Total Mercury Concentrations in Muscle Tissue from Fish Captured during the Spring – Fall 2013 in Michigan Waters of Lake Superior

by

Christine N. Polkinghorne
Kimberly M. Beesley
Thomas P. Markee

Lake Superior Research Institute
University of Wisconsin-Superior
Superior, Wisconsin 54880

for

Great Lakes Indian Fish and Wildlife Commission
P.O. Box 9
Odanah, Wisconsin 54861

February 12, 2014

Introduction

Skinless fillet samples from burbot (*Lota lota*), cisco (*Coregonus artedi*), lake trout (*Salvelinus namaycush*) and whitefish (*Coregonus clupeaformis*) captured during the spring, summer or fall of 2013 from the Michigan waters of Lake Superior were analyzed for total mercury (Hg) content at the University of Wisconsin-Superior's Lake Superior Research Institute (LSRI). Fillets from 98 fish, collected from Lake Superior, were analyzed.

Methods

At the time fish were collected, a Tribal Fisheries Department or GLIFWC Biological Services staff member was present to identify species and measure the total length of each fish. Fish were tagged with a unique number (i.e. a fish identification number) and were frozen within 12 hours of capture. 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 along with a label containing the fish identification number. This fish processing followed SOPs developed by GLIFWC. Sex of the fish was determined during the filleting process. At the LSRI laboratories, the fish were received frozen and in good condition with chain-of-custody documentation. Samples were stored in a freezer at or below -10°C until they were removed and thawed for processing and analysis.

Before processing the fish tissues, all glassware, utensils, and grinders were cleaned according to the appropriate methods (LSRI SOP SA/8 v.7). Each day, the fish to be processed were removed from the freezer and allowed to warm to a flexible, but stiff, consistency. The skinless fillet was passed through a grinder three times. A small amount of the initial tissue that passed through the grinder was collected and discarded (LSRI SOP SA/10 v.6). Due to their small sample size, cisco fillets from Eagle River were processed using liquid nitrogen and following (LSRI SOP SA/38 v. 2). A sub-sample of the ground tissue was placed into a certified clean glass vial and frozen until mercury analysis was conducted. The grinding apparatus was disassembled after each fillet 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 and the sample homogenized using a metal spatula. The remaining tuna was ground in the same manner as the fish fillets. This check was made to ensure that no contamination or loss of mercury was occurring in the grinding process. Three procedural blanks were prepared during this project. The initial procedural blank was prepared on the first day fish were ground for the project and the last procedural blank was generated on the last day fish were processed. The third procedural blank was prepared on an intermediate date when fish were being ground. This intermediate tuna procedural blank was processed on the same day and in the same manner as the cisco from Eagle River (LSRI SOP SA/38 v. 2).

Fish tissues were weighed for mercury analysis following standard operating procedure SOP SA/11 v.6. Prior to the use of the balance for weighing samples, the accuracy of the balance was verified following LSRI SOP GLM/12 v.5. 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 0.21g (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 to 5 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 23 to 168 hours. After the initial drying and weighing, a few of 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 35 percent of the fish analyzed for mercury had moisture content determined.

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 percent of the spike. If a spike

recovery did not fall within the acceptable range, the sample was spiked and analyzed again during the next analysis set.

A quality assurance audit was conducted by the LSRI quality assurance manager during the Lake Superior 2014 project. That report is provided in Appendix C.

Results of Fish Tissue Analyses

Quality Assurance – Three tuna procedural blanks were processed coincident with the grinding of fish collected for the project. A minimum of one of the three procedural blanks was analyzed with each set of mercury samples, There were a total of four analyses resulting in a mean relative percent difference of 21.6 ± 22.7 (Table 1). The relative percent difference values ranged from 0.0 to 45.3%, all were within the acceptable range of < 50%. All of the tuna samples were found to have very low mercury concentrations.

Analysis of dogfish shark tissue DORM-4 was conducted concurrently with fish 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 76.3 to 93.6% with the grand mean and standard deviation of the recoveries being 85.5 ± 5.9 percent of the certified value. All of the DORM-4 reference sample daily mean values were within the acceptance range.

Fish tissues were analyzed for mercury in duplicate ten 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 1.0 to 18.2% with the average and standard deviation of the agreements being 7.1 ± 5.4% (Table 3).

Samples of tissue were spiked in duplicate with known concentrations of mercury prior to digestion. Mean recovery for the 10 spiked samples was 102.1 ± 2.5 percent with the individual average recovery values ranging from 97.2 to 107% (Table 4).

Mercury Analysis – Skinless fillets of 98 fish collected from a total of nine locations in Michigan waters of Lake Superior were analyzed for total mercury concentration. Total mercury concentrations on a wet weight basis (Table 5) ranged from 0.007^{Q} to 0.694 µg Hg/g (parts per million). Concentration differences existed between the species examined, with whitefish having the lowest mercury concentrations and lake trout having higher concentrations of mercury. Cisco and burbot had intermediate concentrations of mercury.

Tissue Moisture Analysis – Percent moisture was measured in 29 of the 98 fish tissues. Moisture analysis took place immediately following grinding of the fillets. The data obtained from drying and weighing the samples twice indicates that drying for 23 hours was sufficient to remove the moisture from the samples used for moisture determination.

Fish muscle tissue had an overall mean moisture value of 76.9 ± 3.4 percent (Table 6). The results for each species were as follows: burbot muscle tissue had a mean moisture value of 82.0 ± 1.4 percent, cisco muscle tissue had a mean moisture value of 76.4 ± 0.9 percent, lake trout muscle tissue had a mean moisture value of 76.7 ± 2.4 and whitefish muscle tissue had a mean moisture value of 73.2 ± 2.6 percent. Of the 29 tissues analyzed for moisture, five were analyzed

in duplicate, all yielding relative percent differences of 0.0 to 0.4 percent. Five 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
1/9/2014	12/23/2013	0.013	0.008	0.011	45.3
1/9/2014	12/30/2013	0.018	0.018	0.018	0.0
1/16/2014	12/23/2013	<0.007	<0.007	<0.007	NC
1/30/2014	1/14/2014	0.014	0.017	0.016	19.4
			Mean ± Std. Dev.		**21.6 ± 22.7**

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 measured value for the reference standard.

	DORM 4-1		DORM 4-2		DORM 4-3		
Date of Analysis	µg Hg/g	% of Certified Value	µg Hg/g	% of Certified Value	µg Hg/g	% of Certified Value	Mean
1/9/2014	0.347	84.7	0.344	83.8	0.344	84.0	84.2
1/16/2014	0.374	91.3	0.378	92.3	0.326	79.6	87.7
1/30/2014	0.343	83.6	0.384	93.6	0.313	76.3	84.5
					Mean ± Std. Dev.		**85.5 ± 5.9**

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

Date of Analysis	Lake Superior Sample Location and Tag Number	Species	µg Hg/g	Duplicate µg Hg/g	Mean µg Hg/g	Relative Percent Difference
1/9/2013	Menge Creek Outlet 33	Burbot	0.092	0.103	0.098	11.3
1/9/2013	Huron Bay 37	Burbot	0.121	0.115	0.118	5.1
1/9/2013	Copper Harbor 11738	Cisco	0.097	0.098	0.098	1.0

Date of Analysis	Lake Superior Sample Location and Tag Number	Species	µg Hg/g	Duplicate µg Hg/g	Mean µg Hg/g	Relative Percent Difference
1/16/2014	Huron Islands 6	Lake Trout	0.185	0.198	0.192	6.8
1/16/2014	Keweenaw Bay 17	Lake Trout	0.138	0.134	0.136	2.9
1/16/2014	Menge Creek Outlet 24	Lake Trout	0.045	0.049	0.047	8.5
1/30/2014	Eagle River 1188	Whitefish	0.036	0.032	0.034	11.8
1/30/2014	Eagle River 1196	Whitefish	0.106	0.108	0.107	1.9
1/30/2014	Black River Harbor 12722	Whitefish	0.028	0.029	0.029	3.5
1/30/2014	Black River Harbor 12733	Whitefish	0.024 [Q]	0.020 [Q]	0.022 [Q]	18.2
				Mean ± Std. Dev.		**7.1 ± 5.4**

