GREAT LAKES INDIAN FISH & WILDLIFE COMMISSION

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• MEMBER TRIBES •

MICHIGAN
Bay Mills Community
Keweenaw Bay Community
Lac Vieux Desert Band

WISCONSIN
Bad River Band
Lac Courte Oreilles Band
Lac du Flambeau Band
Red Cliff Band
St. Croix Chippewa
Sokaogon Chippewa

MINNESOTA
Fond du Lac Band
Mille Lacs Band

To: Neil Kmiecik, Biological Services Director

From: Sara Moses, Environmental Biologist Sara K. Moses

Date: August 13, 2014

Re: Results of Mercury Testing of Walleye Collected During Spring 2013

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 2013 GLIFWC was funded through a U.S. EPA Great Lakes Restoration Initiative (GLRI) grant [GL00E00613-0] to collect and test for mercury up to 360 walleye from inland lakes within the ceded territories. The data collected is used to update GLIFWC's mercury maps, which provide safe walleye consumption advice to our member tribes. The data presented here, along with previous years' data and data shared by the states of WI, MI, and MN was used to update the maps in spring 2014. The maps were last updated in the spring of 2012. 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 by the EPA on June 24, 2011.

A total of 315 walleye were collected from 29 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 43 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 17, 2013 together with results of the QA/QC audit for these analyses (Appendix 1). All analytical QA/QC measures were within their respective acceptance ranges. The QA audit found no instances of deviation from or non-conformance with the project QAPP or applicable LSRI Standard Operating Procedures (SOPs).

Total mercury concentrations on a wet weight basis ranged from 0.046 to 1.82 µg/g (parts per million or ppm). Figure 1 shows the number of walleye falling into each of five mercury concentration ranges. Summary statistics for walleye mercury concentrations by lake can be found in the Table 2. The results of mercury analysis for each individual sample are included in Table 3.

cc: John Coleman, Environmental Section Leader

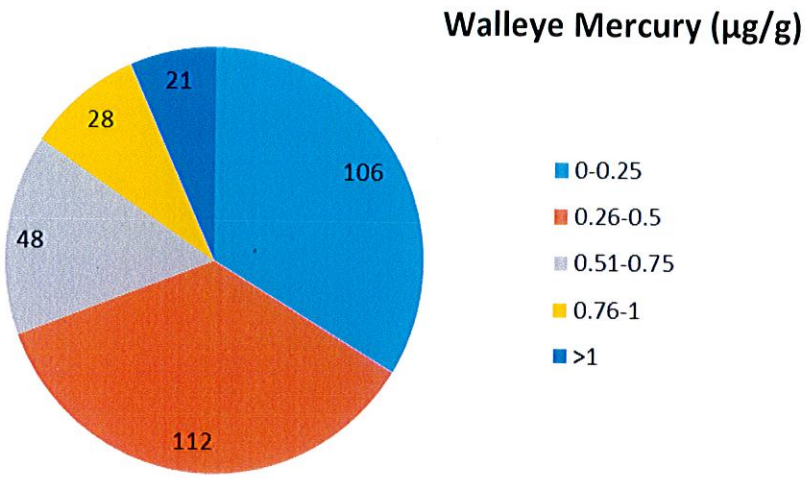

Figure 1: Number of walleye collected during Spring 2013 by mercury content.

Table 1: Number of Walleye Collected from Inland Lakes Planned for Sampling during Spring 2013

STATE	COUNTY	LAKE	Collection Assigned to: 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	Kniskern (LVD)	3	3	3	1	10	83%
MI	ONTONAGON	BOND FALLS FL	Kniskern (LVD)						0%
MN	MILLE LACS	MILLE LACS L	Inland Assessment	3	3	3	3	12	100%
WI	ASHLAND	CAROLINE L	Bad River DNR						0%
WI	ASHLAND	ENGLISH L	Bad River DNR	3	3	2	4	12	100%
WI	ASHLAND	KAKAGON SLOUGH	Bad River DNR	3	3	3	3	12	100%
WI	ASHLAND	L GALILEE	GLIFWC Bio						0%
WI	ASHLAND	MEDER L	Bad River DNR	3	6	3		12	100%
WI	ASHLAND	MINERAL L	V. Stone (BRV)						0%
WI	BAYFIELD	DIAMOND L	J. Stone (RCF)						0%
WI	BAYFIELD	L OWEN	J. Stone (RCF)		3	2		5	42%
WI	BAYFIELD	NAMEKAGON L	Kacizak (STC)						0%
WI	BAYFIELD	PIKE L CHAIN	J. Stone (RCF)	3	3	3	1	10	83%
WI	CHIPPEWA	L WISSOTA	Kacizak (STC)						0%
WI	FOREST	BUTTERNUT L	Inland Assessment	3	3	3	3	12	100%
WI	IRON	GILE FL	Moermond (LDF)						0%
WI	IRON	ISLAND L	Bad River DNR	3	8	1		12	100%
WI	IRON	PINE L	V. Stone (BRV)						0%
WI	IRON	TURTLE-FLAMBEAU FL	V. Stone (BRV)						0%
WI	LINCOLN	RICE R FL CHAIN	Moermond (LDF)	3	3	3	3	12	100%
WI	ONEIDA	BEARSKIN L	Moermond (LDF)	3	3	3	3	12	100%
WI	ONEIDA	SAND L	Moermond (LDF)	3	3	2		8	67%
WI	ONEIDA	SQUIRREL L	Inland Assessment	3	3	3	3	12	100%
WI	ONEIDA	THUNDER L	McGeshick (MLK)						0%
WI	ONEIDA	VIRGIN L	McGeshick (MLK)	3	2	3	1	9	75%
WI	ONEIDA	WILLOW FL	Moermond (LDF)	3	3	3	3	12	100%
WI	SAWYER	L CHETAC	Tuori (LCO)			3		3	25%
WI	SAWYER	L CHIPPEWA	Tuori (LCO)	3	3	5	1	12	100%
WI	SAWYER	WINDIGO L	Tuori (LCO)	3	3	3		9	75%
WI	ST CROIX	CEDAR L	Kacizak (STC)	3	3	3	1	10	83%
WI	VILAS	ALLEQUASH L	Moermond (LDF)	3	3	2	1	9	75%
WI	VILAS	BALLARD L	Moermond (LDF)	3	3	3	3	12	100%
WI	VILAS	BIG MUSKELLUNGE L	Moermond (LDF)	3	4	4	1	12	100%
WI	VILAS	BIRCH L	Moermond (LDF)	3			1	4	33%
WI	VILAS	KENTUCK L	Inland Assessment	3	3	3	3	12	100%
WI	VILAS	LAC VIEUX DESERT	McGeshick (MLK)	3	3	3	3	12	100%
WI	VILAS	OTTER L	Moermond (LDF)						0%
WI	VILAS	PAPOOSE L	Moermond (LDF)	3	3	3	3	12	100%
WI	VILAS	SHERMAN L	Moermond (LDF)	3	3	3	1	10	83%
WI	VILAS	SQUAW L	Inland Assessment	4	5	3		12	100%
WI	VILAS	STAR L	Moermond (LDF)	3	3	3	3	12	100%
WI	WASHBURN	BASS L	Kacizak (STC)						0%
WI	WASHBURN	LONG L	Tuori (LCO)	3	3	6		12	100%
							TOTAL:	**315**	**88%**

Table 2: Summary Statistics by Lake for Length, Weight, and Mercury Concentration (µg/g wet weight) of Walleye Collected from Inland Lakes during Spring 2013

Lake	County	n	Length (Inches)		Weight (Pounds)		Mercury (µg/g ww)	
			Range	Mean	Range	Mean	Range	Mean
L Gogebic	Gogebic (MI)	10	13.2-19.8	16.9	0.63-2.45	1.56	0.08-0.45	0.22
Mille Lacs	Mille Lacs (MN)	12	14.8-24.0	19.1	1.15-4.25	2.30	0.08-0.29	0.15
English L	Ashland	12	14.4-25.2	16.5	1.03-5.67	1.70	0.45-1.47	0.67
Kakagon Slough	Ashland	12	11.7-27.0	18.7	0.51-5.99	2.63	0.08-0.54	0.24
Meder L	Ashland	12	11.9-21.8	16.0	0.54-3.22	1.50	0.24-1.08	0.49
L Owen	Bayfield	5	15.4-19.8	17.4	1.15-2.32	1.70	0.28-0.48	0.31
Pike L Chain	Bayfield	10	12.9-22.9	17.2	0.59-4.22	1.75	0.14-0.45	0.24
Butternut L	Forest	12	13.4-25.7	18.6	0.74-4.88	2.32	0.05-0.56	0.19
Island L	Iron	12	13.7-18.3	15.9	0.67-1.66	1.16	0.38-1.26	0.79
Rice R FL Chain	Lincoln	12	14.2-28.8	18.8	0.83-8.91	2.62	0.23-1.47	0.52
Bearskin L	Oneida	12	12.2-26.1	18.1	0.52-7.52	2.63	0.07-0.30	0.16
Sand L	Oneida	8	12.3-18.2	15.5	0.56-1.98	1.30	0.19-0.93	0.51
Squirrel L	Oneida	12	13.6-26.7	18.7	0.68-6.57	2.59	0.24-0.89	0.38
Virgin L	Oneida	9	12.1-24.0	17.3	0.53-4.67	1.94	0.35-1.26	0.70
Willow FL	Oneida	12	14.6-27.8	18.8	0.90-7.44	2.45	0.59-1.61	1.00
L Chetac	Sawyer	3	18.8-20.1	19.3	2.28-2.87	2.56	0.22-0.28	0.26
L Chippewa	Sawyer	12	12.6-23.5	17.2	0.67-4.98	2.00	0.31-0.92	0.50
Windigo L	Sawyer	9	14.2-19.0	16.7	0.96-2.05	1.43	0.44-1.41	0.87
Cedar L	St Croix	10	12.8-18.7	15.6	0.63-2.06	1.29	0.09-0.30	0.18
Allequash L	Vilas	9	13.0-25.0	16.5	0.69-5.16	1.68	0.22-0.70	0.39
Ballard L	Vilas	12	12.6-26.3	18.4	0.54-6.71	2.43	0.39-1.82	0.82
Big Muskellunge L	Vilas	12	12.4-24.1	17.1	0.51-4.95	1.85	0.14-0.78	0.38
Birch L	Vilas	4	12.2-22.1	14.7	0.26-3.93	1.32	0.32-0.73	0.45
Kentuck L	Vilas	12	13.0-24.2	18.3	0.71-4.75	2.29	0.25-0.73	0.41
Lac Vieux Desert	Vilas	12	13.7-23.0	18.1	0.85-4.85	2.18	0.08-0.28	0.20
Papoose L	Vilas	12	12.8-28.4	18.5	0.54-9.14	2.67	0.39-1.24	0.62
Sherman L	Vilas	10	12.4-26.0	17.1	0.56-5.71	1.84	0.18-0.88	0.41
Squaw L	Vilas	12	12.0-18.7	16.1	0.53-2.10	1.38	0.21-0.73	0.52
Star L	Vilas	12	14.0-26.2	18.8	0.79-6.59	2.35	0.18-0.73	0.36
Long L	Washburn	12	13.4-20.0	17.1	0.70-2.53	1.59	0.09-0.38	0.21

Table 3: Mercury Concentration (µg/g wet weight), Length (inches), Sex, and Weight (Pounds) of Individual Walleye Collected from Inland Lakes during the Spring 2013 Spearing Season

Lake	County	Length (Inches)	Mercury (µg/g ww)	Sample Number	Sex	Age	Date	Weight (Pounds)
L GOGEBIC	GOGEBIC (MI)	19.8	0.449	6857	M	9	5/13/2013	2.06
L GOGEBIC	GOGEBIC (MI)	18.1	0.245	6859	F	4	5/13/2013	0.63
L GOGEBIC	GOGEBIC (MI)	19.4	0.377	6860	F	8	5/13/2013	1.64
L GOGEBIC	GOGEBIC (MI)	14.1	0.098	6861	M	3	5/13/2013	0.85
L GOGEBIC	GOGEBIC (MI)	18.9	0.248	6863	M	7	5/13/2013	1.30
L GOGEBIC	GOGEBIC (MI)	13.2	0.089	6864	M	7	5/13/2013	1.42
L GOGEBIC	GOGEBIC (MI)	17.3	0.201	6866	M	10	5/17/2013	2.45
L GOGEBIC	GOGEBIC (MI)	14.2	0.079	6868	M	8	5/17/2013	2.03
L GOGEBIC	GOGEBIC (MI)	16.8	0.182	6869	M	8	5/17/2013	2.37
L GOGEBIC	GOGEBIC (MI)	17.1	0.199	6870	M	3	5/17/2013	0.82
MILLE LACS	MILLE LACS (MN)	19.9	0.129	1183	U	9	5/30/2013	2.21
MILLE LACS	MILLE LACS (MN)	17.7	0.100	1185	U	6	5/30/2013	1.73
MILLE LACS	MILLE LACS (MN)	22.7	0.231	11278	F	9	5/31/2013	3.75
MILLE LACS	MILLE LACS (MN)	14.8	0.099	11279	M	5	5/31/2013	1.15
MILLE LACS	MILLE LACS (MN)	22.0	0.244	11915	F	9	5/31/2013	2.90
MILLE LACS	MILLE LACS (MN)	16.4	0.092	11917	M	5	5/31/2013	1.43
MILLE LACS	MILLE LACS (MN)	17.0	0.093	11947	M	6	5/31/2013	1.57
MILLE LACS	MILLE LACS (MN)	24.0	0.293	11948	F	10	5/31/2013	4.25
MILLE LACS	MILLE LACS (MN)	19.7	0.142	11973	F	6	5/31/2013	2.45
MILLE LACS	MILLE LACS (MN)	16.2	0.096	11974	M	NA	5/31/2013	2.12
MILLE LACS	MILLE LACS (MN)	18.2	0.080	12273	M	6	5/31/2013	1.44
MILLE LACS	MILLE LACS (MN)	20.4	0.189	12390	F	NA	5/31/2013	2.59
ASHLAND	ENGLISH L	14.8	0.496	11713	M	5	5/17/2013	1.03
ASHLAND	ENGLISH L	14.4	0.531	11714	M	5	5/17/2013	1.08
ASHLAND	ENGLISH L	19.9	1.07	11715	M	13	5/17/2013	2.74
ASHLAND	ENGLISH L	14.9	0.540	11716	M	4	5/17/2013	1.12
ASHLAND	ENGLISH L	16.0	0.548	11717	M	8	5/17/2013	1.31
ASHLAND	ENGLISH L	25.2	1.47	11718	F	12	5/17/2013	5.67
ASHLAND	ENGLISH L	16.6	0.620	11719	M	6	5/17/2013	1.58
ASHLAND	ENGLISH L	15.2	0.450	11720	M	4	5/17/2013	1.20
ASHLAND	ENGLISH L	15.9	0.516	11721	M	6	5/17/2013	1.34
ASHLAND	ENGLISH L	14.7	0.566	11722	M	5	5/17/2013	1.11
ASHLAND	ENGLISH L	15.0	0.551	11723	M	5	5/17/2013	1.20
ASHLAND	ENGLISH L	14.9	0.627	11724	M	4	5/17/2013	1.04
ASHLAND	KAKAGON SLOUGH	22.6	0.295	6872	U	8	5/9/2013	4.45
ASHLAND	KAKAGON SLOUGH	25.0	0.523	6873	U	11	5/9/2013	4.59
ASHLAND	KAKAGON SLOUGH	16.5	0.167	6874	U	5	5/9/2013	1.77
ASHLAND	KAKAGON SLOUGH	18.6	0.228	6875	U	5	5/9/2013	1.86
ASHLAND	KAKAGON SLOUGH	11.7	0.075	6877	U	3	5/9/2013	0.51
ASHLAND	KAKAGON SLOUGH	27.0	0.540	6879	U	11	5/9/2013	5.99
ASHLAND	KAKAGON SLOUGH	11.9	0.094	6880	U	4	5/9/2013	0.56
ASHLAND	KAKAGON SLOUGH	23.6	0.364	6881	U	11	5/9/2013	4.38
ASHLAND	KAKAGON SLOUGH	22.0	0.220	6882	U	8	5/9/2013	3.88
ASHLAND	KAKAGON SLOUGH	15.4	0.124	6883	U	6	5/9/2013	1.41
ASHLAND	KAKAGON SLOUGH	15.6	0.111	6884	U	4	5/9/2013	1.15
ASHLAND	KAKAGON SLOUGH	14.3	0.087	6885	U	4	5/9/2013	1.00
ASHLAND	MEDER L	15.9	0.331	6802	M	6	5/17/2013	1.47
ASHLAND	MEDER L	15.2	0.292	11701	M	4	5/17/2013	1.29
ASHLAND	MEDER L	16.0	0.341	11702	M	4	5/17/2013	1.44
ASHLAND	MEDER L	17.8	0.964	11704	M	11	5/17/2013	1.96
ASHLAND	MEDER L	12.1	0.249	11705	M	4	5/17/2013	0.57
ASHLAND	MEDER L	15.8	0.310	11706	F	4	5/17/2013	1.38
ASHLAND	MEDER L	11.9	0.267	11707	M	4	5/17/2013	0.54
ASHLAND	MEDER L	18.7	0.599	11708	M	7	5/17/2013	2.02
ASHLAND	MEDER L	15.8	0.325	11709	M	5	5/17/2013	1.31
ASHLAND	MEDER L	18.5	0.857	11710	M	8	5/17/2013	2.09
ASHLAND	MEDER L	21.8	1.08	11711	F	11	5/17/2013	3.22
ASHLAND	MEDER L	12.7	0.238	11712	M	3	5/17/2013	0.71
BAYFIELD	L OWEN	19.8	0.480	6806	F	8	5/23/2013	2.32
BAYFIELD	L OWEN	16.5	0.217	6809	F	5	5/23/2013	1.42
BAYFIELD	L OWEN	18.3	0.344	6810	M	6	5/23/2013	1.82
BAYFIELD	L OWEN	17.2	0.279	6811	M	5	5/23/2013	1.81

Lake	County	Length (Inches)	Mercury (µg/g ww)	Sample Number	Sex	Age	Date	Weight (Pounds)
BAYFIELD	L OWEN	15.4	0.204	6824	M	4	5/23/2013	1.15
BAYFIELD	PIKE L CHAIN	22.9	0.214	6769	F	7	5/14/2013	4.22
BAYFIELD	PIKE L CHAIN	12.9	0.176	6770	M	5	5/14/2013	0.59
BAYFIELD	PIKE L CHAIN	21.0	0.267	6772	F	6	5/14/2013	2.74
BAYFIELD	PIKE L CHAIN	20.3	0.294	6773	F	11	5/14/2013	2.67
BAYFIELD	PIKE L CHAIN	14.5	0.187	6775	M	6	5/14/2013	0.79
BAYFIELD	PIKE L CHAIN	15.1	0.221	6776	M	6	5/14/2013	0.93
BAYFIELD	PIKE L CHAIN	17.9	0.454	6778	M	8	5/14/2013	1.71
BAYFIELD	PIKE L CHAIN	13.8	0.138	6779	M	4	5/14/2013	0.74
BAYFIELD	PIKE L CHAIN	15.8	0.234	6780	M	7	5/14/2013	1.12
BAYFIELD	PIKE L CHAIN	18.0	0.194	6781	F	4	5/14/2013	1.99
FOREST	BUTTERNUT L	13.4	0.046	11765	M	5	5/14/2013	0.74
FOREST	BUTTERNUT L	20.4	0.240	11789	F	8	5/14/2013	2.99
FOREST	BUTTERNUT L	14.6	0.070	11928	M	4	5/14/2013	1.00
FOREST	BUTTERNUT L	20.5	0.180	11929	F	9	5/14/2013	2.99
FOREST	BUTTERNUT L	17.2	0.123	11930	F	6	5/14/2013	1.83
FOREST	BUTTERNUT L	14.1	0.078	12306	M	5	5/14/2013	0.84
FOREST	BUTTERNUT L	22.4	0.206	12308	F	10	5/14/2013	3.55
FOREST	BUTTERNUT L	25.7	0.555	12534	F	13	5/14/2013	4.88
FOREST	BUTTERNUT L	17.5	0.156	12659	F	8	5/14/2013	1.60
FOREST	BUTTERNUT L	17.3	0.223	12660	F	8	5/14/2013	1.63
FOREST	BUTTERNUT L	18.0	0.142	12985	F	8	5/14/2013	2.08
FOREST	BUTTERNUT L	22.5	0.228	12987	F	10	5/14/2013	3.70
IRON	ISLAND L	16.7	1.08	6887	M	10	5/17/2013	1.35
IRON	ISLAND L	15.3	0.745	6888	U	5	5/17/2013	1.05
IRON	ISLAND L	16.4	1.19	6889	M	11	5/17/2013	1.24
IRON	ISLAND L	13.7	0.406	6890	M	5	5/17/2013	0.67
IRON	ISLAND L	15.7	0.840	6891	U	6	5/17/2013	1.16
IRON	ISLAND L	15.6	0.648	6892	U	5	5/17/2013	1.10
IRON	ISLAND L	18.3	1.26	6893	F	10	5/17/2013	1.66
IRON	ISLAND L	16.6	0.727	6894	M	7	5/17/2013	1.33
IRON	ISLAND L	14.5	0.542	6895	U	5	5/17/2013	0.91
IRON	ISLAND L	16.5	0.850	6897	M	10	5/17/2013	1.26
IRON	ISLAND L	13.9	0.379	6898	U	6	5/17/2013	0.83
IRON	ISLAND L	17.0	0.870	6899	M	8	5/17/2013	1.41
LINCOLN	RICE R FL CHAIN	14.9	0.384	12871	M	7	5/8/2013	1.06
LINCOLN	RICE R FL CHAIN	14.8	0.229	12872	M	4	5/8/2013	0.85
LINCOLN	RICE R FL CHAIN	17.4	0.361	12873	M	7	5/8/2013	1.69
LINCOLN	RICE R FL CHAIN	16.3	0.371	12874	M	8	5/8/2013	1.45
LINCOLN	RICE R FL CHAIN	14.2	0.265	12875	M	5	5/8/2013	0.83
LINCOLN	RICE R FL CHAIN	16.5	0.260	12876	M	5	5/8/2013	1.58
LINCOLN	RICE R FL CHAIN	21.0	0.520	12877	F	11	5/8/2013	3.53
LINCOLN	RICE R FL CHAIN	18.3	0.361	12878	F	6	5/8/2013	2.19
LINCOLN	RICE R FL CHAIN	19.5	0.350	12879	F	9	5/8/2013	2.59
LINCOLN	RICE R FL CHAIN	28.8	1.47	12880	F	14	5/8/2013	8.91
LINCOLN	RICE R FL CHAIN	22.1	0.903	12881	F	10	5/8/2013	3.47
LINCOLN	RICE R FL CHAIN	22.3	0.754	12882	F	9	5/8/2013	3.27
ONEIDA	BEARSKIN L	12.2	0.071	12751	M	5	5/7/2013	0.52
ONEIDA	BEARSKIN L	13.5	0.125	12752	M	5	5/7/2013	0.75
ONEIDA	BEARSKIN L	13.7	0.095	12753	M	5	5/7/2013	0.72
ONEIDA	BEARSKIN L	15.0	0.136	12754	M	7	5/7/2013	1.01
ONEIDA	BEARSKIN L	20.8	0.194	12755	F	9	5/7/2013	3.26
ONEIDA	BEARSKIN L	15.2	0.116	12756	M	6	5/7/2013	1.19
ONEIDA	BEARSKIN L	15.3	0.173	12757	M	11	5/7/2013	1.31
ONEIDA	BEARSKIN L	18.3	0.225	12758	M	15	5/7/2013	2.00
ONEIDA	BEARSKIN L	26.1	0.182	12759	F	9	5/7/2013	7.52
ONEIDA	BEARSKIN L	19.5	0.125	12760	F	9	5/7/2013	2.49
ONEIDA	BEARSKIN L	230	0.176	12761	F	9	5/7/2013	4.62
ONEIDA	BEARSKIN L	25.0	0.302	12762	F	11	5/7/2013	6.20
ONEIDA	SAND L	12.3	0.230	12977	M	4	5/11/2013	0.56
ONEIDA	SAND L	18.0	0.883	12978	M	12	5/11/2013	1.94
ONEIDA	SAND L	13.4	0.248	12979	M	6	5/11/2013	0.76
ONEIDA	SAND L	12.6	0.188	12980	U	4	5/11/2013	0.66
ONEIDA	SAND L	15.4	0.390	12981	M	9	5/11/2013	1.19
ONEIDA	SAND L	18.2	0.763	12989	U	12	5/11/2013	1.98
ONEIDA	SAND L	17.7	0.932	12990	F	10	5/11/2013	1.95
ONEIDA	SAND L	16.2	0.474	12991	M	12	5/11/2013	1.34
ONEIDA	SQUIRREL L	13.6	0.295	11932	M	5	5/15/2013	0.68

