	Bayfield	15.2	M	0.706
7/13/2012	Siskiwit Lake	12513	Bayfield	16.4	M	0.872
7/12/2012	Siskiwit Lake	12514	Bayfield	12.4	M	0.527
7/12/2012	Siskiwit Lake	12549	Bayfield	18.3	F	1.11
7/12/2012	Siskiwit Lake	12552	Bayfield	13.5	M	0.464

Analysis Date	Lake	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
7/12/2012	Siskiwit Lake	12554	Bayfield	13.8	M	0.541
7/12/2012	Siskiwit Lake	12559	Bayfield	15.6	M	0.692
7/12/2012	Siskiwit Lake	12560	Bayfield	19.0	F	0.923
7/12/2012	Siskiwit Lake	12561	Bayfield	19.9	F	1.04
7/12/2012	Squaw Lake	12059	Vilas	13.4	M	0.486
7/12/2012	Squaw Lake	12231	Vilas	23.7	F	1.03
7/12/2012	Squaw Lake	12548	Vilas	27.2	F	0.924
7/12/2012	Squaw Lake	12557	Vilas	21.9	F	0.653
7/12/2012	Squaw Lake	12591	Vilas	17.8	F	0.882
7/12/2012	Squaw Lake	12592	Vilas	12.7	M	0.574
7/12/2012	Squaw Lake	12593	Vilas	13.5	M	0.512
7/12/2012	Squaw Lake	12594	Vilas	15.2	M	0.594
7/12/2012	Squaw Lake	12595	Vilas	15.7	M	0.581
7/12/2012	Squaw Lake	12596	Vilas	24.4	F	0.786
6/28/2012	Squirrel Lake	12132	Oneida	13.2	M	0.213
6/28/2012	Squirrel Lake	12133	Oneida	13.9	M	0.281
6/28/2012	Squirrel Lake	12134	Oneida	15.1	M	0.178
6/28/2012	Squirrel Lake	12135	Oneida	15.7	M	0.242
6/28/2012	Squirrel Lake	12136	Oneida	15.0	M	0.240
6/28/2012	Squirrel Lake	12137	Oneida	19.5	F	0.273
6/28/2012	Squirrel Lake	12138	Oneida	18.9	F	0.324
6/28/2012	Squirrel Lake	12139	Oneida	23.5	F	0.661
6/28/2012	Squirrel Lake	12140	Oneida	21.9	F	0.448
6/28/2012	Squirrel Lake	12141	Oneida	20.4	M	0.319
6/28/2012	Squirrel Lake	12142	Oneida	21.6	F	0.365
6/28/2012	Squirrel Lake	12143	Oneida	24.7	F	0.696
6/28/2012	Squirrel Lake	12458	Oneida	12.1	M	0.204
9/5/2012	Stone Lake	11757	Washburn	13.9	M	0.201
9/5/2012	Stone Lake	11758	Washburn	23.4	F	0.909
9/5/2012	Stone Lake	11759	Washburn	19.9	F	0.402
9/5/2012	Stone Lake	11760	Washburn	15.8	M	0.242
9/5/2012	Stone Lake	11761	Washburn	19.8	F	0.412
9/5/2012	Stone Lake	11762	Washburn	13.0	M	0.241
9/5/2012	Stone Lake	11763	Washburn	19.3	F	0.495
9/5/2012	Stone Lake	11767	Washburn	16.4	M	0.251
9/5/2012	Stone Lake	11768	Washburn	16.4	M	0.442
9/5/2012	Stone Lake	11769	Washburn	22.6	F	0.521
9/5/2012	Stone Lake	11770	Washburn	24.3	F	0.846
9/5/2012	Stone Lake	11771	Washburn	14.1	M	0.226

Analysis Date	Lake	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
6/28/2012	Tenderfoot Lake	11479	Vilas	23.2	F	0.546
6/28/2012	Tenderfoot Lake	11480	Vilas	18.2	M	0.551
6/28/2012	Tenderfoot Lake	11481	Vilas	18.2	M	0.537
6/28/2012	Tenderfoot Lake	11482	Vilas	14.0	M	0.318
6/28/2012	Tenderfoot Lake	11483	Vilas	16.6	M	0.426
6/28/2012	Tenderfoot Lake	11484	Vilas	14.7	M	0.429
6/28/2012	Tenderfoot Lake	11485	Vilas	17.5	F	0.416
6/28/2012	Tenderfoot Lake	11486	Vilas	14.0	M	0.254
6/28/2012	Tenderfoot Lake	11487	Vilas	23.9	F	0.601
6/28/2012	Tenderfoot Lake	11488	Vilas	26.3	F	0.485
6/28/2012	Tenderfoot Lake	11489	Vilas	16.9	F	0.399
6/28/2012	Tenderfoot Lake	11490	Vilas	18.5	F	0.399
9/5/2012	Trout Lake	11914	Vilas	28.0	U	0.737
9/5/2012	Trout Lake	12953	Vilas	18.0	M	0.154
9/5/2012	Trout Lake	12954	Vilas	13.3	M	0.137
9/5/2012	Trout Lake	12956	Vilas	16.5	M	0.172
9/5/2012	Trout Lake	12958	Vilas	25.4	F	0.349
9/5/2012	Trout Lake	12959	Vilas	23.9	F	0.362
9/5/2012	Trout Lake	12960	Vilas	17.2	M	0.280
9/5/2012	Trout Lake	12961	Vilas	14.6	M	0.127
9/5/2012	Trout Lake	12963	Vilas	18.3	F	0.175
9/7/2012	Trout Lake	12964	Vilas	14.2	M	0.125
9/7/2012	Trout Lake	12965	Vilas	21.2	F	0.318
9/7/2012	Trout Lake	12966	Vilas	16.0	M	0.158
8/1/2012	Turtle-Flambeau Flowage	11912	Iron	23.0	F	0.884
7/13/2012	Turtle-Flambeau Flowage	11964	Iron	14.0	M	0.378
7/13/2012	Turtle-Flambeau Flowage	11965	Iron	14.9	M	0.227
7/13/2012	Turtle-Flambeau Flowage	11966	Iron	12.3	M	0.381
7/13/2012	Turtle-Flambeau Flowage	11967	Iron	15.1	M	0.247
7/13/2012	Turtle-Flambeau Flowage	11968	Iron	15.2	M	0.448
7/13/2012	Turtle-Flambeau Flowage	11969	Iron	16.1	M	0.405
7/13/2012	Turtle-Flambeau Flowage	11970	Iron	18.8	F	0.575
7/13/2012	Turtle-Flambeau Flowage	11971	Iron	20.6	F	0.757
7/13/2012	Turtle-Flambeau Flowage	11972	Iron	18.3	M	0.420
8/1/2012	Twin Lake Chain	11496	Vilas	16.5	M	0.374
8/1/2012	Twin Lake Chain	11498	Vilas	12.7	M	0.123
8/1/2012	Twin Lake Chain	11499	Vilas	16.9	M	0.137
8/1/2012	Twin Lake Chain	11692	Vilas	19.7	F	0.256
8/1/2012	Twin Lake Chain	11694	Vilas	24.3	F	0.218

Analysis Date	Lake	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
8/1/2012	Twin Lake Chain	11695	Vilas	21.1	M	0.271
8/1/2012	Twin Lake Chain	11696	Vilas	18.3	M	0.232
8/1/2012	Twin Lake Chain	11697	Vilas	22.4	F	0.176
8/1/2012	Twin Lake Chain	11698	Vilas	14.9	M	0.108
8/1/2012	Twin Lake Chain	11699	Vilas	17.0	M	0.198
8/1/2012	Twin Lake Chain	11700	Vilas	14.7	M	0.231
8/1/2012	Twin Lake Chain	11788	Vilas	23.1	F	0.324
7/20/2012	Whitefish Lake	12014	Douglas	14.9	M	0.134
7/20/2012	Whitefish Lake	12187	Douglas	16.8	M	0.452
7/20/2012	Whitefish Lake	12263	Douglas	19.8	M	0.505
7/20/2012	Whitefish Lake	12339	Douglas	20.8	M	0.724
7/20/2012	Whitefish Lake	12340	Douglas	17.2	M	0.311
7/20/2012	Whitefish Lake	12341	Douglas	21.2	M	0.525
7/20/2012	Whitefish Lake	12342	Douglas	16.5	M	0.184
7/20/2012	Whitefish Lake	12489	Douglas	18.2	M	0.341
7/20/2012	Whitefish Lake	12535	Douglas	16.1	M	0.115
7/20/2012	Whitefish Lake	12558	Douglas	23.4	F	0.603
7/20/2012	Whitefish Lake	12625	Douglas	14.6	M	0.165
7/20/2012	Whitefish Lake	12626	Douglas	15.6	M	0.207

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

Date	Sample ID		Percent Moisture	Relative Percent Difference
6/1/2012	Lac Courte Oreilles 12968		78.3	
6/1/2012	Lac Courte Oreilles 12971		78.5	
6/1/2012	Lac Courte Oreilles 12981		78.4	
6/1/2012	Lac Courte Oreilles 12978		77.8	
6/1/2012	Dunn Lake 12307		78.8	
6/1/2012	Dunn Lake 12265		81.2	
6/1/2012	Dunn Lake 12261		79.3	
6/4/2012	Lake Gogebic 11755		78.2	
6/4/2012	Lake Gogebic 11751		77.3	
6/4/2012	Lake Gogebic 11748		78.3	
6/4/2012	Lake Gogebic 11748	DUP	78.6	0.5
6/5/2012	Cranberry Lake 11596		79.5	
6/5/2012	Cranberry Lake 11599		78.9	
6/5/2012	Cranberry Lake 11597		80.0	
6/5/2012	Middle Eau Claire Lake 12326		77.6	

