Second Report on Interlab Comparisons and Quality Control/Quality Assurance Data from laboratories participating in the Penobscot Mercury Study

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I. General description of the Quality Assurance/Quality Control (QA/QC) Program for the Penobscot Mercury Study

"Quality control" refers to *procedures that are done on a regular basis* in laboratories to determine the precision and accuracy of the methods used. For mercury and methyl mercury analyses, these procedures typically include the analysis of certified reference materials, internal standard additions of mercury or methyl mercury, measurement of the amount of trace mercury in reagents used in mercury analyses, determination of the precision of duplicate analyses, etc. In addition to *analytical* quality control procedures, there are also *field sampling* quality control procedures. For samples that will be analyzed for mercury, this includes verification of sampling handling techniques through handling of blank water in the same way that sample water is handled, and by taking replicate samples from the same site.

"Quality assurance" refers to a planned system of *review procedures* conducted by *personnel not directly involved* in the laboratory analysis of samples. It also refers to the *reporting system* by which the project laboratories provide information on the routine technical procedures that are used in "quality control". A good reporting system is complete and transparent, i.e., the reviewer can easily discern if standards, duplicates, reference materials, and recovery procedures are being tested out on a routine basis, and if the results of these procedures are within acceptable limits. Acceptable limits can be defined by agencies such as the EPA, by the analytical laboratory(s), or agreed upon between the client and the laboratories.

The two laboratories that routinely analyze samples for the Penobscot Mercury Study are Battelle Marine Sciences Laboratory in Sequim, Washington, and Flett Research Ltd. in Winnipeg, Manitoba. These two laboratories have achieved general accreditation by appropriate national agencies (the National Environmental Laboratory Accreditation Program in the U.S. and the Canadian Association of Environmental and Analytical Laboratories).

A third laboratory, run by Dr. Holger Hintelmann at Trent University in Trent, Ontario, has participated in inter-laboratory comparisons. Dr. Hintelmann is widely acknowledged to be an expert in the field of mercury analytical techniques.

The types of samples taken for the Penobscot Mercury Study include water, sediments, fish and other biota.

The goals of the QA/QC program for this Study are:
1. To monitor the quality control data provided by each laboratory for compliance with the standards set for accuracy and precision, for each type of analyses.
2. To monitor the results of tests of the field handling procedures for mercury samples.
3. To ensure that interlab comparison exercises for mercury in water, sediments, and tissues is carried out at least once a year.
4. To make recommendations to the laboratories, the field crew, and the study leaders regarding any needed improvements in sampling or analytical procedures.

II. Review of Quality Control Procedures and Transparency

The two laboratories that carried out routine analyses of Penobscot Mercury Study samples were Battelle Marine Sciences Laboratory in Sequim, Washington, and Flett Research Ltd. in Winnipeg, Manitoba. Details of the methods used were available, and are included in the Appendices of this report. Both of these laboratories reported their quality control data in a suitably transparent manner, and included these QC data with each set of analytical results provided to the Study. The quality control data included the results of analysis of a reference material suitable to the type of sample, e.g., when sediments were being analyzed, the reference material was a sediment and when biota tissue was being analyzed, the reference material was dogfish or lobster hepatopancreas. The reference materials used for each method are summarized in Appendix B. Also included were the results of spike matrix analyses, where a known spike of mercury or methyl mercury was added to a subsample of the material being analyzed, and the recovery of this known spike was determined. In addition, duplicate analyses were done and reported. Reagent blanks were analyzed for mercury or methyl mercury, depending on the analysis being done. If these quality control measurements were not within the guidelines established, the laboratory repeated the analyses.

In addition to the results for interlab comparison from both laboratories, the analytical reports on routine Penobscot Study samples were also made available to this reviewer, and were reviewed on a regular basis. These reports included results for water, sediment, and biota.

Field sampling records were also made available for review by Normandeau Associates, including the chain of custodyrecords. These were checked against records kept by the analytical laboratories for any problems that might occur in identifying samples sent from the field. Normandeau also made available a copy of their Standard Operating Procedure, with descriptions of the field sampling procedures to be followed. These were reviewed, and some suggestions were made on the procedures for taking blank samples. These suggestions were incorporated.

Overall, it is important to note that both analytical laboratories and Normandeau Associates have made available for review all information that was requested. This transparency in procedural details and in quality control data is essential to proper review, and there have been no problems in this area.

III. Water Sampling and Analyses

Samples were collected in the field by Normandeau Associates. personnel. They were shipped to the analytical laboratory in coolers by courier. Laboratory procedures on receipt of samples were to check the cooler temperature to ensure that it was within the optimal temperature range for unpreserved samples (4±2°C), and to preserve the samples within 48 hours. Freshwater and brackish water were preserved with HCl, added to a final concentration of 0.5%. Seawater samples were preserved with H_2SO_4, added to a final concentration of 0.2%. Total Hg samples were analyzed within 90 days and MeHg samples within 180 days. During Phase I of the Study, routine water analyses were done at Battelle Laboratory.

Analytical precision—analysis of duplicates

The precision of analyses of mercury in water was determined by carrying out duplicate analyses on single samples. (Note: analytical duplicates are different from field replicates, where two different samples are taken from the same site, and sample to sample differences will play a role in the variability of the results. For analytical duplicates, only differences in the analytical operations affect the precision.) All routine water analyses for the Penobscot Study were done at Battelle Laboratory, and the analytical duplicate data are from this lab.

The relative per cent difference (RPD) for each pair of duplicate analyses was calculated (% RPD = ((Sample A- Sample B)/ (average of A & B))*100. The average RPD's for both total mercury and methyl mercury duplicates done during both the May 2007 and July 2007 sampling periods (Table 1) were well within the recommended limit of 24% (EPA Method 1631).

Table 1. Precision in analytical duplicates of unfiltered water samples. Analyses done at Battelle Marine Sciences Laboratory. The recommended EPA limit is +/- 24%.

Sampling Period (2007)	**Total Mercury**		**Methyl Mercury**	
	Average %RPD	**n**	**Average %RPD**	**n**
May 31-June 2	**4.4** +/- 3.2	9	**10.3** +/- 11.2	**8**
July 10-12	**5.2 +/- 3.6**	**12**	**8.9 +/- 7.9**	**14**

Sample handling--Field blank results

The purpose of field blank measurements was to check for contamination of water samples that can occur through contact of the water or sample bottles with 1) the personnel doing the sampling, 2) the sampling equipment, 3) the immediate surroundings, or 4) the inside the coolers in which samples were stored. The general approach was for the analytical laboratory to send "blank water" (water that has undetectable mercury) to the field, where the field crew carried out the same handling procedures with this blank water as are used for handling samples. These "field blank" samples are then analyzed to see if any contamination of the blank water has occurred during this handling. In addition, some bottles of blank water were shipped to the field site but never opened, and shipped back to the laboratory. These blanks are called "trip blanks". They reflect contamination that can occur simply through diffusion of elemental mercury through the Teflon or polypropylene, and/or on opening the bottles in the laboratory on return.

During Phase I, field blanks were part of the regular Penobscot Mercury Study sampling program, and were done usually at a frequency of every fifth sampling site. In 2007, there were two periods of water sampling (May 31-June 2, and July 10-12). Blank water was supplied by Battelle MSL, and mercury analyses were done by Battelle. Field procedures were carried out by Normandeau Associates at field sites on the Penobscot River.

Field Blanks--Methyl mercury in water. Field blank results for methyl mercury in water were acceptable in 2006 (previous report) and also in 2007 (Table 2 below). MeHg was undetectable (<0.0188ng/L) in the blank water sent to the field. After this water was passed through the sampling apparatus (unfiltered blanks), MeHg was just above detection (0.019 to 0.024 ng MeHg/L, Table 2). When this water was passed through both the sampling apparatus and the filter (filtered blanks), MeHg was also just above detection (0.018 to 0.026 ng MeHg/L, Table 2).

For methyl mercury, the field blank concentrations in May were below or just above the detection limit. This is the desired outcome for MeHg field blanks. In July, however, blanks had measureable MeHg. This is surprising, as MeHg is not generally present in sufficient quantities to cause contamination. The trip blanks also showed measureable MeHg (Table 2), so field procedures may not have been the problem. Field blank levels should continue to be watched in the future.

While the MeHg concentrations measured in the blanks were very low, blank concentrations can not be disregarded in data analysis. In regular samples taken in 2007, the average MeHg concentrations were 0.08 to 0.15 ng MeHg/L, depending on the sampling period, and whether the sample was filtered or unfiltered (Table 2). Where the sample concentration is at least 5 times the blank concentration, the blank correction might not be important. However, for samples at the low end of the concentration range (0.02 to 0.10 ng MeHg/L), there may need to be some correction for, or at least acknowledgment of, the concentration of MeHg that could be due simply to sample handling.

Field Blanks--Total mercury in water. On both sampling occasions in 2007, field blank results for total mercury in water were acceptable (Table 2). This had also been the case in the last sampling period of 2006. Earlier in 2006, however, some of the THg field blank results were higher than desired. However, it could not be resolved whether the high THg results were caused by contaminated blank water, or by faulty field procedures, because there were some uncertainties in the record with respect to the mercury level of the blank water sent to the field from Studio Geochimica in early 2006. In the last sampling period of 2006, blanks were done using water sent by Battelle Laboratories, and the field blank results were well within the acceptable range (< 0.5 ng THg/L, previous report).

Total mercury is more likely than MeHg to show up as a contaminant due to sample handling, but the total mercury field blanks demonstrated good handling procedures. Blank values were about 0.2 ng HgT/L, which is below the recommended limit of 0.5 ng HgT/L (EPA Method 1631). Blank values were well below the average values of samples (1 to 4 ng THg/L), and also below the lowest sample values (0.5 to 0.6 ng HgT/L). As with MeHg samples, an acknowledgement of blank contributions is recommended for these very lowest THg sample values.

Trip Blanks for Total and Methyl Mercury in Water. Trip blanks were done in July, 2007, and analyzed for total mercury. These trip blanks had approximately the same increases in MeHg and THg, compared to the original blank water, that the field blanks showed (Table 2). This shows that field procedures added very little in the way of contamination, compared to unavoidable contamination that occurs during shipping and handling in the laboratory.

Table 2. Results for field blanks done in association with water sampling. Values with * are below detection limits (0.0188 ng MeHg/L and 0.188 ng THg/L).

Sampling Period		MeHg ng/L	THg ng/L
May 31-June 2/07	Average, unfiltered blanks, n = 5	0.019	0.213
	Average, filtered blanks, n = 5	0.018	0.192
	Blank Water Sent to Field 4/23/07	0.0188*	0.188*
	Water Sample results (for comparison to blanks)		
	Unfiltered, mean +/- S.D.	0.143 +/- 0.062	2.95 +/- 0.90
	Filtered, mean +/- S.D	0.112 +/- 0.056	1.945 +/- 0.631
	Low samples, filtered, range	0.02 to 0.10	0.7 to 1.5
July 10-12/07	Average, unfiltered blanks, n = 7	0.024	0.241
	Average, filtered blanks, n = 7	0.026	0.224
	Trip Blank Water Sent to Field 7/9/07	0.0188*	0.188*
	Average, Trip Blanks	0.0282	0.2219
	Blank Water Sent to Field 6/28/07	NA	0.188*
	Water Sample Results (for comparison to blanks)		
	Unfiltered, mean +/- S.D.	0.153 +/- 0.173	3.72 +/- 5.75
	Filtered, mean +/- S.D	0.082 +/- 0.061	1.32 +/- 0.48
	Low samples, filtered, range	0.02 to 0.05	0.6 to 1.3

Overall, the field blank results demonstrated no obvious problems in field procedures, with respect to contamination of samples by shipping or sampling procedures. However, the blank results do need to be taken into account when using results from samples when THg or MeHg concentrations are very low.