Lake	County	Length (Inches)	Mercury (µg/g ww)	Sample Number	Sex	Age	Date	Weight (Pounds)
ONEIDA	SQUIRREL L	15.0	0.324	12270	F	7	5/15/2013	1.11
ONEIDA	SQUIRREL L	24.7	0.559	12319	F	13	5/15/2013	5.53
ONEIDA	SQUIRREL L	18.6	0.312	12355	F	9	5/15/2013	2.13
ONEIDA	SQUIRREL L	18.0	0.359	12440	F	7	5/15/2013	1.97
ONEIDA	SQUIRREL L	19.2	0.314	12441	F	8	5/15/2013	2.31
ONEIDA	SQUIRREL L	22.8	0.363	12442	F	10	5/15/2013	4.75
ONEIDA	SQUIRREL L	17.2	0.273	12443	F	7	5/15/2013	1.68
ONEIDA	SQUIRREL L	18.6	0.342	12444	F	7	5/15/2013	2.28
ONEIDA	SQUIRREL L	16.0	0.238	12445	F	7	5/15/2013	1.29
ONEIDA	SQUIRREL L	26.7	0.893	12541	F	12	5/15/2013	6.57
ONEIDA	SQUIRREL L	13.9	0.286	12545	F	5	5/15/2013	0.79
ONEIDA	VIRGIN L	24.0	0.973	12823	U	12	5/15/2013	4.67
ONEIDA	VIRGIN L	16.8	1.08	12827	M	9	5/15/2013	1.49
ONEIDA	VIRGIN L	12.1	0.387	12839	M	8	5/15/2013	0.53
ONEIDA	VIRGIN L	13.8	0.553	12840	M	9	5/15/2013	0.90
ONEIDA	VIRGIN L	13.4	0.349	12842	U	5	5/15/2013	0.86
ONEIDA	VIRGIN L	18.6	0.466	12861	U	8	5/15/2013	2.40
ONEIDA	VIRGIN L	18.7	0.467	12866	M	7	5/15/2013	1.29
ONEIDA	VIRGIN L	21.8	1.26	12867	U	6	5/15/2013	3.75
ONEIDA	VIRGIN L	16.9	0.791	12870	U	8	5/15/2013	1.52
ONEIDA	WLLOW FL	20.0	1.17	12801	M	8	5/18/2013	2.50
ONEIDA	WLLOW FL	22.0	1.53	12802	F	11	5/18/2013	3.30
ONEIDA	WLLOW FL	17.1	0.862	12804	M	9	5/18/2013	1.57
ONEIDA	WLLOW FL	14.6	0.622	12806	M	7	5/18/2013	0.90
ONEIDA	WLLOW FL	18.4	1.01	12992	F	9	5/18/2013	2.13
ONEIDA	WLLOW FL	16.9	0.926	12993	M	9	5/18/2013	1.38
ONEIDA	WLLOW FL	17.5	0.771	12994	M	9	5/18/2013	1.43
ONEIDA	WLLOW FL	14.8	0.885	12995	M	5	5/18/2013	0.98
ONEIDA	WLLOW FL	27.8	1.61	12996	F	13	5/18/2013	7.44
ONEIDA	WLLOW FL	23.3	1.13	12997	F	10	5/18/2013	4.71
ONEIDA	WLLOW FL	18.3	0.587	12998	F	5	5/18/2013	2.07
ONEIDA	WLLOW FL	14.6	0.842	12999	M	5	5/18/2013	0.96
SAWYER	L CHETAC	19.1	0.221	12708	M	10	5/10/2013	2.28
SAWYER	L CHETAC	18.8	0.270	12710	M	11	5/10/2013	2.55
SAWYER	L CHETAC	20.1	0.284	12714	M	12	5/10/2013	2.87
SAWYER	L CHIPPEWA	13.7	0.335	12763	M	4	5/8/2013	0.73
SAWYER	L CHIPPEWA	21.0	0.367	12764	F	8	5/8/2013	3.25
SAWYER	L CHIPPEWA	12.6	0.311	12765	M	7	5/8/2013	0.67
SAWYER	L CHIPPEWA	15.6	0.558	12766	F	9	5/8/2013	1.51
SAWYER	L CHIPPEWA	23.5	0.406	12767	F	10	5/8/2013	4.98
SAWYER	L CHIPPEWA	13.9	0.336	12768	M	4	5/8/2013	0.96
SAWYER	L CHIPPEWA	17.9	0.337	12770	M	7	5/8/2013	1.91
SAWYER	L CHIPPEWA	20.5	0.595	12771	F	9	5/8/2013	3.28
SAWYER	L CHIPPEWA	16.0	0.915	12772	M	10	5/8/2013	1.38
SAWYER	L CHIPPEWA	18.8	0.521	12773	M	11	5/8/2013	2.35
SAWYER	L CHIPPEWA	17.8	0.408	12774	M	9	5/8/2013	1.90
SAWYER	L CHIPPEWA	14.9	0.883	12776	M	8	5/8/2013	1.12
SAWYER	WINDIGO L	18.7	1.17	12701	U	8	5/9/2013	1.91
SAWYER	WINDIGO L	16.4	0.877	12778	U	8	5/9/2013	1.36
SAWYER	WINDIGO L	14.2	0.484	12779	U	4	5/9/2013	0.97
SAWYER	WINDIGO L	14.8	0.440	12782	U	4	5/9/2013	0.96
SAWYER	WINDIGO L	17.3	1.13	12783	U	7	5/9/2013	1.56
SAWYER	WINDIGO L	18.1	1.04	12785	U	12	5/9/2013	1.72
SAWYER	WINDIGO L	19.0	1.41	12786	U	12	5/9/2013	2.05
SAWYER	WINDIGO L	16.8	0.727	12787	U	7	5/9/2013	1.36
SAWYER	WINDIGO L	14.9	0.530	12789	U	5	5/9/2013	1.01
ST CROIX	CEDAR L	13.1	0.090	12819	M	3	5/2/2013	0.73
ST CROIX	CEDAR L	18.2	0.246	12825	M	10	5/1/2013	1.91
ST CROIX	CEDAR L	16.2	0.218	12827	F	5	5/1/2013	1.37
ST CROIX	CEDAR L	14.0	0.110	12828	M	6	5/1/2013	0.88
ST CROIX	CEDAR L	18.7	0.246	12830	M	10	5/3/2013	2.00
ST CROIX	CEDAR L	18.2	0.302	12831	M	9	5/1/2013	2.06
ST CROIX	CEDAR L	15.1	0.123	12833	F	5	5/1/2013	1.12
ST CROIX	CEDAR L	12.8	0.106	12837	M	6	5/1/2013	0.63
ST CROIX	CEDAR L	15.7	0.201	12838	M	6	5/1/2013	1.26
ST CROIX	CEDAR L	14.3	0.130	12839	M	6	5/3/2013	0.99
VILAS	ALLEQUASH L	13.0	0.251	12962	M	5	4/23/2013	0.69
VILAS	ALLEQUASH L	15.3	0.246	12964	M	5	4/23/2013	1.20

Lake	County	Length (Inches)	Mercury (µg/g ww)	Sample Number	Sex	Age	Date	Weight (Pounds)
VILAS	ALLEQUASH L	13.0	0.219	12965	M	4	4/23/2013	0.70
VILAS	ALLEQUASH L	18.4	0.587	12966	F	10	4/23/2013	1.89
VILAS	ALLEQUASH L	15.4	0.281	12968	M	6	4/23/2013	1.08
VILAS	ALLEQUASH L	13.9	0.303	12971	M	4	4/23/2013	0.83
VILAS	ALLEQUASH L	18.0	0.704	12973	M	8	4/23/2013	1.96
VILAS	ALLEQUASH L	25.0	0.604	12974	F	12	4/23/2013	5.16
VILAS	ALLEQUASH L	16.7	0.274	12976	M	5	4/23/2013	1.58
VILAS	BALLARD L	19.9	0.455	12868	M	9	4/24/2013	2.40
VILAS	BALLARD L	18.5	0.863	12901	M	10	4/24/2013	2.07
VILAS	BALLARD L	12.6	0.553	12947	M	7	5/15/2013	0.54
VILAS	BALLARD L	17.8	0.640	12948	M	9	5/15/2013	1.51
VILAS	BALLARD L	15.5	0.390	12949	M	6	5/15/2013	1.11
VILAS	BALLARD L	18.7	0.784	12951	F	10	5/15/2013	2.21
VILAS	BALLARD L	23.1	0.981	12953	M	13	5/15/2013	4.13
VILAS	BALLARD L	25.6	1.82	12956	F	16	5/15/2013	5.86
VILAS	BALLARD L	13.5	0.472	12958	M	8	5/15/2013	0.68
VILAS	BALLARD L	15.9	0.457	12959	M	6	5/15/2013	1.28
VILAS	BALLARD L	26.3	1.82	12960	F	15	5/15/2013	6.71
VILAS	BALLARD L	13.4	0.626	12961	M	7	5/15/2013	0.67
VILAS	BIG MUSKELLUNGE L	17.4	0.453	12935	M	7	5/26/2013	1.51
VILAS	BIG MUSKELLUNGE L	18.2	0.382	12936	M	6	5/26/2013	1.90
VILAS	BIG MUSKELLUNGE L	16.8	0.473	12937	M	9	5/26/2013	1.22
VILAS	BIG MUSKELLUNGE L	18.8	0.382	12938	M	10	5/26/2013	1.98
VILAS	BIG MUSKELLUNGE L	24.1	0.780	12939	F	11	5/26/2013	4.95
VILAS	BIG MUSKELLUNGE L	18.2	0.439	12940	M	6	5/14/2013	2.09
VILAS	BIG MUSKELLUNGE L	15.7	0.277	12941	F	7	5/14/2013	1.20
VILAS	BIG MUSKELLUNGE L	14.9	0.230	12942	M	5	5/14/2013	0.92
VILAS	BIG MUSKELLUNGE L	14.0	0.317	12943	M	6	5/14/2013	0.76
VILAS	BIG MUSKELLUNGE L	17.1	0.293	12944	M	8	5/14/2013	1.42
VILAS	BIG MUSKELLUNGE L	12.4	0.136	12945	M	4	5/14/2013	0.51
VILAS	BIG MUSKELLUNGE L	17.4	0.362	12946	F	8	5/14/2013	3.78
VILAS	BIRCH L	12.3	0.319	12918	U	4	5/15/2013	0.26
VILAS	BIRCH L	12.2	0.388	12922	U	5	5/15/2013	0.55
VILAS	BIRCH L	22.1	0.731	12925	U	8	5/15/2013	3.93
VILAS	BIRCH L	12.3	0.371	12929	U	4	5/15/2013	0.53
VILAS	KENTUCK L	22.8	0.734	9722	F	11	5/13/2013	3.75
VILAS	KENTUCK L	22.5	0.443	9724	F	10	5/13/2013	3.70
VILAS	KENTUCK L	17.5	0.419	9725	M	8	5/13/2013	1.78
VILAS	KENTUCK L	13.0	0.313	9728	M	5	5/13/2013	0.71
VILAS	KENTUCK L	20.3	0.313	9729	F	9	5/13/2013	3.29
VILAS	KENTUCK L	17.1	0.311	9730	M	8	5/13/2013	1.75
VILAS	KENTUCK L	14.1	0.312	9732	M	6	5/13/2013	0.86
VILAS	KENTUCK L	17.2	0.298	9733	F	8	5/13/2013	1.71
VILAS	KENTUCK L	15.8	0.278	9734	M	6	5/13/2013	1.25
VILAS	KENTUCK L	15.0	0.249	9735	M	7	5/13/2013	1.01
VILAS	KENTUCK L	24.2	0.714	9787	F	10	5/13/2013	4.75
VILAS	KENTUCK L	20.6	0.588	9789	F	9	5/13/2013	2.96
VILAS	LAC VIEUX DESERT	17.8	0.223	12841	M	12	5/10/2013	1.85
VILAS	LAC VIEUX DESERT	13.8	0.078	12844	U	5	5/10/2013	0.85
VILAS	LAC VIEUX DESERT	17.0	0.124	12846	M	5	5/10/2013	1.38
VILAS	LAC VIEUX DESERT	23.0	0.281	12849	F	11	5/10/2013	4.85
VILAS	LAC VIEUX DESERT	13.7	0.108	12850	M	5	5/10/2013	0.85
VILAS	LAC VIEUX DESERT	22.5	0.230	12851	F	12	5/10/2013	3.99
VILAS	LAC VIEUX DESERT	22.4	0.179	12852	F	8	5/10/2013	3.92
VILAS	LAC VIEUX DESERT	18.5	0.270	12853	M	10	5/10/2013	1.80
VILAS	LAC VIEUX DESERT	18.4	0.239	12858	M	10	5/10/2013	2.04
VILAS	LAC VIEUX DESERT	14.0	0.210	12859	M	9	5/10/2013	0.89
VILAS	LAC VIEUX DESERT	18.3	0.220	12864	F	9	5/10/2013	1.94
VILAS	LAC VIEUX DESERT	17.6	0.191	12869	M	8	5/10/2013	1.78
VILAS	PAPOOSE L	25.5	0.843	12855	F	13	5/16/2013	5.47
VILAS	PAPOOSE L	23.7	0.839	12856	F	12	5/16/2013	5.22
VILAS	PAPOOSE L	28.4	1.24	12857	F	14	5/16/2013	9.14
VILAS	PAPOOSE L	18.0	0.474	12858	M	8	5/16/2013	1.64
VILAS	PAPOOSE L	16.8	0.581	12859	M	8	5/16/2013	1.49
VILAS	PAPOOSE L	13.7	0.449	12860	M	5	5/16/2013	0.91
VILAS	PAPOOSE L	19.1	0.586	12861	F	7	5/16/2013	2.21
VILAS	PAPOOSE L	18.7	0.649	12862	F	10	5/16/2013	2.23
VILAS	PAPOOSE L	12.8	0.420	12863	U	4	5/16/2013	0.54

Lake	County	Length (Inches)	Mercury (µg/g ww)	Sample Number	Sex	Age	Date	Weight (Pounds)
VILAS	PAPOOSE L	16.5	0.484	12864	M	7	5/16/2013	1.52
VILAS	PAPOOSE L	13.4	0.388	12865	M	5	5/16/2013	0.64
VILAS	PAPOOSE L	15.6	0.453	12866	M	5	5/16/2013	1.02
VILAS	SHERMAN L	12.4	0.184	12869	M	6	5/10/2013	0.56
VILAS	SHERMAN L	13.6	0.255	12870	M	7	5/10/2013	0.71
VILAS	SHERMAN L	13.8	0.312	12871	M	7	5/10/2013	0.88
VILAS	SHERMAN L	15.7	0.419	12872	M	7	5/10/2013	1.16
VILAS	SHERMAN L	15.8	0.379	12873	M	6	5/10/2013	1.29
VILAS	SHERMAN L	16.8	0.357	12879	M	5	5/11/2013	1.44
VILAS	SHERMAN L	18.4	0.447	12880	F	11	5/11/2013	2.14
VILAS	SHERMAN L	26.0	0.875	12881	F	9	5/11/2013	5.71
VILAS	SHERMAN L	18.4	0.498	12882	U	6	5/11/2013	1.86
VILAS	SHERMAN L	19.8	0.393	12883	U	5	5/11/2013	2.66
VILAS	SQUAW L	16.5	0.610	11998	F	8	5/13/2013	1.55
VILAS	SQUAW L	16.5	0.571	12274	M	8	5/10/2013	1.42
VILAS	SQUAW L	13.4	0.512	12275	M	5	5/10/2013	0.78
VILAS	SQUAW L	17.7	0.620	12309	M	7	5/11/2013	1.65
VILAS	SQUAW L	18.7	0.686	12310	F	6	5/10/2013	1.24
VILAS	SQUAW L	17.8	0.727	12311	F	7	5/13/2013	2.10
VILAS	SQUAW L	12.0	0.238	12312	M	5	5/10/2013	0.53
VILAS	SQUAW L	15.6	0.499	12313	F	8	5/10/2013	1.52
VILAS	SQUAW L	12.7	0.209	12314	M	6	5/10/2013	0.67
VILAS	SQUAW L	16.6	0.455	12712	F	5	5/11/2013	1.65
VILAS	SQUAW L	18.6	0.602	12713	M	9	5/10/2013	2.07
VILAS	SQUAW L	16.7	0.534	12714	M	9	5/13/2013	1.35
VILAS	STAR L	24.8	0.608	12884	F	10	5/15/2013	6.59
VILAS	STAR L	26.2	0.312	12888	F	7	5/15/2013	3.51
VILAS	STAR L	23.3	0.484	12889	F	12	5/15/2013	4.50
VILAS	STAR L	18.6	0.384	12890	F	9	5/15/2013	1.98
VILAS	STAR L	14.9	0.236	12891	M	6	5/15/2013	1.01
VILAS	STAR L	14.7	0.180	12892	M	6	5/15/2013	0.84
VILAS	STAR L	15.7	0.209	12893	M	6	5/15/2013	0.99
VILAS	STAR L	19.1	0.373	12894	F	7	5/15/2013	1.98
VILAS	STAR L	21.3	0.350	12895	F	9	5/15/2013	3.29
VILAS	STAR L	15.9	0.267	12896	F	7	5/15/2013	1.26
VILAS	STAR L	14.0	0.176	12897	M	6	5/15/2013	0.79
VILAS	STAR L	16.7	0.725	12898	M	15	5/15/2013	1.41
WASHBURN	LONG L	17.3	0.161	12704	M	5	5/17/2013	1.35
WASHBURN	LONG L	19.6	0.317	12791	M	9	5/17/2013	2.30
WASHBURN	LONG L	18.6	0.257	12793	M	9	5/18/2013	1.82
WASHBURN	LONG L	13.4	0.088	12794	M	5	5/17/2013	0.70
WASHBURN	LONG L	15.9	0.141	12796	M	5	5/17/2013	1.24
WASHBURN	LONG L	18.2	0.287	12797	M	8	5/17/2013	2.01
WASHBURN	LONG L	14.9	0.141	12798	M	5	5/17/2013	1.02
WASHBURN	LONG L	18.1	0.258	12799	M	7	5/17/2013	1.96
WASHBURN	LONG L	19.1	0.209	12800	M	8	5/18/2013	2.20
WASHBURN	LONG L	20.0	0.380	12847	M	10	5/18/2013	2.53
WASHBURN	LONG L	15.5	0.203	12856	M	4	5/17/2013	1.19
WASHBURN	LONG L	14.0	0.119	12857	M	4	5/17/2013	0.77

APPENDIX 1

LSRI Analytical and QA/QC Reports on Mercury Analysis of Walleye Collected and During Spring 2013

Total Mercury Concentrations in Muscle Tissue from Walleye, Northern Pike, and Muskellunge Collected from Inland Lakes and the Kakagon River during Spring 2013

by

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October 17, 2013

Introduction

Skinless fillet samples from walleye (*Sander vitreus*), northern pike (*Esox lucius*), and muscle plugs from muskellunge (*Esox masquinongy*) captured during the spring of 2013 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 fifteen skinless walleye fillets from a total of twenty nine inland lakes and the Kakagon River, sixteen northern pike fillets from Mille Lacs, and twenty four muskellunge muscle plugs from a total of eight inland lakes, collected by tribal spearers and GLIFWC Inland Fisheries assessment crews, were analyzed.

