

**U.S. Fish and Wildlife Service
Maine Field Office
Special Project Report: FY98-MEFO-2-EC**



**Total Mercury and Methyl Mercury in
Freshwater Mussels (*Elliptio complanata*)
from the
Sudbury River Watershed, Massachusetts**

March 2001

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**Total Mercury and Methyl Mercury in
Freshwater Mussels (*Elliptio complanata*)
from the
Sudbury River Watershed, Massachusetts**

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EXECUTIVE SUMMARY

Remedial investigations associated with the Superfund Program of the U.S. Environmental Protection Agency (EPA) found highly elevated levels of mercury in sediment and fish samples from the Sudbury River, Massachusetts.

Our study was conducted 1) to determine total mercury and methyl mercury concentrations in freshwater mussels from the Sudbury River watershed, and 2) to provide the U.S. Environmental Protection Agency and Massachusetts Department of Environmental Protection with site-specific benthic invertebrate data for human health and ecological risk assessments of the river.

In May and July 1995, USFWS personnel collected the freshwater mussels from reference and mercury-impacted locations in free-flowing riverine locations (Cedar Street Bridge, Sherman Bridge) and in impoundments (Whitehall Reservoir, Reservoir #2). All mussels collected were eastern elliptios *Elliptio complanata*. Twenty-eight mussel tissue samples were analyzed for total mercury and methyl mercury at the University of Maine in Orono.

Total mercury concentration in mussel tissue was significantly greater ($p < 0.05$) in the reference reservoir (mean 0.14 ppm, wet weight, Whitehall Reservoir) than in the impacted reservoir (mean 0.08 ppm, Reservoir #2). Total mercury concentrations in mussel tissue were not significantly different ($p > 0.05$) between the two free-flowing riverine locations, Cedar Street Bridge (0.09 ppm) and Sherman Bridge (0.08 ppm). Similarly, methyl mercury concentrations in mussel tissue were not significantly different ($p > 0.05$) between riverine locations (0.043 ppm, 0.037 ppm) or between reservoir locations (0.038 ppm, 0.057 ppm).

Total mercury and methyl mercury results in reservoir mussels were not markedly different from other mussel studies conducted in the Sudbury River watershed (Bock *et al.* 2000, Salazar *et al.* 1996). Mean total mercury concentrations in resident riverine mussels were similar to transplanted mussels at the same riverine locations (Salazar *et al.* 1996). Resident mussels from riverine locations appeared to have a higher methyl mercury concentration than mussels transplanted to the location and exposed for 12-weeks.

PREFACE

This report summarized the analytical results of a screening-level survey of total mercury and methyl mercury in freshwater mussels from the Sudbury River watershed in Massachusetts. Analytical funding for this study was provided by Region 1 of the U.S. Environmental Protection Agency (EPA) under an interagency agreement between the U.S. Fish and Wildlife Service (USFWS) and EPA for technical assistance in the Superfund Program (EPA/IAG No. DW14934248-01-F).

Questions, comments, and suggestions related to this report are encouraged. Written inquiries should refer to Report Number FY98-MEFO-2-EC and be directed to:

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1. INTRODUCTION

The Nyanza Chemical Site in Ashland, Massachusetts, released mercury-contaminated effluent into the Sudbury River from 1917 until 1978. Elevated levels of mercury have been detected in river sediments and fish more than 30 km (19 mi) downstream of the Nyanza site (NUS Corporation 1992). Because of mercury contamination in surface water, sediment, biota, and groundwater, the Nyanza Chemical Site was designated as a National Priority List Superfund Site in 1983. Wiener and Shields (2000) provide a thorough account of the history of Sudbury River mercury contamination and a synthesis of research on transport, environmental fate, and bioavailability.

2. STUDY PURPOSE

Mussels were collected and analyzed to determine total mercury and methyl mercury concentrations in mussels from the Sudbury River watershed and to provide the U.S. Environmental Protection Agency and Massachusetts Department of Environmental Protection with site-specific data for human health and ecological risk assessments of the river.

3. STUDY AREA

Mussels were collected in two riverine locations and two reservoir locations. The reference reservoir for the study was Whitehall Reservoir, a 237-hectare (586-ac) impoundment in the Town of Hopkinton, MA (Figure 1). The mercury-impacted reservoir for the study was Reservoir #2. Reservoir #2 is a 47-hectare (117-ac) impoundment on the Sudbury River located in the Town of Ashland, MA (Figure 2). The Nyanza Chemical site, located approximately 1.2 kilometers (0.75 mi) upstream from Reservoir #2, released mercury-contaminated effluent into the Sudbury River. The reference free-flowing riverine reach was in the vicinity of Cedar Street Bridge in the Town of Hopkinton (Figure 3). Cedar Street Bridge crosses the Sudbury River approximately 6.5 kilometers (4 mi) upstream of the Nyanza Chemical Site. The Sudbury River reach in the vicinity of Sherman Bridge represented the free-flowing impacted riverine area in the study (Figure 4). The reach is located in the Town of Sudbury within the confines of the Great Meadows National Wildlife Refuge. Sherman Bridge is approximately 24 kilometers (15 mi) downstream of the Nyanza Chemical Site.

4. METHODS

4.1 Collection method - Individual mussels and mussel beds were located with viewing buckets. Mussels of the same species and same approximate size were collected by hand. Shells were scrubbed at the respective collection location with ambient water. Individual mussels were measured and weighed (Tables 1 and 2). Mussels were shucked in the field, the tissue of each mussel placed into individual chemical-clean jars, and frozen until analysis.

4.2 Analytical Method - Mussel tissue samples were digested and analyzed for total mercury and methyl mercury at the U.S. Geological Survey, Leetown Science Center Field Station in Orono, Maine, in accordance with methods outlined in Standard Operating Procedures F10.9, F10.11, F10.12, and F10.15 (Appendix). Quality control was accomplished through instrument calibration verification, duplicate analyses, and analysis of matrix spikes. Quality control results were considered within acceptable limits.

4.3 Data Presentations and Statistical Analyses - Contaminant concentrations in Tables 3 and 4 are presented in : g/g (ppm, parts-per-million), **wet weight**. Wet weight concentrations are presented because those units are often used in ecological and human health risk assessments - the principle users of the data in this report. Often in the scientific literature, however, contaminant levels in benthic organisms are presented on a dry weight basis. Although dry weight data are not used in the text of this report, Tables 5 and 6 list and summarize dry weight values.

Statistical analyses were performed with SYSTAT 9.0 (SPSS 1999). Summary statistics include arithmetic means, ranges, and standard deviations. Differences in mussel morphological metrics, total mercury and methyl mercury concentrations by habitat type (i.e., riverine and reservoir) were tested with a two-sample t test. All statements of significance refer to " $p < 0.05$.

5. RESULTS

Sample morphological metrics are summarized in Table 1 (reservoir) and Table 2 (riverine). Total mercury and methyl mercury data in mussel tissue are summarized in Table 3 (reservoir) and Table 4 (riverine).

5.1 Reservoir - There are no significant differences in length, height, breadth, and weight of mussels collected in the two reservoirs. Total mercury levels in mussel tissue from Whitehall Reservoir (reference reservoir) ranged widely from 0.060 : g/g to 0.227 : g/g (mean 0.139 : g/g), while concentrations in Reservoir #2 exhibited less variation (range: 0.027 : g/g - 0.153 : g/g, mean 0.080 : g/g). Methyl mercury concentrations did not show the same pattern as total mercury. In Reservoir #2, MeHg was more variable (range: 0.011 - 0.095 : g/g, mean 0.057 : g/g) than in Whitehall Reservoir (range: 0.018 - 0.066 : g/g, mean 0.038 : g/g).

The ratio of methyl mercury to total mercury was highly variable in reservoir mussels, particularly in mussel from Reservoir #2. In Reservoir #2, percent MeHg of total Hg ranged from 42 % to 142 % (mean 74%). Methyl mercury analyses may exhibit variability of $\pm 20\%$, and a MeHg/Total Hg ratio > 1 is not highly unusual (T. Haines, USGS, personal communication). However, the MeHg/Total Hg ratio of 1.42 observed in a Reservoir #2 sample (R2MS9507-06) is well above the theoretical maximum of 1.20. The MeHg analytical result for this Reservoir #2 sample is questionable. If the improbable Reservoir #2 sample is excluded, the mean percent MeHg of Total Hg is 60%. The percent of MeHg of Total Hg in Whitehall Reservoir averaged 31% with a minimum of 17% and a maximum of

73%.

5.2 Riverine - Mussels collected from Sherman Bridge were significantly larger in all morphological metrics (i.e., length, height, breadth, weight) than mussels collected at Cedar Street Bridge. Although larger mussels were collected at Sherman Bridge, total mercury and methyl mercury concentrations in mussel composite samples were not significantly different between collection sites. Total mercury in mussels ranged from 0.048 : g/g to 0.121 : g/g (mean 0.091 : g/g) at Cedar Street Bridge, the reference station. At Sherman Bridge, the mean total mercury concentration was 0.077 : g/g ($\pm$ 0.0091, range: 0.065 - 0.094 : g/g).

Methyl mercury concentrations were also not significantly different between riverine collection locations. The mean methyl mercury concentration at the reference location was 0.043 : g/g ($\pm$ 0.0211, range: 0.024 - 0.082 : g/g), while at the mercury-impacted location the mean was 0.037 : g/g ($\pm$ 0.0161, range: 0.021 - 0.066 : g/g). Between locations, the mean percent methyl mercury of total mercury appeared similar (Cedar Street Bridge 49%, Sherman Bridge 48%). Among composite samples from each location, however, percent methyl mercury as total mercury varied widely from 20 to 80%.

6. DISCUSSION

Mussels often comprise a large percentage of the total biomass of the aquatic benthic community (Naimo 1995). Mussels are remarkably long-lived organisms. The eastern ellipio (*Elliptio complanata*) may live more than 60 years (S. von Oettingen, USFWS, personal communication), while pearl mussels (*Margaritifera margaritifera*) may live more than 100 years (Bauer 1992). Freshwater mussel have limited home ranges and are relatively sedentary. In one week, tagged *E. complanata* in a Quebec study moved an average of 12 cm in mid-summer (Amyot and Downing 1997). In a study of *E. complanata* in Virginia, tagged mussels moved an average of 2.9 m during one year (Balfour and Smock 1995).