Combined sampling and analytical precision-- Field replicates

Field replicates are independently taken samples, as opposed to analytical duplicates. This means that two separate bottles were taken at each site, for each of the mercury analyses that would be done later (filtered methyl mercury, unfiltered methyl mercury, filtered total mercury, and unfiltered total mercury).

The purpose of the replicate sampling program was to determine the variation that is inherent in taking a sample from waters where currents may cause spatial heterogeneity in surface water concentrations. Good replication is an indication of good sample handling, but it should also be kept in mind that there is natural heterogeneity in water systems, especially for particulates. Thus, it is expected that the reproducibility for filtered waters will be better than for unfiltered waters, where different concentrations of particulates may introduce differences in total concentrations of mercury in samples. Measurements of the mass of particles (total suspended solids, or TSS) was included in this aspect of the study.

Replicate samples of water for mercury analyses were taken in the 5 Phase I sampling periods, from late August, 2006 through July 2007. In addition, for the last 3 sampling periods (October 2006, May 2007 and July 2007), replicate samples were taken for total suspended solids (TSS). Field replicate results for the 2006 water samplings were reported earlier (July 2007), but are included here so that overall study trends can be examined.

In each sampling period, 28 to 38 replicates (pairs) of samples were taken for each analysis. The relative percent difference (RPD = difference between the two replicates divided by the average of the two replicates) was calculated for each pair (Table 3).

Total Mercury. Sample replication for both filtered and unfiltered total mercury was very good, with the relative percent difference (RPD) in replicate samples averaging from 3 to 13% (Table 3). These results are within the EPA guideline of less than 20% RPD in field replicates.

The RPD's for unfiltered samples were not consistently greater or smaller than the RPD's for filtered samples (Table 3).

Table 3. Relative percent difference (RPD) for pairs of replicate water samples analyzed for filtered total mercury and for replicate samples analyzed for unfiltered total mercury.

	THg filtered water Field Replicates		**THg unfiltered water Field Replicates**	
	Average RPD +/- Std Dev	**n**	**Average RPD +/- Std Dev**	**n**
Sep 6-11/06	11.4 +/- 11.1%	33	6.4 +/- 7.5 %	32
Sep 27-Oct 5/06	10.5 +/- 13.0 %	37	5.0 +/- 5.5 %	37
Oct 22-25/06	9.6 +/- 8.1%	35	10.3 +/- 9.0 %	34
May 29-June 1/07	6.7 +/- 8.6 %	35	5.9 +/- 8.1%	35
July 10-12/07	4.1 +/- 3.0 %	35	11.4 +/- 14.9 %	35

Methyl Mercury. The variability for replicate water samples taken for filtered and unfiltered methyl mercury (Table 4) was greater than for filtered and unfiltered total mercury (Table 3). This is somewhat expected because the methyl mercury concentrations are much lower than total mercury concentrations, and more difficult to measure analytically (*analytical* duplicates had RPD's of 9-10% for MeHg in water, compared to 4-5% for THg). However, field replicates showed much greater variation than can be accounted for by analytical variation alone (field replicate RPD's averaged 19 to 23% for filtered samples, and 12 to 33 % for unfiltered MeHg samples, Table 4). The variability is probably not due to contamination, as MeHg is not very abundant in air or on people handling samples. The most likely reason for the variability is heterogeneity in the water being sampled.

The average RPD's were within the range recommended by the EPA (less than 35%). However, some individual replicates obviously fell outside this range, especially for unfiltered MeHg samples (Table 4).

Table 4. Relative percent difference (RPD) for pairs of replicate water samples analyzed for filtered methyl mercury and unfiltered methyl mercury. Analyses by Battelle MSL.

	MeHg filtered water Field Replicates		**MeHg unfiltered water Field Replicates**	
	Average RPD +/- Std Dev	n	Average RPD +/- Std Dev	n
Sep 6-11/06	19.2+/- 30.5 %	38	28.1 +/- 23.8 %	34
Sep 27-Oct 5/06	23.0 +/- 22.5 %	36	25.8 +/- 24.8 %	37
Oct 22-25/06	21.8 +/- 15.2 %	28	33.4 +/- 23.8 %	29
May 29-June 1/07	15.6 +/- 15.8 %	35	12.2 +/- 8.2 %	35
July 10-12/07	18.5 +/- 21.5 %	35	15.9 +/- 15.0%	35

Total Suspended Solids (TSS). TSS is a direct measurement of concentrations of particulate matter in a water sample. In the Penobscot Study, these measurements are used, together with the difference between unfiltered and filtered mercury concentrations, in the calculation of the concentrations of mercury in particles.

Samples were taken for measurement of TSS in the October/06, May/07, and July/07 sampling periods. Blanks are not an issue for this measurement, but reproducibility in waters where turbidity may be heterogeneous is a concern. The relative percent difference for replicate pairs of samples averaged 10.6 to 16.7% in the different sampling periods (Table 5). There are no set criteria for this; rather the RPD's of replicates is useful as an indicator of the variability that must be taken into account in using TSS measurements.

Table 5. Relative percent difference (RPD) for replicate water samples analyzed for total suspended solids. Analyses by Battelle MSL.

	Total Suspended Solids, water Field Replicates	
	Average RPD +/- Std Dev	n
Oct 22-25/06	16.7 +/- 14.1%	34
May 29-June 1/07	10.6 +/- 8.0%	35
July 10-12/07	12.9 +/- 11.6%	35

Comparison of RPD's for different analyses, and over time. The RPD's for replicate pairs of samples were always highest in the results for methyl mercury in water (Figure 1). The RPD's were lowest for THg, with TSS in between.

During the two sampling seasons of Phase I, some trends over time can be discerned. For total mercury, reproducibility in sampling and analyses was consistent throughout all periods. Reproducibility for TSS was also consistently good, considering that particulates can be quite variable in water samples. For methyl mercury, however, reproducibility was slightly better in the last two sampling periods (Figure 1). Taken all together, the data indicate that precision in sample handling and analyses has improved slightly in 2007.

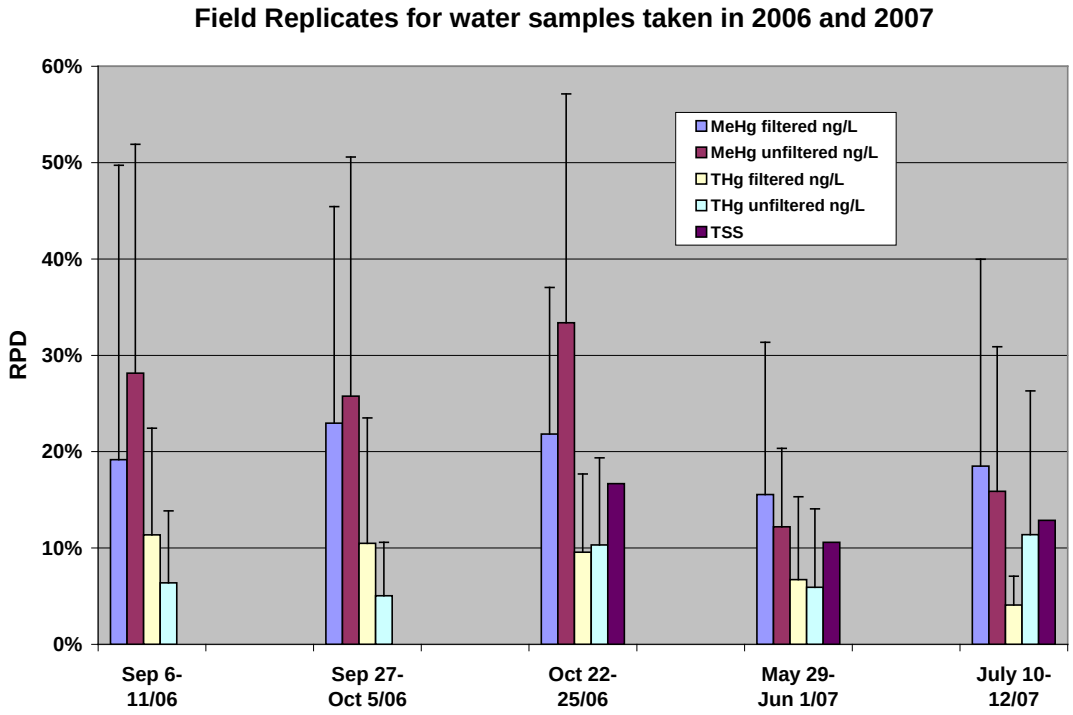

Figure 1. Average relative percent difference in replicate samples taken for total mercury (filtered and unfiltered), methyl mercury (filtered and unfiltered) and total suspended solids. Bars show the mean values, and the vertical lines show 1 standard deviation from the mean.

Interlab comparison on measurement of mercury in water, 2007

In May and July 2007, filtered water samples were taken from sites OV5 (freshwater) and OB2 (estuarine), and sent to the three laboratories that participated in the interlab comparison for measurement of both total and methyl mercury. All three labs used slight variations on EPA Method 1631e for total mercury in water, and EPA Method 1630 for methyl mercury in water. Flett Research and Battelle used Cold Vapor Atomic Flourescence Spectroscopy (CVAFS) for mercury detection, while Trent U. used Isotope Dilution Mass Spectroscopy (IDMS).

Total Mercury in Water. There was very little variability in the results for total mercury in water within each lab (Table 6). The variation among the labs was also small (Table 6, Figure 2), with the % standard deviations on the mean result obtained by all three labs were only 5 to 14% (calculated from data in last column of Table 6).

Table 6. Interlab Comparison results for total mercury in water, 2007.

Average THg ng/L +/- Std Dev.				
	Battelle MSL	**Trent University**	**Flett Research**	**All Labs**
OV5 May	2.65 +/- 0.05	2.21 +/-0.06	2.21 +/- 0.04	2.35 +/- 0.25
OB2 May	1.86 +/- 0.04	1.68+/- 0.07	1.79 +/- 0.09	1.78 +/- 0.09
OV5 July	2.03+/- 0.19	1.62+/- 0.02	1.59 +/- 0.15	1.75 +/- 0.25
OB2 July	1.34 +/- 0.07	1.20 +/- 0.04	1.14 +/- 0.06	1.23 +/- 0.10

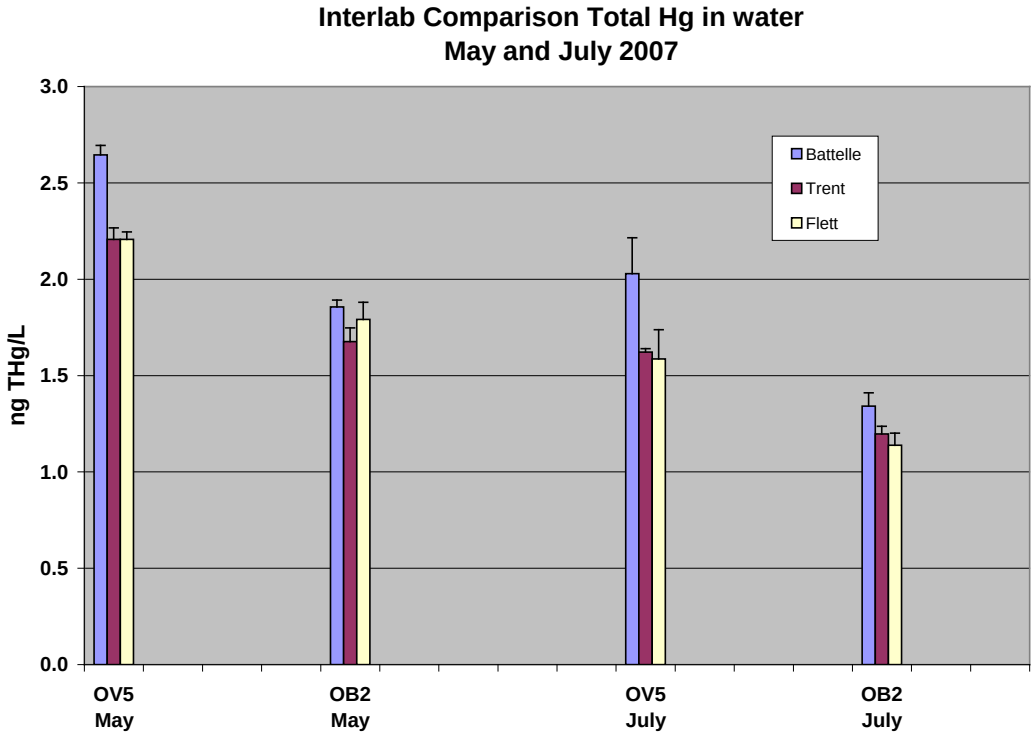

Figure 2. Average THg concentrations for each lab, plus the standard deviation for each lab's results, for the May and July sampling periods in 2007. The % standard deviations ranged from 5.1 to 14.1% of the mean values from all 3 laboratories.