Methods

At the time walleye and northern pike 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. In the case of walleye and northern pike, whole fish with chain-of-custody forms were transferred to the Great Lakes 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 with a label containing the fish identification number. 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 or cliethrum was removed from each walleye or northern pike, respectively, to determine its age.

Muskellunge were harvested by tribal spear fishermen. At the time fish were captured, a trained GLIFWC staff member was present to determine the sex of each fish, measure its total length, and remove the cliethrum for later determination of age. Muskellunge were assigned a unique number and a skin-on anterior dorsal muscle plug sample was collected from each fish (Note: skin was removed from muscle samples prior to analyses). The sample was placed in a Ziploc freezer bag along with a label containing the fish identification number.

At the LSRI laboratories, the fillets and muscle plugs 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 discarded (LSRI SOP SA/10 v.6). The muskellunge muscle plugs and one walleye sample from Birch Lake (12918) were frozen with liquid nitrogen and ground following (LSRI SOP SA/38v.2). The walleye sample was processed using liquid nitrogen due to the small size of the sample that was received. Prior to grinding the musky samples, the skin was removed from the plugs. A sub-sample of the ground tissue was placed into a certified clean glass vial and frozen until mercury analysis was conducted. The grinder or blender was disassembled after each fillet or muscle plug was ground and the unit was washed according to the labware 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 and the tuna was mixed to produce a homogeneous sample. The second portion was ground in the same manner as the fillets or muscle plugs. This check was made to ensure that no contamination or loss of mercury was occurring in the grinding process. Nine 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 seven 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.2). 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 0.007 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 (9-19-12) 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 168 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 32 percent of the walleye, 38 percent of the muskellunge, and 31 percent of the northern pike analyzed for mercury had moisture content determined. Three fish per lake (except in lakes containing less than three fish) were randomly selected for determination of percent moisture. Of these fish, ten percent or greater 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 2013 project on June 20th, 24th and 25th. That report is provided in Appendix C.

Results of Fish Tissue Analyses

Quality Assurance – Nine tuna procedural blanks were processed coincident with the grinding of walleye, northern pike, and muskellunge collected for the project. A minimum of one of the nine procedural blanks was digested with each set of mercury samples for a total of twelve analyses resulting in a mean of 28.8 ± 19.0 relative percent difference (Table 1). The relative percent difference values for samples that had concentrations above the LOQ ranged from15.8 to 38.5%, which were within the acceptable range of <50%. All of the tuna samples with the exception of the sample ground on 8/8/2013 were found to have very low mercury concentrations. Four of the tuna samples had mercury concentrations at or below the limit of detection (0.007 µg/g tissue) so a relative percent difference could not be calculated. Several other procedural blanks had values above the detection limit but below the limit of quantitation. This is likely to have resulted in the higher percent difference values found for two of those samples.

Analysis of dogfish shark tissue DORM-4 was conducted concurrently with walleye, northern pike, and muskellunge tissue analysis (Table 2). The certified mercury concentration for the dogfish tissue was 0.410 ± 0.053 µg Hg/g. The individual recovery values ranged from 74.3 to 90.3% with the grand mean and standard deviation of the recoveries being 82.4 ± 3.8 percent of the certified value. All of the DORM-4 reference sample daily mean values were within the acceptance range. Three individual values (DORM 4-3) from July 17th, July 24th and July 30th were outside the acceptance range but the daily mean was within the acceptance range. Due to larger than usual changes in the absorbance values obtained for the calibration standards analyzed on July 17th, July 24th and July 30th and one of the three DORM-4 Reference

Standards falling outside the acceptance range the entire sets were digested and analyzed again on July 18th and September 6th. No sample data is reported from the analyses done on July 17th, July 24th and July 30th. DORM 4-1 sample digested on September 11th fell just slightly below the acceptable range for recovery but the mean recovery of mercury on that date was 80.8% so the data from the set was acceptable.

Fish tissues were analyzed for mercury in duplicate thirty eight 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 12.8% with the average and standard deviation of the differences being 3.6 ± 2.8% (Table 3).

Samples of tissue were spiked in duplicate with known concentrations of mercury prior to digestion. Mean recovery for the thirty eight spiked samples was 92.8± 9.9 percent with the reported individual average recovery values ranging from 53.2 to 107.3% (Table 4). The Willow Flowage 12996 sample was spiked on two separate occasions. The resulting average recovery for the first time was below the 70% acceptance limit. When this sample was spiked for a second time (9-6-13), closer to 0.2 grams was used and this gave an acceptable spike recovery of 74.0%. The value reported for the Willow Flowage 12996 sample is the value obtained from the 9-6-13 analysis. The relative percent difference between the values (1.54 and 1.61 µg/g) obtained for the sample on the two analysis dates was 4.4 %.

Mercury Analysis – Skinless fillets of 315 walleye collected from 29 inland lakes and the Kakagon River, skinless fillets of 16 northern pike from Mille Lacs, and skin-on muscle plugs of 24 muskellunge from eight inland lakes were analyzed for total mercury concentration. Total mercury concentrations on a wet weight basis (Table 5) ranged from 0.046 to 2.01 µg Hg/g (parts per million).

Tissue Moisture Analysis – Percent moisture was measured in 101 of the 315 walleye tissues, 9 of the 24 northern pike tissues, and 5 of the 16 muskellunge 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.

Walleye, northern pike, and muskellunge muscle tissue had a mean moisture value of 79.2 ± 1.7 percent (Table 6). Of the 115 tissues analyzed for moisture, sixteen were analyzed in duplicate, all yielding relative differences of 0.0 to 0.6 percent. All samples were dried a minimum of an additional 24 hours and reweighed to ensure dryness, all yielding relative percent differences of ≤0.02 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
6/25/2013	6/18/2013	0.007[Q]	<0.007	<0.007	NC
7/2/2013	6/18/2013	0.013[Q]	0.012[Q]	0.013[Q]	8.0
7/10/2013	6/26/2013	<0.007	<0.007	<0.007	NC
7/12/2013	7/9/2013	0.007[Q]	<0.007	<0.007	NC
7/18/2013	7/15/2013	0.018[Q]	0.010[Q]	0.014[Q]	60.9
7/23/2013	7/15/2013	<0.007	<0.007	<0.007	NC
8/28/2013	7/19/2013	0.058	0.042	0.050	32.0
9/4/2013	8/19/2013	0.022[Q]	0.014[Q]	0.018[Q]	44.4
9/4/2013	8-19-13 liquid nitrogen processed	0.017[Q]	0.018[Q]	0.018[Q]	5.7
9/6/2013	7/19/2013	0.062	0.042	0.052	38.5
9/11/2013	8/2/2013	0.014[Q]	0.018[Q]	0.016[Q]	25.0
9/11/2013	8/8/2013	0.430	0.367	0.399	15.8
			Mean ± Std. Dev.		**28.8 ± 19.0**

[Q] Concentration below the limit of quantitation (0.025 µg Hg/g tissue).

Table 2. Mercury Concentrations of Dogfish Shark Tissue (Standard Reference Material DORM-4) Analyzed during Fish Analysis. The Standard Reference has a Certified Mercury Concentration of 0.410 ± 0.053 µg Hg/g Tissue. Data Quality Indicator for accuracy is 75.0 to 125% agreement between the certified concentration and the daily mean value for the reference standard.

Date of Analysis	DORM 4-1		DORM 4-2		DORM 4-3		Mean
	µg Hg/g	% of Certified Value	µg Hg/g	% of Certified Value	µg Hg/g	% of Certified Value	
6/25/2013	0.359	87.5	0.341	83.2	0.370	90.3	87.0
7/2/2013	0.330	80.5	0.349	85.1	0.359	87.5	84.4
7/10/2013	0.316	77.1	0.332	80.9	0.326	79.5	79.2
7/12/2013	0.336	82.0	0.329	80.3	0.318	77.6	80.0
7/18/2013	0.347	84.6	0.342	83.4	0.356	86.9	85.0

Date of Analysis	DORM 4-1		DORM 4-2		DORM 4-3		Mean
	µg Hg/g	% of Certified Value	µg Hg/g	% of Certified Value	µg Hg/g	% of Certified Value	
6/25/2013	0.359	87.5	0.341	83.2	0.370	90.3	87.0
7/2/2013	0.330	80.5	0.349	85.1	0.359	87.5	84.4
7/10/2013	0.316	77.1	0.332	80.9	0.326	79.5	79.2
7/12/2013	0.336	82.0	0.329	80.3	0.318	77.6	80.0
7/18/2013	0.347	84.6	0.342	83.4	0.356	86.9	85.0
7/23/2013	0.366	89.2	0.341	83.3	0.331	80.7	84.4
8/28/2013	0.310	75.7	0.351	85.6	0.338	82.4	81.2
9/4/2013	0.329	80.3	0.338	82.5	0.321	78.3	80.4
9/6/2013	0.334	81.4	0.343	83.6	0.328	80.0	81.7
9/11/2013	0.305	74.3	0.340	83.0	0.349	85.0	80.8
					Mean ± Std. Dev.		**82.4 ± 3.8**

Table 3. Relative Percent Difference for Duplicate Analysis of Total Mercury Content in Skinless Walleye and Northern Pike Fillet Tissue and Skin-on Muskellunge Muscle Plugs. Data quality indicator for precision is <25% relative percent difference.

Date of Analysis	Sample Location and Tag Number	Species	µg Hg/g	Duplicate µg Hg/g	Mean µg Hg/g	Relative Percent Difference
6/25/2013	Bearskin Lake 12756	Walleye	0.115	0.116	0.116	0.9
6/25/2013	Butternut Lake 11789	Walleye	0.246	0.233	0.240	5.4
6/25/2013	Butternut Lake 12985	Walleye	0.141	0.142	0.142	0.7
6/25/2013	Cedar Lake 12839	Walleye	0.132	0.128	0.130	3.1
7/2/2013	Chippewa Flowage 12768	Walleye	0.338	0.333	0.336	1.5
7/2/2013	English Lake 11714	Walleye	0.524	0.538	0.531	2.6
7/2/2013	English Lake 11723	Walleye	0.551	0.550	0.551	0.2
7/2/2013	Island Lake 6897	Walleye	0.849	0.851	0.850	0.2
7/10/2013	Long Lake 12797	Walleye	0.289	0.285	0.287	1.4
7/10/2013	Lake Chetac 12710	Walleye	0.277	0.263	0.270	5.2
7/10/2013	Meder Lake 11702	Walleye	0.344	0.338	0.341	1.8
7/10/2013	Windigo Lake 12778	Walleye	0.879	0.875	0.877	0.5
7/12/2013	Pike Lake Chain 6776	Walleye	0.221	0.221	0.221	0.0
7/12/2013	Squaw Lake 12309	Walleye	0.600	0.639	0.620	6.3
7/12/2013	Kakagon River 6872	Walleye	0.300	0.289	0.295	3.7

Date of Analysis	Sample Location and Tag Number	Species	µg Hg/g	Duplicate µg Hg/g	Mean µg Hg/g	Relative Percent Difference
7/12/2013	Kakagon River 6885	Walleye	0.088	0.085	0.087	3.5
7/18/2013	Allequash Lake 12971	Walleye	0.303	0.303	0.303	0.0
7/18/2013	Ballard Lake 12949	Walleye	0.408	0.371	0.390	9.5
7/18/2013	Big Muskellunge Lake 12936	Walleye	0.378	0.386	0.382	2.1
7/23/2013	Lake Gogebic 6864	Walleye	0.092	0.086	0.089	6.7
7/23/2013	Rice River Flowage 12874	Walleye	0.377	0.364	0.371	3.5
7/23/2013	Sherman Lake 12869	Walleye	0.178	0.189	0.184	6.0
8/28/2013	Kentuck Lake 9730	Walleye	0.319	0.302	0.311	5.5
8/28/2013	Lac Vieux Desert 12844	Walleye	0.073	0.083	0.078	12.8
8/28/2013	Lac Vieux Desert 12864	Walleye	0.216	0.224	0.220	3.6
8/28/2013	Mille Lacs 11974	Walleye	0.096	0.095	0.096	1.0
9/4/2013	Papoose Lake 12860	Walleye	0.459	0.439	0.449	4.5
9/4/2013	Birch Lake 12922	Walleye	0.381	0.394	0.388	3.4
9/4/2013	Virgin Lake 12866	Walleye	0.453	0.481	0.467	6.0
9/4/2013	Willow Flowage 12996 *	Walleye	1.55	1.52	1.54	2.0
9/6/2013	Star Lake 12892	Walleye	0.183	0.176	0.180	3.9
9/6/2013	Squirrel Lake 12270	Walleye	0.334	0.314	0.324	6.2
9/6/2013	Squirrel Lake 12541	Walleye	0.904	0.882	0.893	2.5
9/6/2013	Willow Flowage 12996	Walleye	1.62	1.60	1.61	1.2
9/11/2013	Mille Lacs 11913	Pike	0.197	0.203	0.200	3.0
9/11/2013	Mille Lacs 12909	Pike	0.054	0.051	0.053	5.7
9/11/2013	Big St. Germain 2013M07	Musky	0.717	0.760	0.739	5.8
9/11/2013	Round Lake 2013M18	Musky	1.34	1.40	1.37	4.4
			Mean ± Std. Dev.			**3.6 ± 2.8**

* Indicates that sample was reanalyzed on 9/6/2013 because the spike recovery value on 9/4/2013 was not within the acceptable range.

Table 4. Percent of Mercury Recovered from Walleye and Northern Pike Fillet Tissue and Muskellunge Muscle Plugs Spiked with a Known Concentration of Mercury. Data quality indicator for accuracy is a mean spike recovery of 70 to 130%.

Date of Analysis	Sample Location and Tag Number	Species	Spike #1	Spike #2	Mean Spike Recovery	Std. Dev.
6/25/2013	Bearskin Lake 12756	Walleye	97.4	103.6	100.5	4.4
6/25/2013	Butternut Lake 11789	Walleye	95.6	98.9	97.3	2.3
6/25/2013	Butternut Lake 12985	Walleye	95.1	97.4	96.2	1.6
6/25/2013	Cedar Lake 12839	Walleye	84.2	86.3	85.3	1.5
7/2/2013	Chippewa Flowage 12768	Walleye	93.8	93.0	93.4	0.6
7/2/2013	English Lake 11714	Walleye	85.6	91.1	88.4	3.9
7/2/2013	English Lake 11723	Walleye	83.0	89.8	86.4	4.8
7/2/2013	Island Lake 6897	Walleye	79.9	86.8	83.4	4.9
7/10/2013	Long Lake 12797	Walleye	90.7	98.3	94.5	5.3
7/10/2013	Lake Chetac 12710	Walleye	95.6	94.8	95.2	0.5
7/10/2013	Meder Lake 11702	Walleye	95.9	107.3	101.6	8.1
7/10/2013	Windigo Lake 12778	Walleye	83.6	91.1	87.4	5.3
7/12/2013	Pike Lake Chain 6776	Walleye	95.2	98.5	96.8	2.3
7/12/2013	Squaw Lake 12309	Walleye	88.4	77.1	82.7	8.0
7/12/2013	Kakagon River 6872	Walleye	102.0	98.6	100.3	2.4
7/12/2013	Kakagon River 6885	Walleye	105.1	105.2	105.2	0.0
7/18/2013	Allequash Lake 12971	Walleye	97.9	94.7	96.3	2.3
7/18/2013	Ballard Lake 12949	Walleye	91.8	93.4	92.6	1.1
7/18/2013	Big Muskellunge Lake 12936	Walleye	101.3	98.2	99.8	2.2
7/23/2013	Lake Gogebic 6864	Walleye	101.3	104.7	103.0	2.4
7/23/2013	Rice River Flowage 12874	Walleye	97.9	100.3	99.1	1.7
7/23/2013	Sherman Lake 12869	Walleye	106.1	103.8	104.9	1.6
8/28/2013	Kentuck Lake 9730	Walleye	85.2	84.9	85.1	0.2
8/28/2013	Lac Vieux Desert 12844	Walleye	90.8	91.6	91.2	0.6
8/28/2013	Lac Vieux Desert 12864	Walleye	102.3	106.6	104.4	3.0
8/28/2013	Mille Lacs 11974	Walleye	100.8	98.7	99.7	1.5
9/4/2013	Papoose Lake 12860	Walleye	94.3	99.1	96.7	3.4
9/4/2013	Birch Lake 12922	Walleye	96.8	94.9	95.9	1.3
9/4/2013	Virgin Lake 12866	Walleye	90.4	91.4	90.9	0.7
9/4/2013	Willow Flowage 12996 *	Walleye	64.9	53.2	59.0*	8.3
9/6/2013	Star Lake 12892	Walleye	96.8	100.8	98.8	2.9
9/6/2013	Squirrel Lake 12270	Walleye	99.0	97.9	98.4	0.8
9/6/2013	Squirrel Lake 12541	Walleye	85.2	83.6	84.4	1.1

Date of Analysis	Sample Location and Tag Number	Species	Spike #1	Spike #2	Mean Spike Recovery	Std. Dev.
9/6/2013	Willow Flowage 12996	Walleye	72.5	75.5	74.0	2.1
9/11/2013	Mille Lacs 11913	Pike	95.5	98.2	96.8	1.9
9/11/2013	Mille Lacs 12909	Pike	101.5	101.7	101.6	0.2
9/11/2013	Big St. Germain 2013M07	Musky	81.7	82.9	82.3	0.8
9/11/2013	Round Lake 2013M18	Musky	83.8	73.7	78.8	7.2
			Mean ± Std. Dev.		**92.8 ± 9.9**	

*Sample was reanalyzed on 9/6/2013 due to failing data quality objective.

Table 5. Total Mercury Concentration (Wet Weight) in Walleye and Northern Pike Fillets and Muskellunge Muscle Plugs from Fish Captured during the Spring of 2013.

Analysis Date	Sample Location	Species	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
6/25/2013	Bearskin Lake	Walleye	12751	Oneida	12.2	M	0.071
6/25/2013	Bearskin Lake	Walleye	12752	Oneida	13.5	M	0.125
6/25/2013	Bearskin Lake	Walleye	12753	Oneida	13.7	M	0.095
6/25/2013	Bearskin Lake	Walleye	12754	Oneida	15.0	M	0.136
6/25/2013	Bearskin Lake	Walleye	12755	Oneida	20.8	F	0.194
6/25/2013	Bearskin Lake	Walleye	12756	Oneida	15.2	M	0.116
6/25/2013	Bearskin Lake	Walleye	12757	Oneida	15.3	M	0.173
6/25/2013	Bearskin Lake	Walleye	12758	Oneida	18.3	M	0.225
6/25/2013	Bearskin Lake	Walleye	12759	Oneida	26.1	F	0.182
6/25/2013	Bearskin Lake	Walleye	12760	Oneida	19.5	F	0.125
6/25/2013	Bearskin Lake	Walleye	12761	Oneida	23.0	F	0.176
6/25/2013	Bearskin Lake	Walleye	12762	Oneida	25.0	F	0.302
6/25/2013	Butternut Lake	Walleye	11765	Forest	13.4	M	0.046
6/25/2013	Butternut Lake	Walleye	11789	Forest	20.4	F	0.240
6/25/2013	Butternut Lake	Walleye	11928	Forest	14.6	M	0.070
6/25/2013	Butternut Lake	Walleye	11929	Forest	20.5	F	0.180
6/25/2013	Butternut Lake	Walleye	11930	Forest	17.2	F	0.123
6/25/2013	Butternut Lake	Walleye	12306	Forest	14.1	M	0.078
6/25/2013	Butternut Lake	Walleye	12308	Forest	22.4	F	0.206
6/25/2013	Butternut Lake	Walleye	12534	Forest	25.7	F	0.555
6/25/2013	Butternut Lake	Walleye	12659	Forest	17.5	F	0.156

Analysis Date	Sample Location	Species	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
6/25/2013	Butternut Lake	Walleye	12660	Forest	17.3	F	0.223
6/25/2013	Butternut Lake	Walleye	12985	Forest	18.0	F	0.142
6/25/2013	Butternut Lake	Walleye	12987	Forest	22.5	F	0.228
6/25/2013	Cedar Lake	Walleye	12819	St. Croix	13.1	M	0.090
6/25/2013	Cedar Lake	Walleye	12825	St. Croix	18.2	M	0.246
6/25/2013	Cedar Lake	Walleye	12827	St. Croix	16.2	F	0.218
6/25/2013	Cedar Lake	Walleye	12828	St. Croix	14.0	M	0.110
6/25/2013	Cedar Lake	Walleye	12830	St. Croix	18.7	M	0.246
6/25/2013	Cedar Lake	Walleye	12831	St. Croix	18.2	M	0.302
6/25/2013	Cedar Lake	Walleye	12833	St. Croix	15.1	F	0.123
6/25/2013	Cedar Lake	Walleye	12837	St. Croix	12.8	M	0.106
6/25/2013	Cedar Lake	Walleye	12838	St. Croix	15.7	M	0.201
6/25/2013	Cedar Lake	Walleye	12839	St. Croix	14.3	M	0.130
7/2/2013	Chippewa Flowage	Walleye	12763	Sawyer	13.7	M	0.335
7/2/2013	Chippewa Flowage	Walleye	12764	Sawyer	21.0	F	0.367
7/2/2013	Chippewa Flowage	Walleye	12765	Sawyer	12.6	M	0.311
7/2/2013	Chippewa Flowage	Walleye	12766	Sawyer	15.6	F	0.558
7/2/2013	Chippewa Flowage	Walleye	12767	Sawyer	23.5	F	0.406
7/2/2013	Chippewa Flowage	Walleye	12768	Sawyer	13.9	M	0.336
7/2/2013	Chippewa Flowage	Walleye	12770	Sawyer	17.9	M	0.337
7/2/2013	Chippewa Flowage	Walleye	12771	Sawyer	20.5	F	0.595
7/2/2013	Chippewa Flowage	Walleye	12772	Sawyer	16.0	M	0.915
7/2/2013	Chippewa Flowage	Walleye	12773	Sawyer	18.8	M	0.521
7/2/2013	Chippewa Flowage	Walleye	12774	Sawyer	17.8	M	0.408
7/2/2013	Chippewa Flowage	Walleye	12776	Sawyer	14.9	M	0.883
7/2/2013	English Lake	Walleye	11713	Ashland	14.8	M	0.496
7/2/2013	English Lake	Walleye	11714	Ashland	14.4	M	0.531
7/2/2013	English Lake	Walleye	11715	Ashland	19.9	M	1.07
7/2/2013	English Lake	Walleye	11716	Ashland	14.9	M	0.540
7/2/2013	English Lake	Walleye	11717	Ashland	16.0	M	0.548
7/2/2013	English Lake	Walleye	11718	Ashland	25.2	F	1.47
7/2/2013	English Lake	Walleye	11719	Ashland	16.6	M	0.620
7/2/2013	English Lake	Walleye	11720	Ashland	15.2	M	0.450
7/2/2013	English Lake	Walleye	11721	Ashland	15.9	M	0.516
7/2/2013	English Lake	Walleye	11722	Ashland	14.7	M	0.566
7/2/2013	English Lake	Walleye	11723	Ashland	15.0	M	0.551
7/2/2013	English Lake	Walleye	11724	Ashland	14.9	M	0.627