Date	Sample ID		Percent Moisture	Relative Percent Difference
6/5/2012	Middle Eau Claire Lake 12322		78.1	
6/5/2012	Middle Eau Claire Lake **		77.6	
6/6/2012	Round Lake 11774		78.0	
6/6/2012	Round Lake 11774	DUP	77.2	1.0
6/6/2012	Round Lake 11777		79.1	
6/6/2012	Round Lake 11775		78.3	
6/8/2012	Mille Lacs 12753		79.5	
6/8/2012	Mille Lacs 12757		80.0	
6/8/2012	Mille Lacs 12752		79.4	
6/8/2012	Planting Grounds Lake 11983		80.5	
6/8/2012	Planting Grounds Lake 11986		80.2	
6/8/2012	Planting Grounds Lake 11988		79.7	
6/8/2012	Atkins Lake 12586		77.9	
6/8/2012	Atkins Lake 12556		77.9	
6/8/2012	Atkins Lake 12232		78.4	
6/8/2012	Atkins Lake 12232	DUP	78.3	0.2
6/13/2012	Chippewa Lake 11798		77.0	
6/13/2012	Chippewa Lake 11793		77.8	
6/13/2012	Chippewa Lake 11788		77.9	
6/13/2012	Red Cedar Lake 12372		76.9	
6/13/2012	Red Cedar Lake 12277		78.1	
6/13/2012	Red Cedar Lake 12356		77.7	
6/14/2012	Squirrel Lake 12135		77.8	
6/14/2012	Squirrel Lake 12138		78.8	
6/14/2012	Squirrel Lake 12138	DUP	78.8	0.0
6/14/2012	Squirrel Lake 12141		79.5	
6/14/2012	Shell Lake 12268		78.7	
6/14/2012	Shell Lake 12670		78.6	
6/14/2012	Shell Lake 12316		78.8	
6/26/2012	Tenderfoot Lake 11486		78.0	
6/26/2012	Tenderfoot Lake 11485		79.8	
6/26/2012	Tenderfoot Lake 11490		79.7	
6/26/2012	Presque Isle Lake 12924		79.6	
6/26/2012	Presque Isle Lake 12922		80.0	
6/26/2012	Presque Isle Lake 12922	DUP	80.0	0.0
6/26/2012	Presque Isle Lake 12930		80.3	
6/27/2012	Turtle Flambeau Flowage 11965		79.3	
6/27/2012	Turtle Flambeau Flowage 11972		79.8	
6/27/2012	Turtle Flambeau Flowage 11967		79.5	

Date	Sample ID		Percent Moisture	Relative Percent Difference
6/28/2012	Kakagon Slough 12062		78.4	
6/28/2012	Kakagon Slough 12071		78.0	
6/28/2012	Kakagon Slough 12070		77.6	
7/2/2012	Squaw Lake 12594		79.4	
7/2/2012	Squaw Lake 12594	DUP	79.2	0.2
7/2/2012	Squaw Lake 12548		80.2	
7/2/2012	Squaw Lake 12231		79.4	
7/2/2012	Siskiwit Lake 12559		78.9	
7/2/2012	Siskiwit Lake 12560		81.0	
7/2/2012	Siskiwit Lake 12513		80.6	
7/2/2012	Whitefish Lake 12340		78.1	
7/2/2012	Whitefish Lake 12339		77.2	
7/2/2012	Whitefish Lake 12489		77.3	
7/5/2012	Bearskin Lake 12089		79.5	
7/5/2012	Bearskin Lake 12089	DUP	79.6	0.2
7/5/2012	Bearskin Lake 12767		78.9	
7/5/2012	Bearskin Lake 12547		78.7	
7/5/2012	Butternut Lake 12603		78.9	
7/5/2012	Butternut Lake 12608		80.3	
7/5/2012	Butternut Lake 12609		79.7	
7/6/2012	Big Sand Lake 11287		78.7	
7/6/2012	Big Sand Lake 11288		79.7	
7/6/2012	Big Sand Lake 11290		79.4	
7/6/2012	Connors Lake 12295		77.2	
7/6/2012	Connors Lake 12293		79.1	
7/6/2012	Connors Lake 12293	DUP	79.4	0.3
7/6/2012	Connors Lake 12292		80.2	
7/16/2012	Mamie Lake 12092		79.6	
7/16/2012	Mamie Lake 11491		78.8	
7/16/2012	Mamie Lake 12097		78.6	
7/17/2012	Butternut Lake 11935		78.9	
7/17/2012	Butternut Lake 11939		79.4	
7/17/2012	Butternut Lake 11942		79.6	
7/18/2012	Duck Lake 12188		78.0	
7/18/2012	Duck Lake 12188	DUP	77.9	0.2
7/18/2012	Duck Lake 12189		78.8	
7/18/2012	Duck Lake 12555		77.6	
7/19/2012	Scattering Rice Lake 12943		78.0	
7/19/2012	Scattering Rice Lake 12945		79.0	

Date	Sample ID		Percent Moisture	Relative Percent Difference
7/19/2012	Scattering Rice Lake 12940		78.2	
7/19/2012	Turtle Flambeau Flowage 11912		79.1	
7/23/2012	Twin Lake Chain 11700		77.3	
7/23/2012	Twin Lake Chain 11692		80.1	
7/23/2012	Twin Lake Chain 11696		78.6	
7/24/2012	Eagle Lake 12950		78.8	
7/24/2012	Eagle Lake 12938		78.8	
7/24/2012	Eagle Lake 12938	DUP	79.0	0.2
7/24/2012	Eagle Lake 12946		78.1	
7/26/2012	Big Gibson Lake 11905		78.5	
7/26/2012	Big Gibson Lake 11909		79.8	
7/26/2012	Big Gibson Lake 11907		78.6	
8/2/2012	Annabelle Lake 12194		77.8	
8/2/2012	Annabelle Lake 12391		80.9	
8/2/2012	Annabelle Lake 12193		79.5	
8/3/2012	Oxbow Lake 12915		79.1	
8/3/2012	Oxbow Lake 12913		79.3	
8/3/2012	Oxbow Lake 12913	DUP	79.4	0.1
8/3/2012	Oxbow Lake 12907		80.4	
8/7/2012	Big Arbor Vitae 12904		76.5	
8/7/2012	Big Arbor Vitae 12901		78.2	
8/7/2012	Big Arbor Vitae 11901		76.7	
8/8/2012	Big Lake 11918		80.6	
8/8/2012	Big Lake 11922		80.1	
8/8/2012	Big Lake 11926		81.0	
8/9/2012	Pelican Lake 12782		78.9	
8/9/2012	Pelican Lake 12779		77.7	
8/9/2012	Pelican Lake 12779	DUP	78.2	0.6
8/9/2012	Pelican Lake 12781		78.8	
8/13/2012	Trout Lake 12960		76.9	
8/13/2012	Trout Lake 12963		77.3	
8/13/2012	Trout Lake 12959		77.8	
8/15/2012	Stone Lake 11771		76.7	
8/15/2012	Stone Lake 11767		79.1	
8/15/2012	Stone Lake 11761		78.1	
	Mean ± Std. Dev.		**78.8 ± 1.0**	

** The sample number for the third moisture sample from Middle Eau Claire Lake was not recorded on 6/5/2012.

Appendix A

Determination of 2012 Limit of Detection (LOD) and Limit of Quantitation (LOQ) using a ground tuna sample from June 2, 2011.

Sample	Tissue Type	ng/L	ng Hg	g sample	µg Hg/g
Tuna 2 June 2011 -1	ground tuna	94.4	4.72	0.213	0.022
Tuna 2 June 2011 -2	ground tuna	76.8	3.84	0.203	0.019
Tuna 2 June 2011 -3	ground tuna	81.2	4.06	0.204	0.020
Tuna 2 June 2011 -4	ground tuna	85.6	4.28	0.203	0.021
Tuna 2 June 2011 -5	ground tuna	85.6	4.28	0.211	0.020
Tuna 2 June 2011 -6	ground tuna	90.0	4.50	0.210	0.021
Tuna 2 June 2011 -7	ground tuna	85.6	4.28	0.210	0.020
Tuna 2 June 2011 -8	ground tuna	85.6	4.28	0.209	0.020
				Mean	0.0206
				Std. Dev.	0.00099

2012 LOD = Std. Dev. x t = 0.00099 x 2.998 = 0.0030
2012 LOQ = 10/3 x LOD = 0.0099

2012	**Hg LOD = 0.0030 µg/g**	**LOQ = 0.0099 µg/g**
2011	**Hg LOD= 0.0017 µg/g**	**LOQ= 0.0057 µg/g**
2010	**Hg LOD = 0.0046 µg/g**	**LOQ = 0.0153 µg/g**
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 2012 Walleye Fillets

Analysis Date	Standard Conc. ng Hg/L	Blank Corrected Abs. 1	Blank Corrected Abs. 2	Blank Corrected Mean	Standard Deviation	Slope	Y-Intercept	Correlation
6/12/2012	0	0.0023	0.0021	0.0000	0.0001	2.497 E-05	0.001293	0.9997
6/12/2012	100	0.0026	0.0028	0.0027	0.0001			
6/12/2012	500	0.0134	0.0134	0.0134	0.0000			
6/12/2012	1000	0.0272	0.0258	0.0265	0.0010			
6/12/2012	5000	0.1346	0.1277	0.1312	0.0049			
6/12/2012	10,000	0.2564	0.2405	0.2485	0.0112			
6/15/2012	0	0.0013	0.0000	0.0000	0.0009	2.459 E-05	0.001437	0.9997
6/15/2012	100	0.0032	0.0031	0.0032	0.0001			
6/15/2012	500	0.0142	0.0121	0.0132	0.0015			
6/15/2012	1000	0.0291	0.0238	0.0265	0.0037			
6/15/2012	5000	0.1423	0.1158	0.1291	0.0187			
6/15/2012	10,000	0.2717	0.2182	0.2450	0.0378			
6/19/2012	0	0.0011	0.0003	0.0000	0.0006	2.483 E-05	0.001385	0.9996
6/19/2012	100	0.0029	0.0026	0.0028	0.0002			
6/19/2012	500	0.0139	0.0129	0.0134	0.0007			
6/19/2012	1000	0.0270	0.0260	0.0265	0.0007			
6/19/2012	5000	0.1338	0.1277	0.1308	0.0043			
6/19/2012	10,000	0.2519	0.2422	0.2471	0.0069			
6/28/2012	0	0.0013	0.0001	0.0000	0.0008	2.512 E-05	0.001023	0.9998
6/28/2012	100	0.0028	0.0027	0.0028	0.0001			
6/28/2012	500	0.0137	0.0132	0.0135	0.0004			
6/28/2012	1000	0.0271	0.0257	0.0264	0.0010			
6/28/2012	5000	0.1337	0.1264	0.1301	0.0052			
6/28/2012	10,000	0.2590	0.2421	0.2506	0.0120			
7/12/2012	0	0.0013	0.0012	0.0000	0.0001	2.523 E-05	0.001487	0.9996
7/12/2012	100	0.0023	0.0027	0.0025	0.0003			
7/12/2012	500	0.0122	0.0134	0.0128	0.0008			
7/12/2012	1000	0.0285	0.0293	0.0289	0.0006			
7/12/2012	5000	0.1306	0.1337	0.1322	0.0022			
7/12/2012	10,000	0.2506	0.2522	0.2514	0.0011			
7/13/2012	0	0.0015	0.0014	0.0000	0.0001	2.433 E-05	0.002119	0.9995
7/13/2012	100	0.0027	0.0032	0.0030	0.0004			
7/13/2012	500	0.0135	0.0128	0.0132	0.0005			