A difficulty in interpreting the results of interlab comparisons where natural samples are used is that there is no "true" or "certified" value. However, the use of natural samples is necessary because components in water other than the mercury can affect the outcome of the analyses. A mathematical approach that has been developed for this purpose is the calculation of "z-scores". The value obtained by averaging the results from all three labs (last column, Table 6) is called the "reference value". The z-score calculation is made to quantify how far away each lab was from this reference value, with a z-score of "1" equal to a 5% difference, "2" equal to a 10% difference, and so on. The equation is

$$Z = (\text{lab result-reference value})/0.05$$

One approach to the criterion for acceptability is to use the same degree of difference allowed for replicate water samples, with the idea being that each lab's result is one replicate, and the reference value is the other replicate of the pair. For total mercury in water, this is 25%. In the format used here, this would equal a z-score of 5 or less. All of the z-scores were within the criterion (Table 7).

Table 7. Interlab comparisons for total mercury in water, z-score for each laboratory. Acceptable z-scores are ≤ 5.

	Battelle MSL		**Trent U.**		**Flett Res. Ltd.**	
	Average THg ng/L	z-score	Average THg ng/L	z-score	Average THg ng/L	z-score
OV5 May	2.65	2.49	2.21	1.24	2.21	1.24
OB2 May	1.86	0.92	1.68	1.11	1.79	0.19
OV5 July	2.03	3.24	1.62	1.42	1.59	1.82
OB2 July	1.34	1.89	1.20	0.47	1.14	1.43

Methyl mercury in water. The results from the interlab comparison on methyl mercury in water were not as complete as for total mercury in water. All 3 labs participated successfully in the May inter-comparison, but only two labs were able to complete the July inter-comparison. In the July sampling, difficulties with sample contamination and a laboratory error made the results from one lab not useable. Fortunately, the lab that does the routine water analyses completed both exercises and the results were satisfactory, if more variable than for total mercury.

Variability within each lab was very low (Table 8). The standard deviations on the average value for all labs were 0.6 to 38% of the averages for each sampling site and date (calculated from the last column in Table 8).

Table 8. Interlab comparison results for methyl mercury in water, 2007.

ng MeHg/L Average +/- Std. Dev.				
	Battelle MSL	**Trent U.**	**Flett Research**	**All Labs**
OV5 May	0.20 +/- 0.02	0.18 +/-0.00	0.15 +/- 0.07	0.18 +/- 0.03
OB2 May	0.11 +/- 0.01	0.11 +/- 0.01	0.10 +/- 0.01	0.10 +/- 0.03
OV5 July	0.12 +/- 0.01	0.12 +/- 0.01	*	0.12 +/- 0.00
OB2 July	0.06 +/- 0.02	0.03	**	0.05 +/- 0.0.02

* These results not useable because of methods error in lab

** These results not useable because of obvious contamination in sample bottles

The average results for each lab were not in as close agreement for methyl mercury in water (Table 9; Figure 3) as for total mercury in water (Table 7, Figure 2). This is expected and is reflected in the greater RPD permitted by the EPA for methyl mercury replicates (35%, compared to 25% for total mercury). It should also be noted that the levels of these concentrations were low, close to the method limit of 0.14 ng/L established at Flett Research for reliable quantification.

In evaluating z-scores for MeHg in water, the criterion for acceptability was ≤ 7, reflecting the EPA acceptability level of 35% for RPD between replicate samples for MeHg (1 z-score unit is a difference of 5%). With this criterion, all of the interlab results were acceptable (Table 9).

Table 9. Laboratory intercomparison for methyl mercury in water, z-scores for each lab.

	Battelle MSL		Trent U.		Flett Research Ltd	
	Average MeHg ng/L	z-score	Average MeHg ng/L	z-score	Average MeHg ng/L	z-score
OV5 May	0.20	2.76	0.18	0.69	0.15	3.45
OB2 May	0.11	2.99	0.11	3.21	0.07	6.19
OV5 July	0.12	0.08	0.12	0.08	*	
OB2 July	0.06	5.32	0.03	5.32	**	

* These results not useable because of methods error in lab

** These results not useable because of obvious contamination in sample bottles

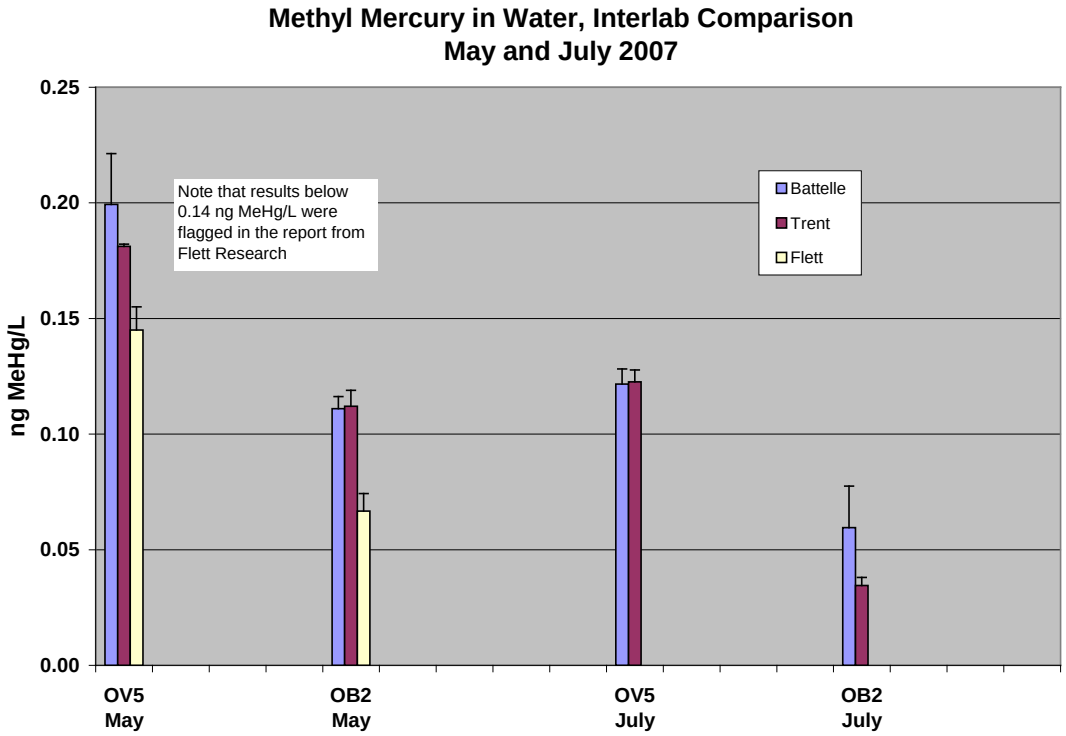

Figure 3. Results of laboratory intercomparison, methyl mercury in water, 2007. Bars show the mean values, and the vertical lines show 1 standard deviation from the mean.

Recommendations with respect to water analyses

There were no problems that surfaced with respect to field handling or analyses of mercury or methyl mercury in water. Several recommendations are made in order to ensure continued good performance and data analysis:

- There needs to be continued high vigilance on field sampling procedures, as a common hazard in trace metal sampling is that field crew personnel become overconfident when there have been no problems! I recommend that an experienced mercury scientist review procedures on site in 2008.
- Concentrations of both methyl mercury and mercury in field blanks should continue to be monitored closely.
- The next lab inter-comparison should be done as soon as water sampling commences in 2008, with special attention to MeHg blanks and samples. Sites chosen should include one site with higher MeHg concentrations than at the two sites used in the 2007 exercise. This would provide comparison of results at concentration levels where the results are generally more reliable for any lab that is carrying out analyses of methyl mercury in water.
- When analyzing the data for water samples, the blank results (about 0.02 ng/L for MeHg and about 0.2 ng/L for THg) should be taken into account, especially when sample concentrations are low (0.02 to 0.10 ng/L for MeHg in water, and 0.2 to 1.0 ng/L for THg in water).

IV. Sediment sampling and analyses

In the previous interlab exercise (2006), no problems were identified in the analyses of total mercury in Penobscot River sediments. However, the results for methyl mercury were quite different from the different laboratories, and this appeared to be related to methodological differences among the laboratories in 2006. These methods have since been extensively tested and investigated by the participating laboratories. Because of this need for methods testing for the methyl mercury analyses, and the lack of this need with respect to total mercury analyses, the QA/QC results for the total mercury in sediments are presented separately from the results for methyl mercury in sediments.

Sediment samples were collected in the field by Normandeau Associates, and were frozen immediately. For routine analyses in Phase I, frozen samples were sent to Battelle MSL in Sequim, WA. For methods testing, samples were sent to Battelle, to Flett Research Ltd., and to Trent University.

IV A. Total mercury in sediments

Analytical precision--Analytical duplicates.

Analytical duplicates were done on one in every ten sediment samples. The relative percent difference (RPD) was calculated for each pair of duplicates taken from a single sediment sample. While there are no set criteria, average RPD's of 4-7% (Table 10) can safely be described as low and indicate no difficulties.

Table 10. Relative percent differences in analytical duplicates for sediment samples collected May 30-June 1, 2007 and July 9-12, and analyzed for total mercury at Battelle Marine Sciences Laboratory.

	May 30-Jun 1/2007	**July 9-12/ 2007**
Average RPD	6.60%	**7.04 %**
Std Dev	4.18 %	**7.31 %**
n	**18**	**15**

Combined sampling and analytical precision--Field replicates.

Field replicates are sediment samples taken independently, at one site. Because two separate cores are taken, the relative per cent difference between samples is expected to be greater than for analytical duplicates (Table 10, above), where both samples came from the same core section. This was the case (Table 11, below). For sediments, contamination is not the same concern as for examining field replicates of water samples, because sediments contain much higher amounts of mercury. Rather, the primary usefulness of these sediment RPD's is

to provide a reference point for statistical expectations on the precision of core data, given the heterogeneity of sediments at a single sampling site. The RPD's found were not higher than expected for core to core variation.

Table 11. Relative percent differences between replicate surface sediment samples taken from two independently taken cores at a single sampling site and analyzed for total mercury.

	May 30-Jun 1/2007	**July 9-12/ 2007**
Average RPD	16.1 %	**30.8 %**
Std Dev	20.5 %	**39.7 %**
n	**20**	**15**

Interlab comparison—Total mercury in sediments.

Three laboratories participated in an inter-lab comparison in 2007. Sediments collected in May 2007 and July 2007 were sent to each lab. The results from the three labs were clearly in good agreement (Figure 4).