Analysis Date	Sample Location	Species	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
7/2/2013	Island Lake	Walleye	6887	Iron	16.7	M	1.08
7/2/2013	Island Lake	Walleye	6888	Iron	15.3	U	0.745
7/2/2013	Island Lake	Walleye	6889	Iron	16.4	M	1.19
7/2/2013	Island Lake	Walleye	6890	Iron	13.7	M	0.406
7/2/2013	Island Lake	Walleye	6891	Iron	15.7	U	0.840
7/2/2013	Island Lake	Walleye	6892	Iron	15.6	U	0.648
7/2/2013	Island Lake	Walleye	6893	Iron	18.3	F	1.26
7/2/2013	Island Lake	Walleye	6894	Iron	16.6	M	0.727
7/2/2013	Island Lake	Walleye	6895	Iron	14.5	U	0.542
7/2/2013	Island Lake	Walleye	6897	Iron	16.5	M	0.850
7/2/2013	Island Lake	Walleye	6898	Iron	13.9	U	0.379
7/2/2013	Island Lake	Walleye	6899	Iron	17.0	M	0.870
7/10/2013	Lake Chetac	Walleye	12708	Sawyer	19.1	M	0.221
7/10/2013	Lake Chetac	Walleye	12710	Sawyer	18.8	M	0.270
7/10/2013	Lake Chetac	Walleye	12714	Sawyer	20.1	M	0.284
7/10/2013	Lake Owen	Walleye	6806	Bayfield	19.8	F	0.480
7/10/2013	Lake Owen	Walleye	6809	Bayfield	16.5	F	0.217
7/10/2013	Lake Owen	Walleye	6810	Bayfield	18.3	M	0.344
7/10/2013	Lake Owen	Walleye	6811	Bayfield	17.2	M	0.279
7/10/2013	Lake Owen	Walleye	6824	Bayfield	15.4	M	0.204
7/10/2013	Long Lake	Walleye	12704	Washburn	17.3	M	0.161
7/10/2013	Long Lake	Walleye	12791	Washburn	19.6	M	0.317
7/10/2013	Long Lake	Walleye	12793	Washburn	18.6	M	0.257
7/10/2013	Long Lake	Walleye	12794	Washburn	13.4	M	0.088
7/10/2013	Long Lake	Walleye	12796	Washburn	15.9	M	0.141
7/10/2013	Long Lake	Walleye	12797	Washburn	18.2	M	0.287
7/10/2013	Long Lake	Walleye	12798	Washburn	14.9	M	0.141
7/10/2013	Long Lake	Walleye	12799	Washburn	18.1	M	0.258
7/10/2013	Long Lake	Walleye	12800	Washburn	19.1	M	0.209
7/10/2013	Long Lake	Walleye	12847	Washburn	20.0	M	0.380
7/10/2013	Long Lake	Walleye	12856	Washburn	15.6	M	0.203
7/10/2013	Long Lake	Walleye	12857	Washburn	14.0	M	0.119
7/10/2013	Meder Lake	Walleye	6802	Ashland	15.9	M	0.331
7/10/2013	Meder Lake	Walleye	11701	Ashland	15.2	M	0.292
7/10/2013	Meder Lake	Walleye	11702	Ashland	16.0	M	0.341
7/10/2013	Meder Lake	Walleye	11704	Ashland	17.8	M	0.964
7/10/2013	Meder Lake	Walleye	11705	Ashland	12.1	M	0.249

Analysis Date	Sample Location	Species	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
7/10/2013	Meder Lake	Walleye	11706	Ashland	15.8	F	0.310
7/10/2013	Meder Lake	Walleye	11707	Ashland	11.9	M	0.267
7/10/2013	Meder Lake	Walleye	11708	Ashland	18.7	M	0.599
7/10/2013	Meder Lake	Walleye	11709	Ashland	15.8	M	0.325
7/10/2013	Meder Lake	Walleye	11710	Ashland	18.5	M	0.857
7/10/2013	Meder Lake	Walleye	11711	Ashland	21.8	F	1.08
7/10/2013	Meder Lake	Walleye	11712	Ashland	12.7	M	0.238
7/10/2013	Windigo Lake	Walleye	12701	Sawyer	18.7	U	1.17
7/10/2013	Windigo Lake	Walleye	12778	Sawyer	16.4	U	0.877
7/10/2013	Windigo Lake	Walleye	12779	Sawyer	14.2	U	0.484
7/10/2013	Windigo Lake	Walleye	12782	Sawyer	14.8	U	0.440
7/10/2013	Windigo Lake	Walleye	12783	Sawyer	17.3	U	1.13
7/10/2013	Windigo Lake	Walleye	12785	Sawyer	18.1	U	1.04
7/10/2013	Windigo Lake	Walleye	12786	Sawyer	19.0	U	1.41
7/10/2013	Windigo Lake	Walleye	12787	Sawyer	16.8	U	0.727
7/10/2013	Windigo Lake	Walleye	12789	Sawyer	14.9	NA	0.530
7/12/2013	Pike Lake Chain	Walleye	6769	Bayfield	22.9	F	0.214
7/12/2013	Pike Lake Chain	Walleye	6770	Bayfield	12.9	M	0.176
7/12/2013	Pike Lake Chain	Walleye	6772	Bayfield	21.0	F	0.267
7/12/2013	Pike Lake Chain	Walleye	6773	Bayfield	20.3	F	0.294
7/12/2013	Pike Lake Chain	Walleye	6775	Bayfield	14.5	M	0.187
7/12/2013	Pike Lake Chain	Walleye	6776	Bayfield	15.1	M	0.221
7/12/2013	Pike Lake Chain	Walleye	6778	Bayfield	17.9	M	0.454
7/12/2013	Pike Lake Chain	Walleye	6779	Bayfield	13.8	M	0.138
7/12/2013	Pike Lake Chain	Walleye	6780	Bayfield	15.8	M	0.234
7/12/2013	Pike Lake Chain	Walleye	6781	Bayfield	18.0	F	0.194
7/12/2013	Squaw Lake	Walleye	11998	Vilas	16.5	F	0.610
7/12/2013	Squaw Lake	Walleye	12274	Vilas	16.5	M	0.571
7/12/2013	Squaw Lake	Walleye	12275	Vilas	13.4	M	0.512
7/12/2013	Squaw Lake	Walleye	12309	Vilas	17.7	M	0.620
7/12/2013	Squaw Lake	Walleye	12310	Vilas	18.7	F	0.686
7/12/2013	Squaw Lake	Walleye	12311	Vilas	17.8	F	0.727
7/12/2013	Squaw Lake	Walleye	12312	Vilas	12.0	M	0.238
7/12/2013	Squaw Lake	Walleye	12313	Vilas	15.6	F	0.499
7/12/2013	Squaw Lake	Walleye	12314	Vilas	12.7	M	0.209
7/12/2013	Squaw Lake	Walleye	12712	Vilas	16.6	F	0.455
7/12/2013	Squaw Lake	Walleye	12713	Vilas	18.6	M	0.602

Analysis Date	Sample Location	Species	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
7/12/2013	Squaw Lake	Walleye	12714	Vilas	16.7	M	0.534
7/12/2013	Kakagon River	Walleye	6872	Ashland	22.6	U	0.295
7/12/2013	Kakagon River	Walleye	6873	Ashland	25.0	U	0.523
7/12/2013	Kakagon River	Walleye	6874	Ashland	16.5	U	0.167
7/12/2013	Kakagon River	Walleye	6875	Ashland	18.6	U	0.228
7/12/2013	Kakagon River	Walleye	6877	Ashland	11.7	U	0.075
7/12/2013	Kakagon River	Walleye	6879	Ashland	27.0	U	0.540
7/12/2013	Kakagon River	Walleye	6880	Ashland	11.9	U	0.094
7/12/2013	Kakagon River	Walleye	6881	Ashland	23.6	U	0.364
7/12/2013	Kakagon River	Walleye	6882	Ashland	22.0	U	0.220
7/12/2013	Kakagon River	Walleye	6883	Ashland	15.4	U	0.124
7/12/2013	Kakagon River	Walleye	6884	Ashland	15.6	U	0.111
7/12/2013	Kakagon River	Walleye	6885	Ashland	14.3	U	0.087
7/18/2013	Allequash Lake	Walleye	12962	Vilas	13.0	M	0.251
7/18/2013	Allequash Lake	Walleye	12964	Vilas	15.3	M	0.246
7/18/2013	Allequash Lake	Walleye	12965	Vilas	13.0	M	0.219
7/18/2013	Allequash Lake	Walleye	12966	Vilas	18.4	F	0.587
7/18/2013	Allequash Lake	Walleye	12968	Vilas	15.4	M	0.281
7/18/2013	Allequash Lake	Walleye	12971	Vilas	13.9	M	0.303
7/18/2013	Allequash Lake	Walleye	12973	Vilas	18.0	M	0.704
7/18/2013	Allequash Lake	Walleye	12974	Vilas	25.0	F	0.604
7/18/2013	Allequash Lake	Walleye	12976	Vilas	16.7	M	0.274
7/18/2013	Ballard Lake	Walleye	12868	Vilas	19.9	M	0.455
7/18/2013	Ballard Lake	Walleye	12901	Vilas	18.5	M	0.863
7/18/2013	Ballard Lake	Walleye	12947	Vilas	12.6	M	0.553
7/18/2013	Ballard Lake	Walleye	12948	Vilas	17.8	M	0.640
7/18/2013	Ballard Lake	Walleye	12949	Vilas	15.5	M	0.390
7/18/2013	Ballard Lake	Walleye	12951	Vilas	18.7	F	0.784
7/18/2013	Ballard Lake	Walleye	12953	Vilas	23.1	M	0.981
7/18/2013	Ballard Lake	Walleye	12956	Vilas	25.6	F	1.82
7/18/2013	Ballard Lake	Walleye	12958	Vilas	13.5	M	0.472
7/18/2013	Ballard Lake	Walleye	12959	Vilas	15.9	M	0.457
7/18/2013	Ballard Lake	Walleye	12960	Vilas	26.3	F	1.82
7/18/2013	Ballard Lake	Walleye	12961	Vilas	13.4	M	0.626
7/18/2013	Big Muskellunge Lake	Walleye	12935	Vilas	17.4	M	0.453
7/18/2013	Big Muskellunge Lake	Walleye	12936	Vilas	18.2	M	0.382
7/18/2013	Big Muskellunge Lake	Walleye	12937	Vilas	16.8	M	0.473

Analysis Date	Sample Location	Species	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
7/18/2013	Big Muskellunge Lake	Walleye	12938	Vilas	18.8	M	0.382
7/18/2013	Big Muskellunge Lake	Walleye	12939	Vilas	24.1	F	0.780
7/18/2013	Big Muskellunge Lake	Walleye	12940	Vilas	18.2	M	0.439
7/18/2013	Big Muskellunge Lake	Walleye	12941	Vilas	15.7	F	0.277
7/18/2013	Big Muskellunge Lake	Walleye	12942	Vilas	14.9	M	0.230
7/18/2013	Big Muskellunge Lake	Walleye	12943	Vilas	14.0	M	0.317
7/18/2013	Big Muskellunge Lake	Walleye	12944	Vilas	17.1	M	0.293
7/18/2013	Big Muskellunge Lake	Walleye	12945	Vilas	12.4	M	0.136
7/18/2013	Big Muskellunge Lake	Walleye	12946	Vilas	17.4	F	0.362
7/23/2013	Lake Gogebic	Walleye	6857	Gogebic	19.8	M	0.449
7/23/2013	Lake Gogebic	Walleye	6859	Gogebic	18.1	F	0.245
7/23/2013	Lake Gogebic	Walleye	6860	Gogebic	19.4	F	0.377
7/23/2013	Lake Gogebic	Walleye	6861	Gogebic	14.1	M	0.098
7/23/2013	Lake Gogebic	Walleye	6863	Gogebic	18.9	M	0.248
7/23/2013	Lake Gogebic	Walleye	6864	Gogebic	13.2	M	0.089
7/23/2013	Lake Gogebic	Walleye	6866	Gogebic	17.3	M	0.201
7/23/2013	Lake Gogebic	Walleye	6868	Gogebic	14.2	M	0.079
7/23/2013	Lake Gogebic	Walleye	6869	Gogebic	16.8	M	0.182
7/23/2013	Lake Gogebic	Walleye	6870	Gogebic	17.1	M	0.199
7/23/2013	Rice River Flowage	Walleye	12871	Lincoln	14.9	M	0.384
7/23/2013	Rice River Flowage	Walleye	12872	Lincoln	14.8	M	0.229
7/23/2013	Rice River Flowage	Walleye	12873	Lincoln	17.4	M	0.361
7/23/2013	Rice River Flowage	Walleye	12874	Lincoln	16.3	M	0.371
7/23/2013	Rice River Flowage	Walleye	12875	Lincoln	14.2	M	0.265
7/23/2013	Rice River Flowage	Walleye	12876	Lincoln	16.5	M	0.260
7/23/2013	Rice River Flowage	Walleye	12877	Lincoln	21.0	F	0.520
7/23/2013	Rice River Flowage	Walleye	12878	Lincoln	18.3	F	0.361
7/23/2013	Rice River Flowage	Walleye	12879	Lincoln	19.5	F	0.350
7/23/2013	Rice River Flowage	Walleye	12880	Lincoln	28.8	F	1.47
7/23/2013	Rice River Flowage	Walleye	12881	Lincoln	22.1	F	0.903
7/23/2013	Rice River Flowage	Walleye	12882	Lincoln	22.3	F	0.754
7/23/2013	Sherman Lake	Walleye	12869	Vilas	12.4	M	0.184
7/23/2013	Sherman Lake	Walleye	12870	Vilas	13.6	M	0.255
7/23/2013	Sherman Lake	Walleye	12871	Vilas	13.8	M	0.312
7/23/2013	Sherman Lake	Walleye	12872	Vilas	15.7	M	0.419
7/23/2013	Sherman Lake	Walleye	12873	Vilas	15.8	M	0.379
7/23/2013	Sherman Lake	Walleye	12879	Vilas	16.8	M	0.357

Analysis Date	Sample Location	Species	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
7/23/2013	Sherman Lake	Walleye	12880	Vilas	18.4	F	0.447
7/23/2013	Sherman Lake	Walleye	12881	Vilas	26.0	F	0.875
7/23/2013	Sherman Lake	Walleye	12882	Vilas	18.4	U	0.498
7/23/2013	Sherman Lake	Walleye	12883	Vilas	19.8	U	0.393
8/28/2013	Kentuck Lake	Walleye	9722	Vilas	22.8	F	0.734
8/28/2013	Kentuck Lake	Walleye	9724	Vilas	22.5	F	0.443
8/28/2013	Kentuck Lake	Walleye	9725	Vilas	17.5	M	0.419
8/28/2013	Kentuck Lake	Walleye	9728	Vilas	13.0	M	0.313
8/28/2013	Kentuck Lake	Walleye	9729	Vilas	20.3	F	0.313
8/28/2013	Kentuck Lake	Walleye	9730	Vilas	17.1	M	0.311
8/28/2013	Kentuck Lake	Walleye	9732	Vilas	14.1	M	0.312
8/28/2013	Kentuck Lake	Walleye	9733	Vilas	17.2	F	0.298
8/28/2013	Kentuck Lake	Walleye	9734	Vilas	15.8	M	0.278
8/28/2013	Kentuck Lake	Walleye	9735	Vilas	15.0	M	0.249
8/28/2013	Kentuck Lake	Walleye	9787	Vilas	24.2	F	0.714
8/28/2013	Kentuck Lake	Walleye	9789	Vilas	20.6	F	0.588
8/28/2013	Lac Vieux Desert	Walleye	12841	Vilas	17.8	M	0.223
8/28/2013	Lac Vieux Desert	Walleye	12844	Vilas	13.8	U	0.078
8/28/2013	Lac Vieux Desert	Walleye	12846	Vilas	17.0	M	0.124
8/28/2013	Lac Vieux Desert	Walleye	12849	Vilas	23.0	F	0.281
8/28/2013	Lac Vieux Desert	Walleye	12850	Vilas	13.7	M	0.108
8/28/2013	Lac Vieux Desert	Walleye	12851	Vilas	22.5	F	0.230
8/28/2013	Lac Vieux Desert	Walleye	12852	Vilas	22.4	F	0.179
8/28/2013	Lac Vieux Desert	Walleye	12853	Vilas	18.5	M	0.270
8/28/2013	Lac Vieux Desert	Walleye	12858	Vilas	18.4	M	0.239
8/28/2013	Lac Vieux Desert	Walleye	12859	Vilas	14.0	M	0.210
8/28/2013	Lac Vieux Desert	Walleye	12864	Vilas	18.3	F	0.220
8/28/2013	Lac Vieux Desert	Walleye	12869	Vilas	17.6	M	0.191
8/28/2013	Mille Lacs	Walleye	1183	Mille Lacs	19.9	U	0.129
8/28/2013	Mille Lacs	Walleye	1185	Mille Lacs	17.7	U	0.100
8/28/2013	Mille Lacs	Walleye	11278	Mille Lacs	22.7	F	0.231
8/28/2013	Mille Lacs	Walleye	11279	Mille Lacs	14.8	M	0.099
8/28/2013	Mille Lacs	Walleye	11915	Mille Lacs	22.0	F	0.244
8/28/2013	Mille Lacs	Walleye	11917	Mille Lacs	16.4	M	0.092
8/28/2013	Mille Lacs	Walleye	11947	Mille Lacs	17.0	M	0.093
8/28/2013	Mille Lacs	Walleye	11948	Mille Lacs	24.0	F	0.293
8/28/2013	Mille Lacs	Walleye	11973	Mille Lacs	19.7	F	0.142

Analysis Date	Sample Location	Species	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
8/28/2013	Mille Lacs	Walleye	11974	Mille Lacs	16.2	M	0.096
8/28/2013	Mille Lacs	Walleye	12273	Mille Lacs	18.4	M	0.080
8/28/2013	Mille Lacs	Walleye	12390	Mille Lacs	20.4	F	0.189
9/4/2013	Papoose Lake	Walleye	12855	Vilas	25.5	F	0.843
9/4/2013	Papoose Lake	Walleye	12856	Vilas	23.7	F	0.839
9/4/2013	Papoose Lake	Walleye	12857	Vilas	28.4	F	1.24
9/4/2013	Papoose Lake	Walleye	12858	Vilas	18.0	M	0.474
9/4/2013	Papoose Lake	Walleye	12859	Vilas	16.8	M	0.581
9/4/2013	Papoose Lake	Walleye	12860	Vilas	13.7	M	0.449
9/4/2013	Papoose Lake	Walleye	12861	Vilas	19.1	F	0.586
9/4/2013	Papoose Lake	Walleye	12862	Vilas	18.7	F	0.649
9/4/2013	Papoose Lake	Walleye	12863	Vilas	12.8	U	0.420
9/4/2013	Papoose Lake	Walleye	12864	Vilas	16.5	M	0.484
9/4/2013	Papoose Lake	Walleye	12865	Vilas	13.4	M	0.388
9/4/2013	Papoose Lake	Walleye	12866	Vilas	15.6	M	0.453
9/4/2013	Birch Lake	Walleye	12918	Vilas	12.3	U	0.319
9/4/2013	Birch Lake	Walleye	12922	Vilas	12.2	U	0.388
9/4/2013	Birch Lake	Walleye	12925	Vilas	22.1	U	0.731
9/4/2013	Birch Lake	Walleye	12929	Vilas	12.3	U	0.371
9/4/2013	Virgin Lake	Walleye	12823	Vilas	24.0	U	0.973
9/4/2013	Virgin Lake	Walleye	12827	Vilas	16.8	M	1.08
9/4/2013	Virgin Lake	Walleye	12839	Vilas	12.1	M	0.387
9/4/2013	Virgin Lake	Walleye	12840	Vilas	13.8	M	0.553
9/4/2013	Virgin Lake	Walleye	12842	Vilas	13.4	U	0.349
9/4/2013	Virgin Lake	Walleye	12861	Vilas	18.6	U	0.466
9/4/2013	Virgin Lake	Walleye	12866	Vilas	18.7	M	0.467
9/4/2013	Virgin Lake	Walleye	12867	Vilas	21.8	U	1.26
9/4/2013	Virgin Lake	Walleye	12870	Vilas	16.9	U	0.791
9/4/2013	Willow Flowage	Walleye	12801	Vilas	20.0	M	1.17
9/4/2013	Willow Flowage	Walleye	12802	Vilas	22.0	F	1.53
9/4/2013	Willow Flowage	Walleye	12804	Vilas	17.1	M	0.862
9/4/2013	Willow Flowage	Walleye	12806	Vilas	14.6	M	0.622
9/4/2013	Willow Flowage	Walleye	12992	Vilas	18.4	F	1.01
9/4/2013	Willow Flowage	Walleye	12993	Vilas	16.9	M	0.926
9/4/2013	Willow Flowage	Walleye	12994	Vilas	17.5	M	0.771
9/4/2013	Willow Flowage	Walleye	12995	Vilas	14.8	M	0.885
9/6/2013	Willow Flowage	Walleye	12996	Vilas	27.8	F	1.61