Analysis Date	Standard Conc. ng Hg/L	Blank Corrected Abs. 1	Blank Corrected Abs. 2	Blank Corrected Mean	Standard Deviation	Slope	Y-Intercept	Correlation
7/13/2012	1000	0.0303	0.028	0.0292	0.0016			
7/13/2012	5000	0.1337	0.1232	0.1285	0.0074			
7/13/2012	10,000	0.2555	0.2302	0.2429	0.0179			
7/20/2012	0	0.0018	0.0014	0.0000	0.0003	2.497 E-05	0.000856	0.9998
7/20/2012	100	0.0024	0.0025	0.0025	0.0001			
7/20/2012	500	0.0129	0.0131	0.0130	0.0001			
7/20/2012	1000	0.0258	0.0260	0.0259	0.0001			
7/20/2012	5000	0.1297	0.1298	0.1298	0.0001			
7/20/2012	10,000	0.2420	0.2552	0.2486	0.0093			
8/1/2012	0	0.0030	0.0030	0.0000	0.0000	2.515 E-05	0.001404	0.9998
8/1/2012	100	0.0031	0.0025	0.0028	0.0004			
8/1/2012	500	0.0149	0.0131	0.0140	0.0013			
8/1/2012	1000	0.0285	0.0257	0.0271	0.0020			
8/1/2012	5000	0.1376	0.1247	0.1312	0.0091			
8/1/2012	10,000	0.2598	0.2419	0.2509	0.0127			
8/17/2012	0	0.0000	0.0012	0.0000	0.0008	2.396 E-05	0.000213	0.9999
8/17/2012	100	0.0025	0.0023	0.0024	0.0001			
8/17/2012	500	0.0115	0.0121	0.0118	0.0004			
8/17/2012	1000	0.0226	0.0251	0.0239	0.0018			
8/17/2012	5000	0.1134	0.1309	0.1222	0.0124			
8/17/2012	10,000	0.2210	0.2565	0.2388	0.0251			
8/21/2012	0	0.0016	0.0016	0.0000	0.0000	2.652 E-05	0.000895	0.9997
8/21/2012	100	0.0027	0.0026	0.0027	0.0001			
8/21/2012	500	0.0139	0.0131	0.0135	0.0006			
8/21/2012	1000	0.0272	0.0270	0.0271	0.0001			
8/21/2012	5000	0.1330	0.1447	0.1389	0.0083			
8/21/2012	10,000	0.2600	0.2669	0.2635	0.0049			
9/5/2012	0	0.0016	0.0015	0.0000	0.0001	2.636 E-05	0.001531	0.9996
9/5/2012	100	0.003	0.0029	0.0030	0.0001			
9/5/2012	500	0.0142	0.0145	0.0144	0.0002			
*9/5/2012	1000	0.0282	-	0.0282	-			
*9/5/2012	5000	0.1389	-	0.1389	-			
*9/5/2012	10,000	0.2623	-	0.2623	-			
9/7/2012	0	0.0013	0.0012	0.0000	0.0001	2.391 E-05	0.001201	0.9997
9/7/2012	100	0.0026	0.0027	0.0027	0.0001			
9/7/2012	500	0.0127	0.0128	0.0128	0.0001			
9/7/2012	1000	0.0255	0.0257	0.0256	0.0001			

Analysis Date	Standard Conc. ng Hg/L	Blank Corrected Abs. 1	Blank Corrected Abs. 2	Blank Corrected Mean	Standard Deviation	Slope	Y-Intercept	Correlation
9/7/2012	5000	0.1235	0.1264	0.1250	0.0021			
9/7/2012	10,000	0.2404	0.2361	0.2383	0.0030			
9/11/2012	0	0.0010	0.0010	0.0000	0.0000	2.339 E-05	0.001708	0.9995
9/11/2012	100	0.0027	0.0025	0.0026	0.0001			
9/11/2012	500	0.0133	0.0128	0.0131	0.0004			
9/11/2012	1000	0.0267	0.0248	0.0258	0.0013			
9/11/2012	5000	0.1295	0.1194	0.1245	0.0071			
9/11/2012	10,000	0.2458	0.2196	0.2327	0.0185			

* An instrument problem occurred after the sample for Trout Lake 12963 was run so no other samples were analyzed on that date. The second 1,000 ng Hg/L, 5,000 ng Hg/L and 10,000 ng Hg/L standards were not analyzed on 9/5/2012.

Appendix C

Quality Assurance Audit Report: 2012 Technical Systems Audit of Great Lakes Indian Fish and Wildlife Commission (GLIFWC) Mercury Testing Project – Spring Walleye

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

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

Audit Dates: 31 July 2012 and 02 August 2012

Closing Discussions with LSRI-GLIFWC Staff: 25 September 2012

Description and Scope of Audit

A technical systems audit (TSA) of the laboratory analysis for the 2012 project *Great Lakes Indian Fish and Wildlife Commission (GLIFWC) Mercury Testing and Updating Tribal-Walleye Consumption Advice*, hereafter referred to as the 2012 GLIFWC Mercury Testing Project, was conducted 31 July 2012 and 02 August 2012. The objectives of the TSA were to review the project quality system documentation, personnel files, equipment/analytical instrumentation calibration and maintenance, and raw data from sample processing and analysis of the spring 2012 walleye samples. The TSA included a procedural audit of walleye sample grinding, digestion, and mercury analysis, which were observed to verify that they were conducted in accordance with LSRI standard operating procedures (SOPs) and with the GLIFWC Mercury Testing Quality Assurance Project Plan (QAPP). The digestion and mercury analysis procedural audit was conducted 31 July 2012, and the sample grinding procedural audit was conducted 02 August 2012. The walleye sample grinding (samples collected in spring 2012 from Annabelle Lake in Vilas County, WI; n=7 samples total) was audited against LSRI SOP SA/10, *v.*6 (and supporting LSRI SOP SA/8, *v.*6). The digestion and mercury analysis was audited against LSRI SOP SA/49, *v.*1 draft (and supporting LSRI SOPs SA/11, *v.*6; GLM/12, *v.*5; and SA/42, *v.*2); samples (n=38) were collected in spring 2012 from Butternut Lake (Forest County, WI), Duck Lake (Barron County, WI), Scattering Rice Lake (Vilas County, WI), Twin Lakes Chain (Vilas County, WI), and Turtle-Flambeau Flowage (Iron County, WI). In addition, the project documentation (GLIFWC Project Laboratory Notebook and 2012 GLIFWC Mercury Testing Project Three-Ring Binder) was reviewed during the procedural audits in order to verify compliance with LSRI's Quality Management Plan and the GLIFWC Mercury Testing QAPP. A draft quality assurance report was sent to LSRI-GLIFWC Project staff on 18 September 2012 and staff members commented on the TSA findings during a closing meeting on 25 September 2012.

The GLIFWC Project Co-Managers at LSRI are Thomas Markee and Christine Polkinghorne, and Kimberly Beesley is a project staff member. Cole Holstrom is the student researcher assisting with the project.

Audit findings in this report are classified as follows (according to the ISO 9001 model):

- **Non-Conformance:** Requires corrective action and may have affected data quality.
- **Deviations:** Area of concern that requires preventative action, as it has the potential for non-conformance. Findings in this category have deviations forms assigned to them.
- **Observations:** Do not require corrective action, but could transform into nonconformance. Observations may provide additional information or explanation of the sample analysis results.
- **Praises or Noteworthy Efforts:** Areas that were observed to be excellent examples of implementation of LSRI's Quality Management Plan and/or the GLIFWC Mercury Testing QAPP, or that show significant improvement from prior audits. Do not require corrective action.
- **Opportunity for Improvement:** Areas identified that can improve process or data quality through implementation of changes.

1 Quality System Documentation

1.1 Audit Findings

1.1.1 Non-Conformance

- No non-conformance findings from audit of quality system documentation.

1.1.2 Deviations

- No deviations found during audit of quality system documentation.

1.1.3 Observations

- The contract between LSRI and GLIFWC has different requirements than stated in the GLIFWC Mercury Testing QAPP. The contract states that the final report is due to GLIFWC ***30 September 2012***, while the QAPP states that the final report is due ***30 October 2012***.
- The balance identification for Mettler Toledo PB303-S was not recorded on the sample weighing datasheet.

1.1.4 Praises/Noteworthy Efforts

- The current GLIFWC Mercury Testing QAPP received final approval on 24 June 2011. The QAPP is stored in the 2012 GLIFWC Mercury Testing Project binder (12-05-24_GLIFWC). The LSRI SOP(s) for each procedure being conducted were found in the laboratory during each procedural audit.

- The Chain of Custody (COC) forms for the spring 2012 samples (received by LSRI in two sets; 24 May 2012 and 13 July 2012) are included in the 2012 GLIFWC Mercury Testing Project binder, and the date that samples were transferred to the LSRI freezer by LSRI-GLIFWC Project staff was recorded on the COC.
    - The minimum/maximum temperature range of the freezer when the samples were added was -24°C to -21.2°C (first set) and -23°C to -19°C (second set), which is in accordance with specified sample handling requirements specified in LSRI SOP SA/10, *v.*6 (i.e., <-10°C). *Note that sample storage temperature is not specified in the GLIFWC Mercury Testing QAPP.*
    - Samples are stored in a locked chest freezer; temperature range at the time of the audit was -20.3°C (-24.7°C to -13.0°C).
- Data and observations were appropriately recorded (i.e., entries in indelible ink, dated, initialed, and error corrections done properly) in the project laboratory notebook and on datasheets stored in the 2012 GLIFWC Mercury Testing Project binder.

1.1.5 Opportunity for Improvement

- It is recommended that the contract between LSRI and GLIFWC reflect the requirements stated in the GLIFWC Mercury Testing QAPP whenever possible to avoid confusion.
- Although the same balance is routinely used for sample weighing, it is important to record the balance identification on the datasheet so that any internal or external reviewer/auditor would be able to look up the balance accuracy verification data, as well as, any maintenance information.
- LSRI SOP SA/49, *v.*1 was in "working draft" form during processing and analysis of the spring 2012 walleye samples, and needs final review and approval prior to the receipt of the 2012 fall walleye samples.
    - Prior to finalizing SA/49, v.1, it is suggested that Step 29 be revised: *"Just prior to analysis of blanks, standards, and samples, add 10 mL of 10% (w/v) hydroxylamine hydrochloride with 10% (w/v) sodium chloride in two 5 mL aliquots, dilute to 50 mL with deionized water using the correct line on the digestion cup, cover with a screw cap and mix sample* ***until no*** *purple or* ***brown color remains.***" The permanganate solution stains the tubes a light brown color, which causes the solution to appear light brown (although the solution is actually clear). For clarification, revise this requirement as follows: "*...mix sample* ***until no*** *purple color or* ***brown precipitate remains.***"
    - As discussed during the closing meeting on 25 September 2012, SA/49 should also include a step to verify that the temperature of the solution in the HotBlock™ is within the correct temperature range for the digestion

procedure. This verification should be conducted each digestion day. It is recommended that a datasheet be developed to record this information over time (i.e., during the course of a project year).

- It is recommended that the existing LSRI SOP for routine maintenance of the FIMS-100 (i.e., 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 detail the methods, materials, and schedules to be used in the routine inspection, cleaning, maintenance, testing, calibration, and use of this instrument.
- A new draft version of LSRI SOP SA/08, *v.*7 exists, but has not been finalized. This SOP should be reviewed and finalized prior to receipt of fall walleye samples.
    - Note that as of 25 September 2012, SA/08, *v.*7 has been reviewed by the GLIFWC Project Co-Managers and is awaiting review by the LSRI QAM prior to finalization.