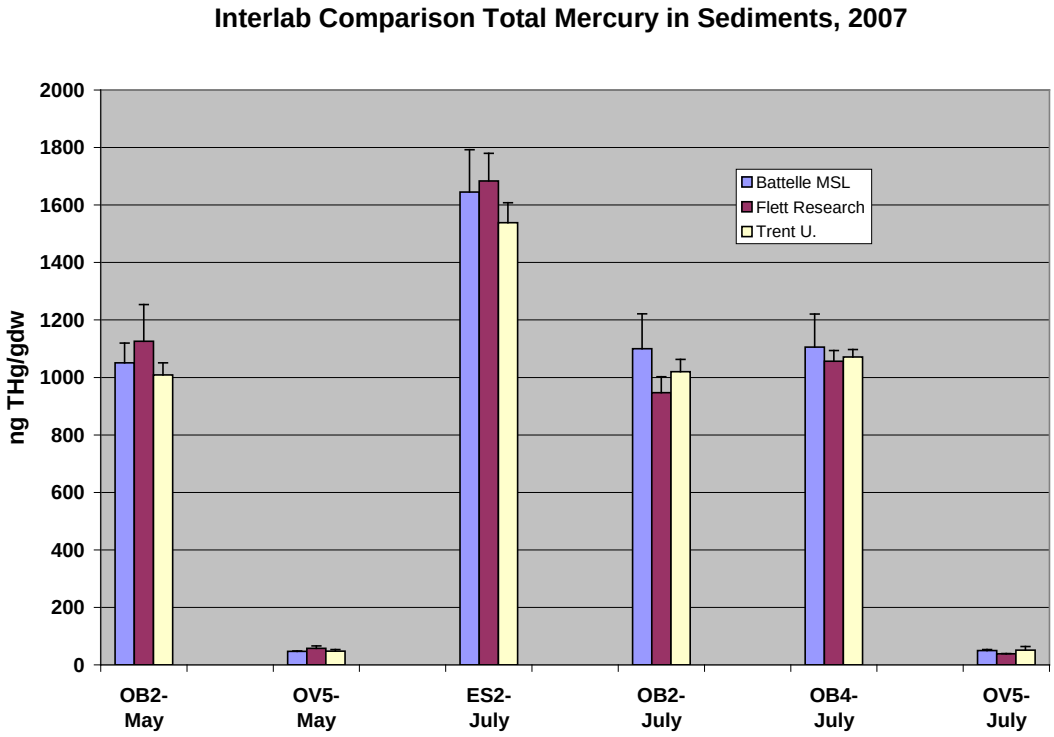

Figure 4. Results for inter-lab comparison on total mercury measurements in sediments. Sediments were collected at sites in the Penobscot River and estuary. Surface sediments (0-3 cm) were used.

The variation in results was small within each lab, as indicated by the standard deviations on the average result from each lab for each site (Table 12).

The z-score is a measure of the difference between each lab's average result, and the average result for all the labs, in units of 5% of the mean result. All z-scores were well within the acceptable range of 5 (Table 12).

Table 12. Numerical results from interlab comparison for total mercury in sediments, 2007.

	Battelle MSL		**Flett Research**		**Trent U.**	
Site	**Site Average ng THg/gdw**	**z-score**	**Site Average ng THg/gdw**	**z-score**	**Site Average ng THg/gdw**	**z-score**
OB2-May	1051 +/- 68.3	0.21	1126 +/- 127.7	1.21	1009 +/- 42.3	1.00
OV5-May	46.7 +/- 1.4	1.55	57.3 +/- 8.7	2.63	47.9 +/- 5.3	1.08
ES2-July	1645 +/- 147.3	0.28	1683 +/- 96.3	0.75	1538 +/- 69.3	1.03
OB2-July	1100 +/- 121.2	1.52	947 +/- 55.4	1.48	1020 +/- 42.8	0.05
OB4-July	1106 +/- 114.6	0.52	1056 +/- 37.0	0.40	1071 +/- 26.4	0.12
OV5-July	50 +/- 3.2	1.54	38 +/- 1.2	3.49	51 +/- 12.7	1.95

IV B. Methyl mercury in sediments

In the previous interlab exercise (2006), results for methyl mercury in sediments differed significantly among labs. Concentrations obtained by Battelle were lower, by as much as a factor of 2, compared to the other two labs. Battelle used extraction as the first step in their method, while Flett Research and Trent U. used distillation. At first, it was thought that the difference might be due to an analytical artifact previously identified for high mercury sediments when these sediments are analyzed using distillation. This artifact arises if some of the inorganic mercury in the sediments is chemically methylated during the distillation process. However, in the case of the Penobscot sediments, 1-2 % of total mercury would have to have been methylated in this way to account for the difference in the results, and previous measurements of this artifact have demonstrated that only 0.01 to 0.03% of inorganic mercury is typically methylated during the distillation step (Hintelmann et al, 1997). Thus, in 2007, a number of methods comparisons and tests were undertaken in order to investigate whether this difference in results from the different laboratories for Penobscot sediments was consistent, and if so, to make an informed choice as to methodology for the routine sediment analyses for methyl mercury.

In the examination of the sediment measurements, attention will be given first to precision of each of the two methods, for analytical duplicates done on a single sample, and for field replicates taken at a single site. Secondly, differences in the magnitude of the concentrations obtained using the two methods will be examined, with the objective of evaluating which method is most appropriate for Penobscot sediments.

Analytical precision--Analytical duplicates.

There are no EPA guidelines for acceptable RPD's for analytical duplicates in sediments (for MeHg in water the limit is 35%). Sediments are generally acknowledged to be more difficult to split reproducibility, because sediment samples are more heterogeneous than water. In any case, in samples taken for the Penobscot Mercury Study, the average RPD's for analytical duplicates were much lower than 35% (Table 13). Also, the precision of analytical duplicates for methyl mercury in sediment was similar for both the extraction and distillation methods. Thus, while giving different answers, one method gave just as consistent results as the other method.

Table 13. The relative percent differences between pairs of analytical duplicates (subsamples taken in the laboratory from one sediment core section). Methyl mercury analyses done at Battelle Marine Sciences Laboratory.

	Extraction		**Distillation**	
	Average RPD	**n**	**Average RPD**	**n**
May 30-Jun 1, 2007	6.0 +/- 5.9 %	6	10.9 +/- 10.8 %	16
Jul 9-12, 2007	10.5 +/- 10.9 %	5	8.6 +/- 8.4 %	16

Combined sampling and analytical precision--Field replicates.

Field replicates are samples taken from independently taken cores, at one site. As expected, the variation was greater for these replicates (Table 14, below) than for analytical duplicates taken from a single sample (Table 13, above). Both extraction and distillation methods showed similar degrees of variation in the results for field replicates (Table 14). There are no specific guidelines for acceptability in variation in field samples for methyl mercury in sediments. Rather, the statistics gathered on replicates is useful in making determinations of whether concentrations measured at different sites are any more different than concentrations measured within one site. This information is also necessary for comparing results on replicate samples analyzed by the two different methods, i.e., the differences need to be greater than the differences shown in Table 14 for one method in order to conclude that the two methods are actually giving different answers.

Table 14. Relative percent differences between pairs of field replicates in sediment samples analyzed for methyl mercury.

	Extraction		Distillation	
	Average RPD	n	Average RPD	n
May 30-Jun 1, 2007	44.7 +/- 29.2 %	5	37.9 +/- 37.0 %	15
July 9-12, 2007	---		35.3 +/- 43.0 %	15

Interlab comparison—Methyl mercury in sediments

Each participating laboratory used both the extraction and distillation methods to measure methyl mercury in the Penobscot interlab sediment samples. These methods differ in the first step, as indicated by their names. In one method, the methyl mercury was initially recovered from the sediments by solvent extraction , while in the other method the methyl mercury was recovered by distilling it out of the wet sediment sample. After this step, however, the methods were essentially the same—the recovered methyl mercury in the extract or distillate was ethylated, and mercury species were measured by Cold Vapor Fluorescent Atomic Spectroscopy (CVFAS) or by Isotope Dilution Mass Spectroscopy (IDMS).

When results were compared for the extraction method only, results from both Battelle MSL and Flett Research Inc. were almost identical in the May 2007 samples (Figure 5. In July, the results from Battelle were consistently higher (Figure 5). For all sites and samples, however, the interlab comparison results were good, with z-scores well within the criterion of 7 (Table 15).

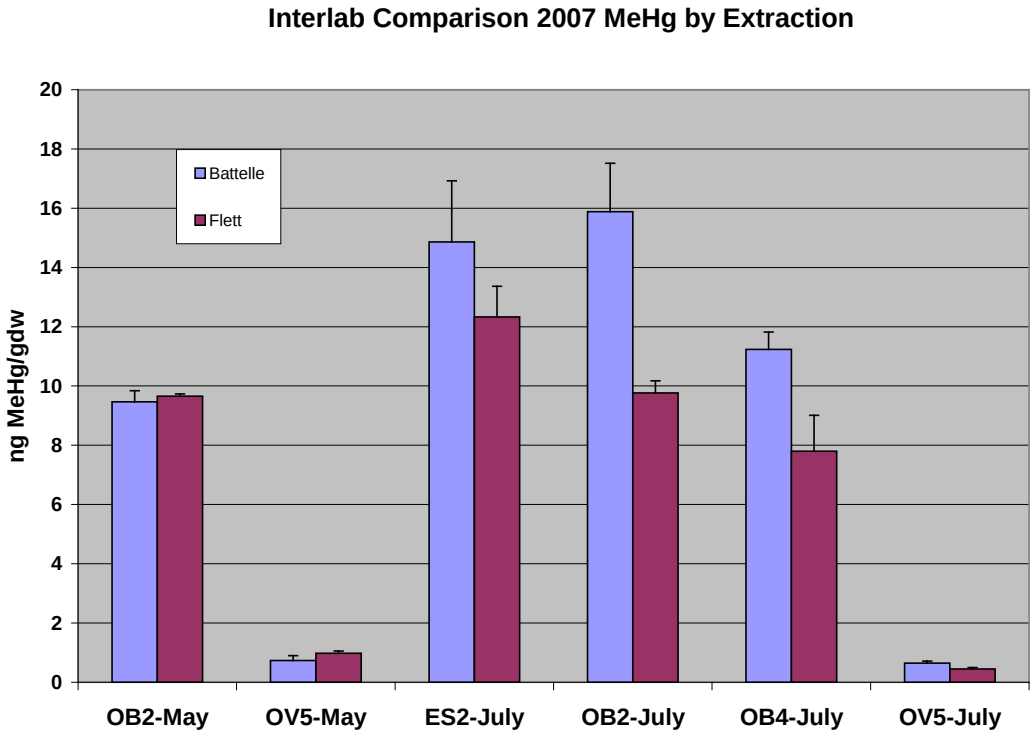

Figure 5. Methyl mercury in sediments, measured by extraction and CVFAS, at Battelle MSL and Flett Research Ltd. Bars show the mean values, and the vertical lines show 1 standard deviation from the mean.

Table 15. Interlab comparison results for methyl mercury in sediments measured by extraction on samples taken in 2007.

	Battelle MSL		Flett Research Ltd.	
Site	**Site Average ng MeHg/gdw**	**z-score**	**Site Average ng MeHg/gdw**	**z-score**
OB2-May	9.46 +/- 0.38	0.20	9.66 +/- 0.08	0.20
OV5-May	0.73 +/- 0.16	2.86	0.98 +/- 0.07	2.86
ES2-July	14.86 +/- 2.06	1.86	12.33 +/- 1.03	1.86
OB2-July	15.88 +/- 1.63	4.77	9.77 +/- 0.40	4.77
OB4-July	11.23 +/- 0.59	3.61	7.80 +/- 1.21	3.61
OV5-July	0.64 +/- 0.07	3.55	0.45 +/- 0.05	3.55

The same samples done by extraction, above, were also done by using distillation as the first step in the analyses (Figure 6). When results were compiled for this method, the Z-scores were within the acceptable range for all sites and dates, for all three labs (Table 16).