[Q] indicates that the measured value is between the level of detection and the level of quantification

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

Date of Analysis	Lake Superior Sample Location and Tag Number	Species	Spike #1	Spike #2	Mean Spike Recovery	Std. Dev.
1/9/2013	Menge Creek Outlet 33	Burbot	104.6	104.1	104.4	0.4
1/9/2013	Huron Bay 37	Burbot	102.1	100.4	101.3	1.2
1/9/2013	Copper Harbor 11738	Cisco	104.7	107.0	105.9	1.6
1/16/2014	Huron Islands 6	Lake Trout	102.8	98.5	100.7	3.0
1/16/2014	Keweenaw Bay 17	Lake Trout	101.0	104.0	102.5	2.1
1/16/2014	Menge Creek Outlet 24	Lake Trout	101.6	100.0	100.8	1.1
1/30/2014	Eagle River 1188	Whitefish	101.7	103.3	102.5	1.1
1/30/2014	Eagle River 1196	Whitefish	104.8	102.4	103.6	1.7
1/30/2014	Black River Harbor 12722	Whitefish	98.0	102.4	100.2	3.1
1/30/2014	Black River Harbor 12733	Whitefish	97.2	101.6	99.4	3.1
		Mean ± Std. Dev.		**102.1 ± 2.5**		

Table 5. Total Mercury Concentration (Wet Weight) in Fillets from Fish Captured in Lake Superior during the Spring-Fall of 2013.

Analysis Date	Lake Superior Sample Location	Species	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
1/9/2014	Huron Islands (MI-4)	Burbot	8	Marquette	28.1	M	0.275
1/9/2014	Huron Islands (MI-4)	Burbot	9	Marquette	24.0	F	0.181
1/9/2014	Menge Creek Outlet (MI-4)	Burbot	28	Baraga	21.2	M	0.051
1/9/2014	Menge Creek Outlet (MI-4)	Burbot	29	Baraga	23.5	M	0.169
1/9/2014	Menge Creek Outlet (MI-4)	Burbot	31	Baraga	23.5	F	0.219
1/9/2014	Menge Creek Outlet (MI-4)	Burbot	33	Baraga	22.5	M	0.098
1/9/2014	Menge Creek Outlet (MI-4)	Burbot	34	Baraga	21.6	M	0.155
1/9/2014	Huron Bay (MI-4)	Burbot	26	Baraga	23.1	F	0.157
1/9/2014	Huron Bay (MI-4)	Burbot	27	Baraga	22.8	F	0.130
1/9/2014	Huron Bay (MI-4)	Burbot	30	Baraga	21.7	F	0.132
1/9/2014	Huron Bay (MI-4)	Burbot	32	Baraga	21.1	M	0.087
1/9/2014	Huron Bay (MI-4)	Burbot	35	Baraga	22.4	F	0.110
1/9/2014	Huron Bay (MI-4)	Burbot	36	Baraga	25.4	M	0.094
1/9/2014	Huron Bay (MI-4)	Burbot	37	Baraga	22.5	M	0.118
1/9/2014	Copper Harbor (MI-3)	Cisco	11728	Keweenaw	17.4	M	0.092
1/9/2014	Copper Harbor (MI-3)	Cisco	11730	Keweenaw	17.3	M	0.079
1/9/2014	Copper Harbor (MI-3)	Cisco	11731	Keweenaw	17.1	F	0.092
1/9/2014	Copper Harbor (MI-3)	Cisco	11732	Keweenaw	17.0	M	0.141
1/9/2014	Copper Harbor (MI-3)	Cisco	11733	Keweenaw	17.3	M	0.089
1/9/2014	Copper Harbor (MI-3)	Cisco	11734	Keweenaw	19.8	F	0.076

Analysis Date	Lake Superior Sample Location	Species	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
1/9/2014	Copper Harbor (MI-3)	Cisco	11735	Keweenaw	18.1	F	0.090
1/9/2014	Copper Harbor (MI-3)	Cisco	11736	Keweenaw	18.4	F	0.117
1/9/2014	Copper Harbor (MI-3)	Cisco	11738	Keweenaw	18.4	F	0.098
1/9/2014	Copper Harbor (MI-3)	Cisco	11739	Keweenaw	20.4	F	0.152
1/9/2014	Copper Harbor (MI-3)	Cisco	11740	Keweenaw	18.0	F	0.069
1/9/2014	Copper Harbor (MI-3)	Cisco	11742	Keweenaw	16.7	M	0.116
1/9/2014	Eagle River (MI-3)	Cisco	1192	Keweenaw	10.1	M	0.173
1/9/2014	Eagle River (MI-3)	Cisco	11729	Keweenaw	11.7	M	0.048
1/9/2014	Eagle River (MI-3)	Cisco	11737	Keweenaw	13.5	M	0.074
1/9/2014	Eagle River (MI-3)	Cisco	11741	Keweenaw	12.4	M	0.060
1/9/2014	Eagle River Shoal (MI-3)	Cisco	6878	Keweenaw	19.3	M	0.122
1/16/2014	Huron Islands (MI-4)	Lake Trout	1	Marquette	29.3	F	0.388
1/16/2014	Huron Islands (MI-4)	Lake Trout	2	Marquette	26.1	F	0.243
1/16/2014	Huron Islands (MI-4)	Lake Trout	3	Marquette	24.9	M	0.215
1/16/2014	Huron Islands (MI-4)	Lake Trout	4	Marquette	21.6	M	0.137
1/16/2014	Huron Islands (MI-4)	Lake Trout	5	Marquette	29.4	F	0.321
1/16/2014	Huron Islands (MI-4)	Lake Trout	6	Marquette	22.9	M	0.192
1/16/2014	Huron Islands (MI-4)	Lake Trout	7	Marquette	31.5	F	0.318
1/16/2014	Huron Islands (MI-4)	Lake Trout	10	Marquette	28.9	M	0.122
1/16/2014	Huron Islands (MI-4)	Lake Trout	11	Marquette	29.3	M	0.298
1/16/2014	Huron Islands (MI-4)	Lake Trout	14	Marquette	22.8	M	0.144

Analysis Date	Lake Superior Sample Location	Species	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
1/16/2014	Huron Islands (MI-4)	Lake Trout	15	Marquette	26.0	M	0.212
1/16/2014	Keweenaw Bay (MI-4)	Lake Trout	13	Baraga	21.1	M	0.072
1/16/2014	Keweenaw Bay (MI-4)	Lake Trout	16	Baraga	19.1	M	0.092
1/16/2014	Keweenaw Bay (MI-4)	Lake Trout	17	Baraga	20.6	M	0.136
1/16/2014	Keweenaw Bay (MI-4)	Lake Trout	20	Baraga	20.9	M	0.067
1/16/2014	Keweenaw Bay (MI-4)	Lake Trout	21	Baraga	24.9	M	0.111
1/16/2014	Keweenaw Bay (MI-4)	Lake Trout	23	Baraga	22.9	M	0.109
1/16/2014	Keweenaw Bay (MI-4)	Lake Trout	25	Baraga	24.3	F	0.132
1/16/2014	Menge Creek Outlet (MI-4)	Lake Trout	12	Baraga	20.1	M	0.097
1/16/2014	Menge Creek Outlet (MI-4)	Lake Trout	18	Baraga	17.3	M	0.062
1/16/2014	Menge Creek Outlet (MI-4)	Lake Trout	19	Baraga	21.9	M	0.097
1/16/2014	Menge Creek Outlet (MI-4)	Lake Trout	22	Baraga	25.1	F	0.030
1/16/2014	Menge Creek Outlet (MI-4)	Lake Trout	24	Baraga	24.5	F	0.047
1/16/2014	Copper Harbor (MI-3)	Lake Trout	6871	Keweenaw	24.7	M	0.162
1/16/2014	Copper Harbor (MI-3)	Lake Trout	12738	Keweenaw	26.7	M	0.244
1/16/2014	Copper Harbor (MI-3)	Lake Trout	12739	Keweenaw	35.3	M	0.694
1/16/2014	Copper Harbor (MI-3)	Lake Trout	12740	Keweenaw	22.8	M	0.187
1/16/2014	Copper Harbor (MI-3)	Lake Trout	12741	Keweenaw	22.2	M	0.107
1/16/2014	Copper Harbor (MI-3)	Lake Trout	12742	Keweenaw	34.6	M	0.568
1/16/2014	Copper Harbor (MI-3)	Lake Trout	12747	Keweenaw	29.3	F	0.287
1/16/2014	Copper Harbor (MI-3)	Lake Trout	12748	Keweenaw	22.8	M	0.083