1.2 Conclusions from Quality System Documentation Audit

Overall project documentation using laboratory notebook 06-07-10-CNP and the 2012 GLIFWC Mercury Testing Project Binder (12-05-24_GLIFWC) was very good, and provided sufficient documentation to follow the samples from receipt at LSRI though mercury analysis. The GLIFWC Mercury Testing QAPP is stored in the Project Binder and was finalized and approved prior to sample analysis. All current versions of the relevant project SOPs were kept in the laboratory where the procedures were carried out, although the mercury analysis SOP was in "working draft" form and should be finalized as soon as possible.

2 Organization and Responsibilities

2.1 Audit Findings

- No audit findings in any category to report; there is a sufficient number of LSRI personnel dedicated to the GLIFWC Mercury Testing Project to maintain the level of quality required by the QAPP.

3 Training and Safety

3.1 Audit Findings

3.1.1 Non-Conformance

- No non-conformance findings from audit of training and safety.

3.1.2 Deviations

- No deviations found during audit of training and safety.

3.1.3 Observations

- Cole Holstrom recently began wearing prescription glasses. At the time of the procedural audit, LSRI did not have comfortable Over-Prescription Safety Glasses (i.e., safety glasses that are designed to fit over prescription eyewear); therefore, Cole had to take off his prescription glasses in order to wear the current safety glasses that LSRI has in stock.
- Cole Holstrom's Certificate of SOP Compliance does not indicate that he read LSRI SOP SA/49, *v.*1 although his Certificate of Training Competency shows that he received training on this procedure. It is likely that SA/49, *v.*1 was erroneously not recorded on the Certificate at the time it was read.

3.1.4 Praises/Noteworthy Efforts

- The LSRI QAM has resumes on file for all LSRI-GLIFWC Project staff; all have been updated within the past 12 months.

3.1.5 Opportunity for Improvement

- Over-Prescription Safety Glasses should be available for LSRI-GLIFWC project staff and student employees.
    - Note that as of 25 September 2012, the UWS Environmental Health and Safety Director provided LSRI with several options for Over-Prescription Safety Glasses that LSRI-GLIFWC project staff and student employees can choose from.
- Verify that Cole Holstrom has read LSRI SOP SA/49, *v.*1; the LSRI QAM will update the Certificate of SOP Compliance accordingly.

3.2 Conclusions from Training and Safety Audit

Resumes are on file for LSRI staff working on the 2012 GLIFWC Mercury Testing Project. GLIFWC Project personnel have read all relevant SOPs (verify that Cole Holstrom has read LSRI SOP SA/49, *v.*1), have completed the LSRI Quality System Orientation, and have taken the UWS Laboratory Health and Safety Training course. All laboratory safety procedures were followed during the TSA. Over-Prescription Safety Glasses should be available for staff and student employees who need them.

4 Equipment and Analytical Instrumentation

4.1 Audit Findings

4.1.1 Non-Conformance

- No non-conformance findings from audit of equipment and analytical instrumentation.

4.1.2 Deviations

- Deviation #2012-GLIFWC-01: The balance used to weigh processed tissue for digestion was calibrated using three ASTM Class 1 weights; however, the lowest verification weight used (i.e., 0.2 g) was greater than that of the Certified Reference Material for Trace Metals (i.e., DORM-3) being measured (i.e., DORM-3 weight was 0.1 g – 0.15 g). According to LSRI SOP GLM/12, *v.*5 – Procedure for Verification of Laboratory Balances, three ANSI/ASTM Class 1 weights must be selected that "bracket the weight being determined". This was discussed with the project staff during the audit, and it was suggested that a 0.1 g verification weight or lower mass be used as the lowest verification weight whenever the Certified Reference Material for Trace Metals is weighed.

4.1.3 Observations

- DORM-3 Certified Reference Material does not have a lot number and expiration date, which is unusual for a certified standard. Can an expiration date be obtained? If yes, it should be written on the container.
    - Note that the DORM-3 Certified Reference Material has an expiration date of September 2016. As of 25 September 2012, this information has been added to the container so that the expiration date is clearly visible to LSRI-GLIFWC project staff and students.
- Following measurement of carrier and reductant flow rates, the collection tubes were placed into the appropriate solution bottles, but the FIAS was not run one more time (as stated in Step 28 of LSRI SOP SA/49, *v.*1 draft).

4.1.4 Praises/Noteworthy Efforts

- Laboratory balance and PerkinElmer FIMS-100 used during sample processing and analysis have a routine, preventative maintenance schedule (as described in LSRI SOP

GLM/12, *v.*5 and LSRI SOP SA/50, respectively), and calibration/maintenance logs are kept for the balances and FIMS-100. Manufacturer's operating manuals are readily available to LSRI-GLIFWC Project staff. Procedural audit and review of maintenance and operational records indicated that the laboratory balances and FIMS-100 were in good operating condition at the time of the audit; the FIMS-100 met the standard curve acceptance criteria (LSRI SOP SA/49, *v.*1 draft).

- Based on the procedural audit conducted 31 July 2012, the mercury standard and spike preparation procedure was in compliance with LSRI SOP SA/42 *v.*1 – *Stock, Standard, and Spike Preparation for Mercury Analysis*.
    - The 10.0 mg/L Hg Sub-Stock was prepared by Kimberly Beesley on 09 July 2012, which was prior to the one month expiration required by LSRI SOP SA/42 *v.*1.
    - The 500 µg/L Hg Sub-Stock was prepared by Kimberly Beesley on 31 July 2012, which was prior to the one week expiration required by LSRI SOP SA/42 *v.*1.
- Limit of Detection and Quantification for 2012 GLIFWC Mercury Testing Project was determined 02 June 2012 (prior to any sample analysis for the project): n=8 samples, LOD = 0.0030 µg Hg/g and LOQ = 0.0099 µg/g.

4.1.5 Opportunity for Improvement

- It is suggested that a new can opener and filet knives be purchased for the project, as this equipment is rusty.
    - As of 25 September 2012, a new can opener and filet knives have been purchased for the project. LSRI-GLIFWC project staff will try to slow down the development of rust by drying this equipment after it has been cleaned and storing it in the laboratory drawers.
- LSRI does not have a finalized pipet verification SOP currently (although a draft version is available), however, verification of pipet accuracy using the draft version of this SOP is strongly encouraged and should be conducted within three months of their use in the GLIFWC Mercury Testing Project.

4.2 Conclusions from Equipment and Analytical Instrumentation Audit

The equipment/analytical instrumentation used in the sample processing and analysis of the spring 2012 walleye samples was found to be in good working order (a new can opener and filet knives have been purchased to replace rusty equipment), with calibration/verification and maintenance activities appropriately recorded in the equipment-specific log books. A 0.1 g or lower-mass verification weight should be used to verify the accuracy of the balance when weighing Certified Reference Material (i.e., DORM-3) for digestion. Verification of pipet accuracy is encouraged and should be conducted within three months of their use in the GLIFWC Mercury Testing Project.

Supplemental Data

I. Completed Technical Systems Audit Checklist for 2012 GLIFWC Mercury Testing Project (Spring Walleye Samples)

II. Results from 2012 GLIFWC Mercury Testing Project Sample Digestion and Analysis Procedural Audit

III. Results from 2012 GLIFWC Mercury Testing Project Sample Grinding Procedural Audit

IV. Completed Deviation Form for Deviation #2012-GLIFWC-01

Appendix D

Standard Operating Procedures (SOPs) Used During Project

Standard Operating Procedure SA/8 v.7

ROUTINE LABWARE CLEANING FOR METALS ANALYSIS

INTRODUCTION

This standard operating procedure (SOP) describes the process used for the routine cleaning of labware and tissue grinding equipment used for metals analysis. The equipment used for tissue grinding (e.g., grinder attachment for KitchenAid™ Stand Mixer, blender, bowls, fillet knife, etc.) must be prepared by following the entire cleaning procedure before the initial use of the equipment if it has not been used for more than one week, as well as, after each use of the equipment. Labware is typically in contact with higher metal concentrations than the equipment used for tissue grinding and, therefore, must be cleaned using a different procedure (i.e., 10% (v/v) nitric acid) than the tissue grinding equipment. In addition, the stronger acid concentration used to clean the labware will cause damage to 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.

DEFINITIONS

Labware: For metals analysis, this refers to 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).

EQUIPMENT LIST

- Aluminum Foil
- Ammonium Hydroxide, Concentrated (Approximately 30%)
- Deionized Water
- Dish Pan
- Fillet Knife
- Gloves
- KitchenAid™ Food Grinder Attachment
- Hydrochloric Acid, Concentrated (Approximately 37%)
- Lab Coat
- Labware to be Washed
- Liquinox® Detergent
- Nalgene® 10-L Carboy, Marked with 1-L Graduations
- Nitric Acid, Concentrated (Approximately 70%)
- pH Indicator Strips
- Plastic Bottles
- Plastic Dish Rack
- Safety Glasses or Goggles
- Sodium Bicarbonate (Baking Soda)
- Spatula (Stainless Steel)
- Stainless Steel Bowls
- Various Labware
- Volumetric Flasks
- Volumetric Pipets
- Wash Bottle
- Washing Brushes

PROCEDURE

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

Note: Equipment should be processed through this entire cleaning procedure before the initial use if it has not been used for more than one week, as well as, after each use.

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

1. Fill a 10-L carboy to the 10-L mark with 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 a minimum of 30 seconds). Used acid should not be returned to the 10-L carboy. Remake the 0.1 M hydrochloric solution every six months or when the supply has been depleted. Unused acid should be stored in a tightly sealed carboy labeled with the contents of the bottle, the date of preparation, and initials of the preparer.

2. Neutralize used or expired acid prior to disposal 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. Pour the neutralized acid down the drain while running cold water. Record the disposal of neutralized acid on the appropriate disposal form or lab notebook.

Cleaning Tissue Grinding Equipment

4. Dismantle the KitchenAid™ food grinder attachment before washing.

5. Scrub all grinding equipment in hot[a] water containing Liquinox® detergent. Replace soapy water as needed during washing process when the water becomes contaminated with fish tissue.

6. Rinse equipment with tap water until there is no presence of soap.

7. Rinse equipment once with deionized water.

8. Soak equipment in 0.1 M hydrochloric acid for a minimum of 30 seconds (be sure acid comes in contact with all surfaces of equipment).

9. Rinse equipment three times with deionized water.

10. Upon drying, cover equipment with aluminum foil to store until used. Note that the filet knife and can opener rust quickly and should be dried by hand after completing the cleaning procedure, covered with aluminum foil, and stored in a drawer.

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

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.

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

[a] In the event that hot water is unavailable (i.e., during UWS Steam Plant shutdown; usually in August), an attempt should be made to obtain hot water from the dechlorinated lab water supply for at least the scrubbing portion of the cleaning. Rinsing can be done with cold water when hot water is unavailable.