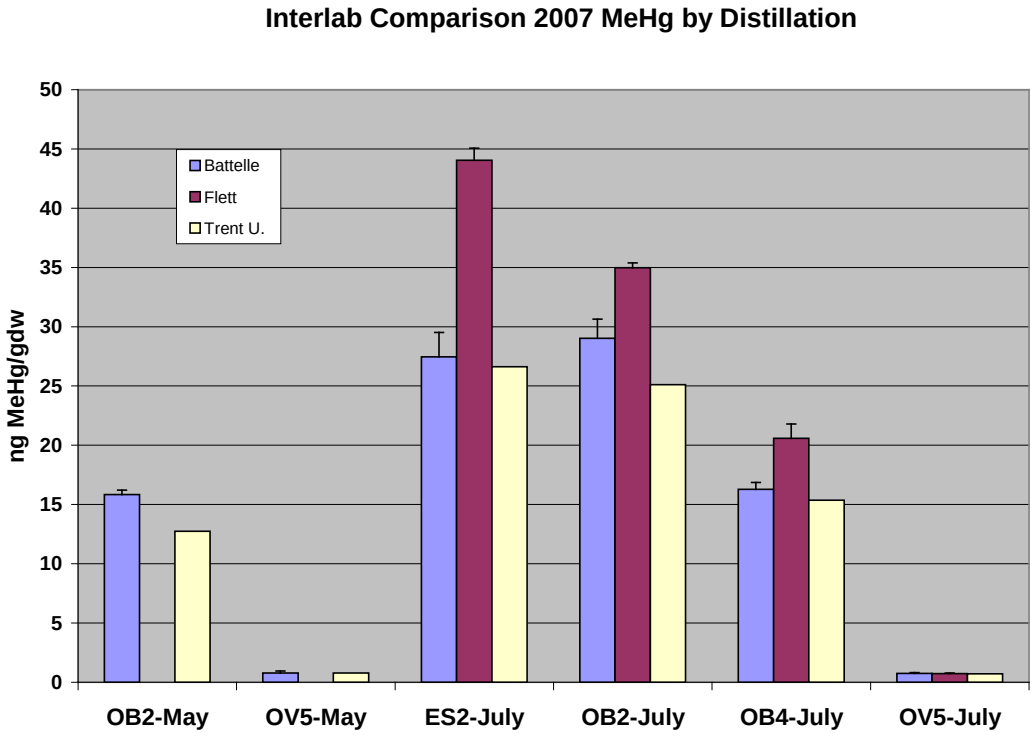

Figure 6. Methyl mercury concentrations measured in Penobscot River sediments by three laboratories, using distillation as the first step in the analysis. Bars show the mean values, and the vertical lines show 1 standard deviation from the mean.

Table 15. Interlab comparison of sediments analyzed for methyl mercury, with the first step being distillation. Samples were all surface sediments, collected in 2007.

	Trent U.		Battelle MSL		Flett Research Ltd.	
	Average (ng/gdw)	z-score	Average (ng/gdw	z-score	Average (ng/gdw	z-score
OB2-May	12.74 +/- 0.51	2.17	15.83 +/- 0.42	2.17		
OV5-May	0.79 +/- 0.11	0.08	0.78 +/- 0.12	0.08		
ES2-July	26.62 +/- 2.58	3.72	27.46 +/- 4.21	3.21	44.04 +/- 2.82	6.93
OB2-July	25.11 +/- 1.17	3.09	29.02 +/- 3.40	0.46	34.98 +/- 6.21	3.55
OB4-July	15.37 +/- 0.90	2.34	16.27 +/- 0.31	1.30	20.58 +/- 1.95	3.65
OV5-July	0.72 +/- 0.08	0.34	0.74 +/- 0.08	0.32	0.73 +/- 0.07	0.02

Thus, evaluations of precision, and interlab comparisons using only one method, did not show any data that would raise concerns. However, when all samples done by both extraction and distillation were compared, results using extraction were obviously lower than distillation. This was true in both 2007 (Figure 7) and 2008 (Figure 8), and when both

methods were done in the same laboratory, or in different laboratories. On average, the results using distillation were about twice as high as the results using extraction (Figure 9).

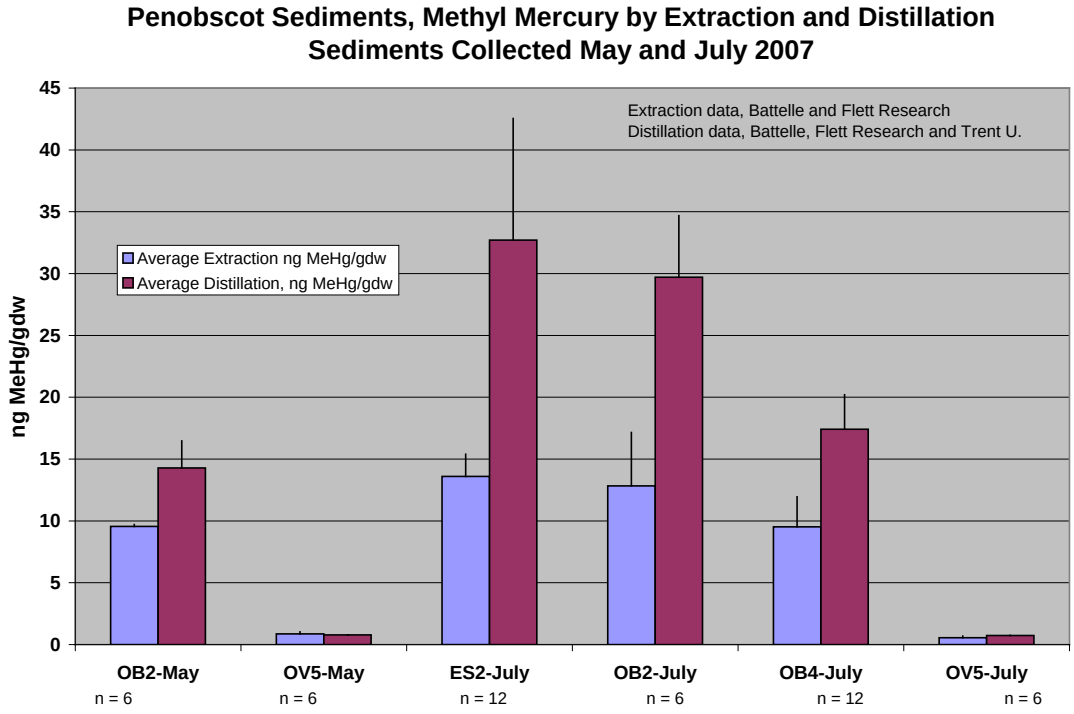

Figure 7. Comparison of methyl mercury results in sediments where the first step in analysis was extraction or distillation. Each bar is the average result, combining data from all labs, and the line above each bar shows 1 standard deviation on the mean.

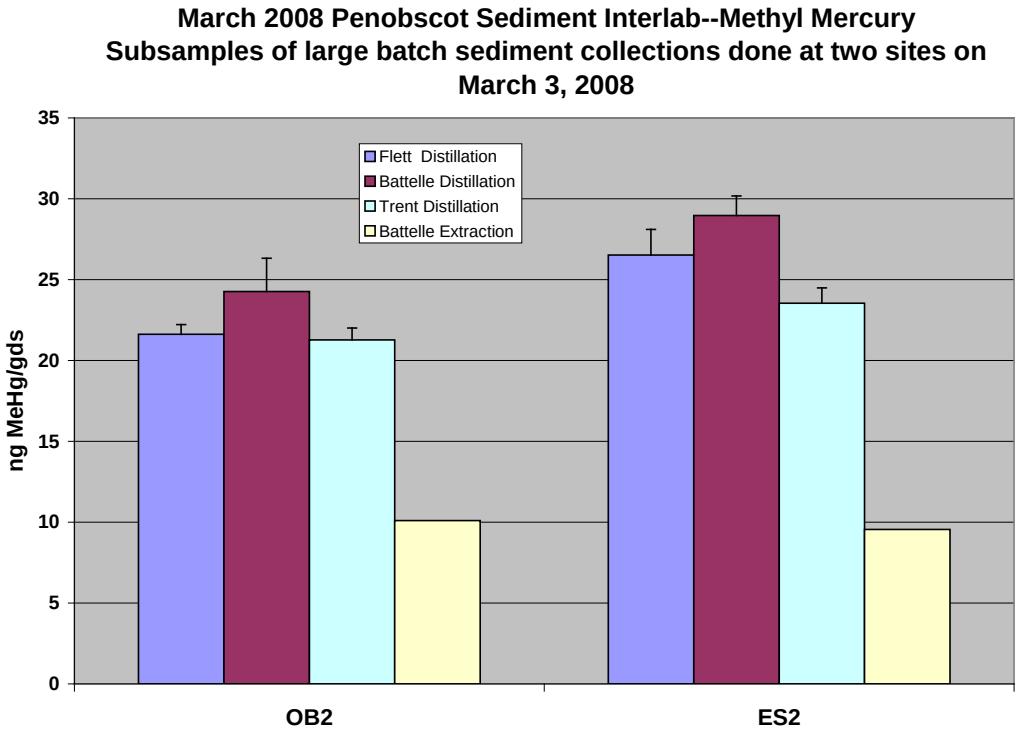

Figure 8. Methyl mercury measured in Penobscot River sediments, by two methods, in 2008. Battelle MSL did measurements by both methods; Flett Research Ltd. and Trent U. did measurements using distillation. Bars show the mean values, and the vertical lines show 1 standard deviation from the mean.

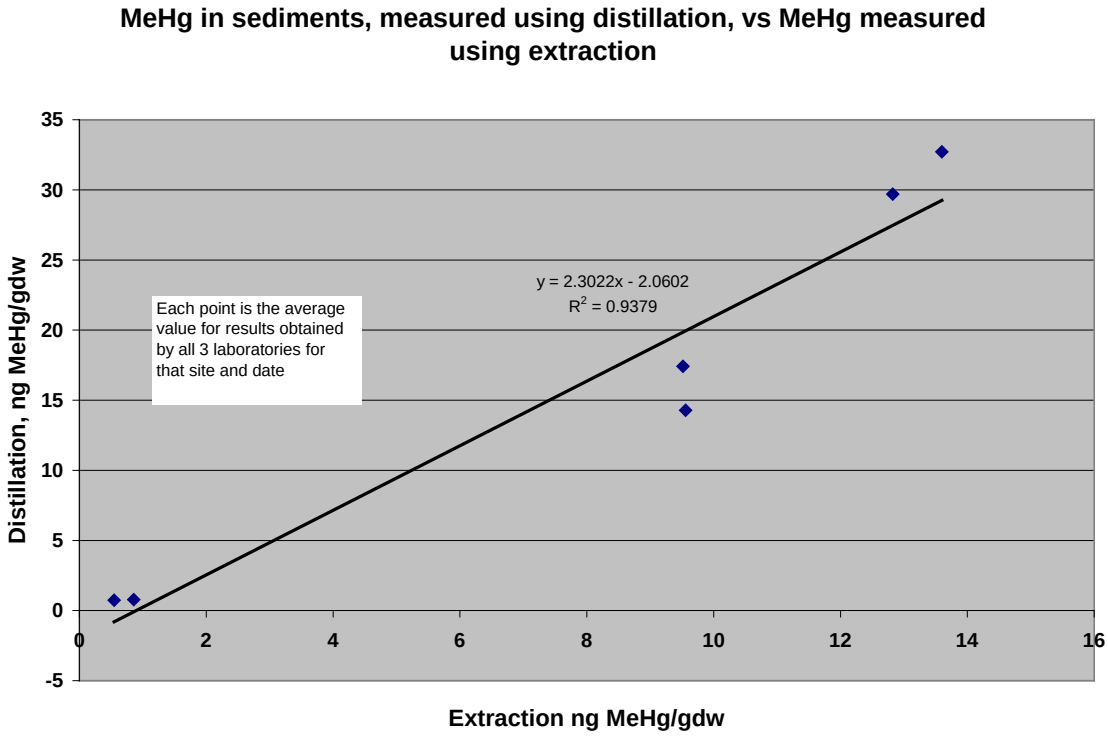

Figure 9. The average distillation result for MeHg in sediments at each site sampled for the interlab comparison in 2007, vs. the average extraction result for the same site.

The data above showed clearly that the distillation method consistently resulted in higher measurements of MeHg concentrations in Penobscot sediments than the extraction method. The magnitude of the difference (about 2X) was greater than would be expected for sample to sample variation (35 to 45%, Table 14). Thus, the next step was to determine if the higher results for distillation could be due to an artifact of the distillation method that has been identified in some situations. This artifact is the chemical methylation of a small portion of the inorganic Hg in the sample, producing MeHg that would not naturally be in the sample (Hintelmann et al, 1997).