Analysis Date	Sample Location	Species	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
9/4/2013	Willow Flowage	Walleye	12997	Vilas	23.3	F	1.13
9/4/2013	Willow Flowage	Walleye	12998	Vilas	18.3	F	0.587
9/4/2013	Willow Flowage	Walleye	12999	Vilas	14.6	M	0.842
9/6/2013	Star Lake	Walleye	12884	Vilas	24.8	F	0.608
9/6/2013	Star Lake	Walleye	12888	Vilas	26.2	F	0.312
9/6/2013	Star Lake	Walleye	12889	Vilas	23.3	F	0.484
9/6/2013	Star Lake	Walleye	12890	Vilas	18.6	F	0.384
9/6/2013	Star Lake	Walleye	12891	Vilas	14.9	M	0.236
9/6/2013	Star Lake	Walleye	12892	Vilas	14.7	M	0.180
9/6/2013	Star Lake	Walleye	12893	Vilas	15.7	M	0.209
9/6/2013	Star Lake	Walleye	12894	Vilas	19.1	F	0.373
9/6/2013	Star Lake	Walleye	12895	Vilas	21.3	F	0.350
9/6/2013	Star Lake	Walleye	12896	Vilas	15.9	F	0.267
9/6/2013	Star Lake	Walleye	12897	Vilas	14.0	M	0.176
9/6/2013	Star Lake	Walleye	12898	Vilas	16.7	M	0.725
9/6/2013	Squirrel Lake	Walleye	11932	Oneida	13.6	M	0.295
9/6/2013	Squirrel Lake	Walleye	12270	Oneida	15.0	F	0.324
9/6/2013	Squirrel Lake	Walleye	12319	Oneida	24.7	F	0.559
9/6/2013	Squirrel Lake	Walleye	12355	Oneida	18.6	F	0.312
9/6/2013	Squirrel Lake	Walleye	12440	Oneida	18.0	F	0.359
9/6/2013	Squirrel Lake	Walleye	12441	Oneida	19.2	F	0.314
9/6/2013	Squirrel Lake	Walleye	12442	Oneida	22.8	F	0.363
9/6/2013	Squirrel Lake	Walleye	12443	Oneida	17.2	F	0.273
9/6/2013	Squirrel Lake	Walleye	12444	Oneida	18.6	F	0.342
9/6/2013	Squirrel Lake	Walleye	12445	Oneida	16.0	F	0.238
9/6/2013	Squirrel Lake	Walleye	12541	Oneida	26.7	F	0.893
9/6/2013	Squirrel Lake	Walleye	12545	Oneida	13.9	M	0.286
9/6/2013	Sand Lake	Walleye	12977	Vilas	12.3	M	0.230
9/6/2013	Sand Lake	Walleye	12978	Vilas	18.0	M	0.883
9/6/2013	Sand Lake	Walleye	12979	Vilas	13.4	M	0.248
9/6/2013	Sand Lake	Walleye	12980	Vilas	12.6	U	0.188
9/6/2013	Sand Lake	Walleye	12981	Vilas	15.4	M	0.390
9/6/2013	Sand Lake	Walleye	12989	Vilas	18.2	U	0.763
9/6/2013	Sand Lake	Walleye	12990	Vilas	17.7	F	0.932
9/6/2013	Sand Lake	Walleye	12991	Vilas	16.2	M	0.474
9/11/2013	Mille Lacs	N. Pike	11280	Mille Lacs	25.2	M	0.097
9/11/2013	Mille Lacs	N. Pike	11281	Mille Lacs	33.3	F	0.169

Analysis Date	Sample Location	Species	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
9/11/2013	Mille Lacs	N. Pike	11282	Mille Lacs	24.2	M	0.089
9/11/2013	Mille Lacs	N. Pike	11743	Mille Lacs	39.3	F	0.197
9/11/2013	Mille Lacs	N. Pike	11744	Mille Lacs	21.0	F	0.146
9/11/2013	Mille Lacs	N. Pike	11913	Mille Lacs	37.1	U	0.200
9/11/2013	Mille Lacs	N. Pike	11976	Mille Lacs	26.5	F	0.166
9/11/2013	Mille Lacs	N. Pike	11977	Mille Lacs	39.3	U	0.292
9/11/2013	Mille Lacs	N. Pike	11978	Mille Lacs	24.3	M	0.077
9/11/2013	Mille Lacs	N. Pike	11996	Mille Lacs	32.0	F	0.211
9/11/2013	Mille Lacs	N. Pike	12645	Mille Lacs	35.4	U	0.165
9/11/2013	Mille Lacs	N. Pike	12789	Mille Lacs	22.2	F	0.109
9/11/2013	Mille Lacs	N. Pike	12908	Mille Lacs	33.5	F	0.156
9/11/2013	Mille Lacs	N. Pike	12909	Mille Lacs	19.9	F	0.053
9/11/2013	Mille Lacs	N. Pike	12910	Mille Lacs	24.5	M	0.145
9/11/2013	Mille Lacs	N. Pike	12911	Mille Lacs	27.9	F	0.187
9/11/2013	Ballard Lake	Musky	2013M01	Vilas	31.3	M	1.72
9/11/2013	Ballard Lake	Musky	2013M02	Vilas	34.8	M	2.01
9/11/2013	Ballard Lake	Musky	2013M03	Vilas	35.8	M	1.57
9/11/2013	Ballard Lake	Musky	2013M04	Vilas	35.6	M	1.63
9/11/2013	Big St. Germain Lake	Musky	2013M05	Vilas	29.3	U	0.151
9/11/2013	Big St. Germain Lake	Musky	2013M06	Vilas	42.0	U	0.804
9/11/2013	Big St. Germain Lake	Musky	2013M07	Vilas	40.0	M	0.739
9/11/2013	Carrol Lake	Musky	2013M08	Oneida	38.6	M	0.599
9/11/2013	Clear Lake	Musky	2013M09	Vilas	42.5	F	0.633
9/11/2013	Clear Lake	Musky	2013M10	Vilas	33.9	M	0.310
9/11/2013	Clear Lake	Musky	2013M11	Vilas	38.0	M	1.48
9/11/2013	Clear Lake	Musky	2013M12	Vilas	36.5	M	0.429
9/11/2013	Irving Lake	Musky	2013M13	Vilas	41.5	M	1.79
9/11/2013	Irving Lake	Musky	2013M14	Vilas	39.0	F	1.70
9/11/2013	Irving Lake	Musky	2013M15	Vilas	34.9	M	1.79
9/11/2013	Irving Lake	Musky	2013M16	Vilas	39.5	F	1.91
9/11/2013	Round Lake	Musky	2013M17	Sawyer	44.7	F	0.865
9/11/2013	Round Lake	Musky	2013M18	Sawyer	44.0	F	1.37
9/11/2013	Sweeney Lake	Musky	2013M19	Oneida	42.0	M	0.898
9/11/2013	Upper Gresham Lake	Musky	2013M20	Vilas	36.7	F	0.288
9/11/2013	Upper Gresham Lake	Musky	2013M21	Vilas	41.0	M	0.916
9/11/2013	Upper Gresham Lake	Musky	2013M22	Vilas	31.3	M	0.111
9/11/2013	Upper Gresham Lake	Musky	2013M23	Vilas	38.5	M	0.403

Analysis Date	Sample Location	Species	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
9/11/2013	Upper Gresham Lake	Musky	2013M24	Vilas	38.5	M	1.31

Table 6. Percent Moisture in Walleye and Northern Pike Fillets and Muskellunge Muscle Plugs (Measured Immediately after Grinding).

Date	Sample Location	Tag Number	Species		Percent Moisture	Relative Percent Difference
6/18/2013	Bearskin Lake	12752	Walleye		79.1	
6/18/2013	Bearskin Lake	12753	Walleye		80.3	
6/18/2013	Bearskin Lake	12755	Walleye		79.0	
6/18/2013	Bearskin Lake	12755	Walleye	DUP	79.0	0.1
6/18/2013	Butternut Lake	11930	Walleye		80.9	
6/18/2013	Butternut Lake	11928	Walleye		78.8	
6/18/2013	Butternut Lake	11789	Walleye		81.2	
6/19/2013	Cedar Lake	12819	Walleye		78.4	
6/19/2013	Cedar Lake	12833	Walleye		78.0	
6/19/2013	Cedar Lake	12825	Walleye		78.8	
6/19/2013	Chippewa Flowage	12768	Walleye		78.2	
6/19/2013	Chippewa Flowage	12768	Walleye	DUP	78.0	0.3
6/19/2013	Chippewa Flowage	12764	Walleye		80.0	
6/19/2013	Chippewa Flowage	12770	Walleye		79.0	
6/20/2013	English Lake	11724	Walleye		78.2	
6/20/2013	English Lake	11724	Walleye	DUP	78.2	0.1
6/20/2013	English Lake	11721	Walleye		78.7	
6/20/2013	English Lake	11713	Walleye		79.1	
6/20/2013	Island Lake	6888	Walleye		80.8	
6/20/2013	Island Lake	6898	Walleye		69.7	
6/20/2013	Island Lake	6889	Walleye		87.0	
6/26/2013	Long Lake (Washburn Co.)	12856	Walleye		76.8	
6/26/2013	Long Lake (Washburn Co.)	12856	Walleye	DUP	77.3	0.6
6/26/2013	Long Lake (Washburn Co.)	12796	Walleye		78.5	
6/26/2013	Long Lake (Washburn Co.)	12704	Walleye		80.1	

Date	Sample Location	Tag Number	Species		Percent Moisture	Relative Percent Difference
6/26/2013	Pike Lake Chain	6775	Walleye		78.8	
6/26/2013	Pike Lake Chain	6780	Walleye		77.7	
6/26/2013	Pike Lake Chain	6781	Walleye		79.1	
6/27/2013	Chetac Lake	12708	Walleye		80.1	
6/27/2013	Meder Lake	11710	Walleye		79.2	
6/27/2013	Meder Lake	11710	Walleye	DUP	79.2	0.1
6/27/2013	Meder Lake	11711	Walleye		79.5	
6/27/2013	Meder Lake	11704	Walleye		79.1	
6/27/2013	Lake Owen	6810	Walleye		78.6	
6/27/2013	Lake Owen	6809	Walleye		78.4	
7/1/2013	Windigo Lake	12782	Walleye		78.7	
7/1/2013	Windigo Lake	12782	Walleye	DUP	78.3	0.5
7/1/2013	Windigo Lake	12778	Walleye		79.5	
7/1/2013	Windigo Lake	12701	Walleye		80.1	
7/1/2013	Squaw Lake	12313	Walleye		79.9	
7/1/2013	Squaw Lake	11998	Walleye		79.4	
7/1/2013	Squaw Lake	12311	Walleye		80.0	
7/9/2013	Kakagon River	6874	Walleye		79.6	
7/9/2013	Kakagon River	6883	Walleye		82.1	
7/9/2013	Kakagon River	6883	Walleye	DUP	81.7	0.5
7/9/2013	Kakagon River	6879	Walleye		82.3	
7/9/2013	Allequash Lake	12964	Walleye		79.0	
7/9/2013	Allequash Lake	12966	Walleye		78.9	
7/9/2013	Allequash Lake	12973	Walleye		78.2	
7/11/2013	Ballard Lake	12958	Walleye		78.6	
7/11/2013	Ballard Lake	12958	Walleye	DUP	78.5	0.2
7/11/2013	Ballard Lake	12948	Walleye		79.0	
7/11/2013	Ballard Lake	12901	Walleye		80.2	
7/11/2013	Big Muskellunge Lake	12944	Walleye		79.1	
7/11/2013	Big Muskellunge Lake	12946	Walleye		80.1	
7/11/2013	Big Muskellunge Lake	12941	Walleye		79.5	
7/15/2013	Rice River Flowage	12872	Walleye		77.7	
7/15/2013	Rice River Flowage	12873	Walleye		78.5	
7/15/2013	Rice River Flowage	12873	Walleye	DUP	78.2	0.4
7/15/2013	Rice River Flowage	12877	Walleye		78.9	
7/15/2013	Lake Gogebic	6870	Walleye		79.3	

Date	Sample Location	Tag Number	Species		Percent Moisture	Relative Percent Difference
7/15/2013	Lake Gogebic	6868	Walleye		80.0	
7/15/2013	Lake Gogebic	6860	Walleye		80.8	
7/15/2013	Squirrel Lake	11932	Walleye		79.4	
7/15/2013	Squirrel Lake	12444	Walleye		80.9	
7/15/2013	Squirrel Lake	12444	Walleye		80.7	
7/15/2013	Squirrel Lake	12442	Walleye		80.3	
7/19/2013	Star Lake	12891	Walleye		79.4	
7/19/2013	Star Lake	12897	Walleye		80.1	
7/19/2013	Star Lake	12890	Walleye		83.1	
7/19/2013	Sand Lake	12980	Walleye		78.4	
7/19/2013	Sand Lake	12980	Walleye	DUP	78.6	0.2
7/19/2013	Sand Lake	12979	Walleye		79.2	
7/19/2013	Sand Lake	12981	Walleye		79.9	
7/19/2013	Sherman Lake	12869	Walleye		80.1	
7/19/2013	Sherman Lake	12871	Walleye		81.0	
7/19/2013	Sherman Lake	12882	Walleye		81.6	
8/2/2013	Ballard Lake	M03	Musky		78.7	
8/2/2013	Big St. Germain Lake	M06	Musky		75.5	
8/2/2013	Big St. Germain Lake	M06	Musky	DUP	75.6	0.1
8/2/2013	Sweeney Lake	M19	Musky		77.4	
8/2/2013	Irving Lake	M13	Musky		78.2	
8/2/2013	Carrol Lake	M08	Musky		76.3	
8/2/2013	Clear Lake	M09	Musky		77.6	
8/2/2013	Round Lake	M17	Musky		76.2	
8/2/2013	Upper Gresham Lake	M21	Musky		78.4	
8/8/2013	Mille Lacs	12789	Northern		80.3	
8/8/2013	Mille Lacs	12789	Northern	DUP	80.1	0.2
8/8/2013	Mille Lacs	12645	Northern		80.0	
8/8/2013	Mille Lacs	11978	Northern		78.8	
8/8/2013	Mille Lacs	11743	Northern		79.2	
8/12/2013	Kentuck Lake	9735	Walleye		78.7	
8/12/2013	Kentuck Lake	9735	Walleye	DUP	78.2	0.6
8/12/2013	Kentuck Lake	9733	Walleye		78.4	
8/12/2013	Kentuck Lake	9730	Walleye		79.0	
8/12/2013	Virgin Lake	12827	Walleye		80.4	
8/12/2013	Virgin Lake	12866	Walleye		79.2	

Date	Sample Location	Tag Number	Species		Percent Moisture	Relative Percent Difference
8/12/2013	Virgin Lake	12861	Walleye		79.7	
8/14/2013	Willow Flowage	12806	Walleye		79.0	
8/14/2013	Willow Flowage	12806	Walleye	DUP	79.1	0.0
8/14/2013	Willow Flowage	12994	Walleye		79.3	
8/14/2013	Willow Flowage	12992	Walleye		79.8	
8/14/2013	Lac Vieux Desert	12853	Walleye		78.9	
8/14/2013	Lac Vieux Desert	12846	Walleye		79.5	
8/14/2013	Lac Vieux Desert	12869	Walleye		79.9	
8/15/2013	Mille Lacs	11279	Walleye		79.4	
8/15/2013	Mille Lacs	11279	Walleye	DUP	79.5	0.1
8/15/2013	Mille Lacs	12273	Walleye		79.2	
8/15/2013	Mille Lacs	11974	Walleye		78.5	
8/15/2013	Birch Lake	12922	Walleye		79.3	
8/15/2013	Birch Lake	12925	Walleye		79.4	
8/19/2013	Papoose Lake	12866	Walleye		78.5	
8/19/2013	Papoose Lake	12866	Walleye	DUP	78.7	0.2
8/19/2013	Papoose Lake	12862	Walleye		81.3	
8/19/2013	Papoose Lake	12863	Walleye		79.9	
			Mean and Std. Dev.		**79.2 ± 1.7**	

Appendix A

Determination of 2013 Limit of Detection (LOD) and Limit of Quantitation (LOQ) using a ground tuna sample from September 19, 2012.

Sample	Tissue Type	ng/L	ng Hg	g sample	ug Hg/g
Tuna 19 Sept 2012 -1	ground tuna	135.9	6.80	0.213	0.032
Tuna 19 Sept 2012 -2	ground tuna	139.3	6.96	0.204	0.034
Tuna 19 Sept 2012 -3	ground tuna	152.7	7.63	0.207	0.037
Tuna 19 Sept 2012 -4	ground tuna	162.7	8.14	0.214	0.038
Tuna 19 Sept 2012 -5	ground tuna	162.7	8.14	0.207	0.039
Tuna 19 Sept 2012 -6	ground tuna	149.3	7.47	0.210	0.036
Tuna 19 Sept 2012 -7	ground tuna	142.6	7.13	0.213	0.033
Tuna 19 Sept 2012 -8	ground tuna	146.0	7.30	0.211	0.035
				Mean	0.0355
				Std. Dev.	0.00245

2013 LOD = Std. Dev. x t = 0.00245 x 2.998 = 0.0073
2013 LOQ = 10/3 x LOD = 0.0245

May 1, 2013	**Hg LOD= 0.0073 µg/g LOQ= 0.0245 µg/g**
May 31, 2012	**Hg LOD = 0.0030 µg/g LOQ = 0.0099 µg/g**
2011	**Hg LOD=0.0017µg/g LOQ=0.0057µg/g**
2010	**Hg LOD = 0.00459 µg/g LOQ = 0.0153 µg/g**
2009	**Hg LOD = 0.00660 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 2013 Walleye and Northern Pike Fillets and Muskellunge Muscle Plugs. Data Quality Indicators for Calibration Curves include a Slope of 2.0-3.0 x 10^{-5} and a Coefficient of Determination of >0.995.

Analysis Date	Standard Conc. ng Hg/L	Blank Corr. Abs.1	Blank Corr. Abs. 2	Blank Corr. Mean	Standard Deviation	Slope	Y-Intercept	Correlation
6/25/2013	0	0.0001	0.0002	0.0000	0.0001	2.9911 E-05	0.001572	0.9998
6/25/2013	100	0.0032	0.0034	0.0033	0.0001			
6/25/2013	500	0.0177	0.0163	0.0170	0.0010			
6/25/2013	1000	0.0331	0.0300	0.0316	0.0022			
6/25/2013	5000	0.1656	0.1459	0.1558	0.0139			
6/25/2013	10,000	0.3138	0.2829	0.2984	0.0218			
7/2/2013	0	0.0002	0.0002	0.0000	0.0000	2.8687 E-05	0.000890	0.9999
7/2/2013	100	0.0029	0.0034	0.0032	0.0004			
7/2/2013	500	0.0150	0.0153	0.0152	0.0002			
7/2/2013	1000	0.0287	0.0307	0.0297	0.0014			
7/2/2013	5000	0.1432	0.1513	0.1473	0.0057			
7/2/2013	10,000	0.2764	0.2962	0.2863	0.0140			
7/10/2013	0	0.0012	0.0009	0.0000	0.0002	2.7604 E-05	0.002504	0.9993
7/10/2013	100	0.0025	0.0031	0.0028	0.0004			
7/10/2013	500	0.0143	0.0142	0.0143	0.0001			
7/10/2013	1000	0.0335	0.0354	0.0345	0.0013			
7/10/2013	5000	0.1445	0.1485	0.1465	0.0028			
7/10/2013	10,000	0.2730	0.2775	0.2753	0.0032			
7/12/2013	0	0.0003	0.0004	0.0000	0.0001	2.8729 E-05	0.001949	0.9997
7/12/2013	100	0.0032	0.0029	0.0031	0.0002			
7/12/2013	500	0.0155	0.0149	0.0152	0.0004			
7/12/2013	1000	0.0345	0.0341	0.0343	0.0003			
7/12/2013	5000	0.1481	0.1491	0.1486	0.0007			
7/12/2013	10,000	0.2854	0.2895	0.2875	0.0029			
7/18/2013	0	0.0006	0.0002	0.0000	0.0003	2.8799 E-05	0.001430	0.9997
7/18/2013	100	0.0030	0.0031	0.0031	0.0001			
7/18/2013	500	0.0153	0.0155	0.0154	0.0001			
7/18/2013	1000	0.0308	0.0301	0.0305	0.0005			
7/18/2013	5000	0.1502	0.1522	0.1512	0.0014			