Mussels are a forage species for many ecological receptors. Glochidia (mussel larvae) and juvenile mussels are prey of fish, birds, and mammals (Martin 1997). Adult mussels are consumed by muskrat, mink, otter, raccoon, and some birds (Toweill and Tabor 1982, Grubb and Coffey 1982, Cummings and Mayer 1992, Strayer and Jirka 1997). Middens of empty mussel shells along the banks of ponds or streams are a typical sign of muskrat, otter, or raccoon foraging. These middens of empty shells can be used to determine mussel species inhabiting a watercourse or waterbody (Cummings and Mayer 1992).

Mussels are particularly well suited for some contaminant investigations because, as filter-feeders and benthic dwellers, they are exposed to contaminants in the water column and sediment (Tessier *et al.* 1984, Naimo 1995). Sedentary, long-lived freshwater mussels have two advantages as a monitoring organism - an inability to avoid a pollutant, and their exposure in a natural environment to a pollutant over a long period of time (Foster and Bates 1978).

6.1 Total Mercury (Hg) in Mussels

Total mercury concentration in mussel tissue was significantly greater ($p < 0.05$) in the reference reservoir (mean 0.14 : g/g, Whitehall Reservoir) than in the mercury-impacted reservoir (mean 0.08 : g/g, Reservoir #2). Total mercury concentrations in mussel tissue were not significantly different between the two free-flowing riverine locations (Cedar Street Bridge 0.09 : g/g, Sherman Bridge 0.08 : g/g).

Two other studies of total mercury and mussels from the Sudbury River watershed in Massachusetts were conducted during the same time period as this study (1994 and 1995, see Table 7). In Bock *et al.* (2000), *E. complanata* collected below the Nyanza Site in Reservoir #2 had total Hg concentrations ranging from 0.05 : g/g to 0.09 : g/g. In the second study (Salazar *et al.* 1996), mussels from Lake Massasecum, Bradford, New Hampshire, were transplanted to the Sudbury River and deployed for 12-weeks in an *in-situ* exposure study. The initial concentration of total Hg in *E. complanata* prior to transplant was 0.12 : g/g. After a 12-week deployment in Reservoir #2, these mussels had a total Hg concentration of 0.13 : g/g.

The mean total mercury concentration reported in our Reservoir #2 mussel collections (0.08 : g/g) is similar to results reported by Bock *et al.* (2000) and less than the end-of-test concentration reported by Salazar *et al.* (1996) after their 12-week transplant exposures. The mean total mercury concentration reported in our Whitehall Reservoir mussel collections (0.14 : g/g) is similar to the pre-transplant concentrations reported by Salazar *et al.* (1996, 0.12 : g/g) and higher than their end-of-test concentration (0.10 : g/g).

Mean total mercury concentrations in resident riverine mussels (Cedar Street Bridge 0.09 : g/g, Sherman Bridge 0.08 : g/g) were similar to concentrations in mussels transplanted by Salazar *et al.* (1996) to the same riverine locations and exposed for 12 weeks (Cedar Street Bridge 0.11 : g/g, Sherman Bridge 0.09 : g/g).

6.2 Methyl Mercury (MeHg) in Mussels

Methyl mercury concentrations in mussel tissue were not significantly different between the riverine locations (mean 0.043 : g/g Cedar Street Bridge, mean 0.037 : g/g Sherman Bridge) or the reservoir locations (mean 0.038 : g/g Whitehall Reservoir, mean 0.057 : g/g Reservoir #2).

Results from our study of reservoirs in the Sudbury River watershed were compared to two other studies of methyl mercury and *E. complanata* that were conducted during the same time period (1994 and 1995, see Table 7). MeHg concentrations in mussels from two Reservoir #2 sites on the Sudbury River were 0.054 : g/g and 0.052 : g/g (Bock *et al.* 2000). These methyl mercury concentrations were similar to the levels detected in our study. After a 12-week exposure of transplanted mussels, Salazar *et al.* (1996) reported a mean end-of-test methyl mercury concentration of 0.043 : g/g in

Reservoir #2, a level less than Bock *et al.* (2000) and our results. Resident Whitehall Reservoir mussels had a mean methyl mercury concentration (0.038 : g/g) similar to the end-of-test levels reported by Salazar *et al.* (1996, 0.041 : g/g).

Resident mussels from Cedar Street Bridge (mean 0.043 : g/g) appeared to have a higher methyl mercury concentration than mussels transplanted to the location and exposed for 12-weeks (0.033 : g/g, Salazar *et al.* 1996). Similarly, resident mussels from Sherman Bridge (mean 0.037 : g/g) appeared to have a higher methyl mercury level than mussels transplanted and exposed for 12 weeks at the same location (0.027 : g/g, Salazar *et al.* 1996).

7. SUMMARY

The total mercury concentration in mussel tissue was significantly higher ($p < 0.05$) in the reference reservoir (mean 0.14 : g/g, Whitehall Reservoir) than in the impacted reservoir (mean 0.08 : g/g, Reservoir #2). Total mercury concentrations in mussel tissue were not significantly different ($p > 0.05$) in the two free-flowing riverine locations (Cedar Street Bridge and Sherman Bridge). Similarly, methyl mercury concentrations in mussel tissue were not significantly different ($p > 0.05$) in the riverine locations or in the reservoir locations.

Total mercury and methyl mercury results in reservoir mussels were not markedly different from other mussel studies conducted in the Sudbury River watershed (Bock *et al.* 2000, Salazar *et al.* 1996). Mean total mercury concentrations in resident riverine mussels were similar to transplanted mussels at the same riverine locations (Salazar *et al.* 1996). Resident mussels from riverine locations appeared to have a higher methyl mercury concentration than mussels transplanted to the same location and exposed for 12-weeks.

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Figures

Figure 1. Collection Location at Whitehall Reservoir (reference area)

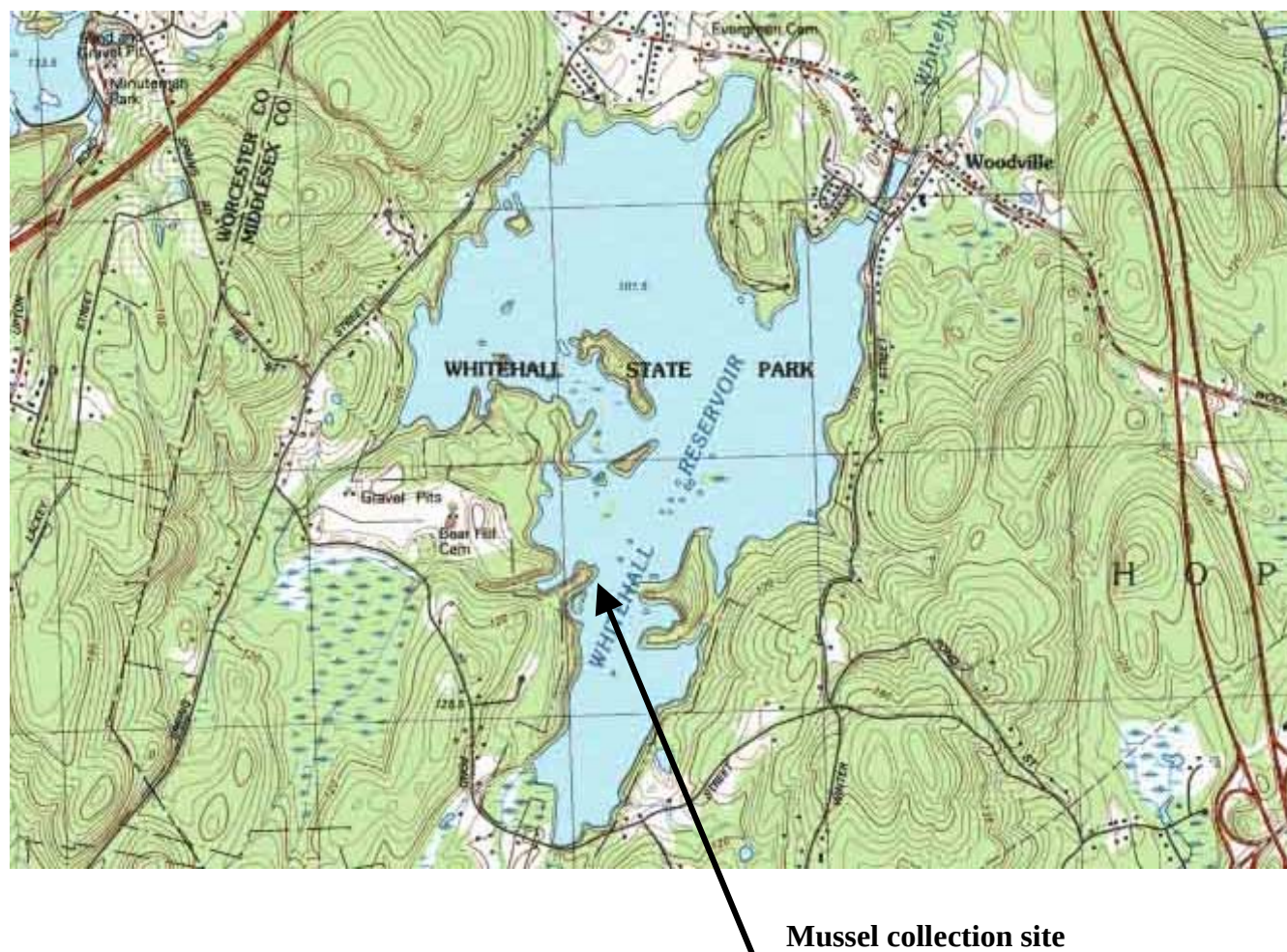
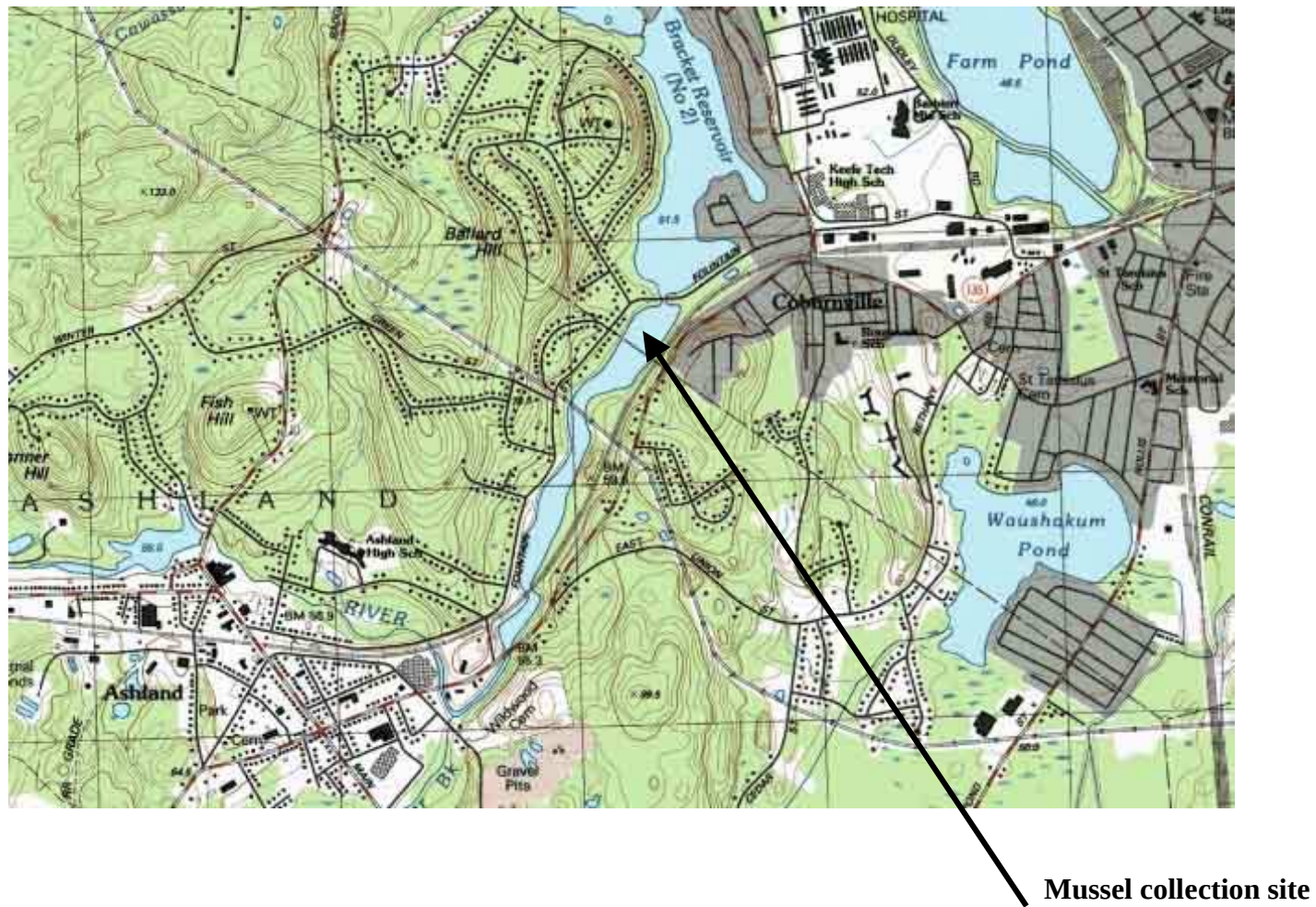