Analysis Date	Lake Superior Sample Location	Species	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
1/16/2014	Copper Harbor (MI-3)	Lake Trout	12750	Keweenaw	22.8	M	0.069
1/30/2014	Money Bay (MI-3)	Lake Trout	12737	Keweenaw	29.5	M	0.469
1/30/2014	Eagle River Shoal (MI-3)	Lake Trout	12743	Keweenaw	25.2	M	0.161
1/30/2014	Eagle River Shoal (MI-3)	Lake Trout	12745	Keweenaw	22.7	M	0.130
1/30/2014	Eagle River (MI-3)	Whitefish	1137	Keweenaw	20.1	M	0.068
1/30/2014	Eagle River (MI-3)	Whitefish	1186	Keweenaw	22.5	F	0.055
1/30/2014	Eagle River (MI-3)	Whitefish	1188	Keweenaw	18.0	M	0.034
1/30/2014	Eagle River (MI-3)	Whitefish	1189	Keweenaw	19.8	F	0.047
1/30/2014	Eagle River (MI-3)	Whitefish	1190	Keweenaw	19.8	M	0.057
1/30/2014	Eagle River (MI-3)	Whitefish	1191	Keweenaw	19.3	M	0.041
1/30/2014	Eagle River (MI-3)	Whitefish	1193	Keweenaw	18.5	F	0.027
1/30/2014	Eagle River (MI-3)	Whitefish	1194	Keweenaw	19.1	M	0.047
1/30/2014	Eagle River (MI-3)	Whitefish	1195	Keweenaw	20.5	M	0.075
1/30/2014	Eagle River (MI-3)	Whitefish	1196	Keweenaw	20.9	M	0.107
1/30/2014	Eagle River (MI-3)	Whitefish	1197	Keweenaw	17.1	F	0.030
1/30/2014	Eagle River (MI-3)	Whitefish	1198	Keweenaw	20.8	M	0.045
1/30/2014	Eagle River (MI-3)	Whitefish	1199	Keweenaw	21.4	M	0.047
1/30/2014	Eagle River (MI-3)	Whitefish	1200	Keweenaw	21.2	M	0.063
1/30/2014	Eagle River (MI-3)	Whitefish	6876	Keweenaw	20.4	F	0.096
1/30/2014	Eagle River (MI-3)	Whitefish	12734	Keweenaw	21.3	F	0.095
1/30/2014	Black River Harbor (MI-2)	Whitefish	12720	Gogebic	23.5	U	0.019^{Q}

Analysis Date	Lake Superior Sample Location	Species	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g
1/30/2014	Black River Harbor (MI-2)	Whitefish	12721	Gogebic	20.4	U	0.016 [Q]
1/30/2014	Black River Harbor (MI-2)	Whitefish	12722	Gogebic	20.9	U	0.029
1/30/2014	Black River Harbor (MI-2)	Whitefish	12723	Gogebic	18.6	U	0.014 [Q]
1/30/2014	Black River Harbor (MI-2)	Whitefish	12724	Gogebic	21.2	U	0.016 [Q]
1/30/2014	Black River Harbor (MI-2)	Whitefish	12725	Gogebic	20.6	U	0.025
1/30/2014	Black River Harbor (MI-2)	Whitefish	12726	Gogebic	23.9	U	0.033
1/30/2014	Black River Harbor (MI-2)	Whitefish	12727	Gogebic	18.6	U	0.010 [Q]
1/30/2014	Black River Harbor (MI-2)	Whitefish	12728	Gogebic	19.0	U	0.014 [Q]
1/30/2014	Black River Harbor (MI-2)	Whitefish	12729	Gogebic	20.1	U	0.015 [Q]
1/30/2014	Black River Harbor (MI-2)	Whitefish	12730	Gogebic	17.6	U	0.018 [Q]
1/30/2014	Black River Harbor (MI-2)	Whitefish	12731	Gogebic	27.6	U	0.029
1/30/2014	Black River Harbor (MI-2)	Whitefish	12732	Gogebic	17.9	U	0.020 [Q]
1/30/2014	Black River Harbor (MI-2)	Whitefish	12733	Gogebic	23.2	U	0.022 [Q]
1/30/2014	Black River Harbor (MI-2)	Whitefish	12735	Gogebic	18.1	U	0.007 [Q]
1/30/2014	Black River Harbor (MI-2)	Whitefish	12736	Gogebic	18.6	U	0.013 [Q]

[Q] indicates that the measured value is between the level of detection and the level of quantification

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

Date	Lake Superior Sample Location	Species	Tag Number		Percent Moisture	Relative Percent Difference
12/23/2013	Huron Islands	Burbot	8		83.3	
12/23/2013	Menge Creek Outlet	Burbot	29		83.1	
12/23/2013	Menge Creek Outlet	Burbot	34		83.5	
12/23/2013	Huron Bay	Burbot	35		80.6	
12/23/2013	Huron Bay	Burbot	35	DUP	80.7	0.0

Date	Lake Superior Sample Location	Species	Tag Number		Percent Moisture	Relative Percent Difference
12/23/2013	Huron Bay	Burbot	26		81.1	
12/30/2013	Copper Harbor	Cisco	11730		75.1	
12/30/2013	Copper Harbor	Cisco	11742		77.1	
12/30/2013	Copper Harbor	Cisco	11742	DUP	77.2	0.0
12/30/2013	Copper Harbor	Cisco	11734		75.6	
12/30/2013	Eagle River	Cisco	11729		77.2	
12/30/2013	Eagle River Shoal	Cisco	6878		76.1	
12/30/2013	Eagle River Shoal	Lake Trout	12743		75.1	
12/30/2013	Money Bay	Lake Trout	12737		74.5	
1/2/2014	Huron Islands	Lake Trout	4		78.2	
1/2/2014	Huron Islands	Lake Trout	14		80.2	
1/2/2014	Huron Islands	Lake Trout	14	DUP	80.3	0.1
1/2/2014	Huron Islands	Lake Trout	15		74.5	
1/2/2014	Menge Creek Outlet	Lake Trout	12		75.0	
1/2/2014	Menge Creek Outlet	Lake Trout	22		80.8	
1/3/2014	Copper Harbor	Lake Trout	6871		74.9	
1/3/2014	Copper Harbor	Lake Trout	12748		76.7	
1/3/2014	Copper Harbor	Lake Trout	12748	DUP	77.1	0.4
1/3/2014	Copper Harbor	Lake Trout	12742		75.7	
1/3/2014	Keweenaw Bay	Lake Trout	23		73.1	
1/3/2014	Keweenaw Bay	Lake Trout	13		76.8	
1/3/2014	Keweenaw Bay	Lake Trout	16		77.6	
1/13/2014	Eagle River	Whitefish	1197		75.4	
1/13/2014	Eagle River	Whitefish	1197	DUP	75.4	0.0
1/13/2014	Eagle River	Whitefish	1190		75.1	
1/13/2014	Eagle River	Whitefish	1191		72.8	
1/14/2014	Black River Harbor	Whitefish	12722		70.3	
1/15/2014	Black River Harbor	Whitefish	12727		69.0	
1/16/2014	Black River Harbor	Whitefish	12732		74.2	
			Mean and Std. Dev.		**76.9 ± 3.4**	

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= .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 2014 Lake Superior Burbot, Cisco, Lake Trout, and Whitefish fillets. Indicators for Calibration Curves include a Slope of 2.0-3.0x10^{-5}and a Coefficient of Determination of >0.995.