7/18/2013	10,000	0.2833	0.2898	0.2866	0.0046			
7/23/2013	0	0.0001	0.0001	0.0000	0.0000	2.6558 E-05	0.002897	0.9992
7/23/2013	100	0.0031	0.0031	0.0031	0.0000			
7/23/2013	500	0.0151	0.0148	0.0150	0.0002			
7/23/2013	1000	0.0325	0.0326	0.0326	0.0001			
7/23/2013	5000	0.1431	0.1431	0.1431	0.0000			
7/23/2013	10,000	0.2743	0.2548	0.2646	0.0138			
8/28/2013	0	0.0001	0.0002	0.0000	0.0001	2.6687 E-05	0.001116	0.9999
8/28/2013	100	0.0030	0.0028	0.0029	0.0001			
8/28/2013	500	0.0137	0.0154	0.0146	0.0012			
8/28/2013	1000	0.0263	0.0306	0.0285	0.0030			
8/28/2013	5000	0.1304	0.1440	0.1372	0.0096			
8/28/2013	10,000	0.2454	0.2878	0.2666	0.0300			
9/4/2013	0	0.0000	0.0002	0.0000	0.0001	2.7896 E-05	0.002072	0.9994
9/4/2013	100	0.0034	0.0031	0.0033	0.0002			
9/4/2013	500	0.0155	0.0154	0.0155	0.0001			
9/4/2013	1000	0.0304	0.0305	0.0305	0.0001			
9/4/2013	5000	0.1473	0.1509	0.1491	0.0025			
9/4/2013	10,000	0.2800	0.2745	0.2773	0.0039			
9/6/2013	0	0.0001	0.0002	0.0000	0.0001	2.8990 E-05	0.001837	0.9996
9/6/2013	100	0.0034	0.0032	0.0033	0.0001			
9/6/2013	500	0.0159	0.0159	0.0159	0.0000			
9/6/2013	1000	0.0317	0.0312	0.0315	0.0004			
9/6/2013	5000	0.1517	0.1543	0.1530	0.0018			
9/6/2013	10,000	0.2878	0.2894	0.2886	0.0011			
9/11/2013	0	0.0000	0.0001	0.0000	0.0001	2.7848 E-05	0.001320	0.9997
9/11/2013	100	0.0029	0.0032	0.0031	0.0002			
9/11/2013	500	0.0136	0.0155	0.0146	0.0013			
9/11/2013	1000	0.0271	0.0323	0.0297	0.0037			
9/11/2013	5000	0.1314	0.1599	0.1457	0.0202			
9/11/2013	10,000	0.2586	0.2959	0.2773	0.0264			

Appendix C

Quality Assurance Audit Report: Technical Systems Audit of Great Lakes Indian Fish and Wildlife Commission (GLIFWC) Mercury Testing Project – 2013 Spring Walleye Samples

Auditee: Lake Superior Research Institute (LSRI) staff and students assigned to GLIFWC Mercury Testing Project
Auditor: Kelsey Prihoda, LSRI Quality Assurance Manager (QAM)
Audit Dates: 20, 24, and 25 June 2013
Closing Discussions with LSRI-GLIFWC Staff: 25 – 31 July 2013

Description and Scope of Audit

A technical systems audit (TSA) of the laboratory analysis for the project *Great Lakes Indian Fish and Wildlife Commission (GLIFWC) Mercury Testing and Updating Tribal-Walleye Consumption Advice*, hereafter referred to as the GLIFWC Mercury Testing Project, was conducted 20, 24, and 25 June 2013. The objectives of the TSA were to review the project quality system documentation, personnel files, and equipment/analytical instrumentation calibration and maintenance from sample processing and analysis of the spring 2013 walleye samples. The TSA included a procedural audit of 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 sample grinding procedural audit was conducted 20 June 2013, and the digestion and mercury analysis procedural audit was conducted 24-25 June 2013. The sample grinding (samples collected from English Lake in Ashland, WI) was audited against LSRI SOP SA/10, *v.*6 (and supporting LSRI SOP SA/8, *v.*7). The digestion and mercury analysis was audited against LSRI SOP SA/49, *v.*1 (and supporting LSRI SOPs SA/11, *v.*6; GLM/12, *v.*5; and SA/42, *v.*2). In addition, the project documentation (GLIFWC Project Laboratory Notebook and 2013 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 25 July 2013 and staff members commented on the TSA findings during a comment period on 25 – 31 July 2013.
The GLIFWC Project Manager at LSRI is Christine Polkinghorne, and Kimberly Beesley is a project staff member. Student research assistants on the project include Cole Holstrom, Colton Kennedy, Olivia Krause, and Ethan Lawler.

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.

Quality System Documentation

Audit Findings

Non-Conformance

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

Deviations

- No deviations found during audit of quality system documentation.

Observations

- No observations to report.

Praises/Noteworthy Efforts

- The GLIFWC Mercury Testing QAPP received final approval on 24 June 2011. The QAPP is stored in the current GLIFWC Mercury Testing Project binder (13-06-14_GLIFWC). The current revisions of LSRI SOP(s) for each procedure being conducted were found in the laboratory during each procedural audit.
- The Chain of Custody (COC) form for the spring 2013 walleye samples is included in the 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 sample handling and storage procedures were in accordance with the QAPP and with sample handling requirements specified in LSRI SOP SA/10, *v.*6.
    - Samples are stored in a locked chest freezer; temperature range at the time of the audit was -20.5°C (-23.7°C to -17.7°C), which is in accordance with SA/10, *v.*6.
- 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 GLIFWC Mercury Testing Project binder.
- LSRI SOP SA/50 – *Routine Maintenance for FIMS-100* has been updated by project staff and is awaiting final review by the LSRI QAM (as of 25 July 2013). Once this SOP has been finalized, all project SOPs will have been updated within the past two years.

Opportunity for Improvement

- No findings to report

Conclusions from Quality System Documentation Audit

Overall project documentation using laboratory notebook 13-06-14_GLIFWC and the GLIFWC Mercury Testing Project Binder (12-9-10_GLIFWC) was very good, and provided sufficient documentation to follow the samples from receipt at LSRI through mercury analysis and reporting. 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. The FIMS-100 maintenance SOP has been updated by project staff and is awaiting final review by the LSRI QAM (as of 31 July 2013).

Organization and Responsibilities

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. Descriptions of the GLIFWC Mercury Testing Project organization and personnel responsibilities are listed in the QAPP.

Training and Safety

Audit Findings

Non-Conformance

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

Deviations

- No deviations found during audit of training and safety.

Observations

- The LSRI QAM received verbal confirmation that Olivia Krause, Ethan Lawler, and Colton Kennedy have read the appropriate SOPs assigned to them for this project and have completed hands-on training. Their Certificates of Completion and Competency should be provided to the LSRI QAM when complete (i.e., SOPs and training for multiple projects are documented on one form).

Praises/Noteworthy Efforts

- Christine Polkinghorne, Kimberly Beesley, and Cole Holstrom have read the appropriate SOPs assigned to them, and have adequate training/experience to perform routine procedures.
- Each staff member was appropriately outfitted with personal protective equipment during the audit.

Opportunity for Improvement

- The LSRI QAM has resumes on file for all LSRI-GLIFWC Project staff and students except Colton Kennedy.
    - *Follow-up: A reminder was sent via e-mail to Colton on 24 July 2013 to provide a copy of his most current resume to the LSRI QAM as soon as possible.*
- All LSRI-GLIFWC Project staff and students have completed UWS Laboratory Health and Safety Training except Colton Kennedy. On 23 May 2013, the LSRI QAM sent him an e-mail message asking him to contact Carol Lindberg and set up computer-based training this summer. No response to that e-mail message was received.
    - *Follow-up: A reminder was sent via e-mail to Colton on 24 July 2013 to set up computer-based safety training as soon as possible.*

Conclusions from Training and Safety Audit

Resumes are on file for LSRI staff and students working on the GLIFWC Mercury Testing Project, with the exception of Colton Kennedy. The LSRI QAM sent a reminder e-mail message to Colton on 24 July 2013. GLIFWC Project personnel have read all relevant SOPs and received hands-on training on these SOPs (verbal confirmation received for Olivia Krause, Ethan Lawler, and Colton Kennedy). All LSRI-GLIFWC staff have completed the LSRI Quality System Orientation, and all except Colton Kennedy have taken the UWS Laboratory Health and Safety Training course (a reminder e-mail message was sent to Colton on 24 July 2013). All laboratory safety procedures were followed during the TSA.

Equipment and Analytical Instrumentation

Audit Findings

Non-Conformance

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

Deviations

- No deviations to report from audit of equipment and analytical instrumentation.

Observations

- The laboratory balance was verified accurate prior to sample grinding on 20 June 2013 by Kimberly Beesley (as documented in laboratory notebook 05-9-26_BAL). Prior to sample digestion on 24 June 2013, the balance was again verified to be accurate using 0.020 g, 2.000 g, and 100.000 g ASTM Class I weights.

Praises/Noteworthy Efforts

- Kimberly Beesley calibrated the 1-5 mL Finnpipette at 5 mL and also calibrated the 10-100 µL adjustable-volume pipette prior to spring 2013 walleye sample grinding, digestion, and analysis.

- The 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. 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.
- Based on the procedural audit conducted 24 June 2013, 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 from Fisher Mercury Reference Standard Solution (lot: 115065; exp: 12/2013) by Kimberly Beesley on 24 June 2013, 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 24 June 2013, which was prior to the one week expiration required by LSRI SOP SA/42 *v.*1.
- Limit of Detection (LOD) and Quantification (LOQ) for 2013 GLIFWC Mercury Testing Project was determined by Kimberly Beesley on 01 May 2013 (prior to any sample analysis for the project) using a ground tuna sample from 19 September 2013: n=8 samples, LOD = 0.0073 µg Hg/g and LOQ = 0.0245 µg/g.
- Carrier and reductant flow rates were recorded on the sample analysis datasheet, where they were easily verified by the LSRI QAM to be within the acceptance ranges (i.e., carrier flow rate was 10.5 mL/min; reductant flow rate was 6.0 mL/min).

Opportunity for Improvement

- It may be worthwhile to evaluate whether the acceptance range for the slope of the calibration curve should change (i.e., slightly increase) based on data from 2013 and due to the change in the acceptance range for the 5000 ng/L Hg standard response (i.e., 0.12 to 0.17).
- Should LSRI SOP SA/49, *v.*1 be revised to prepare more than 900 mL of reductant solution for a full set of 40 samples? During the audit on 25 June 2013, the stannous chloride solution ran out and caused the 10,000 ng/L Hg standard to have a falsely inaccurate response.
    - *Follow-up: LSRI SOP SA/49, v.1 already contains the procedure for preparing 1 L of reductant solution. Project staff will now be preparing 1 L of this solution, following the procedure in the SOP, to ensure a sufficient volume is always available for a full set of 40 samples.*

Conclusions from Equipment and Analytical Instrumentation Audit

The equipment/analytical instrumentation used in the sample processing and analysis of the spring 2013 walleye samples was found to be in good working order, with calibration/verification and maintenance activities appropriately recorded in the equipment-

specific log books. The pipettes used for the project were calibrated (at the volumes typically used for project measurements) prior to sample digestion and analysis. Mercury standard and spike preparation was found to be in accordance with LSRI SOP SA/42, *v.*1. The 2013 LOD and LOQ were determined prior to project sample analysis. It is suggested that project staff evaluate whether the calibration curve acceptance range should change based on recent historical data from 2013. During future analyses, project staff will prepare 1 L of reductant solution (following LSRI SOP SA/49) for a full set of 40 samples to ensure a sufficient volume is always available.

Supplemental Data

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

II. Results from GLIFWC Mercury Testing Project Sample Grinding Procedural Audit

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

Appendix
D

Standard Operating Procedures (SOPs) Used During Project

Standard Operating Procedure SA/8v.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[1] 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.

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[1] 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.

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

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 hot1 water containing Liquinox® detergent.

16. Rinse the labware with hot1 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/10v.6
SAMPLE GRINDING FOR METALS ANALYSIS

INTRODUCTION

This Lake Superior Research Institute (LSRI) standard operating procedure (SOP) describes the method used for grinding biological tissue, typically fish tissue, into homogeneous samples for metals analysis. The Kitchen Aid™ food grinder attachment and labware used to grind the tissue are cleaned using the 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.

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.

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.

PROCEDURE

Grinding Tissue Samples

1. If the grinding equipment has not been used for more than one day, 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 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.

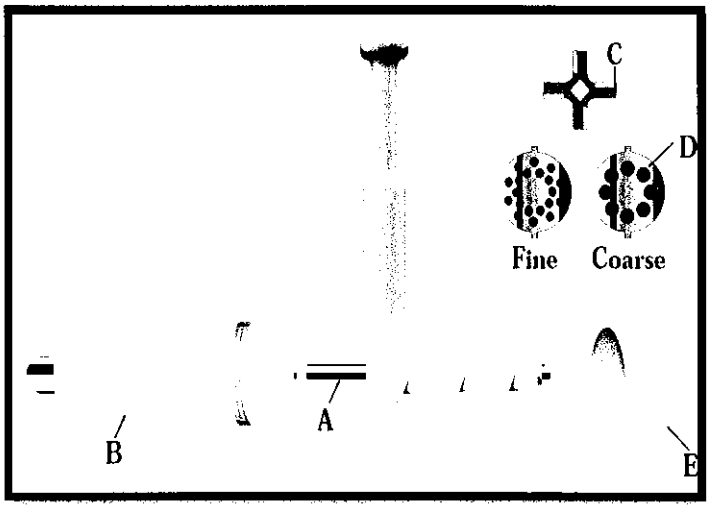

Figure 1. Assembly of KitchenAid™ Stand Mixer Food Grinder Attachment.

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.

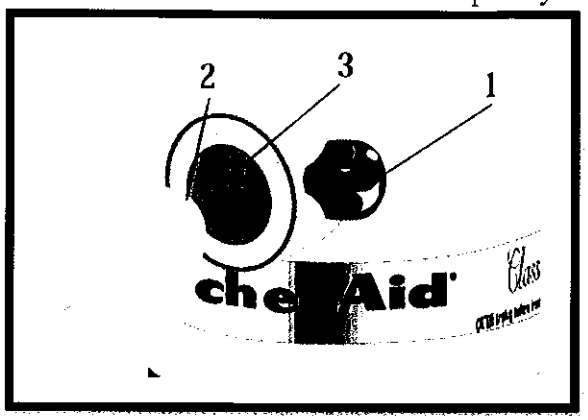

Figure 2. Connection of the Assembled Food Grinder Attachment to the KitchenAid™ Stand Mixer.

7. 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.

8. 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.

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

10. 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.

11. Wash the food grinder 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.

12. Continue to grind each sample by repeating Steps 4 to 11.

Preparing the Procedural Blank

13. 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.

14. 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 Steps 9 and 10. Label this procedural blank as "Tuna before Grinding" and include the date of processing. The unground blank is included with the analysis set.

15. Grind the remainder of the tuna as a procedural blank following the procedure outlined in Steps 5 to 11. 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/11v.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!
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Standard Operating Procedure GLM/12v.5
PROCEDURE FOR VERIFICATION OF LABORATORY BALANCES

INTRODUCTION

This standard operating procedure (SOP) describes the method used for routine maintenance and verification of laboratory balances. This SOP applies to those precision or analytical balances used for accurate weighing. Examples include preparation of reconstituted water for culturing, *S. capricornutum* media, analytical standards and samples, stock solutions, and balances used for weighing test organisms and weighing filters for the determination of total suspended solids concentrations. This SOP does not apply to those balances that are used for non-accurate weighing, such as during the preparation of various diets for test organisms reared at the Lake Superior Research Institute, weighing whole fish collected for metals analysis, or weighing large quantities of salt for large-volume salt water preparation. Balance verification checks must be conducted each day before the first use of a balance, or when weighing outside the range of standard weights used to verify the balance on that day. If the results fall outside of the designated acceptance limits for the balance (see Appendix 2), the balance should not be used until it has been calibrated by an LSRI senior staff member (or a service technician, if necessary) and meets the calibration limits.

Laboratory balances are verified using ANSI/ASTM Class 1 weights. The Class 1 weights are ***never*** to be touched with the hands. Always use the forceps supplied with the weights. Care should be taken to avoid scratching or getting dirt, oil, or moisture on the weights. Improper use or care of the weights may affect the calibration and could result in declassification of the weights.

The frequency with which precision/analytical balances must be serviced by an outside vendor is project-specific, and will be specified in the project planning documentation (i.e., Quality Assurance Project Plan). In addition, recertification frequency of ANSI/ASTM Class 1 weights used to verify balance accuracy is dependent upon the project and will be specified in the project planning documentation.

DEFINITIONS

Accuracy: How closely an instrument measures the true or actual value of the variable being measured.

ANSI/ASTM Class 1 Weights: Weights that can be used as a reference standard in calibrating other weights and that are appropriate for calibrating high precision analytical balances with a readability as low as 0.1 mg to 0.01 mg.

Calibration: An *adjustment* of an instrument based on comparison to materials with known or certified values.

Verification: A *check* of instrument accuracy with an external known source.

REFERENCES

Mettler Toledo Operating Instructions Manual for B-S Line of Balances. April 2001. Mettler-Toledo GmBH, Laboratory and Weighing Technologies, CH-8606 Greifensee, Switzerland.

National Institute of Standards and Technology Handbook 44, 2007 Edition. Specifications, Tolerances, and Other Technical Requirements for Weighing and Measuring Devices. National Institute of Standards and Technology, Weights and Measures Division, Gaithersburg, MD.

EQUIPMENT LIST

- ANSI/ASTM Class 1 Weights (usually Troemner)
- Balance Accuracy Tolerances for NIST Class I Balances (Appendix 2)
- Balance Routine Maintenance and Verification Datasheet (Appendix 1)
- Forceps for Weights
- Gloves
- Laboratory Balance Log Book and/or Three-Ring Binder (may use one log book/binder for labs with multiple balances)
- Laboratory Precision or Analytical Balance (usually Mettler Toledo)

PROCEDURE

Before beginning to use balance, check to see that it has been serviced by an outside vendor within the time frame specified in the project planning documentation. This can be done by checking the sticker applied to the balance by the servicing company. Also, confirm that the Class 1 weights have been certified within the time frame specified in the project planning documentation. The date of most recent certification will be noted on the box containing the weights.

1. Ensure that the balance is located in a stable, vibration-free position that is free from direct sunlight, excessive temperature fluctuations, and drafts. If the balance is not located in an area where the greatest accuracy of weighing can be achieved, move the balance to a stable bench in an area that is protected against drafts and is as far away as possible from doors, windows, radiators, or air conditioning units. Alternatively, a marble slab or similar device may be used to reduce vibration on laboratory benches.

2. Clean the draft shield, weighing pan, and bottom plate ***before verification and after using the balance***:

    2.1. Use a soft-fiber (hair) brush or dry Kimwipe™ to sweep away loose debris.
    2.2. If necessary, <u>remove</u> the weighing pan from the balance and clean with a damp Kimwipe™ or wash the weighing pan.
    2.3. If using a moist Kimwipe™ for cleaning, be sure balance pan is dry before using.

3. Ensure that the balance is level by checking the level gauge/spirit (usually located at the back of the balance). Level the balance if needed using the adjustable leveling feet (located either on the front or back of the balance); the balance is level when the air bubble is in the middle

of the level gauge/spirit.

4. Remove any load from the weighing pan and turn on the balance. Check the zero on the balance and, if necessary, adjust to read zero (using the "Tare" or "Zero" key) with no load.

5. Select three ANSI/ATSM Class 1 weights, which bracket the weight being determined. The Class 1 weights typically present in LSRI laboratories are: 20 mg, 200 mg, 2 g, and 20 g, therefore, if the sample weight is about 0.5 g, use the 20 mg, 200 mg, and 2 g weights.

6. Place the lowest-mass weight on the pan using the forceps. When the display stabilizes, read and record the mass (as grams) of the weight on the "Balance Routine Maintenance and Verification Datasheet" (Appendix 1). The verification datasheets should be kept in a three-ring binder located in an easily accessible area of the laboratory.

7. Repeat Step 6 with the middle- and highest-mass weights. Record the verification check date, results (pass or fail according to Step 8 below), and your initials in the laboratory notebook/three-ring binder for the balance(s) used.

8. Compare the values obtained with the actual value for the weights. If the difference is larger than the balance accuracy tolerance (see acceptable accuracy tolerances in Appendix 2), do not use the balance. Report the problem to the lab supervisor. The supervisor should recheck the balance and recalibrate, if necessary. If the balance is unable to be calibrated, a professional service and calibration should be scheduled. If the balance is found to be out of specification, a note should be attached to warn others not to use it. Record any maintenance (cleaning, etc.) and calibration activities in the laboratory notebook/three-ring binder.

9. Weigh test materials, samples, etc. following the appropriate SOP, if one exists. For example, follow LSRI/SOP/SA/11 - *Sample Weighing for Metals Analysis* (issued 1992) if weighing biological tissue samples for metals analysis. When applicable, record the mass of the material(s) weighed in the balance laboratory notebook or on a project-specific datasheet or notebook.

10. When weighing is completed, turn balance off. Clean the balance housing, weighing pan, and bottom plate after using a laboratory balance according to Step 2 of this procedure. In addition, clean the counter around the balance.

11. Technical specifications for each balance can be found in the operator's manual for that particular balance. The original copy of operator's manuals for every balance at LSRI should be kept on file by the LSRI Quality Assurance Manager. If needed, a copy of the operator's manual can be made and kept next to the balance (i.e., in the three-ring binder).