One approach to evaluating the contribution of chemical methylation of inorganic Hg during distillation is to add isotopic inorganic Hg to the sample at the beginning of the distillation process. This was done at Trent U. in 2007, with the result that only 0.01 to 0.06% of the inorganic Hg added was converted to methyl mercury (Table 17). The average methylation rate was 0.03%, for 27 samples. Conversion of inorganic mercury isotope additions was also measured in 2008, with the average rate again being 0.03% (data not shown).

Table 17. Percentages of conversion of isotopic (^{200}Hg) inorganic Hg that was added to Penobscot sediments at the beginning of the distillation step. Analyses done at Trent University.

Sample ID	Station	Description	Date of Sampling	Ambient THg	^{200}Hg spike	^{200}MeHg	200Hg methylated (artifact)
				ng/gdw	ng	ng	%
MA-OV5-A	OV5	freshwater	11-Jul-07	0.72	3.1	0.000	0.00%
MA-OV5-A				0.72	31	0.003	0.01%
MA-OV5-B	OV5	freshwater	11-Jul-07	0.80	3.1	0.000	0.01%
MA-OV5-C	OV5	freshwater	11-Jul-07	0.64	3.1	0.001	0.03%
MA-OB2-A	OB2	tidal river	10-Jul-07	23.72	31	0.005	0.01%
MA-OB2-A				29.15	310	0.057	0.02%
MA-OB2-B	OB3	tidal river	10-Jul-07	24.63	31	0.010	0.03%
MA-OB2-B							
MA-OB2-C	OB4	tidal river	10-Jul-07	24.26	31	0.014	0.04%
MA-OB2-C				24.01	31	0.006	0.02%
MA-OB2-C				24.48	310	0.044	0.01%
MA-ES2-A	ES2	estuary	09-Jul-07	24.42	31	0.005	0.02%
MA-ES2-B	ES2	estuary	09-Jul-07	26.91	31	0.013	0.04%
MA-ES2-B				28.68	310	0.070	0.02%
MA-ES2-C	ES2	estuary	09-Jul-07	24.00	31	0.017	0.06%
MA-ES2-D	ES2	estuary	09-Jul-07	27.01	31	0.004	0.01%
MA-ES2-E	ES2	estuary	09-Jul-07	25.56	31	0.007	0.02%
MA-ES2-E							
MA-ES2-F	ES2	estuary	09-Jul-07	29.60	31	0.017	0.05%
MA-ES2-F				32.32	310	0.098	0.03%
MA-OB4-A	OB4	tidal river	09-Jul-07	14.60	31	0.021	0.07%
MA-OB4-B	OB4	tidal river	09-Jul-07	15.15	31	0.008	0.03%
MA-OB4-C	OB4	tidal river	09-Jul-07	16.36	31	0.016	0.05%
MA-OB4-D	OB4	tidal river	09-Jul-07	14.75	31	0.010	0.03%
MA-OB4-D				16.99	310	0.091	0.03%
MA-OB4-E	OB4	tidal river	09-Jul-07	13.71	31	0.006	0.02%
MA-OB4-E				13.37	31	0.001	0.00%
MA-OB4-E				16.73	310	0.095	0.03%
MA-OB4-F	OB4	tidal river	09-Jul-07	14.18	31	0.008	0.03%

In the isotopic method above, only a small amount of additional inorganic mercury is added to the sample, because the measurement method is very sensitive. A second approach to examining the chemical methylation of inorganic Hg during distillation is to add fairly large amounts of inorganic Hg, enough to measure chemically any increase in methyl mercury caused by these additions. This was done at both Battelle MSL and at Flett Research Inc.

Rates of conversion ranged from 0 to 0.09% of the added Hg (data not shown), which was similar to the isotopic approach (Table 17, above).

Could this small rate of chemical methylation of inorganic Hg in Penobscot sediment samples account for the higher concentrations of MeHg found with the distillation method, compared to the extraction method? This was examined by calculating the average increases in MeHg values obtained by distillation, compared to extraction, and dividing this difference by the total Hg concentration at each site (most of the total Hg in these sediments was inorganic Hg, as MeHg concentrations were very low, compared to THg concentrations, Table 18). These increases were 0.40 to 1.65% of total Hg, except in the case of OV5 sediments sampled in May 2007, where there was almost no difference in MeHg measured by the two methods (Table 18). Given the direct measurements of inorganic Hg conversion rates during distillation of Penobscot sediments (Table 17), as well as data from other studies on this phenomenon (0.030 to 0.036%, Hintelmann et al, 1997), it is unlikely that this type of conversion by itself could explain the much higher values obtained using distillation, compared to extraction, on Penobscot sediments (Table 18).

Table 18. Methyl mercury in sediments measured by using extraction or distillation as the first step at each site, and the difference in the two method results expressed as a % of total mercury in sediments at each site.

Site (2007)	Site average (all labs) Extraction, ng MeHg/gdw	Site average (all labs) Distillation, ng MeHg/gdw	Distillation minus Extraction, ng MeHg/gdw	Site average (all labs) ng THg/gdw	Distillation minus Extraction, divided by THg, %
OB2-May	9.56 +/- 0.14	14.29 +/- 2.19	4.73	1062	0.45%
OV5-May	0.86 +/- 0.17	0.78 +/- 0.00	-0.07	51	0.00%
ES2-July	13.60 +/- 1.79	32.71 +/- 9.82	19.11	1622	1.18%
OB2-July	12.82 +/- 4.32	29.70 +/- 4.97	16.88	1022	1.65%
OB4-July	9.52 +/- 2.43	17.41 +/- 2.79	7.89	1078	0.73%
OV5-July	0.55 +/- 0.14	0.73 +/- 0.01	0.19	46	0.40%

In summary, the distillation method for MeHg in sediments resulted in higher values for MeHg concentrations in Penobscot than did the extraction method, and the higher values could not be accounted for by measurements of chemical methylation during the distillation procedure. Thus, the extraction method did not appear to be recovering all the MeHg that was in the sediments, because the distillation method recovered much more. Clearly, the use of extraction underestimates the concentration of MeHg in Penobscot sediments.

Interestingly, all three labs used the same reference material (IAEA-405, an estuarine sediment widely used as a reference for mercury analyses), and analyses of this material by both methods gave results within the guidelines for recovery of MeHg from sediment samples. This is important because laboratories depend on analysis of reference materials to

alert them to potential problems in their measurement methods. Also, the initial comparison of extraction vs. distillation during the development of these methods for measurement of MeHg in sediments (Horvat et al 1993) did not show differences in results. Thus, the clear difference between the extraction and distillation methods for Penobscot sediments was not expected, and could only be discovered through the thorough methods comparison that was carried out.

The extensive comparison of the results from both methods on Penobscot sediments clearly showed that use of extraction on these sediments resulted in under-measurement of MeHg concentrations. There are very few other examples of studies where a large number of locations and sediment types have been investigated for possible differences in MeHg concentrations obtained by these two analytical methods. Most investigations use one method and a reference material. Therefore it is not possible to say whether Penobscot sediments are unique in the characteristic of under-measurement by extraction. What is clear is that methodological considerations are very important for measurement of MeHg in these sediments.

The rest of this section is concerned with the decision making process in choosing the best method for routine sediment analysis for methyl mercury in the Penobscot system. The extraction method was initially chosen for MeHg in sediments because many of the Penobscot samples are high in total Hg, and it was considered desirable to avoid the possibility that artifact conversion of inorganic Hg might occur during methyl mercury analyses. All samples taken in 2006 were analyzed by this method. After discussion with the analysts, the Study Panel, and the Project Leader, it was decided that distillation should be the method of choice for methyl mercury measurements on Penobscot sediment samples collected in after 2006. Also, while all work to date has shown that chemical methylation during distillation was very small, this should be continued to be monitored, to quantify the contribution of this chemical methylation on a continuing basis.

While it is usually not desireable to change methods during a study, the evidence that the extraction method must not be recovering all of the methyl mercury in the Penobscot sediment samples could not be ignored. In order to deal with the change in methods, and to retain the ability to compare 2006 data with samples collected later, it was also decided that 20% of samples done after 2006 would be done using both extraction and distillation, for year to year comparison. This decision resulted in the accumulation of a large number of samples done by both methods in 2007, and showed that the difference in results seen in the interlab comparison exercises continued consistently in later analyses. In May, 2007, results for MeHg in sediments by the distillation and extraction methods were significantly and linearly related to each other, with distillation results 2.4 times higher on average than extraction results (Figure 10, below). The same linearity was seen in July 2007, but with distillation averaging 1.8 times higher (Figure 11, below). This consistency means that results for samples done by only one method or the other can be compared approximately by using a factor of 2, with distillation results about 2 times greater than extraction results.

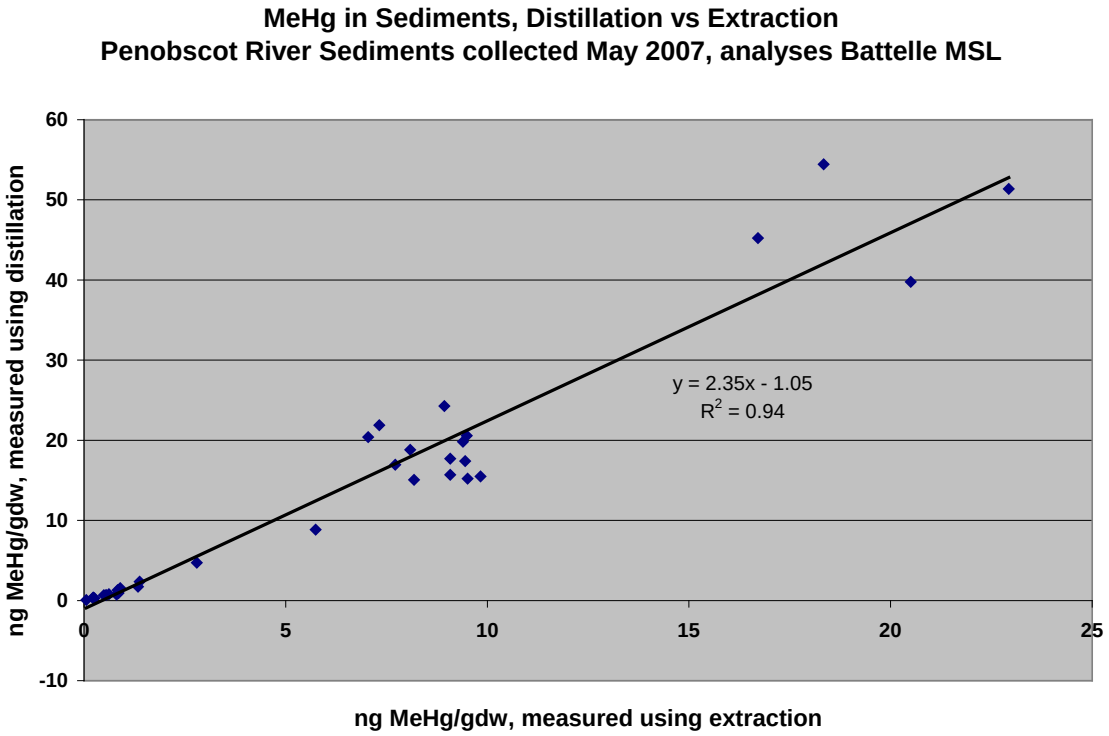

Figure 10. Comparison of MeHg concentrations measured in Penobscot River sediments collected in May 2007, and analyzed using both extraction and distillation as the first step in the analytical method.