Analysis Date	Standard Conc. ng Hg/L	Blank Corrected Abs. 1	Blank Corrected Abs. 2	Blank Corrected Mean	Standard Deviation	Slope	Y-Intercept	Corr.
1/9/2014	0	0.0001	0.0001	0.0000	0.0000	2.8092E-05	0.001769	0.9996
1/9/2014	100	0.0034	0.0034	0.0034	0.0000			
1/9/2014	500	0.0154	0.0155	0.0155	0.0001			
1/9/2014	1000	0.0309	0.0296	0.0303	0.0009			
1/9/2014	5000	0.1487	0.1475	0.1481	0.0008			
1/9/2014	10,000	0.2834	0.2761	0.2798	0.0052			
1/16/2014	0	0.0003	0.0003	0.0000	0.0000	2.8327E-05	0.002588	0.9996
1/16/2014	100	0.0031	0.0036	0.0034	0.0004			
1/16/2014	500	0.0155	0.0161	0.0158	0.0004			
1/16/2014	1000	0.0343	0.0352	0.0348	0.0006			
1/16/2014	5000	0.1473	0.1495	0.1484	0.0016			
1/16/2014	10,000	0.2861	0.2808	0.2835	0.0037			
1/30/2014	0	0.0001	0.0002	0.0000	0.0001	2.8241E-05	0.003066	0.9991
1/30/2014	100	0.0033	0.0036	0.0035	0.0002			
1/30/2014	500	0.0151	0.0160	0.0156	0.0006			
1/30/2014	1000	0.0337	0.0358	0.0348	0.0015			
1/30/2014	5000	0.1509	0.1534	0.1522	0.0018			
1/30/2014	10,000	0.2854	0.2772	0.2813	0.0058			

Appendix C

Quality Assurance Audit Report: Technical Systems Audit of Great Lakes Indian Fish and Wildlife Commission (GLIFWC) Mercury Testing Project – 2013 Lake Superior 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: 13 – 16 January 2014
Closing Discussions with LSRI-GLIFWC Staff: 24 February – 25 February 2014

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 13 – 16 January 2014. 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 2013 Lake Superior fish 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 14 January 2014, and the digestion and mercury analysis procedural audit was conducted 15 – 16 January 2014. The sample grinding 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.*2 (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 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 23 February 2014 and staff members commented on the TSA findings during an informal comment period (via e-mail) on 24 February – 25 February 2015.
The GLIFWC Project Manager at LSRI is Christine Polkinghorne, and Kimberly Beesley is a project staff member. Cole Holstrom is an undergraduate research assistant on the project.
Audit findings in this report are classified as follows (according to the ISO 9001 model):

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

Testing QAPP, or that show significant improvement from prior audits. Do not require corrective action.

- **Opportunity for Improvement:** Areas identified that can improve process or data quality through implementation of changes.

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 (13-12-02_GLIFWC). The current revisions of LSRI SOPs for each procedure being conducted were found in the laboratory during each procedural audit.
- The Chain of Custody (COC) form for the 2013 Lake Superior 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 -20.6°C (-22.9°C to -17.9°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.
- Several project SOPs have exceeded or are approaching the two-year review date. These SOPs should be reviewed by project personnel at LSRI and updated as needed:
    - LSRI/SOP/SA/10 – Sample Grinding for Metals Analysis (*v*.6 final 03 February 2012)
    - LSRI/SOP/SA/11 – Sample Weighing for Metals Analysis (*v*.6 final 22 February 2012)
    - LSRI/SOP/SA/42 – Stock, Standard, and Spike Preparation for Mercury Analysis (*v*.2 final 03 April 2012)

Opportunity for Improvement

- The project datasheet "Mercury Master Daily Analysis Form" does not have a title or version on the datasheet itself. This datasheet is an exact copy of the MS Excel file into which the data are entered, but the hard copy of the datasheet needs additional traceability.

Conclusions from Quality System Documentation Audit

Overall project documentation using laboratory notebook 12-09-06_GLIFWC and the GLIFWC Mercury Testing Project Binder (13-12-02_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. Three project SOPs are nearing the two-year review date, and should be reviewed by project personnel as soon as possible. The project datasheet "Mercury Master Daily Analysis Form" should have a title and version (number, date, or both) associated with it.

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

- No observations to report.

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.
- The LSRI QAM has current resumes on file for all LSRI-GLIFWC Project staff and students.

Opportunity for Improvement

- No improvement in training and safety needed.

Conclusions from Training and Safety Audit

Resumes are on file for LSRI staff and students working on the GLIFWC Mercury Testing Project. LSRI-GLIFWC Project personnel have read all relevant SOPs and received hands-on training on these SOPs. All LSRI-GLIFWC staff and students have completed the LSRI Quality System Orientation and the UWS Laboratory Health and Safety Training course. 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

- LSRITemp Drive (i.e., LSRI's local area network) is not always accessible on the FIMS-100 computer due to an issue with the Windows XP operating system on the FIMS-100 computer connecting to the LSRI server, which has a Windows 7 operating system. Data are saved to a flash drive instead of to the network, which is backed up every 24 hours.
    - *Follow-up (12 February 2014): This issue will be resolved prior to subsequent sample analysis for this project. Windows 7 compatible software will be purchased (a new FIMS has already been purchased), and the current FIMS-100 computer will have the Windows 7 operating system installed on it.*

Praises/Noteworthy Efforts

- The adjustable-volume pipettes used during sample grinding, digestion, and analysis of the 2013 Lake Superior samples were verified for accuracy (and calibrated, if needed) prior to project activities. Pipette verification records are stored in the project three-ring binder.
- 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 15 January 2014, the mercury standard and spike preparation procedure was in compliance with LSRI SOP SA/42 *v.*2 – *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: 127668; exp: 01/2015) by Kimberly Beesley on 17 December 2013, which was prior to the one month expiration required by LSRI SOP SA/42 *v.*2.
    - The 500 μg/L Hg Sub-Stock was prepared by Kimberly Beesley on 15 January 2014, which was prior to the one week expiration required by LSRI SOP SA/42 *v.*2.
- 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 11 mL/min; reductant flow rate was 6 mL/min).