APPENDIX 1

BALANCE MAINTENANCE AND VERIFICATION DATASHEET

LSRI Balance Routine Maintenance and Verification Datasheet

Date	Initials	Balance Model/ID Number	Cleaned Balance? (Y/N)	Leveled Balance? (Y/N)	Zeroed Balance? (Y/N)	Low Weight		Middle Weight		High Weight		Within Tolerance Range? (Y/N)
						Weight Used (g)	Balance Read (g)	Weight Used (g)	Balance Read (g)	Weight Used (g)	Balance Read (g)	

APPENDIX 2

BALANCE ACCURACY TOLERANCE RANGES

LSRI Balance Accuracy Tolerances* for NIST Class I** Balances

Verification Mass Value	Balance Readability:		
	0.001 g (1 mg)	0.0001 g (0.1 mg)	0.00001 g (0.01 mg)
	Balance Tolerance Range (g)	Balance Tolerance Range (g)	Balance Tolerance Range (g)
300 g	299.925-300.075	299.9250-300.0750	299.92500-300.07500
200 g	199.950-200.050	199.9500-200.0500	199.95000-200.05000
100 g	99.975-100.025	99.9750-100.0250	99.97500-100.02500
50 g	49.987-50.013	49.9875-50.0125	49.98750-50.01250
30 g	29.992-30.008	29.9925-30.0075	29.99250-30.00750
20 g	19.995-20.005	19.9950-20.0050	19.99500-20.00500
10 g	9.997-10.003	9.9975-10.0025	9.99750-10.00250
5 g	4.997-5.003	4.9987-5.0013	4.99875-5.00125
3 g	2.997-3.003	2.9992-3.0008	2.99925-3.00075
2 g	1.997-2.003	1.9995-2.0005	1.99950-2.00050
1 g	0.997-1.003	0.9997-1.0003	0.99975-1.00025
500 mg	0.497-0.503	0.4997-0.5003	0.49987-0.50013
300 mg	0.297-0.303	0.2997-0.3003	0.29992-0.30008
200 mg	0.197-0.203	0.1997-0.2003	0.19995-0.20005
100 mg	0.097-0.103	0.0997-0.1003	0.09997-0.10003
50 mg	0.047-0.053	0.0497-0.0503	0.04997-0.05003
30 mg	0.027-0.033	0.0297-0.0303	0.02997-0.03003
20 mg	[a]Below Min. Capacity	0.0197-0.0203	0.01997-0.02003
10 mg	[a]Below Min. Capacity	0.0097-0.0103	0.00997-0.01003
5 mg	[a]Below Min. Capacity	0.0047-0.0053	0.00497-0.00503
3 mg	[a]Below Min. Capacity	0.0027-0.0033	0.00297-0.00303
2 mg	[a]Below Min. Capacity	[a]Below Min. Capacity	0.00197-0.00203
1 mg	[a]Below Min. Capacity	[a]Below Min. Capacity	0.00097-0.00103

*Balance tolerances are ¼ of the 0.1% tolerance as stated in the United States Pharmacopeia (USP) code or 3 times the balance readability, whichever is larger.

**Class I accuracy as determined by NIST Handbook 44-2007 Tables 3 and 8.

[a]Minimum balance capacity is the mass below which the acceptable error is greater than ±10%.

Standard Operating Procedure SA/35v.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.

Standard Operating Procedure SA/37v.1
PROCEDURES FOR CALCULATING MERCURY CONCENTRATIONS USING COLD VAPOR MERCURY ANALYSIS

INTRODUCTION

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

EQUIPMENT

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

PROCEDURE

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

Conversion from µg/mL to ng/mL

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

Concentration of Mercury Sub-Stocks

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

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

Amount of Mercury in each Standard Solution

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

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

Amount of Mercury in each Sample

$$y = mx + b$$

Where, m = Slope of Linear Regression Line

b = Intercept of Linear Regression Line

y = Mean Blank-Corrected Peak Height for Sample of Interest

x = Amount (ng) of Mercury in Sample of Interest

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

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

Concentration of Mercury in each Biological Tissue Sample

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

Standard Operating Procedure SA/38v.2
HOMOGENIZATION OF TISSUES FOR METALS ANALYSIS USING LIQUID NITROGEN

INTRODUCTION

This standard operating procedure (SOP) describes the method for blending tissue samples into homogenous samples, which is based on the procedure used by EnChem, Inc. (1997). This SOP is applicable to preparation of tissue samples (i.e. clams, snails, fish fatty tissue, fish skin, fish muscle plugs, insects, plants) and other samples that are too small to be homogenized using a meat grinder. Liquid nitrogen is used to freeze the tissue sample, which is then processed in a blender to obtain a more homogenous sample than is obtained with a meat grinder. The blender and labware used in this procedure must be cleaned following the procedure outlined in *LSRI SOP SA/08 - Routine Labware Cleaning for Metals Analysis.* Sample vials for storage of homogenized samples are ordered certified pre-cleaned. The proper safety equipment must be worn during the entire grinding procedure; this includes gloves, safety glasses and lab coats.

REFERENCES

EnChem, Incorporated. 1997. Preparation of Tissues for Analytical Determination in the Laboratory. Madison, WI.

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

EQUIPMENT LIST

- Can Opener
- Certified Pre-Cleaned Glass Vials
- Dry Wash Cloths (to protect skin against the cold metal bowls/pitcher/spatulas)
- Fillet Knives
- Glass Cutting Board
- Gloves
- Industrial Strength Blender (two speeds with a stainless steel pitcher)
- KimWipes
- Liquid Nitrogen
- Liquid Nitrogen Dewars
- Safety Glasses
- Samples
- Spatula
- Stainless Steel Bowls
- Tuna Fish, Canned (typically packed in water)

SAMPLE HANDLING REQUIREMENTS

2. After samples are received, they must be stored in a freezer at < -10°C. After homogenization, samples must be stored in a freezer at < -10°C until digestion.

3. Care must be taken when using liquid nitrogen. Liquid nitrogen is -196 °C and causes rapid freezing on contact with living tissue.

PROCEDURE

Preparing the Procedural Blank

1. 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.

2. Open a can of tuna (typically packed in water) and drain the liquid from the can. Homogenize the tissue with a spatula and transfer a portion to a certified-clean sample container labeled "Tuna before Grinding" and include the date of processing and your initials. The unground tuna blank is included with an analysis set.

3. Place the remainder of the tuna sample in a stainless steel bowl. Homogenize the tuna sample by following Steps 5 and 6 below. Place the tuna in a certified-clean sample vial using a spatula and label the tuna fish as "Tuna after Grinding" and include the date of processing and your initials. The ground tuna blank is included with the same analysis set as the unground tuna blank prepared on the same day.

Homogenizing the Samples

4. Remove the sample from the freezer. Larger samples should be cut into approximately ¼-inch cubes on a clean, glass cutting board using a sharp fillet knife. If the sample has skin, such as musky muscle plugs, remove and discard the skin (unless the skin is to be analyzed separately) prior to cubing the sample. The smaller the cubes are, the more quickly and thoroughly the sample will freeze. Smaller samples can be placed directly into the stainless steel bowl and frozen with liquid nitrogen.

5. Place the sample into a stainless steel bowl. Pour liquid nitrogen over the sample until the sample is frozen solid. When this occurs, the sample typically breaks free from the bowl easily.

6. Pre-cool the blender pitcher just prior to adding the frozen tissue sample to be homogenized. The pitcher is cooled by the addition of a small volume of liquid nitrogen (<100 mL). Transfer the frozen sample into a pre-cooled blender pitcher and pulse the blender until the sample is broken into very small pieces of approximately uniform size. Place the homogenized sample into an appropriately labeled certified-clean sample vial. Place the sample in the freezer (< -10°C) until digestion.

7. Wash the blender pitcher parts, stainless steel bowls and spatulas following SOP *SA/08 – Routine Labware Cleaning for Metals Analysis* before homogenizing the next sample.

When reassembling the blender pitcher for the next sample, it is important to dry all parts using a KimWipe to prevent the moving parts from freezing when liquid nitrogen is added to the pitcher.

8. Continue to homogenize samples following Steps 4-7.

Standard Operating Procedure SA/42v.2
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) $KMnO_4$

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/49v.2
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 swirl the HotBlock™ rack holding the digestion cups after 15.0 mL is added.

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 1000 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.

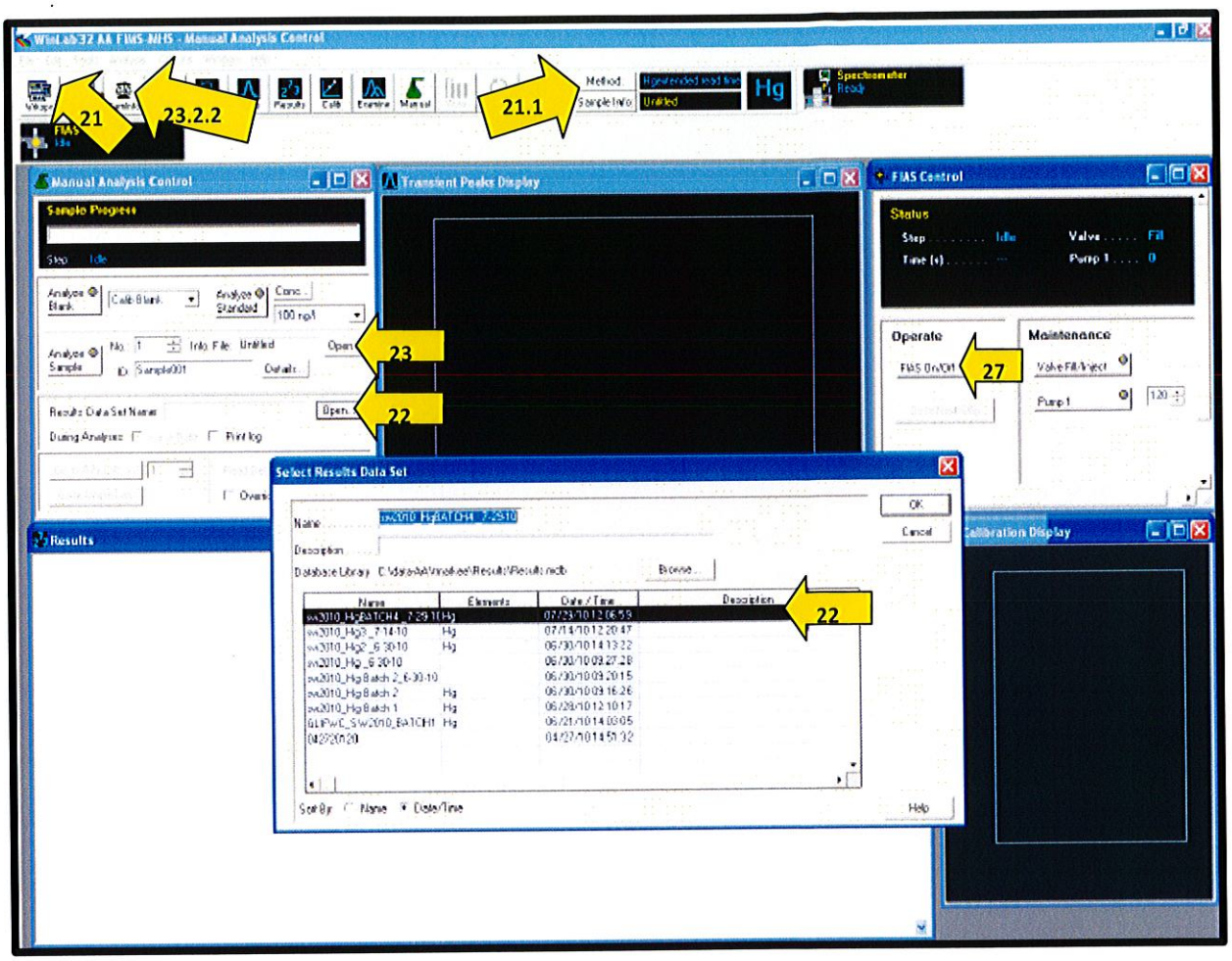

Figure 1. Screen shot of the control window of the WinLab32™ for AA software. The yellow arrows indicate areas of importance and the corresponding steps referenced within this SOP.

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.

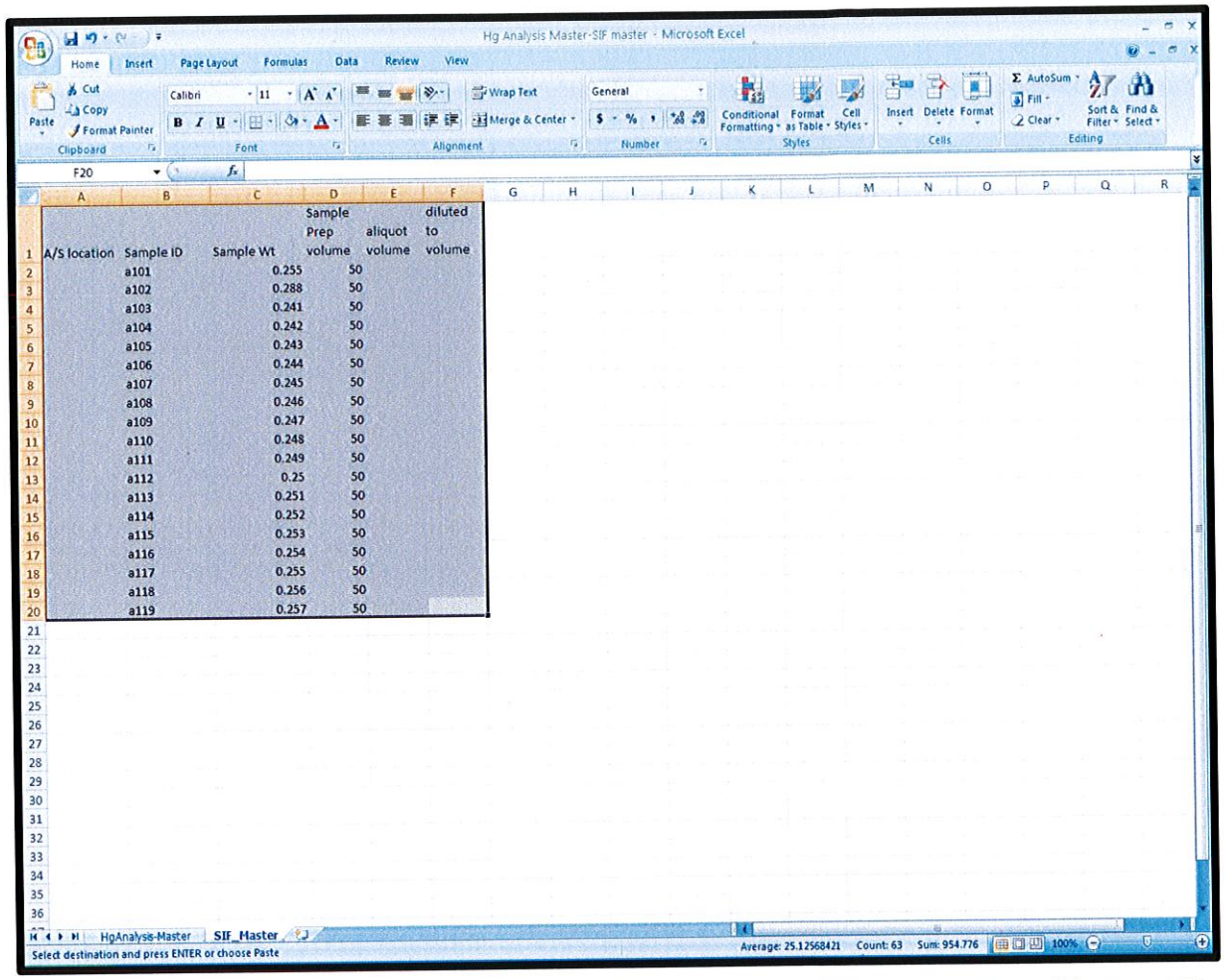

Figure 2. Preparation of a Sample Information File (SIF, in WinLab32™ for AA software) from an MS Excel file. Using MS Excel to create the SIF is ideal if a project MS Excel file has been previously prepared.

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).

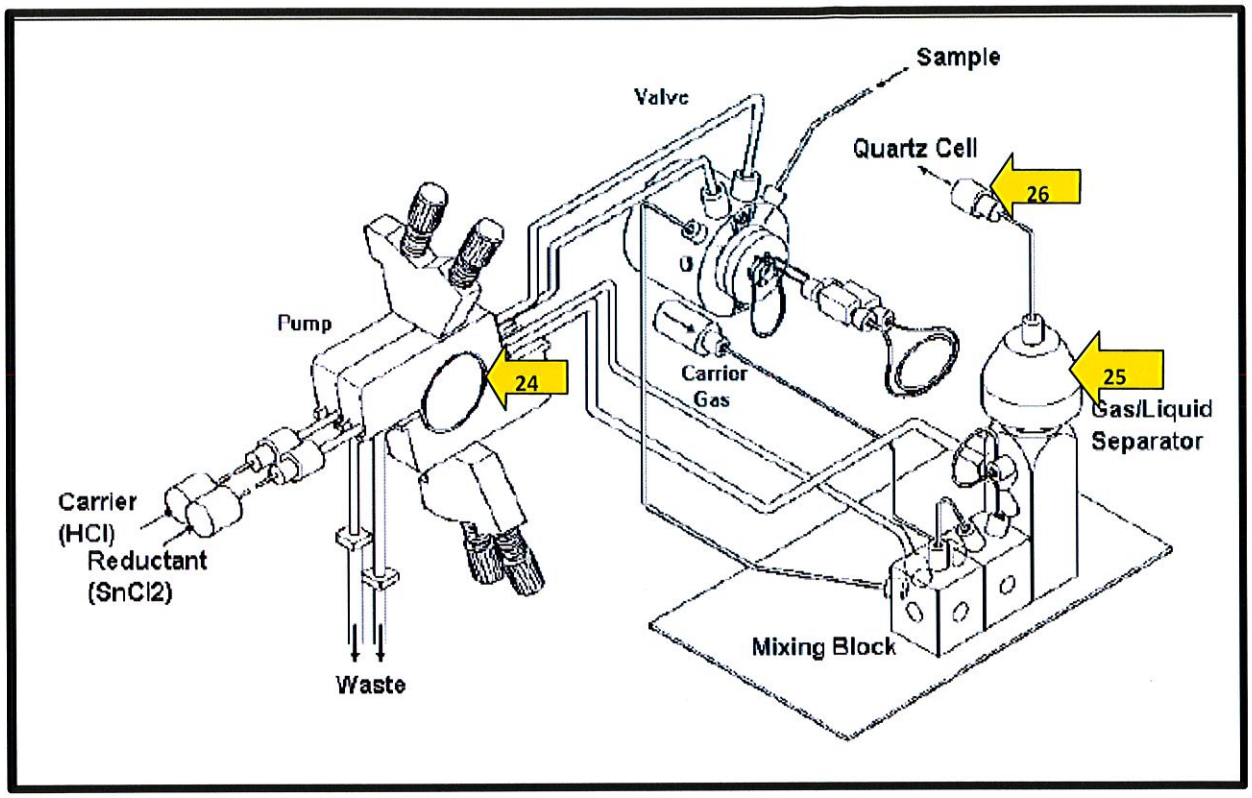

Figure 3. Diagram of the PerkinElmer FIMS-100. The yellow arrows indicate important areas of the instrument that need attention according to the referenced sections of this SOP.

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 project notebook and in the project MS Excel file. 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 presence of bubbles in the waste line must be verified each analysis day; the waste flow rate must be adjusted if no bubbles are observed in the waste line.

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 **0.12 and 0.17.**

    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 10 samples may be analyzed between standards so that the samples can be kept together and in order. For example, if the sample set contains 50 samples, including duplicates and spikes, the set should be run in the following order:

    - First set of standards
    - Certified Reference Material
    - ~10 samples
    - Blank
    - Lowest standard (100 ng/L)
    - ~10 samples
    - Certified Reference Material
    - 500 ng/L standard
    - ~10 samples
    - 1000 ng/L standard
    - ~10 samples
    - 5000 ng/L standard
    - ~10 samples
    - 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/50v.1
ROUNTINE MAINTENANCE FOR THE FIMS-100 MERCURY ANALYSIS SYSTEM

INTRODUCTION

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

REFERENCES

PerkinElmer Instruments. 1994. FIMS Flow Injection Mercury System- Installation, Maintenance and System Description Manual.

PerkinElmer Instruments. 1994. FIMS Flow Injection Mercury System – Setting Up and Performing Analyses Manual.

EQUIPMENT LIST

- FIMS-100
- Lab Coat
- Gloves
- Safety Glasses
- FIMS-100 Record Book
- PerkinElmer *FIMS-100 Installation, Maintenance and System Description Manual*
- PerkinElmer *FIMS Flow Injection Mercury System – Setting Up and Performing Analyses Manual*
- Spare Parts for FIMS-100
- 7 mm Wrench
- Hex/Allen Wrench
- Small Flathead Screwdriver
- Valve Dismantling Tool
- Silicone Vacuum Grease
- Silicone Spray Lubricant
- Kimwipes®
- Methanol
- Forceps
- 2- 50 mL Graduated Cylinders
- Deionized Water

PROCEDURE

General Preventative Maintenance

1. Wipe up spills immediately for safety reasons and to avoid contaminating new samples.

2. After each use, wipe over the instrument's 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 routine and non-routine maintenance performed in the FIMS-100 Record Book.

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

    a. Turn off the FIMS-100.
    b. Remove the filter cover and filter (Figure 1).
    c. Insert a new filter (Part Number B050-2706).
    d. Firmly press the filter cover back in place.
    e. Place a piece of tape on the filter cover stating the date the filter was replaced.
    f. Record in the FIMS-100 Record Book the date that the filter was replaced.

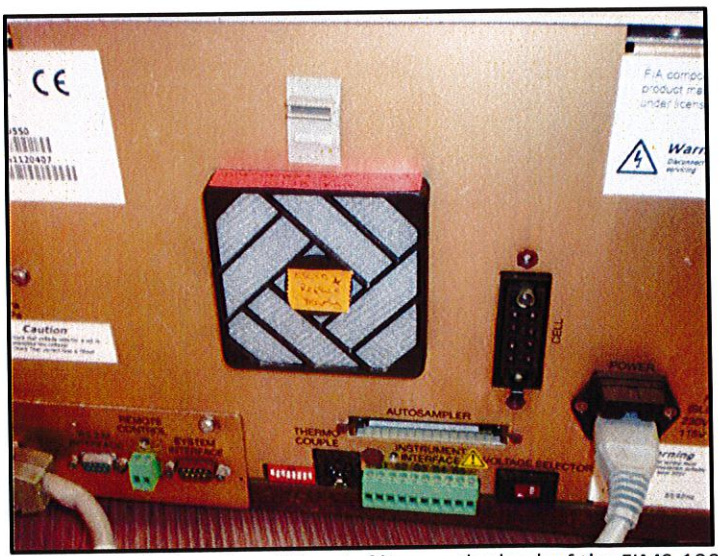

Figure 1. Photograph showing the location of the air filter on the back of the FIMS-100 Mercury Analyzer.