11. Prepare the acid 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. Given the corrosive nature of the nitric acid fumes, the minimal amount of 10% nitric acid required should be prepared.

12. Store unused acid in a tightly-sealed carboy labeled with the contents of the bottle, the date of preparation, and initials of the preparer.

13. After use, neutralize the acid prior to disposal 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.

14. Pour the neutralized acid down the drain while running cold water. Record the disposal of neutralized acid on the appropriate disposal form or lab notebook.

Labware Cleaning

15. Scrub the labware thoroughly in hot[a] water containing Liquinox® detergent.

16. Rinse the labware with hot[a] water until there is no presence of soap.

17. Rinse the labware once with deionized water.

18. Fill a container with 10% nitric acid (place spatulas in a beaker of 10% nitric acid being sure to use only the side that has been submerged for weighing samples). Be sure the portion of the labware that comes into contact with the sample or standard is completely covered and filled with acid (e.g., fill volumetric flasks with acid). Allow the labware to soak for a minimum of 1 minute.

19. Empty the acid from the container back into the acid storage carboy.

20. Rinse the labware a minimum of three times with deionized water.

21. 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.

Standard Operating Procedure SA/10 v.6

SAMPLE GRINDING FOR METALS ANALYSIS

INTRODUCTION

This standard operating procedure (SOP) describes the method used for grinding biological tissue, typically fish tissue, into homogeneous samples for metals analysis. 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/08, issued 1992). The proper safety equipment must be worn during the entire grinding procedure, including gloves, safety glasses, and lab coat.

EQUIPMENT LIST

- Beaker or Stainless Steel Bowls
- Certified-Clean Sample Containers
- Fillet Knife
- Freezer (Set at < -10°C)
- Gloves
- Kitchen Aid™ Food Grinder Attachment
- Kitchen Aid™ Stand Mixer
- Lab Coat
- Label Tape
- Permanent Marker
- Procedural Blank (i.e., Canned Tuna Fish; see Project Planning Documentation)
- Project-Specific Laboratory Notebook
- Safety Glasses
- Spatula
- Tissue Samples to be Ground

SAMPLE HANDLING REQUIREMENTS

1. After samples have been received, they should be stored in a freezer at <-10°C.

REFERENCES

Kitchen Aid™ Stand Mixer and Food Grinder Attachment Manuals.

Lake Superior Research Institute. 1992. LSRI/SOP/SA/08 – Routine Labware Cleaning for Metals Analysis.

PROCEDURE

Grinding Tissue Samples

1. If the grinding equipment has not been used the previous day, wash the grinder (or food grinding attachment of the stand mixer) and labware by following the procedure in *LSRI/SOP/SA/8- Routine Labware Cleaning for Metals Analysis* prior to grinding any samples.

2. 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 in a project-specific laboratory notebook.

3. Remove the samples to be ground from the storage freezer and allow to partially thaw (i.e., until tissue samples are pliable) prior to grinding.

4. If necessary, cut the sample into small pieces that will fit through the food grinder attachment of the stand mixer.

5. Assemble the food grinder attachment as follows (Figure 1):

5.1. Insert the grind worm (Figure 1, A) into the grinder body (Figure 1, B).
5.2. Place the knife (Figure 1, C) over the square shank at the exposed end of the grind worm.
5.3. Place the fine grinding plate (Figure 1, D) over the knife, matching the tabs of the plate with the notches of the grinder body.
5.4. Place the ring (Figure 1, E) on the grinder body, and turn the ring by hand until it is secured.

Figure 1. Assembly of KitchenAid™ Stand Mixer Food Grinder Attachment.

6. Connect the food grinder attachment to the stand mixer as follows (Figure 2):

6.1. Loosen the attachment knob (Figure 2, 1) by turning counterclockwise.
6.2. Remove the attachment hub cover and insert attachment shaft housing (Figure 2, 2) into the attachment hub (Figure 2, 3) making sure that the attachment power shaft fits into the square hub socket. When the attachment is properly seated, the pin on the attachment will fit into the notch on the hub rim.
6.3. Tighten attachment knob until attachment is completely secured to mixer.

Figure 2. Connection of the Assembled Food Grinder Attachment to the KitchenAid™ Stand Mixer.
6. Pass the sample through the food grinder attachment of the stand mixer, discarding the first few grams of tissue that come through. The speed setting on the grinder should be adjusted to the most effective setting (e.g., high speeds are needed for small samples so that the tissue will pass through the grinder without becoming stuck). Collect the tissue in a beaker or bowl.

7. Pass the collected tissue through the food grinding attachment of the stand mixer a second and third time and collect in the same beaker or bowl.

8. Thoroughly mix the tissue with a spatula to ensure homogeneity.

9. 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.

10. Wash the food grinding attachment of the stand mixer and labware by following the procedure in *LSRI/SOP/SA/08- Routine Labware Cleaning for Metals Analysis* prior to grinding the next sample.

11. Continue to grind each sample by repeating Steps 3 to 10.

Preparing the Procedural Blank

12. Prepare an appropriate procedural blank based on the type of tissue being ground. For example, canned tuna fish from a commercial supplier can be used as a procedural blank when grinding fish tissue samples. The frequency of processing procedural blanks, as well as, acceptance criteria and corrective actions are specified in the Quality Assurance Project Plan or other project planning documentation.

13. When using tuna, drain the liquid from the can. Homogenize the tissue with a spatula and transfer a portion to a certified-clean sample container following Step 9. Label this procedural blank as "Tuna before Grinding" and include the date of processing. The unground blank is included with the analysis set.

14. Grind the remainder of the tuna as a procedural blank following the procedure outlined in Steps 6 to 10. Label this procedural blank as "Tuna after Grinding" and include the date of processing. The ground blank is included with the analysis set.

Standard Operating Procedure SA/11 v.6

SAMPLE WEIGHING FOR METALS ANALYSIS

INTRODUCTION

This standard operating procedure (SOP) describes the method used to weigh processed biological tissue samples, typically fish tissue samples, for mercury or other 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/08 - 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/goggles, and lab coat.

REFERENCES

Lake Superior Research Institute. 1995. LSRI/SOP/GLM/12 - Procedure for Verifying Calibration of Laboratory Balances.

Lake Superior Research Institute. 1992. LSRI/SOP/SA/08 – Routine Labware Cleaning for Metals Analysis.

Lake Superior Research Institute. 1992. LSRI/SOP/SA/10 – Sample Grinding for Metals Analysis.

Lake Superior Research Institute. 1999. LSRI/SOP/SA/38 – Preparation of Tissues for Analytical Determinations using Liquid Nitrogen.

EQUIPMENT LIST

- Datasheet (see Appendix 1) and/or Project-Specific Laboratory Notebook
- Deionized Water
- Gloves
- Ground/Processed Samples
- KimWipes®
- Lab Coat
- Permanent Marker
- Polypropylene Digestion Vessels (from a commercial supplier such as Environmental Express)
- Safety Glasses/Goggles
- Spatula
- Top-Loading or Analytical Balance (must be capable of reading to at least 0.001 g)

REAGENTS

- **Nitric Acid (10% v/v):** Add 100 mL of concentrated nitric acid to 900 mL of deionized water. This solution should be prepared in a laboratory hood. The preparer must wear a lab coat, gloves and safety glasses/goggles.

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. Label clean, polypropylene digestion vessels with the appropriate sample number and collection site name.

3. Check the level of the balance and adjust if necessary. Clean the balance pan by removing any foreign materials with a soft brush. Record the balance ID number on the appropriate datasheet (see Appendix 1 for example) or in a project-specific laboratory notebook.

4. 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).

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

6. 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.

7. Record the weight of the sample on the appropriate datasheet (see Appendix 1 for example) or in a project-specific laboratory notebook. The date and initials of the individual performing the procedure must also be recorded.

8. Wipe the spatula clean with a KimWipe®. Rinse the spatula with deionized water and place the spatula in 10% (v/v) nitric acid to soak for at least one minute. Remove the spatula from the 10% nitric acid, rinse with deionized water and wipe with a KimWipe® prior to using the spatula on another sample.

9. Repeat Steps 5 to 8 for all tissue samples to be weighed.

APPENDIX 1

EXAMPLE SAMPLE TISSUE WEIGHING DATASHEET

Date of Sample Weighing/Initials:

Balance ID:

Sample ID	Bl. Corr. Signal	ng/L (FIMS Calc)	ng/L (our calc)	ng Hg	g sample	Calculated μg/g
SAMPLE			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE dup			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE spk 1			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE spk 2			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE			#DIV/0!	#DIV/0!		#DIV/0!
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SAMPLE dup			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE spk 1			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE spk 2			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE			#DIV/0!	#DIV/0!		#DIV/0!
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SAMPLE			#DIV/0!	#DIV/0!		#DIV/0!

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.

APPENDIX 1. STUDENT'S *t*-DISTRIBUTION CHART

Note: Chart displays only the 99% probability values for values of $t_{(n-1)}$ up to 21.

DF = n-1	0.01
1	31.82052
2	6.96456
3	4.54070
4	3.74695
5	3.36493
6	3.14267
7	2.99795
8	2.89646
9	2.82144
10	2.76377
11	2.71808
12	2.68100
13	2.65031
14	2.62449
15	2.60248
16	2.58349
17	2.56693
18	2.55238
19	2.53948
20	2.52798
21	2.51765

Accessed from StatSoft, Inc. (http://www.statsoft.com/textbook/sttable.html) 11/04/2009.

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

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

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/08 - Routine Labware Cleaning for Metals Analysis* (issued 1992).

REFERENCES

Lake Superior Research Institute. 1992. LSRI/SOP/SA/08 – Routine Labware Cleaning for Metals Analysis.

Lake Superior Research Institute. 1992. LSRI/SOP/SA/11 – Sample Weighing for Metals Analysis.

EQUIPMENT LIST

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

PROCEDURE

Mercury (Hg) Sub-Stock Preparation: 10.0 mg/L Hg Sub-Stock

1. Add ~60 mL deionized (DI) water to a 100-mL volumetric flask.

2. Into the flask, add the following:
- 1.00 mL (i.e., using an adjustable-volume, 100-1000 µL pipette) of a 1000 mg/L mercury stock/reference solution
- 1 mL trace metal grade concentrated HCl
- 100 µL 5% (w/v) KMnO4

3. Dilute to 100 mL with deionized water and mix thoroughly by inverting flask to prepare the 10.0 mg/L Hg sub-stock.

4. Label this solution with the concentration, date prepared, initials, and date of expiration as it must be remade **once a month**. The stock solution is stored at room temperature.