Opportunity for Improvement

- The spatulas used to weigh ground tissue samples for digestion were cleaned according to LSRI SOP SA/11, *v.*6, except that immediately after weighing the spatula was not wiped clean with a KimWipe™. This step may not be very necessary, as it appears little tissue remains on the spatula after weighing. Does the SOP need to be revised to remove this step?
    - *Follow-up: LSRI SOP SA/11,* v.*6 will be revised to remove the requirement of wiping the spatula with a KimWipe™ after weighing.*
- During analysis, the first set of standards that was analyzed had a calibration curve with a slope that was outside of the acceptance range. This issue seemed to be solved by turning the instruments on in the order specified in LSRI SOP SA/49, *v.*2, and then opening the FIMS-100 software *after* the instrument was warmed up for 15-minutes. Should LSRI SOP SA/49, *v.*2 be revised to specify that the FIMS-100 software not be turned on until the instrument has been warmed up?
    - *Follow-up: LSRI SOP SA/49,* v.*2 will be revised to specify that the software be turned on after the instrument warm up time.*

- The beaker of deionized water that the sample aspiration tube and the lines from reductant and carrier solutions were placed into at the end of analysis looked cloudy. Should this beaker be changed with some frequency during/after analysis each day?
    - *Follow-up: The beaker with DI water for rinsing is etched from the stannous chloride, so the water always appears cloudy for this reason. The deionized water in the beaker is changed at the beginning of each analysis day. LSRI SOP SA/49,* v.*2 will be revised to include this step.*
- At the end of analysis, the FIMS-100 tubing was flushed once, rather than twice (as specified in LSRI SOP SA/49, *v.*2).

Conclusions from Equipment and Analytical Instrumentation Audit

The equipment/analytical instrumentation used in the sample processing and analysis of the 2013 Lake Superior 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 2013-2014 LOD and LOQ were determined prior to project sample analysis. Project staff may consider revising LSRI SOP SA/11, *v.*6 to remove the step that requires wiping the spatula used to weigh ground tissue immediately after weighing. In addition, project staff may consider revising LSRI SOP SA/49, *v.*2 to specify that the computer be turned on before the FIMS-100, since this seems to have made a difference in the outcome of previous analyses. Finally, LSRI SOP SA/49, *v.*2 may need to be revised in order to specify some frequency of refreshing the deionized water (rinse) beaker.

Supplemental Data

I. Completed Technical Systems Audit Checklist for GLIFWC Mercury Testing Project (Lake Superior Samples)

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

Results from GLIFWC Mercury Testing Project Sample Digestion and Analysis Procedural Audit, Revision 1

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.

[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 hot water containing Liquinox® detergent.

16. Rinse the labware with hot 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.

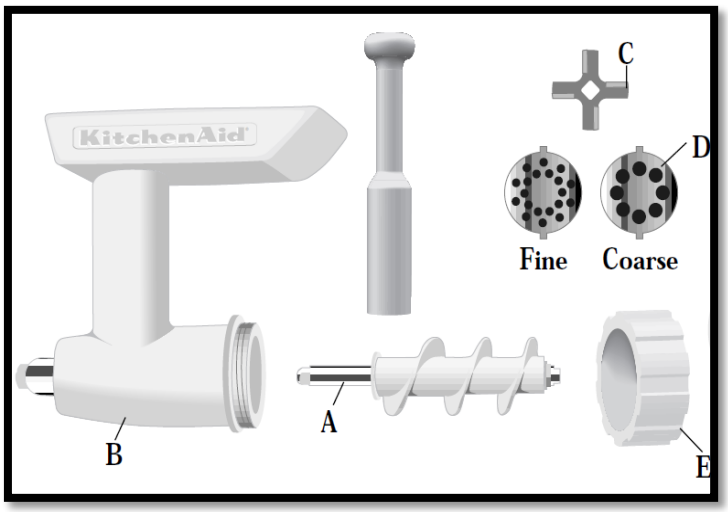

Figure 1. Assembly of KitchenAid™ Stand Mixer Food Grinder Attachment.

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.

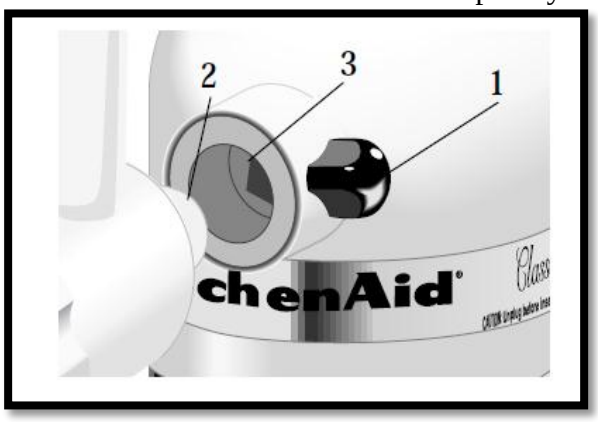

Figure 2. Connection of the Assembled Food Grinder Attachment to the KitchenAid™ Stand Mixer.

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!
SAMPLE			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE			#DIV/0!	#DIV/0!		#DIV/0!
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SAMPLE			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE dup			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE spk 1			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE spk 2			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE			#DIV/0!	#DIV/0!		#DIV/0!
SAMPLE			#DIV/0!	#DIV/0!		#DIV/0!
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!

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 < -10ºC. 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 ºC 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) $KMnO_4$

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.

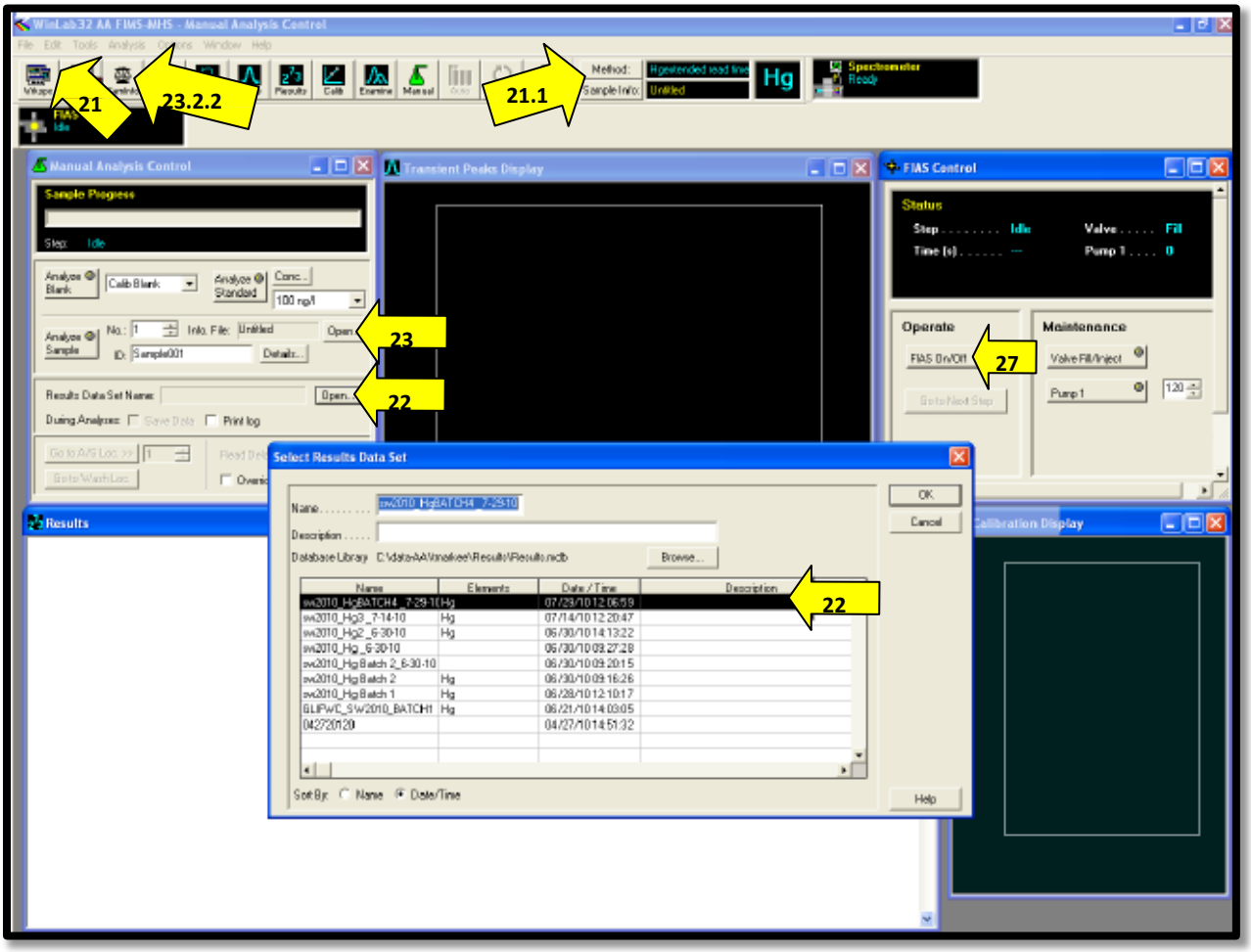


Figure 1. Screen shot of the control window of the WinLab32™ for AA software. The yellow arrows indicate areas of importance and the corresponding steps referenced within this SOP.