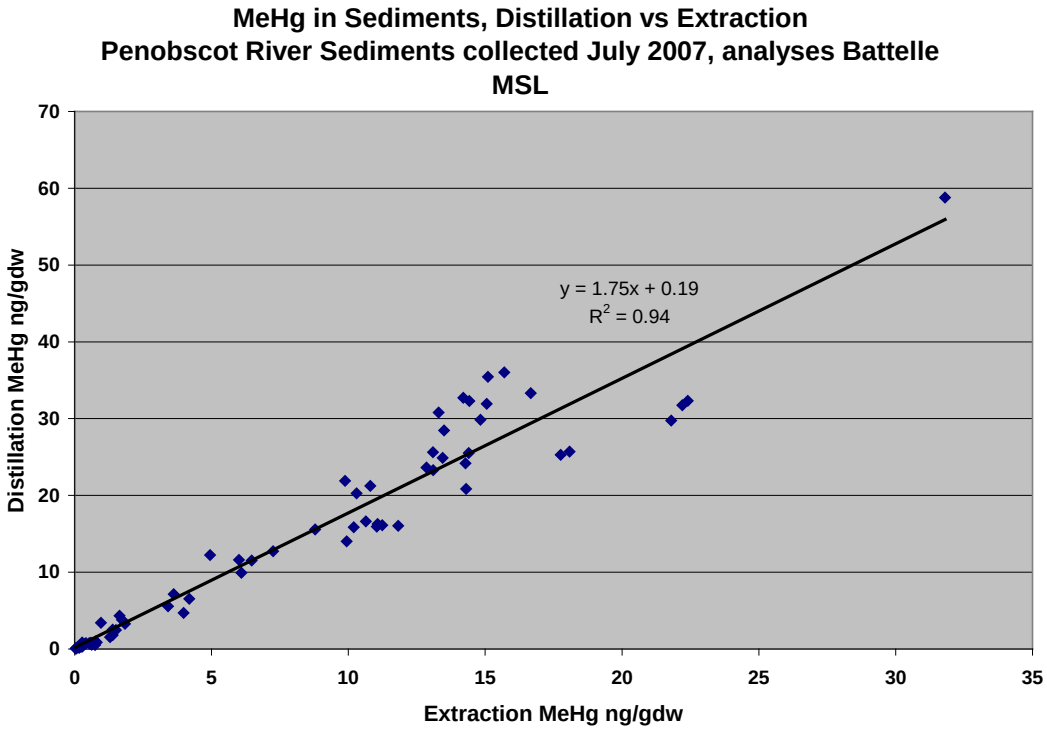

Figure 11. Comparison of MeHg concentrations measured in Penobscot River sediments collected in July 2007, and analyzed using both extraction and distillation as the first step in the analytical method.

Recommendations with respect to sediment samples

There were no changes recommended with respect to total mercury analysis. For methyl mercury analyses the following recommendations were made and have been carried out:

- Distillation should be the method of choice for analysis of methyl mercury in Penobscot sediments.
- Chemical methylation of inorganic mercury during distillation should continue to be monitored.
- For river and estuarine sediment samples taken in 2007, it was recommended that 20% of samples should be done by both extraction and distillation, for comparison to 2006 sediment data obtained used extraction. This need not apply to wetland samples, because none were taken in 2006. It should also not be necessary to do this in 2008.

V. Tissues

A large variety of biological tissues have been collected during Phase I of the Penobscot Mercury Study. Total mercury in tissues was measured after acid digestion, or thermal decomposition. Methyl mercury was measured after a potassium hydroxide digestion (see Appendix E for details of methods).

Analytical Precision--Analytical duplicates

Data for precision (relative percent difference) of analytical duplicates done on tissue samples were compiled for a randomly chosen subset of these tissue types (Table 19), which included *Nereis* (worms), bird eggs and blood, and a variety of mammalian tissues (blood, fur, liver, muscle and brain).

Table 19. Relative percent difference (RPD) for analytical duplicates of randomly selected tissue types.

Tissue	Laboratory	Analysis	Mean % RPD	n
Nereis 2006	Flett Research	THg	7.6 +/- 8.7	**17**
Nereis 2006	Flett Research	MeHg	9.1+/- 9.7	**37**
Bird eggs 2006	Battelle	THg	3.8 +/- 3.3	**5**
Bird blood 2006	Battelle	THg	5/5 +/- 4.0	**5**
Bird eggs 2007	Battelle	THg	2.8 +/- 2.2	**6**
Bird blood 2007	Battelle	THg	5.7 +/- 3.9	**3**
Mammal tissues 2006	Battelle	THg	5.2 +/- 5.5	**15**
Mammal tissues 2006	**Battelle**	**THg**	**6.3 +/- 4.0**	**8**

The relative percent differences between analytical duplicates were all less than 10%. While there are no specific guidelines for this statistic, this degree of analytical agreement seems very adequate for the purpose of the data being collected. It is important that this statistic be compiled for each tissue type that is analyzed, and that it be compared to the variation among individual samples, and among years and sites, i.e., any differences seen that are less than the analytical RPD would not be considered as real differences among samples.

Interlab Comparison—Tissues.

Tissue samples were sent to the three participating laboratories in May 2007. In 2007, two laboratories reported results for total mercury in tissues (Figure 10), and three laboratories reported results for methyl mercury (Figure 11). In 2008, three laboratories reported results for both total mercury and methyl mercury (Figures 12 and 13). Tissue types were *Mytilus edulis* (blue mussel), *Nereis* (worm), *Mya arenaria* (soft-shelled crab) *Carcinus maenas* (common shore crab), scallops, lobster and fish.

All tissue results, for both total mercury and methyl mercury, were obviously in very good agreement in both years (Figures 12-15).

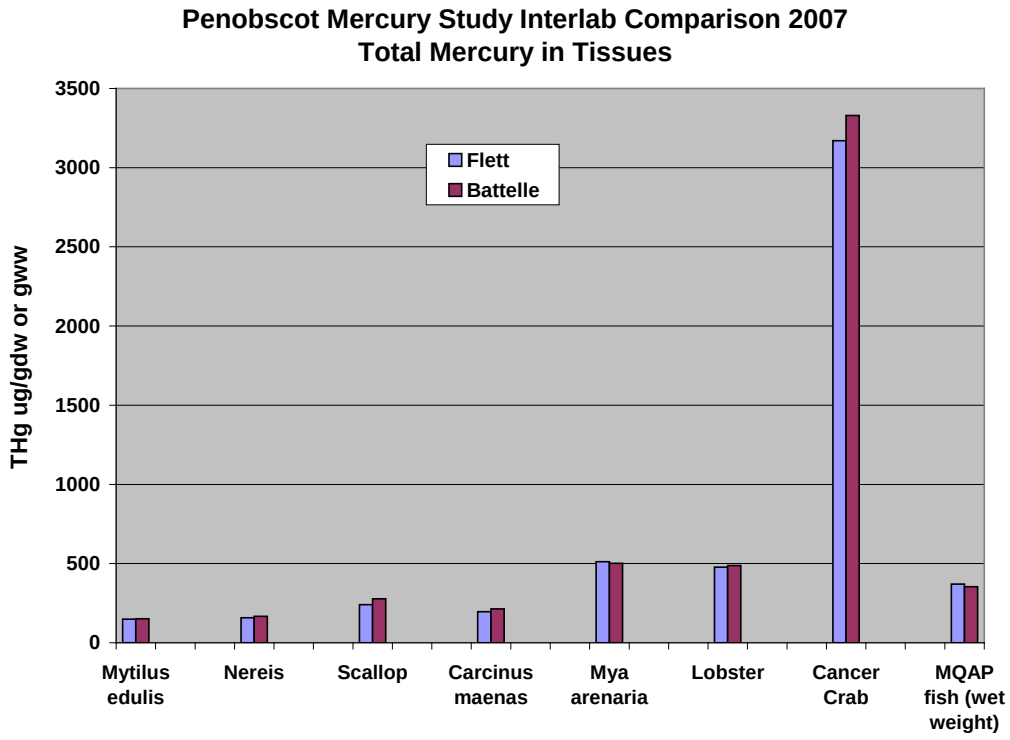

Figure 12. Total mercury in tissue, in interlab comparison tissue samples. Samples were collected in September and October, 2006, prepared as dry powders at Flett Research Ltd, except for the fish sample, which was sent as wet tissue. Samples were sent to laboratories in May 2007.

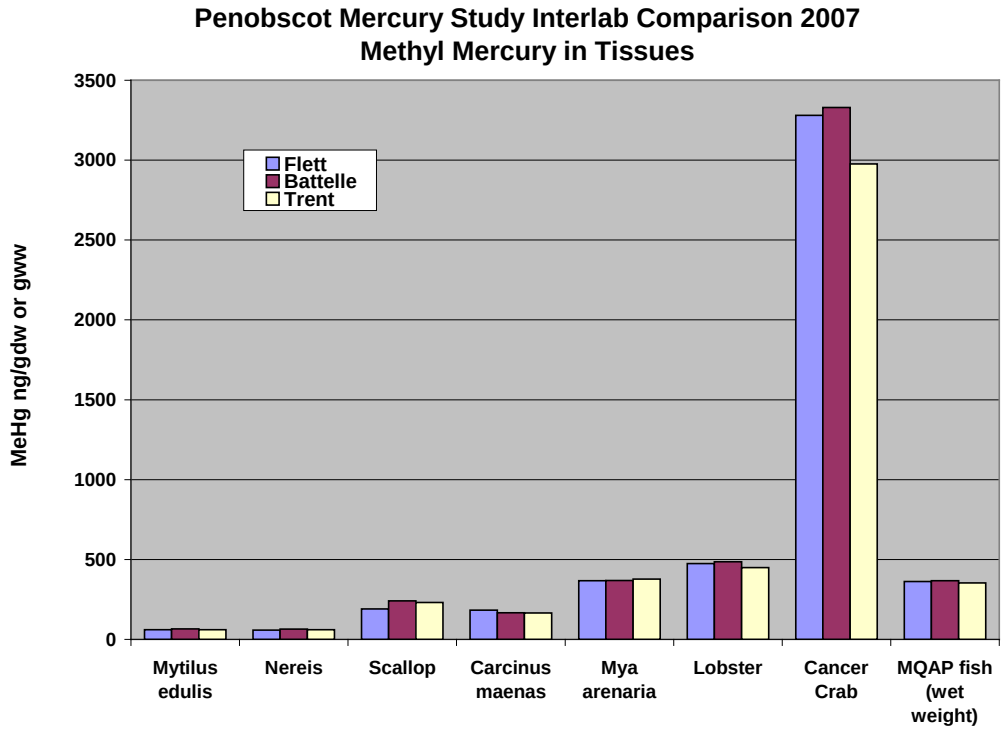

Figure 13. Methyl mercury concentrations in tissues, interlab comparison. Samples were collected in September and October, 2006, prepared as dry powders at Flett Research Ltd, except for the fish sample, which was sent as wet tissue. Samples were sent to laboratories in May 2007.