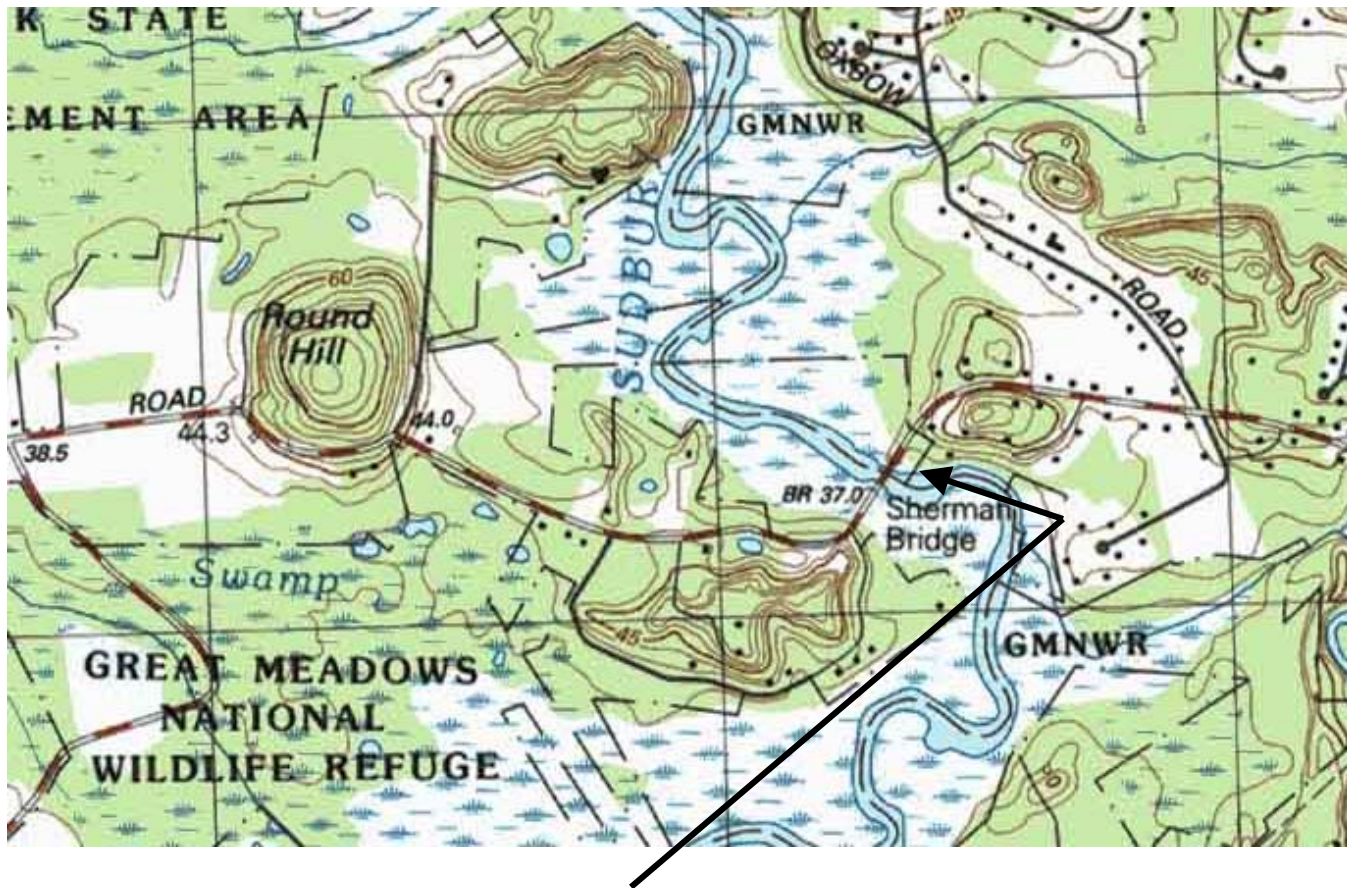
Figure 2. Collection Location at Reservoir #2 (Hg-impacted area)



A topographic map of the Sudbury area. The map features contour lines indicating elevation, with labels for '105' and '85.3'. Key locations include 'Southville', 'Cordaville', and 'Hopkinton'. A river labeled 'Sudbury' flows through the area. A black arrow points to a specific location on the map, near the intersection of a road and a stream. Other labels include 'WT', 'BM', 'CEDAR', 'Buck', and 'Bo'.

A topographic map showing contour lines and a stream. A black arrow points to a specific location on the map.

Figure 4. Collection Location at Sherman Bridge (Hg-impacted area)



Mussel collection site

Tables

Table 1. Morphological Metrics for Reservoir Mussels.

Sample No.	Location	Length (mm)	Height (mm)	Breadth (mm)	Weight (g)
Reference Reservoir (n=7)					
WRMS9505-03	Whitehall Reservoir	83.7	46.9	26.3	66.4
WRMS9505-06	Whitehall Reservoir	84.5	48.5	25.7	64.7
WRMS9505-09	Whitehall Reservoir	72.2	37.0	24.7	35.2
WRMS9505-10	Whitehall Reservoir	71.4	38.5	20.6	30.1
WRMS9507-08	Whitehall Reservoir	79.7	44.3	25.0	49.5
WRMS9507-09	Whitehall Reservoir	82.8	42.2	23.0	46.0
WRMS9507-10	Whitehall Reservoir	<u>79.8</u>	<u>43.5</u>	<u>24.4</u>	<u>50.2</u>
Mean		79.2	43.0	24.2	48.9
SD		4.95	3.86	1.77	12.58
Impacted Reservoir (n=6)					
R2MS9505-06	Reservoir #2	79.5	42.5	21.1	40.2
R2MS9505-07	Reservoir #2	75.7	40.9	21.7	39.8
R2MS9507-04	Reservoir #2	91.1	48.2	29.4	84.0
R2MS9507-06	Reservoir #2	92.9	52.7	29.5	86.3
R2MS9507-07	Reservoir #2	88.1	50.3	27.1	70.4
R2MS9507-12	Reservoir #2	<u>79.9</u>	<u>45.5</u>	<u>26.3</u>	<u>59.1</u>
Mean		84.5	46.7	25.9	63.3
SD		6.46	4.16	3.35	18.76

Table 2. Morphological Metrics for Riverine Mussels.