Mercury (Hg) Sub-Stock Preparation: 500 µg/L Hg Sub-Stock

5. Add ~60 mL of deionized water to a 100-mL volumetric flask.

6. Into the flask, add the following:

- 5.00 mL (i.e., using an adjustable-volume, 1-5 mL pipette) of the 10.0 mg/L Hg substock solution prepared in Steps 1 - 4
- 0.5 mL trace metal grade concentrated HCl
- 100 µL 5% (w/v) KMnO4

7. Dilute to 100 mL with deionized water and mix thoroughly by inverting flask to prepare a 500 µg/L Hg sub-stock.

8. Label this solution with the concentration, date prepared, initials, and expiration date as it must be remade **once a week**. The stock solution is stored at room temperature.

Mercury Standards Preparation

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

10. Pipet the volumes of deionized water and 500 µg/L Hg sub-stock into digestion vessels according to the table below (Table 1). Mercury concentrations of standards are based on the final volume (50 mL) of standard at the time of analysis.

11. Use an adjustable-volume, 10-100 µL or 100-1000 µL micropipette to deliver all water volumes and 500 µg/L Hg sub-stock Hg volumes less than 1 mL.

12. 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 500 µg/L Hg Sub-Stock	Volume of DI Water
Blank	0	1.00 mL
100	10 µL	990 µL
500	50 µL	950 µL
1000	100 µL	900 µL
5000	500 µL	500 µL
10,000	1.00 mL	0 mL

Mercury Spike Preparation

13. Spike a minimum of 10% of samples analyzed for mercury in duplicate.

14. Prepare each mercury spike by using an adjustable-volume micropipette to deliver 500 µL of 500 µg/L Hg sub-stock into a digestion vessel containing a known weight of fish/tissue (i.e., weighed following the procedure outlined in *LSRI/SOP/SA/11*).

Waste Disposal

15. All mercury waste from rinsing pipettes, beakers, etc. should be disposed of in a 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/49 v.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 (AA) detection (Lobring and Potter 1991). Note that the abbreviation 'FIMS' used in this procedure stands for 'Flow-Injection Mercury System', and the abbreviation 'FIAS' stands for 'Flow-Injection Analysis System'.

REFERENCES

Lake Superior Research Institute. 1992. LSRI/SOP/SA/10 – Sample Grinding for Metals Analysis.

Lake Superior Research Institute. 1992. LSRI/SOP/SA/11 – Sample Weighing for Metals Analysis.

Lake Superior Research Institute. 1999. LSRI/SOP/SA/38 – Preparation of Tissues for Analytical Determinations using Liquid Nitrogen.

Lake Superior Research Institute. 2002. LSRI/SOP/SA/42 – Stock, Standard, and Spike Preparation for Mercury Analysis.

Lake Superior Research Institute. 2005. LSRI/SOP/SA/46 – Processing Several Large Fish into one Homogenous Fish Composite.

Lake Superior Research Institute. 2007. LSRI/SOP/SA/50 – Routine Maintenance for FIMS-100.

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.

Perkin Elmer FIMS Flow Injection Mercury System Manuals. (Installation Maintenance System Description and Setting Up and Performing Analyses).

EQUIPMENT LIST

- 10 mg/L Mercuric Nitrate Sub-Stock for FIMS-100 Analysis (see *LSRI/SOP/SA/42*)
- 1000 µg/mL Purchased Mercuric Nitrate Stock
- 500 µg/L Mercuric Nitrate Sub-Stock for FIMS-100 Analysis (see *LSRI/SOP/SA/42*)
- Balance, Top Loading or Analytical (must be capable of reading to 0.001 g)
- Beakers

- Certified Reference Material for Trace Metals (i.e., DORM-3)
- Deionized Water
- FIMS-100 (PerkinElmer) Mercury Analyzer
- FIMS-100 Record Notebook
- HotBlock™ (Environmental Express) and HotBlock™ Racks
- Hydrochloric Acid, Trace Metals Grade
- Hydroxylamine Hydrochloride, Reagent Suitable for Mercury Determination
- Kimwipes®
- Lab Coat
- Nitric Acid, Trace Metals Grade
- Pipets/Pipettors
- Polypropylene Digestion Cups and Covers
- Potassium Permanganate, Certified ACS
- Potassium Persulfate, Reagent Suitable for Mercury Determination
- Procedural Blanks
- Repipet Dispensers, 10 mL and 1-5 mL
- Safety Glasses and Goggles
- Samples (prepared following *LSRI/SOP/SA/10*, *LSRI/SOP/SA/38*, or *LSRI/SOP/SA/46*)
- 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)

REAGENTS

- **10% (w/v) Hydroxylamine Hydrochloride with 10% (w/v) Sodium Chloride:** Dissolve 200 g of hydroxylamine hydrochloride and 200 g of sodium chloride in 2 L of deionized water. Prepare solution as needed; expiration is six months from the date of preparation. Store solution at room temperature.
- **3% (v/v) Hydrochloric Acid (Carrier Solution):** Add 300 mL of trace metal grade hydrochloric acid to 10 L of deionized water. Prepare solution as needed; expiration is six months from the date of preparation. Store solution at room temperature.
- **5% (w/v) Potassium Permanganate:** Dissolve 100 g of potassium permanganate in 2 L of deionized water. Prepare solution as needed; expiration is six months from the date of preparation. Store solution at room temperature.
- **5% (w/v) Potassium Persulfate:** Dissolve 100 g of potassium persulfate in 2 L of deionized water. Prepare solution as needed; expiration is six months from the date of preparation. Store solution at room temperature.
- **5% (w/v) Stannous Chloride in 3% (v/v) Hydrochloric Acid (Reductant Solution)**: Dissolve 50 g of stannous chloride in 1 L of 3% (v/v) Hydrochloric Acid. **This solution must be prepared daily.** Dispose of any unused solution as acid/base waste at the end of mercury analysis.

PROCEDURE

Sample and Standard Preparation

1. Turn the HotBlock™ on. Verify the digestion solution temperature by placing a digestion tube containing 50 mL deionized water into the HotBlock™. Allow the tube to remain in the HotBlock™ for a minimum of 30 minutes after the unit has reached the set-point temperature (i.e., 115°C ±5°C). Record the location of the tube in the HotBlock™ and measure and record the temperature of the water in the digestion tube on the Microsoft Excel "Mercury Master Daily Analysis Form". The temperature of the water in the digestion cup should be 90°C ±5°C. If not, adjust the temperature setting on the HotBlock™ until the temperature of the water is within the accepted range. A different location in the HotBlock™ should be chosen each time a digestion is performed.

2. Prepare samples for mercury digestion and analysis following the appropriate LSRI 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*).

3. Weigh samples, including a set of procedural blanks, using the procedure outlined in *LSRI/SOP/SA/11 – Sample Weighing for Metals Analysis*. A minimum of 10% of the samples must be weighed in duplicate for duplicate analysis.

4. Weigh an appropriate mass of Certified Reference Material for Trace Metals (i.e., DORM-3) using the procedure outlined in *LSRI/SOP/SA/11 – Sample Weighing for Metals Analysis*. An appropriate mass is one in which the analyzed Certified Reference Material will fall within the range of the standard curve. For a set of mercury samples, Certified Reference Material samples should be prepared and analyzed in a ratio of one Certified Reference sample per 15 tissue samples. Typically, one set contains up to 40 samples.

5. Prepare standards and spikes for mercury digestion and analysis following *LSRI/SOP/SA/42 – Stock, Standard, and Spike Preparation for Mercury Analysis*. Two sets of standards should be prepared for each set of mercury samples. In addition, 10% of the samples should be spiked in duplicate.

Sample Digestion

Note: The addition of acids and digestion of samples must be conducted in a fume hood. Proper personal protective clothing (e.g., gloves, lab coat, and safety goggles) must also be worn.

6. 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.

7. Place the racks containing the sample digestion cups into the HotBlock™. Allow samples to digest for approximately 15 minutes or until all the tissue is dissolved.

8. Turn off the HotBlock™, remove the HotBlock™ rack containing the digestion cups from the HotBlock™, and allow contents to cool to room temperature in the fume hood.

9. Add 15.0 mL of 5% (w/v) potassium permanganate to each digestion cup in 5.0 mL increments, gently swirling the HotBlock™ rack holding the digestion cups after each addition.

10. Ensure that the samples remain purple in color for at least 15 minutes. If not, add additional 5% (w/v) potassium permanganate solution (maximum of 5 mL) to the samples. If additional 5% (w/v) potassium permanganate is added to a sample, an equal amount should be added to one set of standards and a blank.

11. 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.

12. Allow the digestion cups to react overnight at room temperature to oxidize organic mercury compounds to inorganic mercury ions.

13. The samples can be stored covered in the fume hood, and will remain stable for up to three days before analysis. Samples are typically analyzed the day following the digestion process.

Sample Analysis Preparation

14. Prepare the carrier and reductant solutions (see "Reagents" section):

    14.1. **Carrier Solution:** 3% (v/v) hydrochloric acid.
    14.2. **Reductant Solution:** 5% (w/v) stannous chloride in 3% (v/v) hydrochloric acid. The volume of 5% stannous chloride prepared will depend on the number of samples to be analyzed. For a full set of 40 samples, prepare 900 mL of Reductant Solution. **This solution must be prepared daily.**
    14.3. If the samples appear to be producing excessive foam during analysis (not typical), 10 mL of Silicon Defoaming Agent may be added per liter reductant solution.

15. Turn on computer and printer.

16. Turn on Nitrogen (set pressure at 400 kPa or 60 psi).

17. Turn on FIMS-100 Mercury Analyzer and allow it to warm up for a minimum of 10 minutes.

18. Press Ctrl+Alt+Del on computer keyboard and enter "Barstow 9B" for the username and "fims100" as the password, while "BARS 9B-9061" shows in the LOG ONTO window.

19. If a Microsoft (MS) Excel file has been created for the project and stored on the "LSRItemp"

Drive, access the file by clicking on the "LSRItemp" Drive shortcut on the desktop and using your personal log-in information when prompted. For example, in the username window enter: "uwsuper\username" followed by your personal password in the password window. Minimize the MS Excel window until it is needed.

20. Double click on the **WinLab32 for AA** icon on the computer desktop.

21. Click on **Wrkspc** icon (Figure 1) and double-click on the **Hg Analysis.ffm** workspace to choose it.

    21.1. Click on the Method button and double click on Hg extended RT5000, which is the correct method for analysis. The method will then show in the **Manual Analysis Control** window.

22. In the **Manual Analysis Control** window near the Results Data Set Name click **open** and enter a new name or choose a file in the list (e.g., DateProject, see Figure 1). Be sure that the **save data** box is checked.