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):

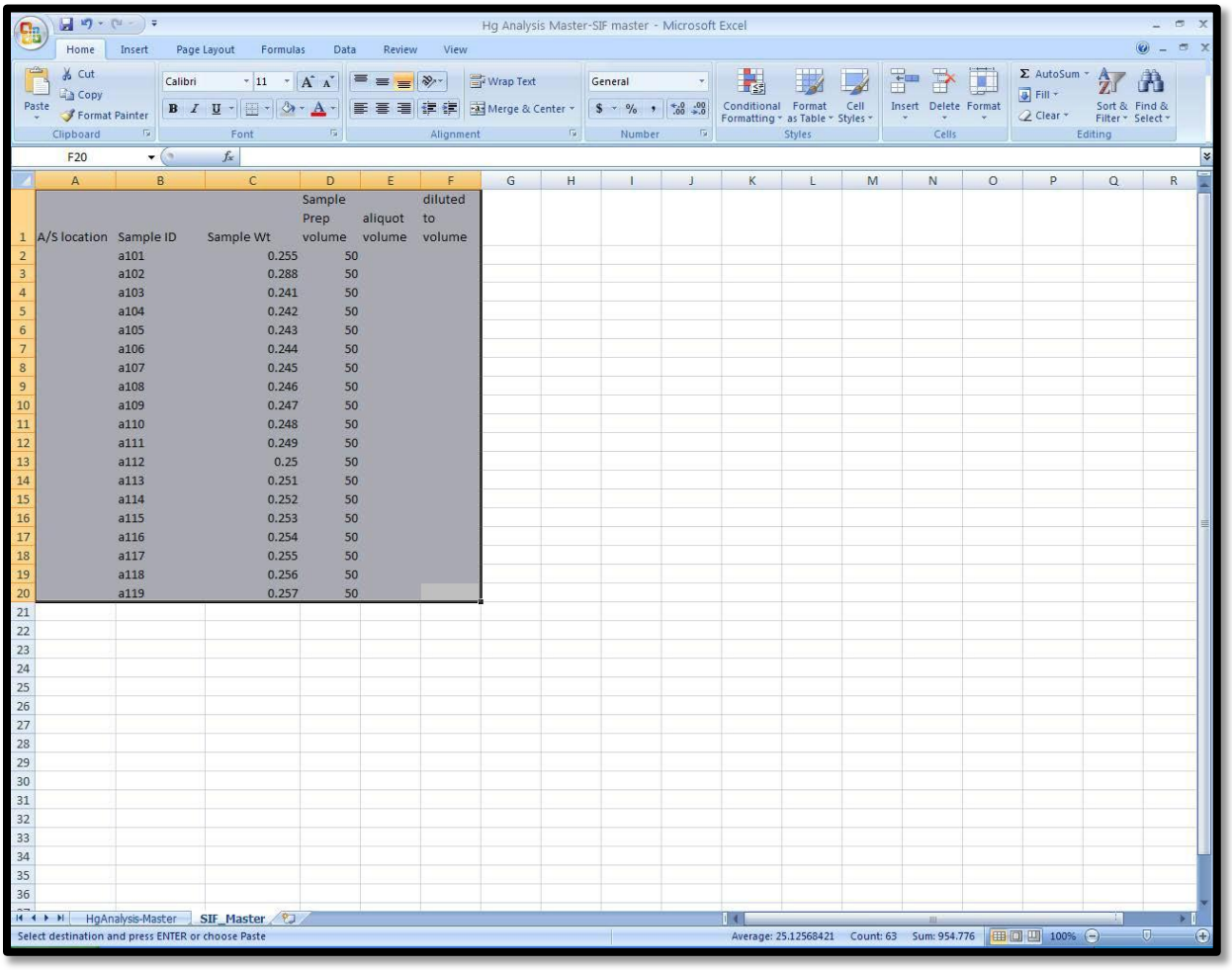
        23.2.1. Highlight the rows in the Excel file to be added to the SIF, and copy (Ctrl+C). Note that Sample ID names must contain less than 25 characters.

        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.



	A	B	C	D	E	F
1	A/S location	Sample ID	Sample Wt	Sample Prep volume	aliquot volume	diluted to volume
2		a101	0.255	50		
3		a102	0.288	50		
4		a103	0.241	50		
5		a104	0.242	50		
6		a105	0.243	50		
7		a106	0.244	50		
8		a107	0.245	50		
9		a108	0.246	50		
10		a109	0.247	50		
11		a110	0.248	50		
12		a111	0.249	50		
13		a112	0.25	50		
14		a113	0.251	50		
15		a114	0.252	50		
16		a115	0.253	50		
17		a116	0.254	50		
18		a117	0.255	50		
19		a118	0.256	50		
20		a119	0.257	50		

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).