Sample No.	Location	Length (mm)	Height (mm)	Breadth (mm)	Weight (g)
Reference Riverine (n=8)					
CBMS9505-02	Cedar St. Bridge	82.0	39.9	25.7	55.2
CBMS9505-04	Cedar St. Bridge	76.3	40.7	27.0	50.8
CBMS9505-09	Cedar St. Bridge	75.7	44.7	23.4	45.3
CBMS9505-11	Cedar St. Bridge	84.3	43.4	25.0	53.7
CBMS9507-03	Cedar St. Bridge	82.8	45.9	24.5	57.3
CBMS9507-08	Cedar St. Bridge	78.1	43.5	26.4	56.9
CBMS9507-09	Cedar St. Bridge	70.5	39.4	22.5	35.7
CBMS9507-10	Cedar St. Bridge	<u>69.5</u>	<u>38.8</u>	<u>21.5</u>	<u>35.7</u>
Mean		77.4	42.0	24.5	48.8
SD		5.15	2.49	1.79	8.38
Impacted Riverine (n=7)					
SBMS9505-02	Sherman Bridge	113.7	58.3	39.6	154.1
SBMS9505-03	Sherman Bridge	103.2	52.5	30.5	102.0
SBMS9505-04	Sherman Bridge	101.5	55.8	32.7	107.5
SBMS9505-05	Sherman Bridge	92.1	47.8	26.7	70.2
SBMS9507-02	Sherman Bridge	113.7	62.2	37.2	153.5
SBMS9507-03	Sherman Bridge	110.6	56.8	37.5	139.0
SBMS9507-04	Sherman Bridge	<u>117.4</u>	<u>58.8</u>	<u>39.2</u>	<u>155.4</u>
Mean		107.5	56.0	34.8	126.0
SD		8.25	4.34	4.53	30.73

Table 3. Total Hg and MeHg in Reservoir Mussels, ug/g (ppm) WW**WET WEIGHT**

Sample No.	Total Hg ug/g	Methyl Hg ug/g	% Total as Methyl
Reference Reservoir			
WRMS9505-03	0.130	0.031	24
WRMS9505-06	0.186	0.040	22
WRMS9505-09	0.153	0.029	19
WRMS9505-10	0.227	0.066	29
WRMS9507-08	0.105	0.018	17
WRMS9507-09	0.116	0.042	36
WRMS9507-10	<u>0.060</u>	<u>0.044</u>	<u>73</u>
Mean	0.139	0.038	31
SD	0.0508	0.0141	
Impacted Reservoir			
R2MS9505-06	0.027	0.011	42
R2MS9505-07	0.072	0.054	74
R2MS9507-04	0.078	0.056	73
R2MS9507-06	0.067	0.095	142
R2MS9507-07	0.081	0.051	63
R2MS9507-12	<u>0.153</u>	<u>0.077</u>	<u>50</u>
Mean	0.080	0.057	74
SD	0.0374	0.0258	

Table 4. Total Hg and MeHg in Riverine Mussels, ug/g (ppm) WW.**WET WEIGHT**

Sample No.	Total Hg ug/g	Methyl Hg ug/g	% Total as Methyl
Reference Riverine			
CBMS9505-02	0.080	0.032	40
CBMS9505-04	0.048	0.035	71
CBMS9505-09	0.092	0.043	47
CBMS9505-11	0.084	0.029	35
CBMS9507-03	0.121	0.024	20
CBMS9507-08	0.103	0.024	23
CBMS9507-09	0.102	0.073	72
CBMS9507-10	<u>0.095</u>	<u>0.082</u>	<u>87</u>
Mean	0.091	0.043	49
SD	0.0200	0.0211	
Impacted Riverine			
SBMS9505-02	0.094	0.021	23
SBMS9505-03	0.076	0.029	38
SBMS9505-04	0.067	0.024	35
SBMS9505-05	0.065	0.024	37
SBMS9507-02	0.083	0.066	80
SBMS9507-03	0.077	0.053	70
SBMS9507-04	<u>0.078</u>	<u>0.042</u>	<u>54</u>
Mean	0.077	0.037	48
SD	0.0091	0.0161	

Table 5. Total Hg and MeHg in Reservoir Mussels, ug/g (ppm) DW.**DRY WEIGHT**

Sample No.	% Moisture	Total Hg ug/g	Methyl Hg ug/g	% Total as Methyl
Reference Reservoir				
WRMS9505-03	86.50	0.964	0.228	24
WRMS9505-06	82.40	1.057	0.228	22
WRMS9505-09	85.40	1.045	0.198	19
WRMS9505-10	81.00	1.193	0.348	29
WRMS9507-08	88.29	0.898	0.151	17
WRMS9507-09	82.02	0.645	0.232	36
WRMS9507-10	<u>90.88</u>	<u>0.657</u>	<u>0.478</u>	<u>73</u>
Mean	85.21	0.923	0.266	31
SD		0.1913	0.1024	
Impacted Reservoir				
R2MS9505-06	90.71	0.289	0.123	42
R2MS9505-07	86.08	0.518	0.386	74
R2MS9507-04	89.14	0.715	0.518	73
R2MS9507-06	81.26	0.358	0.508	142
R2MS9507-07	88.07	0.676	0.428	63
R2MS9507-12	<u>80.82</u>	<u>0.799</u>	<u>0.403</u>	<u>50</u>
Mean	86.01	0.559	0.394	74
SD		0.1872	0.1312	

Table 6. Total Hg and MeHg in Riverine Mussels, ug/g (ppm) DW.**DRY WEIGHT**

Sample No.	% Moisture	Total Hg ug/g	Methyl Hg ug/g	% Total as Methyl
Reference Riverine				
CBMS9505-02	88.60	0.702	0.277	40
CBMS9505-04	92.70	0.662	0.473	71
CBMS9505-09	85.70	0.645	0.300	47
CBMS9505-11	87.40	0.665	0.231	35
CBMS9507-03	75.00	0.485	0.098	20
CBMS9507-08	76.76	0.444	0.103	23
CBMS9507-09	79.33	0.494	0.355	72
CBMS9507-10	<u>77.68</u>	<u>0.424</u>	<u>0.369</u>	<u>87</u>
Mean	82.90	0.565	0.276	49
SD		0.1063	0.1214	
Impacted Riverine				
SBMS9505-02	84.40	0.603	0.136	23
SBMS9505-03	85.10	0.507	0.195	38
SBMS9505-04	85.10	0.452	0.160	35
SBMS9505-05	86.10	0.464	0.171	37
SBMS9507-02	76.85	0.358	0.287	80
SBMS9507-03	81.09	0.406	0.282	70
SBMS9507-04	<u>79.70</u>	<u>0.384</u>	<u>0.206</u>	<u>54</u>
Mean	82.62	0.454	0.205	48
SD		0.0771	0.0544	

Table 7. Comparison of Total Hg and MeHg results in mussel tissue studies of the Sudbury River watershed, ug/g WW.

Location	Total Hg	MeHg	Study
Whitehall Reservoir	0.139	0.038	This Study
	0.100	0.041	Salazar <i>et al.</i> 1996 (Stn 1)
Reservoir #2	0.080	0.057	This Study
	0.130	0.043	Salazar <i>et al.</i> 1996(Stn 3)
	0.059	0.054	Bock <i>et al.</i> 2000 (Stn 4)
Cedar Street Bridge	0.091	0.043	This Study
	0.110	0.033	Salazar <i>et al.</i> 1996 (Stn 2)
Sherman Bridge	0.077	0.037	This Study
	0.094	0.027	Salazar <i>et al.</i> 1996 (Stn 6)

ug/g = ppm, WW = wet weight

All studies used Eastern Elliptio (*Elliptio complanata*)

Mussels from this study and Bock *et al.* 2000 based on analysis of resident mussels. Salazar *et al.* 1996 data from 12-week *in situ* deployment of transplanted mussels.

Analytical Methods

Standard Procedure for Sample Digestion With the CEM MARS-X Microwave

I. EQUIPMENT

- CEM MARS-X microwave accelerated reaction system
- 14 HP-500 Plus digestion vessels (CEM corp.)
- Analytical balance (accurate to 0.001 g)
- Micropipette (up to 2000 μ L)
- 14, 50 mL glass volumetric flasks
- 14 plastic funnels
- Plastic spatulas (for sample transfer)
- 14, 60 mL bottles of polyethylene composition (or suitable substitute)

II. REAGENTS

- 1000 ng Hg/mL 10% HNO₃ stock solution
- Concentrated Trace Grade Nitric Acid (HNO₃)
- 30 % Hydrogen Peroxide (H₂O₂)

III. PROCEDURE

- Set up a digestion log in the appropriate project book with the following format:

Acid Digestion of 99' DEP Wood Samples Using MARSX Microwave (Wood Method)

9-26-99 MP

Sample ID	Digestate ID	Bomb ID	Sample Wt. (g)	Bomb Wt. (g)	Total Wt. (g)	Final Wt. (g)
SRM 1575	9926401	2	0.264	162.3	178.2	177.7
Blank	9926402	3	-----	158.6	173.5	174.4
011E/spk.	9926403	4	0.485	157.3	175.7	174.8
011E	9926404	5	0.492	157.4	175.4	175.2
012M	9926405	6	0.475	158.3	175.0	174.6
Etc....	9926406	7...probe	0.479	157.6	176.2	175.3

1. Placed new rupture discs in each vessel
2. Added 25 mL of 30 % H₂O₂ and 10 mL of concentrated HNO₃ to each vessel
3. Spiked 9926403 w/ 0.250 mL of 1000 ng Hg/mL 10 % HNO₃
4. Placed in microwave and ran using Wood Method
5. Diluted to 50 mL w/ DIW and stored in 60 mL poly-bottles w/ green tape
6. Noticed unusual green coloration of 9926408
7. Particulate matter visible in 9926405 after digestion

Prep Notes (Keep these notes in mind as you read through and follow the actual procedure):

- Be sure to properly title each digestion with the sample type, microwave used and method used
- Be sure to initial and date each digestion
- Digestate ID's are derived from the Julian date on which the digestion is performed (9926401, 99 = year of digestion, 264 = Julian date, 01 = sample designator)
- Note the order of samples (SRM first, Blank second, Spike third followed by a duplicate of the sample that was spiked)
- Maximize each digestion, use all 14 vessels whenever possible which means a total of 11 samples can be run in addition to the necessary SRM, Blank and Spike for each run

- Record each step that is carried out in the digestion procedure and note any irregularities in the procedure such as unusual pressure or temp readings, unusual colors, particulate matter in the digestate, spilled sample, etc...(see example above)
- Be sure to calibrate both the H₂O₂ and spike pipette to ensure that they are delivering 2.000 mL and 0.250 mL respectively (do this using Sartorius balance and DIW...1 mL water=1 g)
- Take out spike before starting the digestion, this will ensure it has completely thawed by the time you need to use it
- Use no more than 0.500 g of any sample until you have established that greater sample will not damage the vessels (check to make sure pressure of a sample weight greater than 0.500 g does not exceed 325 psi)
- Always use a sample in the probe to protect against microwave damage from excess pressure
- Be sure to add the 2 mL of 30 % H₂O₂ and 10 mL of concentrated Trace Grade HNO₃ to all vessels (this includes the SRM, Blank, Spike and all samples)...if additional purity is needed use Optima Grade HNO₃ and ULTREX H₂O₂ in place of above
- Bomb Weight is the weight of the empty bomb with no sample in (minus Kevlar liner), Total Weight is the weight of the bomb with sample and reagents in it (H₂O₂, HNO₃, spike, etc...), Final Weight is the weight of the vessels once they have been removed from the microwave (post digestion) (these are all obtained using the OHAUS Scout Scale...accurate to 0.1g). *Probe bomb weight should be taken without the load disc and with the Teflon cap over the pressure probe connection*

Digestion Procedure:

- Set-up digestion procedure in the lab notebook
- Replace rupture discs in all vessels
- Remove 1000 ng Hg/mL 10 % HNO₃ from the freezer and place in hood to thaw
- Record bomb weight, then transfer just the top section of the vessel (flip vessel top upside down and place sample in depression) to the SETRA scale, tare and add appropriate sample.... record sample weight (repeat for all vessels)
- Add 10 mL of HNO₃ and 2 mL of 30 % H₂O₂ to the vessel (add 0.250 mL of Spike for the Spiked sample at this time) (repeat for all vessels)
- Record Total Weight of the entire vessel (repeat for all vessels)
- Place Kevlar liner over Teflon vessel, then place in the vessel containment unit, hand tighten bolt on top and then turn an additional 1/3 turn using wrench (repeat for all vessels except **probe**: *before wrench tightening attach pressure sensor to the probe, rotate into correct position then wrench tighten*)
- Place in the carousel in the proper order
- Place top on carousel (optional, helps give vessels support, especially when using <14 vessels)
- Insert temperature cable into the probe vessel (BE CAREFUL; this is a fiber optic probe which is fairly flexible however it can not withstand sharp bends and rough handling.... KEEP IN STORAGE BOX WHEN NOT IN USE)
- Turn the microwave on (insert the exhaust hose into the hood at this point)
- Insert the carousel into the microwave being sure that the carousel is properly sitting on the carousel platform
- Rotate the carousel to where carousel position 6 is lined up with the arrows on the front of the microwave using the rotate button on the keypad
- Insert the pressure probe into right side of microwave using only the large diameter seal for hand placement
- Insert the temperature probe into the probe opening on the top of the microwave (there is a blinking red light that can be seen through the correct opening...the probe will snap in)
- Rotate the carousel 360° in each direction to ensure that the pressure and temperature cables do not touch (perform this using the rotate carousel button on the microwave control panel)
- From "CEM Method Menu" (if this is not displayed press "Home") select "digestion directory", then select the appropriate method to match the sample. The selected method will now be displayed at the bottom of the "Home" screen

- Press START
- Keep an eye on pressure and temperature readings throughout the procedure to ensure that the temperature does not exceed the maximum temperature for the method and that the pressure does not exceed 320 psi
- **ERROR MESSAGES** ; the following are three common error messages that may be displayed throughout the procedure causing the system to shut down:
 - **Pressure/Temperature Drop Error, Pressure or Temperature has dropped too rapidly**
 - Press 9 (continue)...if the problem persists the carousel may have to be removed, temp probe reinstalled and method run again.
 - **Warning, cannot Zero ESP-1500 Plus, Run Aborted**
 - Press 2 (zero and continue)...
 - **EST-300 Plus Error, Communications Failure**
 - Press 1 (reset)...if the problem persists there may be problems with the temperature probe. If you have to press 1 more than once throughout a method, abort method and consult a service representative from CEM
- When method has ended allow the temperature to cool below 80° C...if internal pressure is high then allow the pressure to drop to at least 60 psi before removing
- To remove from the microwave, first disconnect the pressure sensor from the microwave, then CAREFULLY grasp the temperature probe on the black ring near its microwave insertion point and pull straight down (do not jerk the probe out). PLACE TEMPERATURE PROBE BACK IN STORAGE BOX
- Vent all vessels in the hood prior to unscrewing the tops
- Replace Teflon cap on the pressure probe connection
- Remove the Teflon vessels and record Final Weight
- Carefully pour contents of vessel (digestate) into a 50 mL volumetric flask (using plastic funnel) rinse both the inside of vessel bottom and tops 3 times with DIW using the low flow DIW squirt bottle being sure not to exceed 50 mL total volume, bring to 50 mL total volume with DIW then carefully transfer to a 60 mL poly-bottle (or other appropriate bottle) labeled with the appropriate digestate ID
- Place 5 mL of concentrated HNO₃ and 5 mL of DIW into empty vessel
- Run Clean Cycle, this is performed same as above, just select Clean Method instead of a sample method (no weights need to be recorded)
- Dump contents of “cleaned” vessels into waste container labeled 50 % Nitric Acid in the fume hood
- Rinse the Teflon vessels 3 times with DIW and place upside down on adsorbent bench paper to dry (KEEP KEVLAR SUPPORT SLEEVES DRY)

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ALKALINE DIGESTION

I. GENERAL

This procedure is used for digestion of sediment and biological samples for the determination of methyl mercury content. It generally follows the procedures of Liang *et al.* (1996).

II. EQUIPMENT

1. Drying Oven, 75°C
2. 15 mL screw top Teflon vials or glass vials
3. Pipette
4. Analytical balance

III. PROCEDURE

Solution: 25% KOH (Potassium hydroxide) dissolved in methanol.

1. Label then weigh empty digestion vials.
2. Set oven at 75°C
3. Weigh sample into vial (SRMs or any freeze dried material < 0.1 g, wet tissue < 0.5 g) and record weight.
4. Add 2 -15 mL 25%KOH in methanol to the vial and record weight of KOH solution. The amount of 25%KOH is determined by the amount of sample used. The more sample used the more 25%KOH needed to digest the sample.
5. Break up clumps either by shaking or vortexing.
6. Weigh vials.
7. Put vials in 75°C oven for 3- 5 hours.
8. Shake or vortex samples at regular intervals while they are in the oven.
9. Allow samples to cool to room temperature.
10. Weigh digestion vials.
11. Return samples to 75°C oven until analysis.

IV. REFERENCES

Liang, L., M. Horvat, E. Cernichiari, B. Gelein, and S. Balogh. 1996. Simple solvent extraction technique for elimination of matrix interferences in the determination of methylmercury in environmental and biological samples by ethylation-gas chromatography-cold vapor atomic fluorescence spectrometry. *Talanta* 43: 1883-1888.

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**TOTAL MERCURY ANALYSIS USING THE MERLIN COLD VAPOR
ATOMIC FLUORESCENCE SPECTROMETER**

1.0 Scope and Application:

This method is for the determination of total mercury in water and solid matrixes using the Merlin fluorescence spectrometer.

2.0 Reagents:

- 2.1 Reagent Water (DIW): Water in which mercury is not detected by this method; 18 M ultrapure deionized water starting from a prepurified source.
- 2.2 Stannous Chloride (2% SnCl₂): Dissolve 10 grams SnCl₂ in 500 mL of 10% trace metal grade hydrochloric acid (add 50 mL HCl to ~ 400 mL DIW and bring to 500 mL total volume with DIW). This solution should be prepared daily.
- 2.3 Nitric and Hydrochloric Acids: Trace metal grade from Fisher Scientific.
- 2.4 10% Nitric Acid: Dilute 100 mL HNO₃ to 1000 mL with DIW.
- 2.5 Mercury Stock/Working Standards:
- a. 100 ppb (100ng/mL) Hg in 10% HNO₃: Add 0.1 mL of the 1000 ppm stock standard from Aldridge Scientific and 100 mL HNO₃ to ~ 800 mL of DIW in a 1000 mL volumetric flask. Bring to 1000 mL final volume with DIW. Mix well before proceeding. This solution should be stable for at least 1 month.
- b. Calibration Standards:

<u>Standard Conc., ppb</u>	<u>Vol. of 100ppb std., mL</u>	<u>Final vol.10% HNO₃, mL</u>
0.1	0.1	100
0.5	0.5	100
1.0	1.0	100
2.5	2.5	100
3.0	3.0	100

Concentrations can be varied based upon samples to be run. These are stored in the hood in the volumetric flasks used to make them (volumetric flasks should not be washed in order to help maintain stability of the standards). Calibration standards should be prepared daily.

2.6 Secondary QC Check Standard:

- a. 100 ppb (100ng/mL) Hg in 10% HNO₃: Add 0.1 mL of the 1000 ppm stock standard from Spex Industries and 100 mL HNO₃ to ~ 800 mL of DIW in a 1000 mL

volumetric flask. Bring to 1000 mL final volume with DIW. Mix well before proceeding.

This solution should be stable for at least 1 month.

- b. 1 ppb (1ng/mL) Check Standard: Add 1 mL of the 100 ppb stock standard and 10 mL HNO₃ to ~ 90 mL of DIW in a 100 mL volumetric flask. Bring to 100 mL final volume with DIW. This solution should be re-made daily.

3.0 Equipment List:

- 3.1 Merlin Cold Vapor Atomic Fluorescence Spectrometer.
- 3.2 PSA 20.100 Serial Sampler.
- 3.3 PSA Automatic Hydride Generator.
- 3.4 Volumetric Flasks.
- 3.5 Graduated Cylinders.
- 3.6 Glass screw-top sample vials with Teflon-lined caps.
- 3.7 Gases:
 - a. Grade V Argon with moisture trap for sample purge. Main flow is regulated prior to the instrument by an external flowmeter.
 - b. Reagent grade nitrogen for Nafion tube purge.
- 3.8 12" Nafion tube to decrease moisture in the sample stream.
- 3.9 Computer:
- 3.10 Water System:

4.0 Sample Preparation:

See the appropriate sample preparation SOP.

5.0 Procedure:

- 5.1 Turn on argon and nitrogen flows.
- 5.2 Turn on the instrument and associated accessories. The detector is left on all of the time.
- 5.3 Load the windows software by double-clicking on the 'Avalon' icon. There is a DOS version as well - it is loaded by pressing '1' for the first option when the computer boots up in DOS.
- 5.4 Make reagents and standards at this point to allow the system to warm up.
- 5.5 Select or create a new method from the 'Library' option and ensure that instrumental parameters are correct.
- 5.6 Empty standard vials and re-fill with new standard solutions. These vials are labeled and stored in the hood filled with the corresponding standard concentration - they need not be washed or changed unless there is indication of a problem.
- 5.7 Standards can be run as part of the run 'batch' or as a separate analysis option under 'Calibration').
- 5.8 Samples can be set up in a batch using the autosampler or run individually as single analyses.
- 5.9 Most samples will need to be diluted. Start with a level biased low to help avoid contaminating the sample train. If samples are of the same matrix, the dilution level can be assumed to be the same for all. Any results not bracketed by the standards should be re-run at the end.