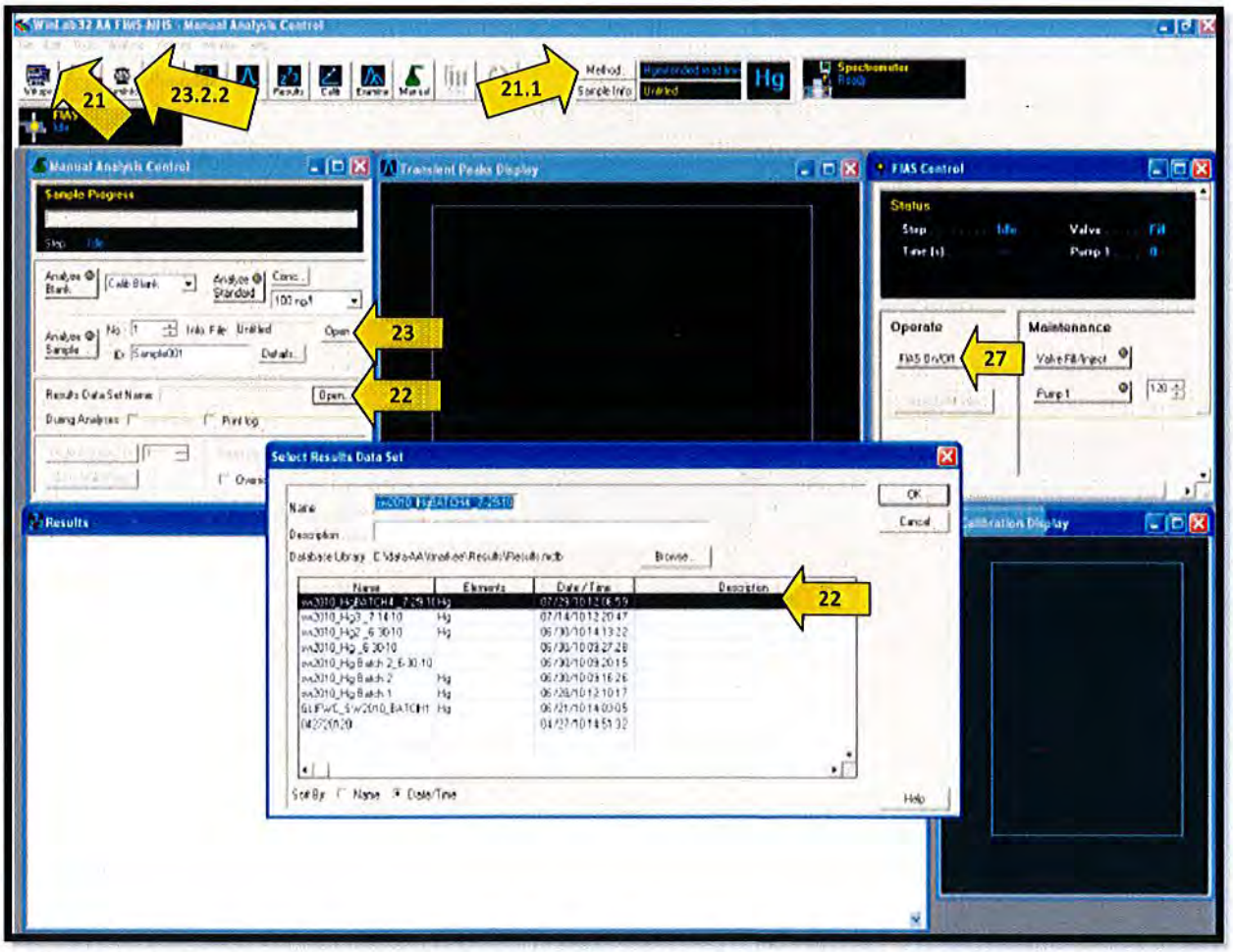

Figure 3. Screen shot of the control window of the WinLab32™ for AA software. The yellow arrows indicate areas of importance and the corresponding steps referenced within this standard operating procedure.

23. Choose or prepare the Sample Information File using WinLab32™ for AA software (SIF, Figure

1).

23.1. 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 (Info File) field in the **Manual Analysis Control** window.

23.2. To prepare an MS Excel file with the same format as a SIF (Figure 2):

23.2.1. Highlight the rows in the Excel file to be added to the SIF, and copy (Ctrl+C). Note that Sample ID names must contain less than 25 characters.

23.2.2. 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).

23.2.3. Close the Sample Information Editor window.

23.2.4. 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.

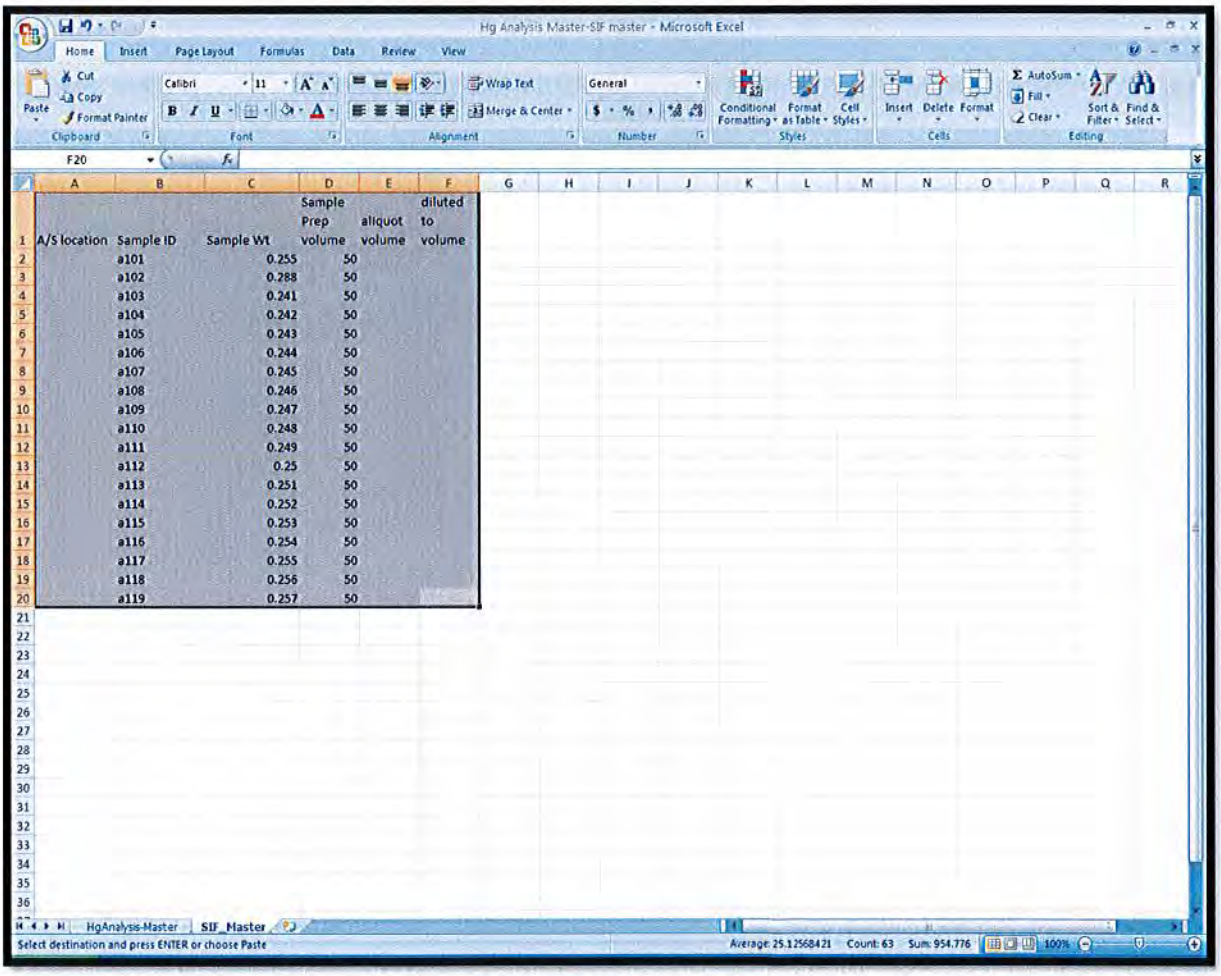

Figure 4. Preparation of a Sample Information File (SIF, in WinLab32™ for AA software) from an MS Excel file. Using MS Excel to create the SIF is ideal if a project MS Excel file has been previously prepared.

24. 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).

25. Check Gas/Liquid Separator cover to see that it has been tightened (Figure 3).

26. Attach tubing from Gas/Liquid Separator to the FIMS-Absorbance [Quartz] Cell (Figure 3).

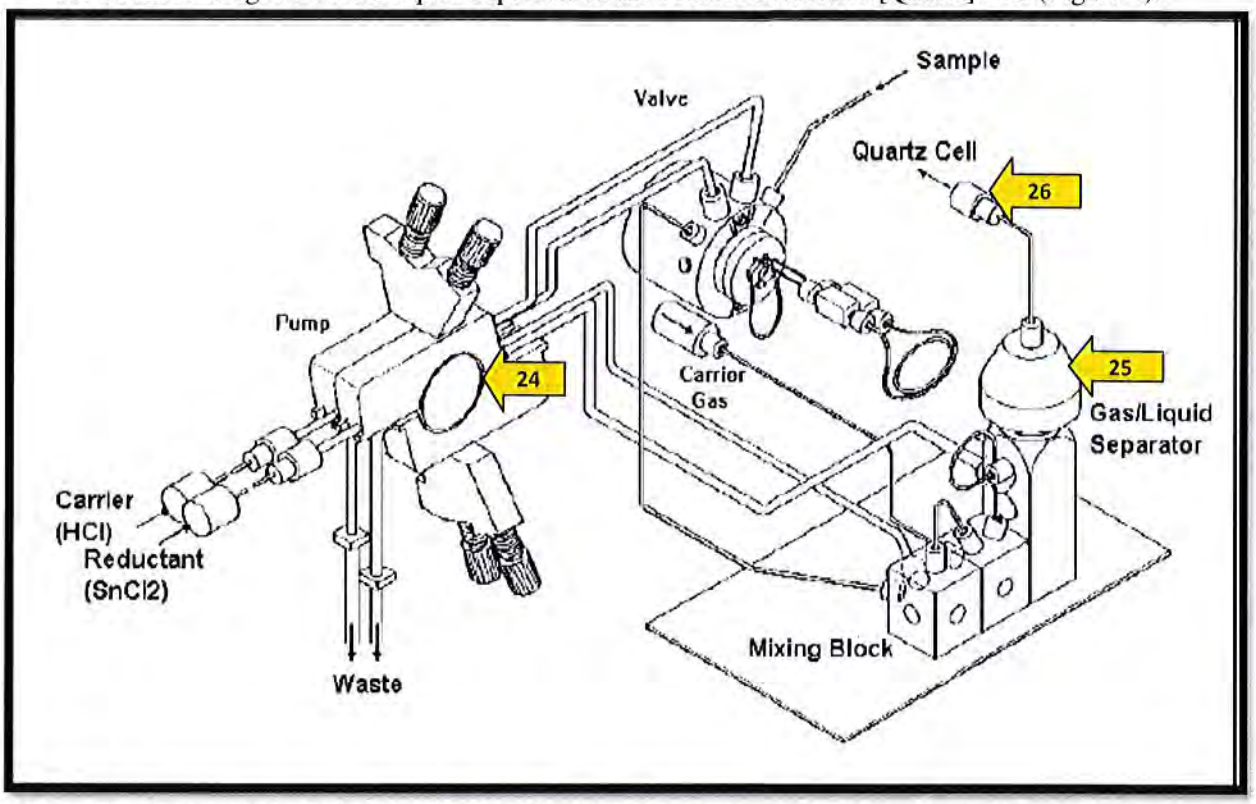

Figure 5. Diagram of the PerkinElmer FIMS-100. The yellow arrows indicate important areas of the instrument that need attention according to the referenced sections of this SOP.