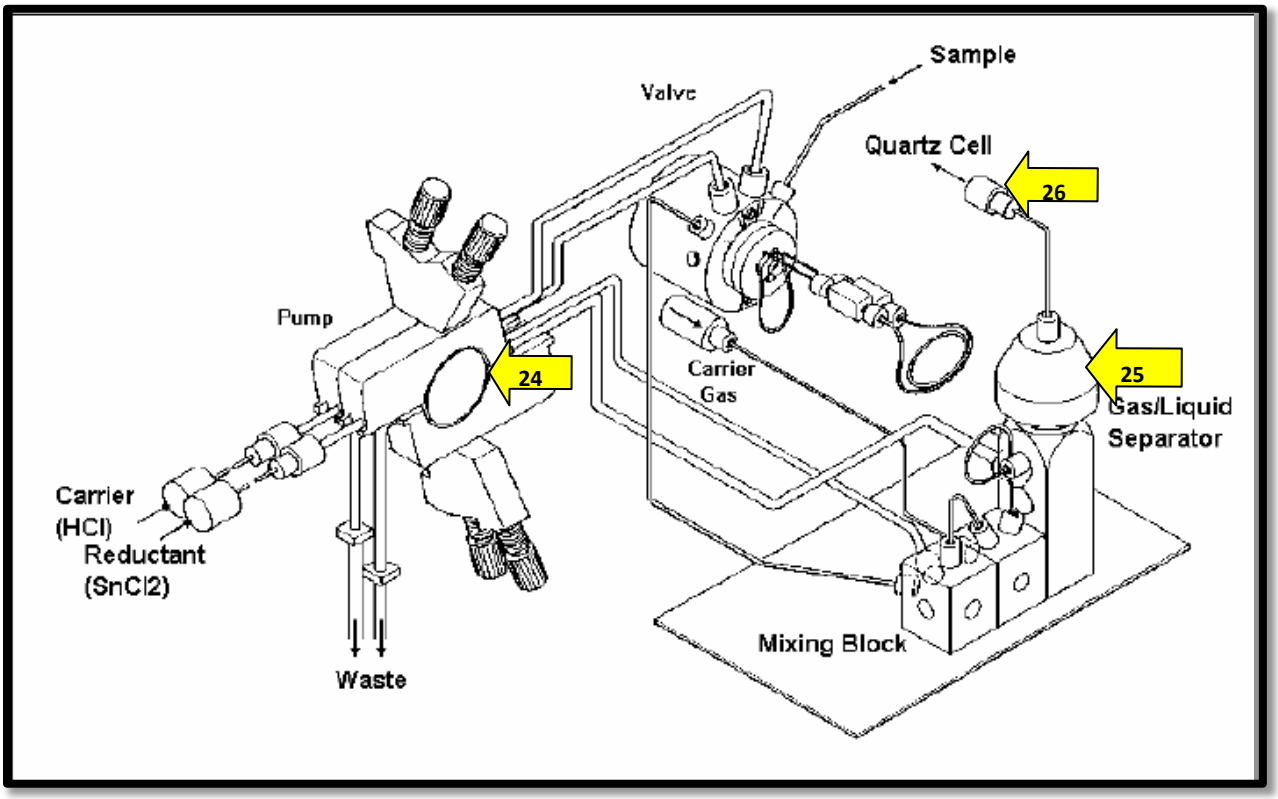


Figure 3. Diagram of the PerkinElmer FIMS-100. The yellow arrows indicate important areas of the instrument that need attention according to the referenced sections of this SOP.

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.

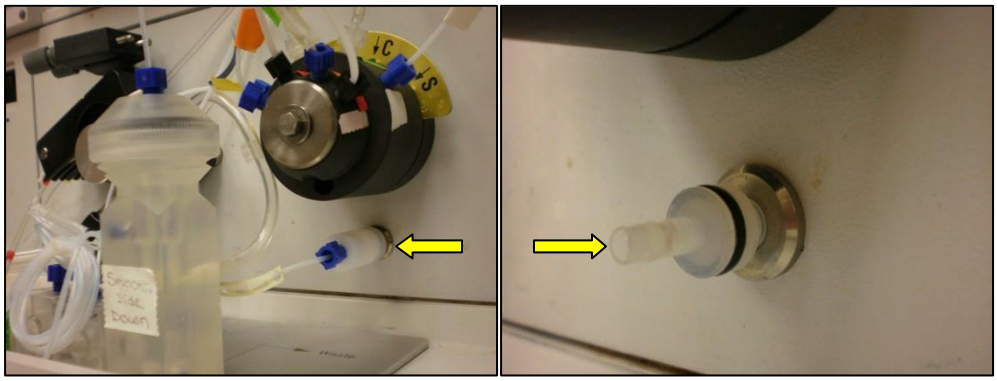


Figure 3A and 3B. Photograph showing the location of the non-return valve (3A) and the rubber sleeve covering the holes on the valve insert.

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.

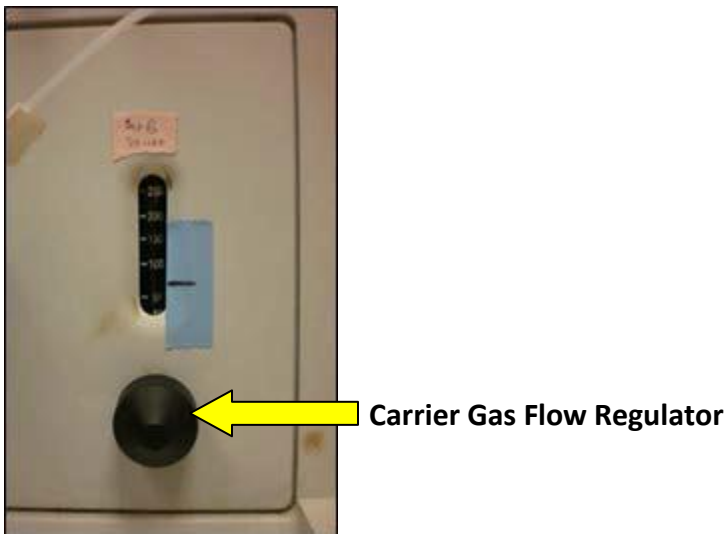


Figure 4. Photograph showing the Carrier Gas Flow Gauge and Carrier Gas Flow Regulator.

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.

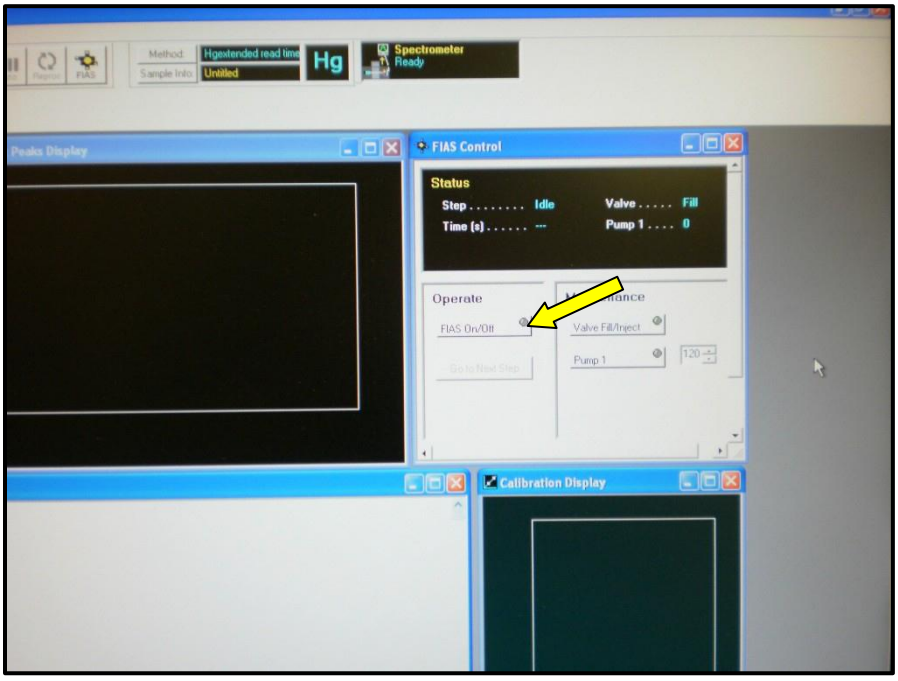


Figure 5. Photograph showing the screen image for initiating the FIAS to check carrier and reductant flow rates.

    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.

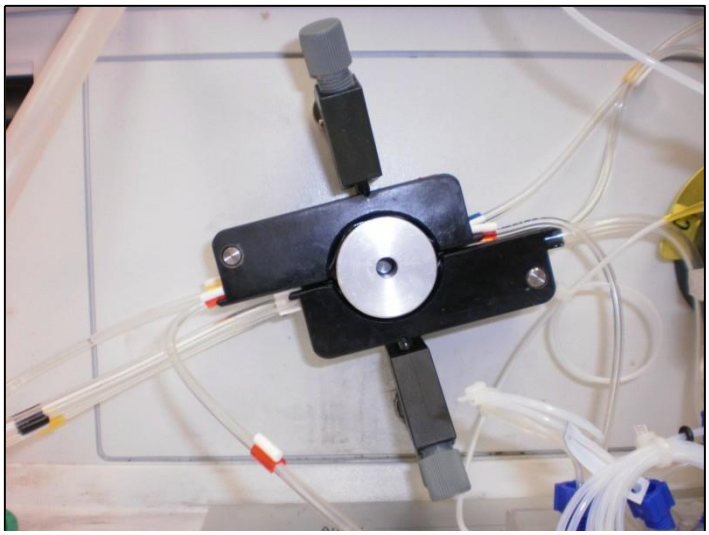


Figure 6. Photograph showing the location of the knobs which can be adjusted to change the flow rate of the pump.

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).