- 5.10 Turn on the peristaltic pump by depressing the button on the front of the unit. Establish flow in the tubing by pulling the metal arms of the pump forward ~3/4 of the way. Too much tension can close off the tubing as well as decrease tubing life. The flow from the reagent containers can be used to determine the optimum tension position - it wants to be smooth with the SnCl_2 channel ~ 1/2 the HNO_3 channel.
 - It is recommended that the tubing be replaced before each run. Tubing used for solid matrices and/or high level samples should not be used for water analysis or low-level samples.
- 5.11 Connect dryer tube (sample line) to the back of the detector.
- 5.12 Check the waste container and empty if necessary (neutralize contents).
- 5.13 Allow the reagents to flush the system for 15-20 minutes prior to beginning analysis.
- 5.14 Type sample information into the 'batch' file option found under 'analysis' and print out.
- 5.15 Fill autosampler based upon the above printout.
- 5.16 Standard and sample vials must be filled to the same height in the vial. Capillary action in the sampling probe causes slight variations in the amount of sample taken up.
- 5.17 Analyze standards with either the 'new curve' option or as part of the batch run. A curve will be automatically graphed once all standards have been run. This should be very linear. If the standards need to be re-run, re-fill vials and flush probe with the 10% reagent blank for a few seconds to avoid carryover into the blank standard analysis.

Note: To print peaks: there is a very short window of time allowed for this after each standard. A command key must be pressed to send the peak scan to the printer during this window, otherwise the scan is lost. It is recommended to print out peaks occasionally for QC and troubleshooting purposes.
- 5.18 Once an acceptable calibration curve is generated and printed, the batch file created above can be run. Under 'analyze', choose the 'batch' option. Begin analysis by addressing each computer check prompt as it appears.
- 5.19 Monitor the system periodically as it is running to ensure the probe is being inserted into the vials and that the reagents have sufficient volume.
- 5.20 Make note of any sample that will need to be re-run. These should be run at the end as opposed to holding until another run. This is so that all information concerning this sample and its associated batch (blanks, etc.) are together, making calculations and QC related determinations easier.
- 5.21 Clean-up and shut down:
 - a. Flush all 3 channels with DIW for few minutes.
 - b. Flush all channels with 20% sodium hydroxide (NaOH) for 15 - 20 minutes (this step is especially important as it is the only way to remove any SnCl_2 buildup. If this is not removed, it will scavenge mercury from the sample train, thus lowering results.
 - c. Follow this step with a 5 minute DIW flush, then a 10 minute 10% HNO_3 rinse.
 - d. Lastly, flush 5 minutes with DIW and then allow the tubing to pump dry.
 - e. Periodically, the quartz U-tube assembly will need to be carefully removed and cleaned more rigorously (i.e. immersing the entire quartz assembly in 20% NaOH for 30 minutes, rinsing with DIW then soaking in 25-30% HNO_3 for at least an hour - this step may be extended to standing overnight if time is a problem).

- f. Neutralize contents of the waste container and flush down the sink.
 - g. Autosampler vials are first washed with soap and water then immersed in a 50% HNO₃ acid bath for 24 hours.
- 5.22 Print out Results.
- 5.23 Shut down the system by turning off all components except the detector.

7.0 Analytical Quality Control:

An analytical batch is a set of not more than 10 samples analyzed with the same instrument calibration and QC data. Calibration standards should bracket the expected sample concentration range. An analytical batch must contain the required QC in order for generated data to be considered valid. All daily activities and problems are to be entered in the log book.

7.1 QC Sample:

A calibration QC is a sample at a concentration between 25-75% of the maximum value of the calibration range and prepared from a source independent of the calibration standard. The QC sample is used to ensure the analytical process is in control. This sample is run immediately after calibration and is expected to be within accuracy objectives. Additionally, QC sample concentrations are plotted on a control chart.

7.3 Blanks:

- a. Reagent Blanks: These blanks include all chemicals and reagents used in the analysis. Purging reagents with mercury-free nitrogen or argon can reduce contamination to acceptable levels.
- b. Bottle Blanks: These are sample bottles filled with DIW at the time of sample splitting and/or receipt and are treated as a sample. These blanks are preserved as for the associated samples and allowed to stand not less than 12 hours prior to analysis.
- c. Field Blanks: This is a bottle filled with DIW and shipped with each set of samples. If the mercury concentration is equal to or greater than the MDL or is greater than one-fifth the level found in associated samples, the source of contamination needs to be identified and corrective action taken.

7.4 Analytical Duplicates:

Analytical duplicates are samples that have been collected, preserved, and prepared as one sample, but analyzed at separate times. These are analyzed at a frequency of 10% of the total sample number and are used to establish analytical precision and error. The relative percent difference (RPD) of each sample is calculated and recorded on the appropriate control chart. %RPD should be +/- 24.

7.5 Matrix Spikes (MS) and Matrix Spike Duplicates (MSD):

These are used to assess problems that may be associated with a given matrix. A sample (blanks may not be used) is spiked, in duplicate as follows:

- a. If the analysis is to be checked against a regulatory concentration limit, the spike level shall be at that limit or at 1-5 times higher than the background concentration of the sample, whichever is higher.
- b. If the analysis is not to be checked against a limit, the spike shall be at the concentration of the low-level working standard or at 1-5 times the background concentration, whichever is higher.

Calculate the relative percent difference (RPD) between the MS and MSD using the concentrations found in the MS and MSD. If the RPD does not meet the acceptance criteria of +/- 24%, the system is judged to be out of control. The problem must be identified and corrected, and the analytical batch reanalyzed.

$$RPD = 200 \times \frac{(D1 - D2)}{(D1 + D2)}$$

Where:

D1 = conc. in the MS sample

D2 = conc. in the MSD sample

Express the accuracy assessment as a percent recovery interval from P-2s to P+2s, where 'P' is the average percent recovery and 's' is the standard deviation of the percent recovery. This should be updated after every 5 to 10 new accuracy measurements.

7.6 Method Detection Limit (MDL):

Measurement of the method detection limit on a yearly basis is necessary in order to provide regular assessment of instrument/method performance, as well as a quantifiable concentration that will indicate when a measured value is above zero and is in fact detectable by the instrument. The MDL is defined as the minimum concentration of a substance that can be measured and reported with a 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte. The concentration of the low-level check standard should be 3 to 5 times the required detection limit. The detection limit is determined by analysis of seven replicates at a concentration 3 to 5 times the estimated detection limit and calculated by the student T-test as outlined in the 40 CFR part 136, Appendix B.

The laboratory must produce an MDL less than or equal to 0.2 ng/L or one-third the regulatory compliance limit, whichever is greater.

7.7 Control Charts:

Control charts are used to track variation of a process or analysis over time. The variation from the expected mean should exhibit a random distribution.

Variation from the expected value may indicate instrument or human error.

Persistent variation in one direction indicates bias. Control charts are expected to be within ± 2 standard deviations from the expected mean. The following general guidelines are useful for interpreting control charts. The results are expected to be distributed randomly about the mean or central line.

One point outside of the control limits is acceptable. Two consecutive points require investigation. Three or more points outside of the control limits mandate corrective action.

7.8 Laboratory Practices:

These include:

- a. Analytical balances are serviced yearly by a factory-authorized representative.
- b. Automated pipettors are checked on a monthly basis and prior to making primary standards.
- c. When a new calibration standard is made, it is compared to the standard being replaced to ensure consistency. Acceptance: 5%.
- d. Purified water system quality is monitored and conductivity is checked prior to use. There is a conductivity meter built into the still for continuous, on-line verification.
- e. Fume hood efficiency is metered and verified annually by University of Maine Office of Facilities Management.
- f. The drying oven temperature is documented daily. Adjustments are made as necessary. Acceptance: $75^{\circ}\text{C} \pm 3^{\circ}\text{C}$ or $50^{\circ}\text{C} \pm 3^{\circ}\text{C}$ if used for sample prep.
- g. All containers are labeled. Chemicals are dated and coded when received and expiration dates checked. All solutions prepared are labeled with contents, date prepared, and initials of analyst. The chemicals and solutions are also entered in the appropriate log books; solutions are given a unique code based upon the date made.

h. Routine paperwork and reagent tracking: Reagents are coded when received. When used, this code is noted in the 'comments' column in the reagent/standard log book.

i. A log is kept for gas tanks. The date changed and tank ID number are recorded.

8.0 Instrument Maintenance:

All maintenance is recorded in the instrument log book.

8.1 Routine Maintenance:

Routine maintenance is performed according to the instrument manufacturers recommendation. All maintenance is recorded in the maintenance logbook and in the runlog for the particular day.

9.0 Calculations:

9.1 Percent Relative Difference (%RPD) (for samples other than MS / MSD:

$$((\text{Sample} - \text{Duplicate}) / ((\text{Sample} + \text{Duplicate})/2)) \times 100 = \%RPD$$

9.2 Spike Recovery (%R):

$$((\text{Spiked Aliquot} - \text{Sample}) / \text{True Spike Value}) \times 100 = \%R$$

9.3 Response Factor (RF):

$$(\text{SnCl}_2 \text{ corrected area} / \text{standard conc.})$$

9.4 RSD:

$$100 \times (\text{std. dev.} / \text{ave. RF})$$

10.0 Notes:

10.1 Low or variable Peak Heights:

- Check U-tube for any deposits or cloudiness. This, in all likelihood, is due to SnCl₂. Clean as described in 5.21.
- Check tubing and connections for gas and/or liquid leakage.
- Check that the pump tubing has not slipped off the rollers of the pump. Tubing will travel if the tension is not tight enough.
- Check that standard concentrations are correct.
- Check for blockage in the tubing, especially at connections.

10.2 Sample probe freezes:

- a. Re-initialize the autosampler using the 'autosampler' option under 'accessories'.
- b. If 'a' does not work, turn off the autosampler and computer and re-load the software as in the beginning.
- c. Check that the sampler tubing is long enough for the sample locations being analyzed.

10.3 Calibration blank high:

- a. Flush tubing with 10% HNO₃ for a few minutes prior to re-analyzing.
- b. Replace tubing and/or sample probe.
- c. Vial has become contaminated.

10.4 Paying close attention to technique, cleanliness of the analytical environment, and regular routine maintenance of the instrument will alleviate many progressive, long-term problems.

10.5 Spike calculations: Enter the SRM/spk weight as 1.000g in the Merlin program. This will enable you to find the **weight**, not the concentration, of Hg in the SRM/spk

1. Calculate the weight of Hg in the SRM sample by multiplying the SRM sample concentration by the weight of sample used in SRM/spk
2. Subtract this value from the weight of Hg in SRM/spk
3. This value should be approximately equal to the 250 ng of Hg that was added via the spike...calculate spike recovery.

$$\% \text{ Recovery} = \frac{(C_1 - C_2) * 100}{(C_1 + C_2)/2}$$

C₁ = Spike weight before digestion

C₂ = Spike weight after digestion

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METHYLMERCURY ANALYSIS USING THE BROOKS RAND COLD VAPOR ATOMIC FLUORESCENCE SPECTROMETER

1.0 Scope and Application:

This method is for determination of methyl mercury in environmental samples by ethylation, purge and trap, desorption, and cold-vapor atomic fluorescence detection. It generally follows the analytical procedures in EPA method 1630, AMethyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, andCVAFS.