27. With all three collection tubes (sample, carrier, and reductant) in clean deionized water, run FIAS (Flow Injection for Atomic Spectroscopy) once by clicking on the **FIAS on/off** button in the FIAS Control Window (Figure 1). Be sure that the waste tubing is in a waste collection container labeled "FIMS Waste".

28. Check the carrier and reductant flows. Place the carrier and reductant collection tubes into their appropriately labeled graduated cylinders and fill to 50 mLwith deionized water. In the FIAS Control Window, click **FIAS On/Off** under the **Operate** tab. Observe the volume withdrawn from each graduated cylinder over 1 minute. Carrier volume should be between **9 and 11 mL/min** and reductant should be about half the carrier flow (**5 to 7 mL/min**). Record both the carrier flow and reductant flow in the FIMS-100 Maintenance Log Book. If needed, flow rates may be adjusted by turning the top knobs (clockwise to increase flow) on the pump magazine pressure adjustment levers.

29. The waste flow rate should be set slightly higher than the flow rate into the gas/liquid separator. If it is not, liquid may get into the quartz cell. If the waste flow is higher than the flow into the gas/liquid separator, bubbles will appear in the waste outlet tube of the gas /liquid separator. The bottom knobs control the waste flow, and the waste flow rate should be checked and adjusted as needed periodically (i.e., prior to the start of project sample analysis).

30. 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.

31. Just prior to analysis of blanks, standards, and samples, add 10 mL of 10% (w/v) hydroxylamine hydrochloride with 10% (w/v) sodium chloride in two 5 mL aliquots, dilute accurately to 50 mL with deionized water using the correct line on the digestion cup, cover with a screw cap and mix sample until no purple color remains and any brown precipitate dissolves. The sample tube may appear brown due to staining from the chemical reagents. Be sure to loosen the cap periodically to vent the sample. Safety glasses and gloves must be worn during this step.

Sample Analysis

32. Rinse the sample aspiration tube with deionized water and place in the blank solution. In the Manual Analysis Control Window 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.

33. Rinse the sample aspiration tube with deionized water and place in the lowest standard. Choose appropriate standard concentration from the drop down menu in the Manual Analysis Control Window near the Analyze Standard button. Click on **analyze standard** and allow instrument time to complete triplicate analysis. In the appropriate MS 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.

34. Prior to analyzing samples check the following parameters:

    34.1. The slope of the calibration curve must fall between **2.0×10^{-5} to 3.0×10^{-5}** and the correlation coefficient (r^2) must be greater than or equal to **0.995.**

    34.2. Review peak shape. The peak maximum should appear 5-10 seconds after the beginning of the read time and the signal should return to the baseline before the read time ends. If the peak is appearing too early, the carrier gas flow should be decreased. If the peak is appearing too late, the carrier gas flow should be increased. Generally, a flow in the range of **40-70 mL/min** is suitable.

    34.3. The 5000 ng/L standard must give a response between **0.10 and 0.15**.

    34.4. **If these checks do not fall in the acceptable range, check carrier and reductant flows, waste flows, and/or perform other maintenance as needed** (see *LSRI/SOP/SA/50 – Routine Maintenance for FIMS-100*).

35. Rinse the sample aspiration tube with deionized water and place in appropriate sample. Check that the sample ID in the ID field of the Manual Analysis Control Window is correct. Click on "analyze sample" and allow instrument time to complete triplicate analysis. Enter the mean Blank Corrected Signal and Percent Relative Standard Deviation (%RSD) values into the appropriate Excel file for that project. Repeat this step for each of the samples to be analyzed. Note that the **%RSD of the samples must be less than or equal to 5% for samples having concentration more than twice the limit of quantification (LOQ)** for that year. If the % RSD is greater than 5%, the sample must be reanalyzed.

36. The second blank, second set of standards, and Certified Reference Material should be run as they were above, sometime in between samples, to check the precision and stability of the instrument. 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. For example, if the sample set contains 52 samples, including duplicates and spikes, the set should be run in the following order:

- First set of standards
- ~13 samples
- Blank
- Lowest standard (100 ng/L)
- Certified Reference Material
- ~13 samples
- Next two standards (500 ng/L and 1000 ng/L)
- Certified Reference Material
- ~13 samples
- 5000 ng/L standard
- Certified Reference Material
- 10,000 ng/L standard

Completion of Analysis

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

38. Flush/clean tubing with deionized water by running FIAS two times. This is accomplished by clicking the FIAS on/off button in the FIAS Control Window.

39. 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.

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

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

42. Unscrew line from FIMS-absorbance cell.

43. Unscrew the gas/liquid separator cover and, using forceps to handle filter, dry filter with a Kimwipe®. Replace filter and loosely put the cover back on.

44. Print report. Choose **File** → **Utilities** → **Data Manager** → Choose the data set for that day → Click **Report** → **Use Existing Design** and select **Browse** to choose **hg.rep** → **Open** → **Next** → Select all the samples for that date or choose **today only** → 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.

45. Save the MS Excel file to the appropriate project folder.

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

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

48. Any sample or standard remaining in the digestion tubes after the analysis has been completed should be collected in a container labeled "Waste Samples/Standards from Mercury Analysis" and disposed of in accordance with the rules and regulations of the UWS Environmental Health and Safety Office.

Standard Operating Procedure SA/50

ROUTINE MAINTENANCE FOR FIMS-100

INTRODUCTION

This procedure is used for the routine maintenance of the PerkinElmer FIMS-100 (Flow Injection Mercury System) to ensure optimal performance of the instrument. The proper safety equipment must be worn during the entire cleaning procedure. This includes gloves, goggles and lab coat.

EQUIPMENT LIST

- FIMS-100
- Lab Coat
- Gloves
- Goggles
- FIMS-100 Record Book
- PerkinElmer FIMS-100 Installation, Maintenance and System Description Manual
- Spare Parts for FIMS-100

PROCEDURE: General Preventative Maintenance

1. Wipe up spills immediately for safety reasons and to avoid contaminating new samples.
2. Wipe over instrument outer surfaces with a clean cloth moistened with a dilute solution of laboratory detergent.
3. Record daily usage in FIMS-100 Record Book, including date, project, analyst, number of injections and hours of use.
4. Record any maintenance performed in the FIMS-100 Record Book.

PROCEDURE: Spectrophotometer Maintenance

1. Measure and record the absorbance of the FIMS-cell window in the FIMS-100 Record Book regularly .
   a. Switch on FIMS analysis system
   b. Start AA WinLab application
   c. Open Continuous Graphics window (Cont on toolbar)
   d. Remove FIMS-cell from the cell compartment
   e. Click on Autozero in the Continuous Graphics window
   f. Install the FIMS-cell in the cell compartment
   g. The absorbance reading in the Continuous Graphics window is the absorbance of the FIMS-cell window. Clean windows should have an absorbance of about 0.75. If the absorbance is greater than this, the

windows should be cleaned. Refer to Installation, Maintenance and System Description Manual page 2-10.

2. Install a new air filter yearly or more often in a dusty environment. Refer to Installation, Maintenance and System Description Manual page 2-19.

PROCEDURE: Fluid System Maintenance

1. Following analysis, rinse the fluid system with deionized water.
2. To reduce wear on pump tubes, place one drop of silicone oil on the part of the tube in contact with the pump rollers. Release tension on the pump tubes when analysis is completed.
3. Wipe pump rollers with a dry lint free cloth.
4. Inspect all fluid tubes daily during periods of instrument usage for damage such as kinks or clogs. Install new tubes as necessary.

PROCEDURE: Carrier Gas System Maintenance

1. Periodically check the nonreturn valve. If the rubber sleeve shows signs of deterioration, fit a new one. See page 2-18 of Installation, Maintenance and System Description Manual.
2. Carrier gas flow should read 40-70 mL/min on the Carrier Gas Flow Gauge.

PROCEDURE: Carrier and Reductant Flows

1. If peak shape is abnormal or the 6000 µg/L Hg standard gives an absorbance of less than 0.12, the carrier and reductant flows should be checked and flows recorded in the FIMS-100 Record Book.
2. Adjust the carrier and reductant flows to produce a ratio of carrier flow to reductant flow of 2:1 with a carrier flow between 9 and 11 mL/min. Record the flows in the FIMS-100 Record Book.
    a. Fill a graduated cylinder with deionized water.
    b. Place the carrier tube inlet in the graduated cylinder.
    c. After running the FIAS for 1 minute note the decrease in volume. The flow should be between 9-11 mL/minute.
    d. Repeat with reductant tube. The flow should be between 5-7 mL/min.
    e. If the flows are not within the acceptable range, adjust the pressure on the appropriate pump tube until the flow is within the range.
    f. If the desired flow is not attained by adjusting the pressure on the pump tubes, it suggests that there is an obstruction in a delivery tube.

Standard Operating Procedure SA/51 v.4

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/moisture from the researchers' hands. The addition of oils/moisture will cause an error in the pan weight.

DEFINITIONS

Gravimetric: Of or pertaining to measurement by weight.

REFERENCES

Lake Superior Research Institute. 1995. LSRI/SOP/GLM/12 – Procedure for Verifying Calibration of Laboratory Balances.

EQUIPMENT LIST

- Aluminum Weigh Pans
- Analytical Balance (i.e., capable of weighing to 0.001 g)
- ASTM/ANSI Class 1 Weights
- Balance Brush
- Desiccation Container with Dry Desiccant
- Drying Oven (60°C ± 10°C)
- Forceps
- Laboratory Notebook and/or Datasheet (see Appendix 1)
- Spatula

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), which should take approximately 3-5 minutes.

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 a 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. In addition, a minimum of 10% of the samples must be dried a second time. Dry a second time, desiccate, and re-weigh to prove that constant dry weight (i.e., the weight change is less than 4% of the first dry 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:

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

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

APPENDIX 1

TISSUE MOISTURE DETERMINATION DATASHEET

Sample ID	Sample Date	Pan ID	Weigh Pan Drying Time		Pan Wt. (g)	Pan + Wet Tissue Wt. (g)	Weigh Pan + Wet Tissue Drying Time		Pan + Dry Tissue Wt. #1 (g)	Weigh Pan + Dry Tissue Drying Time[2]		Pan + Dry Tissue Wt. #2 (g)
			IN Oven Date/Time	OUT of Oven Date/Time			IN Oven Date/Time	OUT of Oven Date/Time		IN Oven Date/Time	OUT of Oven Date/Time	

[2] A minimum of 10% of the samples must be dried for a second time, desiccated, and reweighed.