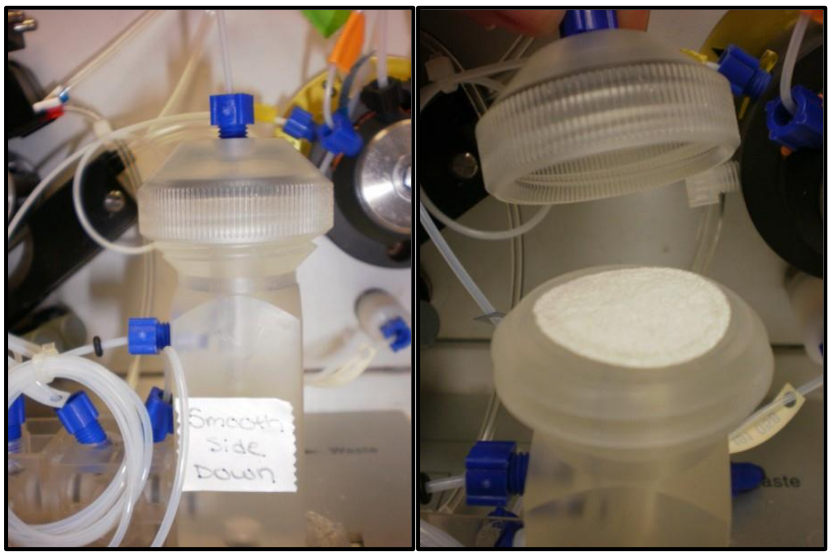


Figure 7A and 7B. Photograph showing the gas/liquid separator and the filter in placement in the gas/liquid separator.

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).

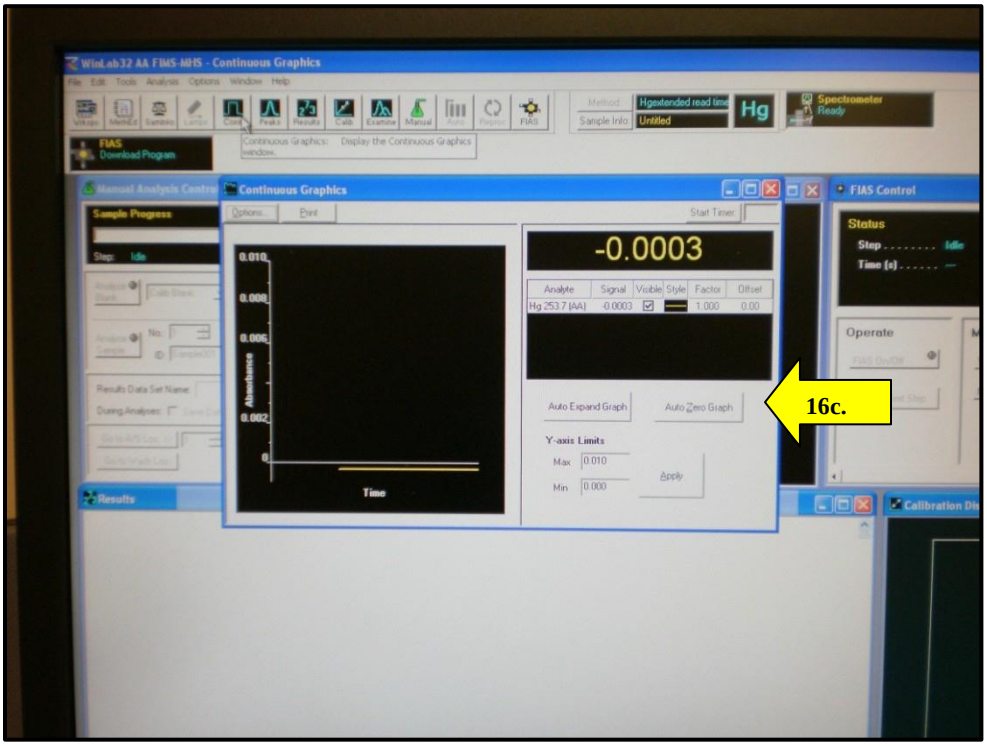


Figure 8. Photograph showing the screen image for measuring the absorbance of the FIMS-cell window.

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).

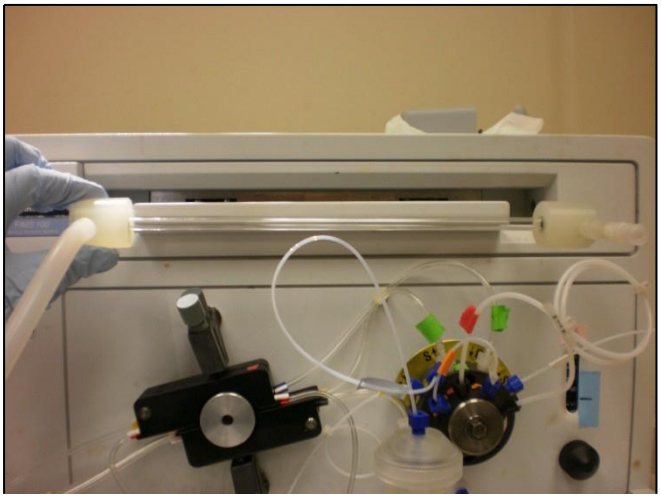


Figure 9. Photograph showing the FIMS-cell after it has been removed from the instrument.

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.

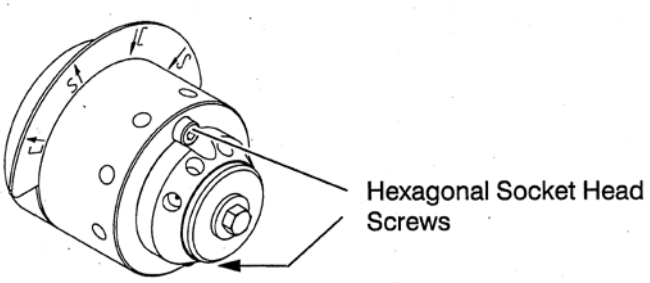
21. Wipe pump rollers with a dry lint-free cloth.

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.



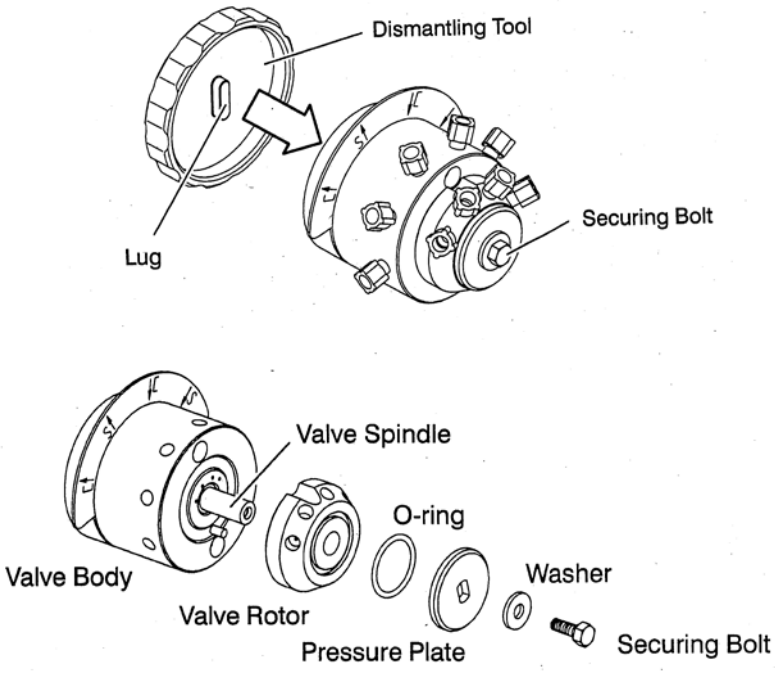


Figure 10. This series of diagrams shows how to remove the FIAS-valve from the motor mount, dismantle the FIAS- valve and separate the FIAS-valve components.

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:

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

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

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.