2.0 Reagents:

2.1 Reagent Water: Water in which mercury is not detected by this method; 18 M ultrapure deionized water starting from a prepurified (distilled, R.O., etc) source.

2.2 Sodium acetate buffer: Dissolve 136 grams sodium acetate (CH_3COONa) in 59ml high purity glacial acetic acid (CH_3COOH) and dilute to 500 mL with low-Hg distilled deionized water (DIW). Store in a clean FEP Teflon bottle.

2.3 Potassium Hydroxide 2% (KOH): Dissolve 2 grams KOH in 100 mL low-Hg DIW. Store in a clean FEP bottle.

2.4 Sodium tetraethylborate 2% ($\text{NaB}(\text{CH}_2\text{CH}_3)_4$) or (NaBEt₄):

Note: Read MSDS sheets before handling.

Store 1 gram bottles of NaBEt₄ in a freezer until needed.

- Place the following inside a glove bag in the laminar flow hood: 1) one 1 gram bottle of NaBEt₄; 2) a clean FEP Teflon bottle containing 100 mL solution of 2% KOH in low-Hg DIW.
- Attach a Hg-free nitrogen gas line to the glove bag. Seal and fill the bag with nitrogen.
- Insert hands in the gloves and open the NaBEt₄. Transfer some of the 2% KOH from the FEP bottle into the container of NaBEt₄ then back into the Teflon bottle. Repeat this procedure several times in order to completely transfer the NaBEt₄. Close all containers.
- The 100 mL solution of NaBEt₄ is then divided into 2 mL aliquots, stored in 3 mL Teflon vials, and frozen until needed.
- Preparation of ethylating agent:
 - Remove one Teflon vial of NaBEt₄ from the freezer, cover, and place in laminar flow hood until it just begins to thaw. At this point it is ready to use. Store in a cooler with two ice packs in-between aliquot removal. Note the number of the vial on the run sheet.
 - The ethylating agent in each vial will last from 5 to 6 hours. After this time period, a new vial is removed from the freezer and used.

2.5 Standards:

- Stock Methyl Mercury Standard: 10 ng/ml methyl mercury chloride in isopropyl alcohol. Presently, this is purchased from Accustandard.

- b) Working Methyl Mercury Standard: Dilute 10 mL of the stock standard to 100 mL with 2% HCl. The actual concentration of this solution will vary based upon the stock standard source. Presently (Feb 1997), this is 0.8 ng/ml and is corrected for during the final calculations. This standard is stored, double-bagged, in refrigerator #1 at all times.
- c) Calibration Standards: These are dilutions of the working standard made directly in the bubblers in DIW.
- 2.6 Hydrochloric Acid: Trace-metal grade from Fisher Scientific.
- 2.7 Nitrogen : Standard laboratory grade further purified by removal of mercury using a gold-coated sand trap.
- 2.8 Argon: Grade V (ultra high-purity, GC grade) inert gas further purified by removal of mercury using a gold-coated sand trap. An oxygen trap is inserted, in-line, just before the analyzer flowmeter.

3.0 Equipment List:

- 3.1 Brooks-Rand Cold Vapor Atomic Fluorescence Spectrometer, model 2.
- 3.2 3 - 220 mL bubblers with 4-way stopcocks; 1 - 125 mL round-bottom bubbler with 4-way stopcock.
- 3.3 4 flowmeters - Cole parmer.
- 3.4 2 nafion drying tubes.
- 3.5 3 veriac transformers.
- 3.6 GC oven with cryogenic GC column: phase: ov-3, 15%; support: 60/80 mesh chromosorb w-aw-dmcs (see appendix).
- 3.7 Pyrolytic organo-mercury breakdown column.
- 3.8 Nichrome wire coil for carbotrap heating.
- 3.9 Carbotraps with Teflon end plugs.
- 3.10 Automatic pipetters.
- 3.11 Gold-coated quartz sand traps with Teflon end plugs.
- 3.12 Computer: Dell Dimension p75t pentium.
- 3.13 Quicklog data acquisition software program.
- 3.14 1 argon regulator.
- 3.15 2 nitrogen regulators.
- 3.16 Milli-Q RG ultra pure water system; Millipore corporation.

4.0 Procedure:

4.1 Brooks-Rand Analyzer:

Set >Gain= at 8.5 and >Background= at approximately 4.90. Allow a stabilization period of at least 2 minutes, then adjust the output reading on the front of the analyzer to approximately 12 mV using the background knob. The gain may be adjusted based upon the expected sample concentrations.

High purity argon is used as the carrier gas for the analysis phase:

- Flowrate: 30 ml/min.
- The flowmeter on the analyzer is used only to meter flow. The actual flowrate is controlled by a flowmeter located before the carbotrap and GC oven. Flow is adjusted with this secondary meter until the analyzer meter (flowmeter #NO32-15) reads 50 units.
- a gold trap is placed in-line just prior to the argon entering the flowmeter to trap any

mercury contamination in the gas. Gold traps are cleaned periodically by heating as for total mercury analysis (the analyzer configuration is different than for methyl analysis; see >Total Mercury Analysis SOP).

4.2 Ethylation Setup (in hood):

a. Bubblers:

Between runs, these are stored in the hood, filled with 0.4% HCl. Prior to use, they must be rinsed thoroughly with low-Hg water and bubbler >stems= leached and filled with DIW at least twice. These steps are important as the ethylating agent is pH sensitive.

b. 2 Nafion drying tubes (24@ in length):

Bubblers are run in pairs, 2 per drying tube.

c. Nitrogen gas flows:

Gas flow is >in= the top and >out= the bottom.

1. Tank #1 (appr. 18 psi):

This tank is used to flush the nafion drying tubes of water or acid vapors that could destroy the carbotraps; Flowrate: 1029 ml/min.

Flowmeter used: #1388 at a setting of 25 units.

2. Tank #2 (appr. 12-15 psi):

This tank is connected to the bubblers via 2 flowmeters at a flowrate of 120 ml/min:

Flowmeters used:

#12-10 at 22 units.

22-13 at 36 units.

Flow to the bubblers is controlled using the individual shut-off valves on the tank regulator.

- As for the argon, a gold trap is placed in-line prior to the nitrogen entering the flowmeters and must be cleaned periodically by heating (see >Total Mercury Analysis SOP).

4.3 Variac Transformer Settings:

a. Transformer A:

Setting: 17.5.

This controls the pyrolytic cell temperature of 900 EC. This need not be turned on until the morning of analysis. Allow a 1 hour warmup. This transformer setting can be adjusted as the cell and coil age.

b. Transformer B:

Setting: 19.5.

This maintains the GC oven temperature of 110E C. The oven requires a long warm-up period; preferably it is turned on the day before an analysis is to be done with an argon flow of appr. 15-20 units. Typically, this is left on most of the time.

c. Transformer C:

Setting: 10.0. This controls the carbotrap heating temperature of 350EC. The length of heating is controlled by the timer clock mounted on the wall. For methyl analysis, this time is 45 seconds. Turn on day of analysis.

5.0 Sample collection:

Note: Blank carbotraps prior to use by heating them for 1 minute as described for sample analysis.

This step does not need to be saved in a file.

Solutions containing methyl mercury should be kept in the dark when not in use.

- 5.1 Turn on nitrogen tank #1 a few minutes before beginning collections. It remains on for the duration of the run.
- 5.2 Rinse bubblers :
 - a. Before first use, rinse and fill bubblers with DIW. Allow bubbler stems to fill. Remove tops, wait for stems to drain and return to bubblers. When stems are again full, remove, drain, and rinse bubblers. They are now ready for use.
 - b. In-between samples, rinse bubblers three times with DIW. Immerse bubbler stems in a container filled with DIW. Once stems are full, remove and allow them to drain. Bubblers are now ready for use.
 - c. Once bubblers are rinsed, add either 100 mL of DIW or distillate - see 5.4 a. and b.
- 5.3 Add 200 μ L of the acetic acid buffer to the bubblers (this is to be done prior to sample or standard addition; change tip between distillates). The solution pH after this addition should be appr. 4.9 ; pH paper is a quick, accurate testing method: remove bubbler top and allow a drop to fall onto a strip of pH paper; do not immerse paper in sample. Add more buffer if necessary.
- 5.4 Add the appropriate amount of sample/standard to a sufficient amount of low-Hg DIW in one set of bubblers. The final volume should be appr. 100 mL in all analyses.
 - a. Standards:
 The working standards are dilutions made from the 1 ng/mL MeHg standard and must bracket the expected sample concentration range. The following amounts are typical:
 Distillations: 10, 25, 50, 100 μ L.
 Alkaline Digestions: 10, 25, 50, 100, 250, 500, 1000 μ L.
 - b. Samples:
 1. Distillations:
 Distillations are quantitatively poured into the bubblers. The Teflon vials in which they were stored are rinsed at least twice with appr. 20 mL of low-Hg DIW. The rinsate is then added to the appropriate bubbler - do not exceed appr. 100 mL total volume in bubbler.
 2. Alkaline Digestions:
 Leave samples in oven except when pipetting. Do not agitate samples. Weigh samples on the morning of analysis. It is best not to use more than 50 μ L of sample due to possible interferences, etc.
 Analytical/pipetting techniques are critical to quality data. Samples are kept in a 75°C oven until needed. Prior to pipetting a sample, equilibrate the pipettor with several pumps of the headspace gas (methanol vapors) to avoid vapor pressure occurring and pushing the sample back out of the tip. Do not immerse the tip any farther than absolutely necessary to avoid adhesion to the outside of the tip (do not sample rinse the tip). Eject the aliquot into the bubbler, beneath the surface of the water and rinse tip 3 times with bubbler water; again, immerse the tip only as far as necessary. Use the smallest pipette tip possible. Serial dilutions in methanol (10:1) can be used for low concentration samples.
- 5.5 Add 50 μ L of the 1%NaBEt₄/2%NaOH solution to the bubblers, rinsing the tip with the bubbler solution. Clamp reaction vessel and bubbler top together.
- 5.6 Mix gently and let stand 10 min (check that the 4-way valve is closed - vertical).
- 5.7 Connect nitrogen lines (gas in top).