6. Install a new mercury absorber cartridge yearly, by following the method below:

    a. The mercury absorber cartridge is located at the end of a piece of silicon tubing that is attached to the left-hand nipple on the FIMS-cell (Figure 2).

b. Remove the old mercury absorber cartridge by pulling it off of the end of the silicon tube.
c. Push the new mercury filter on to the end of the silicon tube.
d. Place a piece of tape on the mercury filter stating the date the mercury filter was replaced.
e. Record in the FIMS-100 Record Book the date that the mercury filter was replaced.

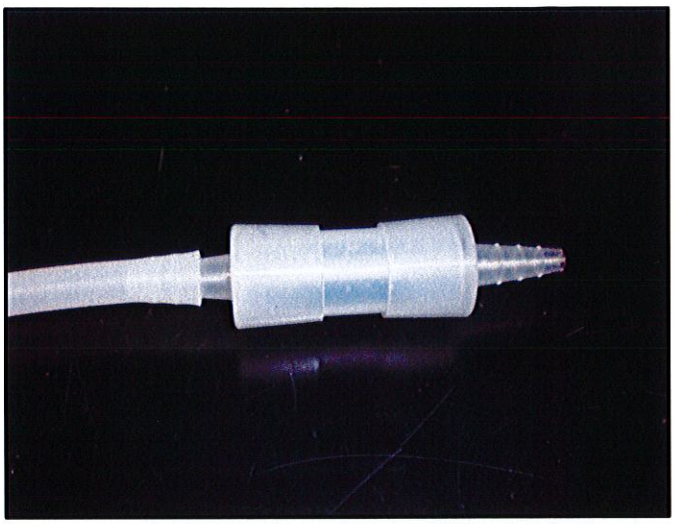

Figure 2. Photograph showing the mercury filter attached to the silicon tube.

7. To perform the remaining routine maintenance tasks in this SOP, turn on the computer. Press Ctrl+Alt+Del on the computer keyboard and enter "Barstow 9B" for the username and "fims100" for the password, while "Bars 9B-9061" shows in the LOG ONTO window.

8. Turn on the nitrogen gas (set pressure at 400 kPa) and the FIMS-100 Mercury Analysis System. Allow the system to warm up for a minimum of 10 minutes.

9. On the desktop, double click on the icon named "WinLab 32 for AA" to open the WinLab application.

Carrier Gas System Maintenance

10. Periodically check the non-return valve located under the FIAS (Flow Injection Atomic Spectroscopy) valve (Figure 3A and 3B). If the rubber sleeve shows signs of deterioration, fit a new one (see page 2-18 of the *Installation, Maintenance, and System Description Manual*). The rubber sleeve should just cover the holes on the valve and not extend beyond the valve tip as this may alter the gas flow.

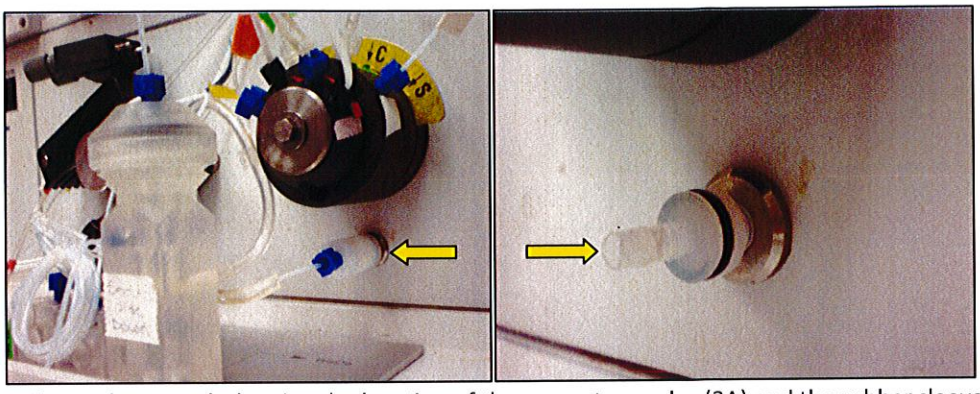

Figure 3A and 3B. Photograph showing the location of the non-return valve (3A) and the rubber sleeve covering the holes on the valve insert.

11. Carrier gas flow should read 40-70 mL/min on the Carrier Gas Flow Gauge while the FIAS pump is running (Figure 4). When the FIAS pump is not running, the carrier gas flow should rest between 70-100 mL/min. If the flow seems to fluctuate outside of this range it can be adjusted using the carrier gas flow regulator (refer to the *Installation, Maintenance and System Description Manual* page 1-25). If this does not work then the flow meter may need to be cleaned.

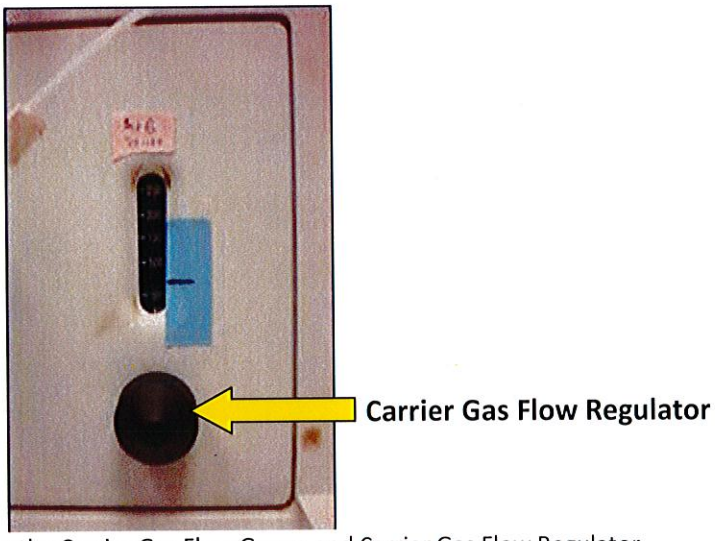

Figure 4. Photograph showing the Carrier Gas Flow Gauge and Carrier Gas Flow Regulator.

a. The flow meter can be cleaned by removing it from the FIMS-100 (this must be done while the instrument is off and unplugged) and soaking the flow tube and ball in methanol. These parts must then be allowed to dry. Silicone high vacuum grease must be applied to O-rings on each end of the flow tube to prevent leaks. The flow meter must then be reassembled and reinstalled in the FIMS-100.

Carrier and Reductant Solution Flows

12. Prior to analysis, the carrier and reductant solution flows should be checked and flows recorded in the FIMS-100 Record Book.

13. Adjust the carrier and reductant flows to produce a ratio of carrier flow to reductant flow of approximately 2:1 with a carrier flow between 9 and 11 mL/min and the reductant flow between 5 and 7 mL/min.

    a. Place the carrier tube inlet in the graduated cylinder labeled "carrier" and the reductant tube inlet in the graduated cylinder labeled "reductant." Bring the deionized water level in the graduated cylinders to the 50 mL mark. In the **FIAS Control Window**, click on the **FIAS On/Off** under the **Operate** tab to start the FIAS (Figure 5). After running the FIAS for one minute, note the decrease in volume. The flow should be 9-11 mL/minute for the carrier tube and 5-7 mL/minute for the reductant tube.

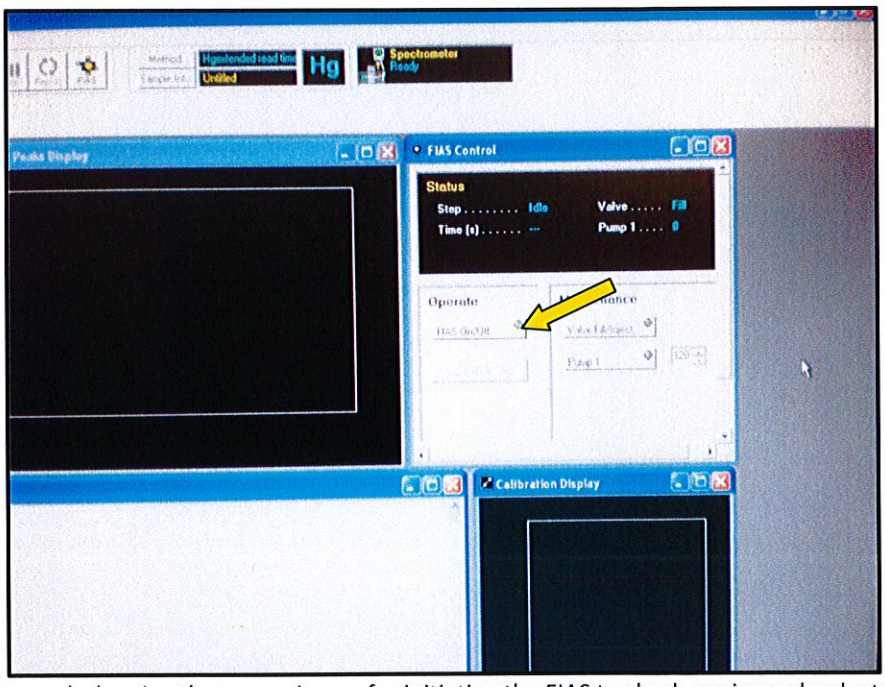

Figure 5. Photograph showing the screen image for initiating the FIAS to check carrier and reductant flow rates.

    b. If the flows are not within the acceptable range, adjust the pressure on the appropriate pump tube by turning the top knobs (clockwise to increase flow) on the pump magazine pressure adjustment levers until the flow is within the range (Figure 6).

    c. 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. The tube will need to be removed and flushed with deionized water or replaced.

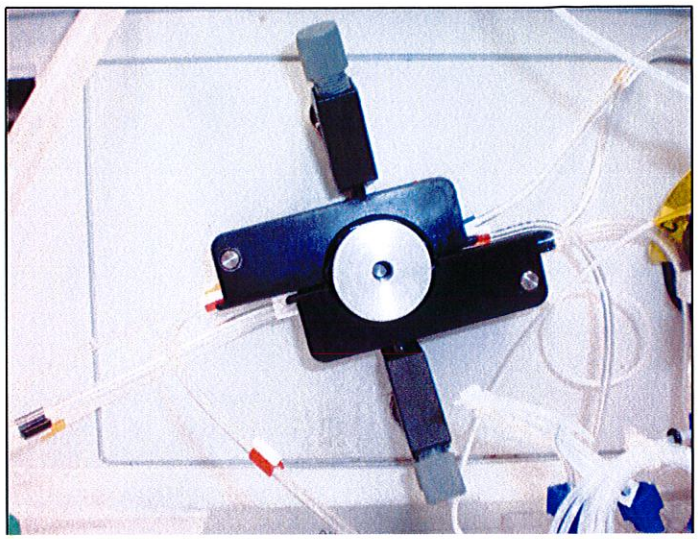

Figure 6. Photograph showing the location of the knobs which can be adjusted to change the flow rate of the pump.

14. During analysis, if the peak shape is abnormal, does not return to baseline, or the 5000 ng/L Hg standard gives an absorbance that is not between 0.12 and 0.17, the carrier and reductant flows should be checked again and flows recorded in the FIMS-100 Record Book.

Gas/Liquid Separator Maintenance

15. Prior to analysis, unscrew the gas/liquid separator cover and place a clean, dry polytetrafluoroethylene (Teflon, PTFE) membrane filter (smooth side down) on the separator block (Figures 7A and 7B), then replace the separator cover (refer to the *Installation, Maintenance and System Description Manual* page 1-22).

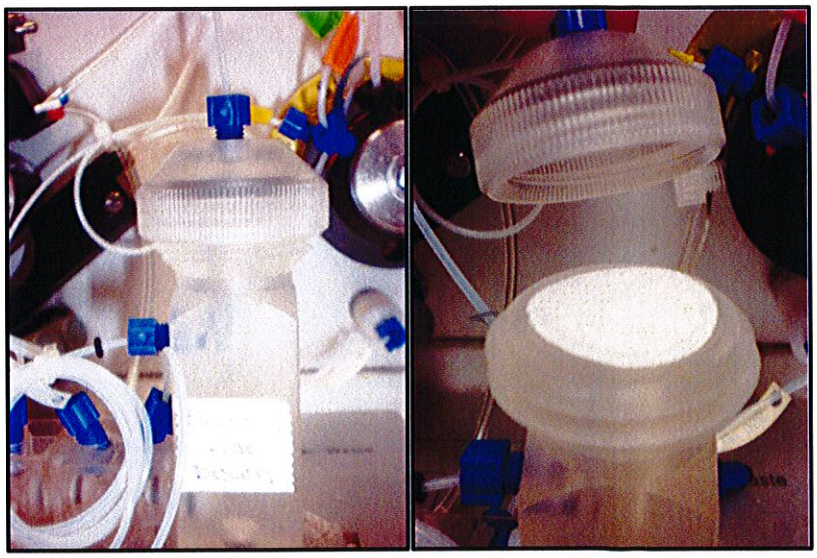

Figure 7A and 7B. Photograph showing the gas/liquid separator and the filter in placement in the gas/liquid separator.

a. During analysis, monitor the liquid bubbling in the separator block below the filter. If the bubbling is excessive and it appears that the bubbles are reaching the filter this will cause the filter to become saturated. The filter must remain clean and dry for successful analyses.
b. If the filter is saturated, remove the filter using a forceps and dry it with a Kimwipe®. Dry out the separator block and cover. Place a dry filter on the separator block and replace the cover.
c. If the separator cover becomes saturated, verify that the sample transfer tubing connecting the separator cover to the FIMS cell does not contain moisture.
d. If there is moisture in the tubing (part number: 198-097) replace the tubing and verify that the cell and cell windows are clean and dry.
e. If moisture is visible in the cell or on the cell windows refer to "FIMS-Cell Maintenance" section below for proper cleaning techniques.

Spectrophotometer Maintenance

16. Measure and record the absorbance of the FIMS-cell window in the FIMS-100 Record Book regularly.

a. In the WinLab32 application, open the Continuous Graphics window ('**Cont**' on toolbar).
b. Remove the FIMS-cell from the cell compartment.
c. Click on **Autozero** in the **Continuous Graphics** window (Figure 8).

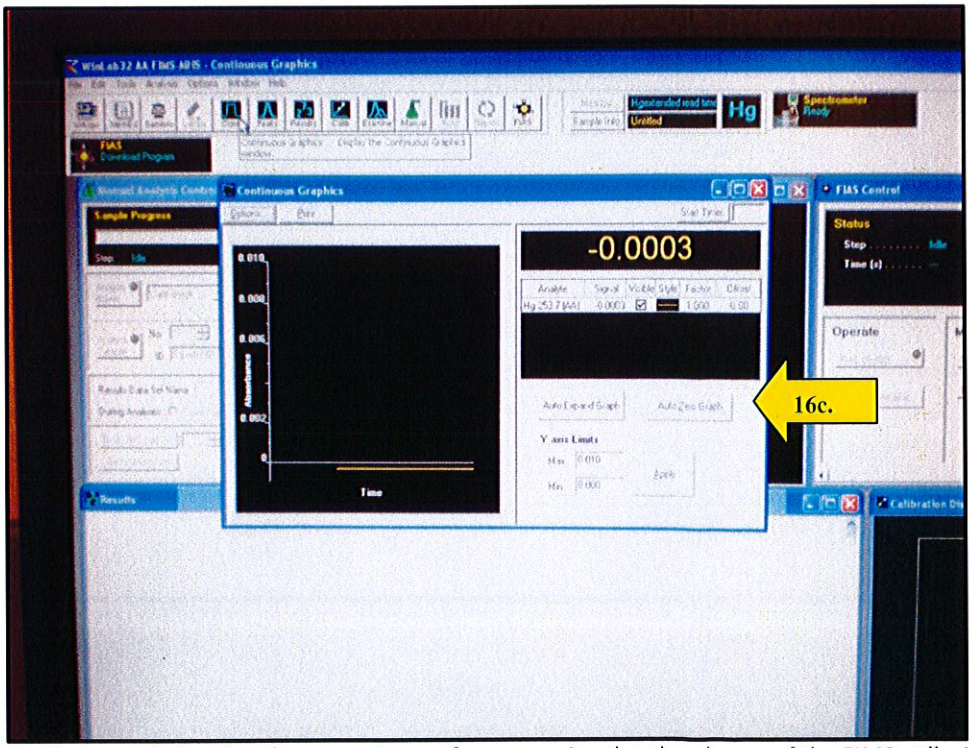

Figure 8. Photograph showing the screen image for measuring the absorbance of the FIMS-cell window.

d. Install the FIMS-cell back in the cell compartment.
e. The absorbance reading in the **Continuous Graphics** window is the absorbance of the FIMS-cell window. Clean windows should have an absorbance between 0.03 and 0.07. If the absorbance is greater than this, the windows should be cleaned. Refer to the Installation, Maintenance, and System Description manual page 2-10.

FIMS-Cell Maintenance

17. If there is a decrease in sensitivity (not attributable to factors such as unsuitable analytical parameters or instrument settings, or incorrectly prepared or contaminated solutions) or if moisture is visible in the cell or on the cell windows the cell and/or cell windows must be removed and cleaned (Figure 9).

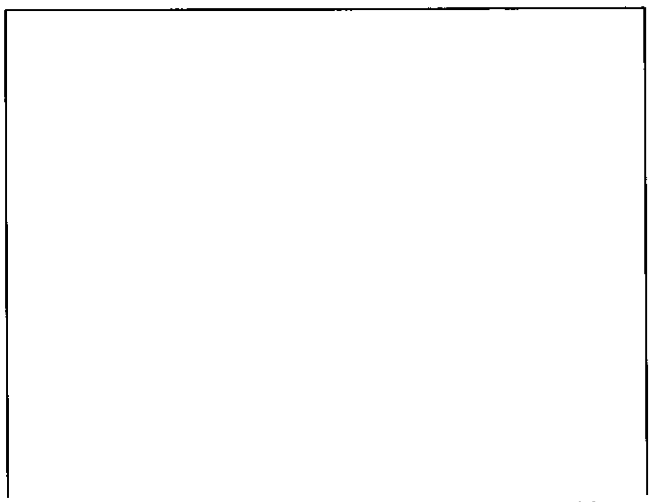

Figure 9. Photograph showing the FIMS-cell after it has been removed from the instrument.

a. Be sure that there is no analysis in progress, remove the FIMS-cell. Pull and simultaneously twist the cell window assemblies off the ends of the FIMS-cell.
b. Carefully use a small screwdriver to remove the outer O-ring that surrounds the window and carefully remove the cell window. Clean the windows with deionized water and dry with a Kimwipe® (refer to the *Installation, Maintenance and System Description Manual* page 2-9). If moisture has entered the cell, attempt to dry it with a Kimwipe®. If this is not feasible, the cell may need to air dry and analysis will temporarily be suspended.
c. After cleaning and drying the cell and/or cell window, carefully fit the cell windows and window assemblies to the FIMS-cell. Re-install the FIMS-cell in the Spectrometer (refer to the *Installation, Maintenance and System Description Manual* page 2-8 and 2-9).
d. Once the FIMS-cell is installed in the Spectrometer, measure the absorbance of the windows. If the absorbance in not within the acceptable range the cell/cell windows may need to be cleaned more thoroughly using a soft, lint-free cloth moistened with spectroscopic grade alcohol (see page 2-10 of *Installation, Maintenance and System Description Manual*). If the cleaning process still fails to produce an acceptable

absorbance the cell windows may need to be replaced.

Fluid System Maintenance

18. To reduce wear on pump tubes, spray a small amount of silicone lubricant on the part of the tube in contact with the pump rollers prior to analysis of samples and standards (Figure 6).

19. Following analysis, rinse the fluid system with deionized water. This is done by placing the sample, carrier, and reductant tubes in deionized water and running the FIAS. The fluid system should be rinsed twice while in deionized water and once while being held out of the water so that air is allowed to pass through the system. After the system is flushed with air, the sample, carrier and reductant tubes should be placed back into deionized water until the next use.

20. Release tension on the pump tubes when analysis and tube rinsing is completed.

21. Wipe pump rollers with a dry lint-free cloth.

22. Inspect all fluid tubes daily during periods of instrument usage for damage such as kinks, leaks or clogs. Install new tubes as necessary.

FIAS-Valve Maintenance

23. Observe the FIAS valve prior to and during analysis for any clogs that may be present. A clog may be indicated by uncharacteristic absorbance results.

    a. Pause the analysis if during analysis it is thought that there might be a clog in the valve or valve tubing. Disconnect all the tubes from the valve and pump deionized water through each channel of the valve and through all tubing that is connected to the valve.
    b. If this is not sufficient in removing the clog then remove the valve from the pump unit and dismantle the valve (refer to the *Installation, Maintenance and System Description Manual* pages 2-14 through 2-16; Figure 10). Once the valve is dismantled, clean each individual part and again pump water though the valve channels and components.

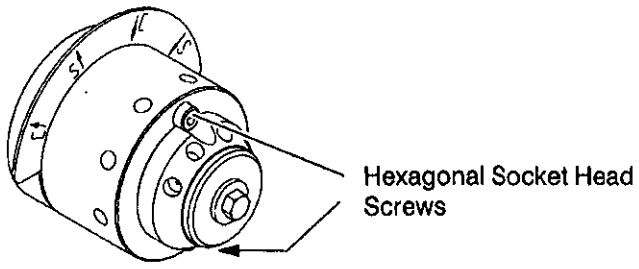

Figure 10. This series of diagrams shows how to remove the FIAS-valve from the motor mount, dismantle the FIAS- valve and separate the FIAS-valve components.

c. To reassemble the valve and reattach the valve to the pump unit refer to the *Installation, Maintenance and System Description Manual* pages 2-14 and 2-17. Apply silicone vacuum grease to the O-ring located in the valve rotor prior to reassembly to prevent leakage.

Standard Operating Procedure SA/51v.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:

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

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

APPENDIX 1

TISSUE MOISTURE DETERMINATION DATASHEET

Tissue Moisture Determination Datasheet (Laboratory Balance ID: _____)

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.