- 5.8 After 10 mins, place a carbotrap on the end of each drying tube (remove both caps) with constriction toward bubblers.
- 5.9 Begin aeration by turning on the regulator valves and opening the 4-way bubbler valves. Aerate for 20 minutes.
- 5.10 When completed, turn off the nitrogen flow at the regulator and close the 4-way valves. Remove traps and cap the ends immediately.
- 5.11 Bubbler tops and flasks are rinsed 3 times with low-Hg DIW as described in step 5.2.

6.0 Carbotrap Analysis:

Carbotraps for one set of bubblers are analyzed while the second set is being aerated. It is important to develop a consistent pattern to performing the steps of this method in order to facilitate sample processing.

6.1 Data Storage (DOS version):

- a. To activate the Quick Log program for data collection, choose the >shut down and restart in DOS= option found through the >Start= icon for Windows 95.
- b. Type Acd/QL@ at the DOS prompt. Enter.
- c. Type Ago@. Enter.
- d. The program automatically boots up in the desired configuration.
- e. Click on the TEXT.TXT icon and select >save as=. Type in the file name. The name must be changed prior to analysis of each carbotrap for later identification in the peak search program. Typically, files are named using month-day formats. ex: Dec30A, Dec30B, etc.

Data Storage (Windows version):

- a. Double-click on the >Shortcut to Workbench.exe= icon.
- b. Hit >Enter= to remove information window.
- c. Under >File= click on >open=.
- d. Double-click on >Methyl1.wbw= file.
- e. To blank traps: start timer (1 min.) and click on the solid arrow icon directly beneath >File= in the upper left.
To analyze sample traps: double-click on >Write 00= module, click on >file name= box, type in desired file name, and click on >OK= for both windows. Start timer (45 sec) and note peak height at 1.60 on chart.
- f. When finished, choose >exit= under >file=.
- g. Area determination is the same as described in section 6.3 except sample files are found in the >wbw= file, not the >QL= file.

6.2 Trap Analysis:

- a. Adjust flowrate of Brooks-Rand analyzer to 50 units with external flowmeter.
- b. Turn off argon at regulator.
- c. Remove carbotrap from spring clamp, disconnect gas lines, and slide heating coil over untaped end.
- d. Connect taped end of new carbotrap to gas lines (constriction downstream - to left). Slide heating coil over opposite end and connect the remaining gas line. Place trap in clamp. Be sure heating coil covers the carbotrap completely.
- e. Cap ends of removed trap.
- f. Turn on argon.

- g. Set up file to save data as above.
- h. Set heating coil timer for 45 seconds and immediately start the collection program (For DOS: click on >off= and >restart= the peak chart) .
- i. Make sure the elapsed time has finished before changing the file name. This also allows time for the trap to cool. Traps may be changed once the desired peak is complete.
- j. Note the mV reading for the MeHg peak (read as methylethylmercury) which occurs at 2 minutes on the computer chart.
- k. When all carbotraps have been analyzed, quit the program and do not save changes to AUTOBOOT at the prompt.

6.3 Area Determination:

- a. Peaks may be integrated during the run if using the windows version.
 - b. Double-click on the >Peak Search= icon.
 - c. Under the file header click on >import= followed by >mercury machine=.
 - d. Double-click on drive >c= then on >QL= for DOS version or >wbfw= for windows version.
 - e. Double-click on desired file name.
 - f. Click on >OK=.
 - g. Click on >yes=.
- Type in >500= points in place of 1000 if allowed, wait for hourglass to disappear, and hit enter.
- h. Click on first icon in command line.
 - i. Click on icon in lower left of screen.
 - j. When integration is complete, find the desired peak number using the data format and peak center location (at approximately 100 on the graph). Scroll down to analyte area listing and note area equivalent to the peak number from above on the run sheet.
 - k. Once all areas have been calculated, exit to windows and call up the appropriate file in excel.
 - l. Copy the necessary headers from the >formula= sheet onto the calculation sheet (note: there are separate sheets and different calculations for alkaline digestions and distillations).
 - m. Fill in all necessary information; be sure to change position numbers in all formulas accordingly.
 - n. Save and print out the day=s run. Make 2 copies: 1 for hard copy files and 1 for QC data entry. The original is attached to all information pertaining to the day=s run and filed, back to front by date, in a loose-leaf ring binder.

7.0 Analytical Quality Control:

An analytical batch is a set of not more than 20 samples analyzed with the same instrument calibration and QC data. Calibration standards should bracket the expected sample concentration range. An analytical batch must contain the required QC in order for generated data to be considered valid. All daily activities and problems are to be entered in the log book.

7.1 QC Sample:

A calibration QC is a sample at a concentration between 25-75% of the maximum value of the calibration range and prepared from a source independent of the calibration standard. The QC sample is used to ensure the analytical process is in control. This sample is run immediately after calibration and is

expected to be within accuracy objectives. Additionally, QC sample concentrations are plotted on a control chart.

7.2 Detection Limit QC Sample:

This is at a concentration approximately 4 - 6 times the expected method detection limit and is run once per analytical batch. Detection limit samples are prepared in a similar manner to QC samples. The results document instrument detection limit capabilities.

7.3 Laboratory and Reagent Blanks:

Laboratory blanks are made from reagent grade water and are carried through the entire sample preparation procedure - 1 per sample batch. Reagent water blanks include all chemicals and reagents used in the analysis of samples. The level of analyte in the blank should be less than the detection limit for that matrix. A High@ blanks (greater than the detection limit) are regarded as evidence of contamination and all relevant samples are reprocessed when possible, or flagged if re-analysis is not feasible.

7.4 Analytical Duplicates:

Analytical duplicates are samples that have been collected, preserved, and prepared as one sample, but analyzed at separate times. These are analyzed at a frequency of 5% of the total sample number and are used to establish analytical precision and error. The relative percent difference (RPD) of each sample is calculated and recorded on the appropriate control chart. %RPD should be .

7.5 Matrix Spike Samples (Analytical Spikes):

A matrix spike (or fortified sample) is prepared by spiking a sample duplicate with a known amount of analyte. The spike source is typically the working standard. This is used to determine the effects of the matrix on the recovery of the analyte. The spike concentration is typically 3 to 5 times greater than the expected level of the analyte in the sample. The spike should be added in as small a volume as possible and can be considered negligible to the total volume of the sample. The matrix spike recovery objective is 80-120 %. The formula for matrix spike recovery is found in section 7.0.

7.6 Method Detection Limit (MDL):

Measurement of the method detection limit on a yearly basis is necessary in order to provide regular assessment of instrument/method performance, as well as a quantifiable concentration that will indicate when a measured value is above zero and is in fact detectable by the instrument. The MDL is defined as the minimum concentration of a substance that can be measured and reported with a 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte. The concentration of the low-level check standard should be 3 to 5 times the required detection limit. The detection limit is determined by analysis of seven replicates at a concentration 3 to 5 times the estimated detection limit and calculated by the student T-test as outlined in the 40 CFR part 136, Appendix B.

7.7 Control Charts:

Control charts are used to track variation of a process or analysis over time. The variation from the expected mean should exhibit a random distribution. Variation from the expected value may indicate instrument or human error. Persistent variation in one direction indicates bias. Control charts are expected to be within +/- 2 standard deviations from the expected mean. The following general guidelines are useful for interpreting control charts. The results are expected to be distributed randomly about the mean or central line.

One point outside of the control limits is acceptable. Two consecutive points require investigation. Three or more points outside of the control limits mandate corrective action.

7.8 Laboratory Practices:

These include:

- a. Analytical balances are serviced yearly by a factory-authorized representative.
- b. Certified weights are used to check the accuracy of a balance on a monthly basis. The weights are kept in a dessicator between usage.
- c. Automated pipettors are checked on a monthly basis and prior to making primary standards.
- d. When a new calibration standard is made, it is compared to the standard being replaced to ensure consistency. Acceptance: 5%.
- e. Purified water system quality is monitored and conductivity is checked prior to use. There is a conductivity meter built into the still for continuous, on-line verification.
- f. Fume hood efficiency is metered and verified annually by University of Maine Office of Facilities Management.
- g. The drying oven temperature is documented daily. Adjustments are made as necessary. Acceptance: 75 C +/- 2 C.
- h. All containers are labeled. Chemicals are dated and coded when received and expiration dates checked. All solutions prepared are labeled with contents, date prepared, and initials of analyst. The chemicals and solutions are also entered in the appropriate log books; solutions are given a unique code based upon the date made.
- i. Routine paperwork and reagent tracking: Reagents are coded when received. When used, this code is noted in the >comments= column in the reagent/standard log book.
- j. A log is kept for gas tanks. The date changed and tank ID number are recorded.

8.0 Instrument Maintenance:

All maintenance is recorded in the instrument log book.

8.1 Routine Maintenance:

Routine maintenance is performed according to the instrument manufacturers recommendation. All maintenance is recorded in the maintenance logbook and in the runlog for the particular day.

9.0 Calculations:

9.1 Distillations:

X-axis = Area Y-axis = pg MeHg as Hg

MeHg as Hg, pg = (Area*Slope) +/- Intercept

MeHg as Hg, ng/L = ((Hg, pg/1000)/volume distilled, mL)*1000(wet weight)

9.2 Alkaline Digestions:

X-axis = Area Y-axis = pg MeHg as Hg

MeHg as Hg, pg = (Area*Slope) +/- Intercept

Sample Injected, g = (sample wt, g/digest vol, mL)*Injection vol, mL

MeHg as Hg, ng/g = (Hg, pg/1000)/sample injected, g (wet weight)

9.3 Methylene Chloride Extraction:

X-axis = Area Y-axis = pg MeHg as Hg

MeHg, pg = (slope*area)+/- Intercept

MeHg, pg/g = MeHg, pg/Total grams used

MeHg as Hg, ng/g = MeHg, pg/g / 1000

9.4 Percent Relative Difference (%RPD):

$$((\text{Sample} - \text{Duplicate}) / ((\text{Sample} + \text{Duplicate})/2)) \times 100 = \%RPD$$

9.5 Spike Recovery:

$$((\text{Spiked Aliquot} - \text{Sample}) / \text{True Spike Value}) \times 100 = \%R$$

10.0 References:

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APPENDIXES

1. Brooks Rand LTD. Parts List; October, 1996
2. GC column technical and ordering information.
3. Sample of methyl analysis run sheet.
4. Diagrams of physical setup.