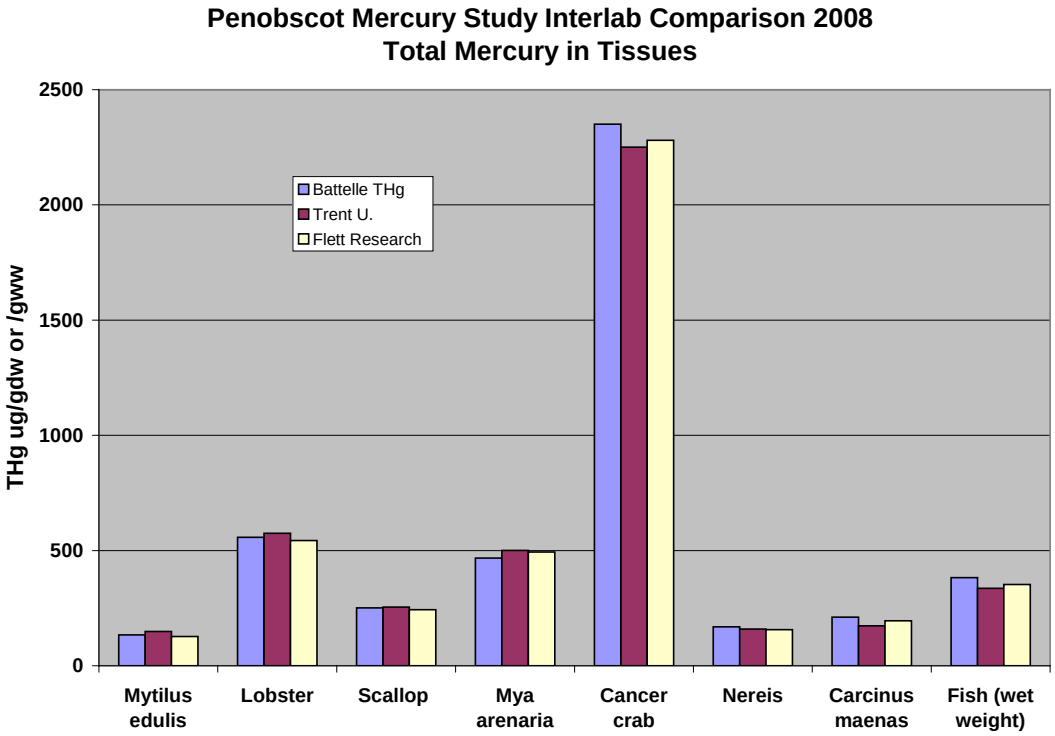

Figure 14. Total mercury in tissue, in interlab comparison tissue samples. Samples were collected in 2008 and prepared as dry powders at Flett Research Ltd, except for the fish sample, which was sent as wet tissue. Samples were sent to laboratories in June, 2008.

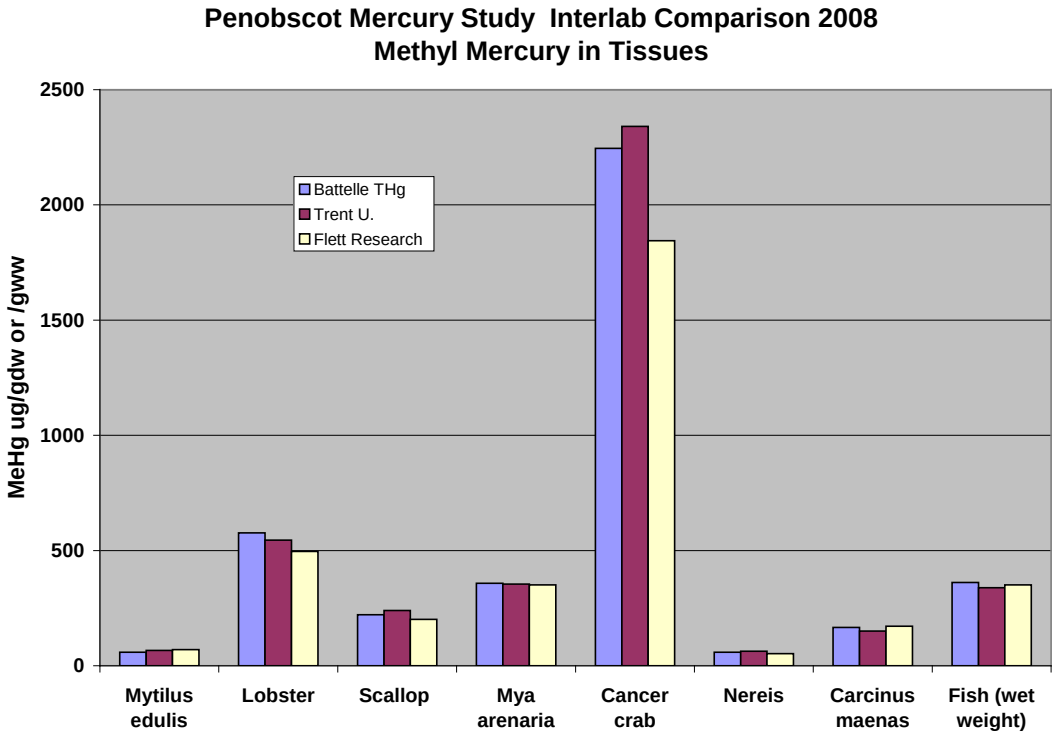

Figure 15. Methyl mercury in tissue, in interlab comparison tissue samples. Samples were collected in 2008 and prepared as dry powders at Flett Research Ltd, except for the fish sample, which was sent as wet tissue. Samples were sent to laboratories in June, 2008.

Recommendations with respect to tissue samples

The duplicate data on analysis of tissue samples, and the interlab comparison, indicated that there were no problems in this area. The interlab comparisons in both 2007 and 2008 demonstrated excellent agreement. The only recommendation is that analytical duplicate data should be compiled for each tissue type, as part of overall data analysis of samples.

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Appendix A. Summary of methods used by the laboratories.

Sample Type and analysis	**Battelle Marine Science Laboratory**	**Flett Research Ltd.**	**Trent U.**
Total Hg in water	EPA 1631e, oxidation and reduction, purge and trap, Cold Vapor Atomic Fluorescence Spectropscopy (CVAFS)	EPA 1631e, oxidation and reduction, purge and trap, Cold Vapor Atomic Fluorescence Spectropscopy (CVAFS)	**Oxidation and reduction, Isotope Dilution Mass Spectroscopy (IDMS)**
MeHg in water	EPA 1630, distillation, ethylation, purge and trap, CVAFS	EPA 1630, distillation, ethylation, purge and trap, CVAFS	**distillation, ethylation, purge and trap, IDMS**
Total Hg in sediments	EPA 7473, Thermal decomposition, CVAS	EPA 1631e, Digestion, purge and trap, CVAFS	**Acid digestion, $SnCl_2$ reduction, cold vapor flow, IDMS**
MeHg in sediments	Extraction and ethylation or distillation and ethylation, purge and trap, CVAFS, (adaptation of EPA 1630)	Extraction and ethylation or distillation and ethylation, purge and trap, CVAFS, (adaptation of EPA 1630)	**Distillation, ethylation, purge and trap, IDMS**
Total Hg in tissues	EPA 7473 CVAA or EPA 1631e CVAF	EPA 7473 CVAA with Direct Mercury Analyzer (DMA-80) or EPA 1631e after acid digestion	**Acid digestion, $SNCl_2$ reduction, cold vapor flow, IDMS**
MeHg in tissues	**KOH digestion, followed by EPA 1630 (ethylation, purge and trap, CVAFS)**	**KOH digestion, followed by EPA 1630 (ethylation, purge and trap, CVAFS)**	**KOH digestion, ethylation, purge and trap, IDMS**

Appendix B. Summary of reference materials used in the analyses of samples.

Sample Type and analysis	Battelle Marine Science Laboratory	Flett Research Ltd.	Trent U.
Total Hg in water	NIST 1641d	Baker QCS	**ORMS-3**
MeHg in water (no certified reference material available)	Laboratory preparation (DORM-2, diluted)	Laboratory preparation; Alfa standard (purchased)	**Laboratory preparation**
Total Hg in sediments	IAEA-405	MESS-2, NRC	**MESS-3**
MeHg in sediments	IAEA-405	IAEA-405	**IAEA-405**
Total Hg in tissues	DOLT-2	DORM-2 or DORM-3	**DORM-3**
MeHg in tissues	**DOLT-2**	**DORM-3**	**TORT-2, DORM-3**

NIST = National Institute of Standards and Technology (U.S.)
DORM = dogfish muscle, National Research Council (NRC), Canada
DOLT = dogfish liver, NRC, Canada
IAEA = International Atomic Energy Agency
TORT = lobster hepatopancreas, NRC, Canada
MESS = Marine sediment, Beaufort Sea, NRC, Canada
ORMS = Ottawa River water spiked with mercury, NRC, Canada

Appendix C. Details of Water analyses methods used by Battelle Marine Sciences Laboratory and Flett Research Ltd.

Total Mercury in water: Both laboratories used variations of EPA Method 1631e "Mercury in water by oxidation, purge and trap, and Cold Vapor Atomic Fluorescence Spectrometry (CVFAS)". In this method, all mercury is first oxidized to Hg(II), and then reduced to Hg^0 by addition of stannous chloride ($SnCl_2$). The gaseous mercury is then purged with gas, and trapped onto gold-coated sand traps. This Hg is then thermally desorbed onto a second trap and desorbed again, or may be directly desorbed into the analytical (fluorescence) cell for quantification.

Battelle Marine Sciences Laboratory.
Analytical Method: All samples stipulated for total mercury were analyzed by EPA Method 1631e.
Detection Limit: For total mercury in water, the achieved detection limit was 0.188 ng THg/L.
Quality Assurance Material: The standard reference material used for total mercury in water was NIST 1641d (1590000 +/- 1800 ng THg/L).
Recovery, accuracy and precision objectives: The accuracy of results for the standard reference material, and of ongoing precision sample, must be within +/- 23% of the expected value. The range of recovery of mercury standard added to samples (spike matrix recoveries) must be within 71-125%. The relative percent difference (RPD) for duplicate analyses of the same sample must be $\leq$ 21%.

Flett Research Ltd.
Analytical Method: EPA 1631e, Total Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectroscopy (CVAFS) (FR internal method #T00120, version 3).
Detection Limit: Minimum detection limit **(**MDL) = 0.04 ng Hg/L (based on 7 replicates of analytical blanks (99% confidence level)). The practical method limit (ML) of 0.5 ng/L, as stated in Method 1631e, has been adopted for our laboratory to reflect occasional elevated bottle blanks (< 0.5 ng/L) observed in reused acid-cleaned Teflon bottles filled with DI water.
Quality assurance material: OPR (Ongoing Precision Reference) solutions, which are large batches of water made up with a THg concentration within the range of usual samples. Each batch is large enough to provide a reference sample that is run on multiple consecutive dates, to check for day to day variance in analytical results, and variance within one day. Recovery must be within 77-133% of the expected value, and it is done once per every 10 samples. A second reference solution is Baker Quality Control Solution (QCS), with a certified concentration of 1000 ng/L. Recovery on this solution must be within 85-115% of the expected value.
Matrix effects: Recovery of total mercury spikes added to samples should be within 71-125% of the expected value, with RPD between duplicates <24%. Spike Matrix additions are done at a rate of once for every 10 samples.
Precision: The relative percent difference (RPD) between duplicate analyses must be <24%.

Estimated uncertainty: The estimated uncertainty of this method has preliminarily been determined to be ± 14.7 % @ 95 % confidence at a concentration level of 0.2 - 50 ng/L.

Methyl mercury in water:

Battelle Marine Sciences Laboratory

Analytical Method: All samples stipulated for methylmercury were distilled by the method of Horvat, et al., 1993 for methyl mercury. Samples were analyzed for methyl mercury by EPA Method 1630. Methylmercury in the distilled sample was ethylated and then purged onto carbon traps as a means of preconcentration and interference removal. The ethylated methylmercury was thermally desorbed into a fluorescence cell. Fluorescence (peak area) is proportional to the quantity of methylmercury collected, which is quantified using an average response factor as a function of the quantity of sample purged.
Detection Limit: For methyl mercury in water, the achieved detection limit was 0.0188 ng MeHg/L.
Quality Assurance Material: There is no certified reference material for methyl mercury in water. Battelle routinely uses DORM-2 tissue, diluted to a suitable concentration.
Recovery, accuracy and precision objectives: The accuracy of measurement of standard reference materials, or of ongoing precision samples, must be within +/- 33% of the expected value. The relative precision (relative percent difference) of analytical duplicates must be ≤ 35%. The range of recovery of methyl mercury standard added to samples (spike matrix recovery) must be with 65-135% of the expected concentration.

Flett Research Ltd.

Analytical Method: EPA (proposed) Method 1630; FR internal method # M10110 (Version 3): Methyl Mercury in water by distillation, ethylation, purge and trap, and CVAFS.
Detection Limit: MDL = 0.048 ng/L; ML = 0.14 ng/L. The method detection limit (MDL) is calculated to be the concentration equivalent to approximately three times the standard deviation of replicate measurements of the analyte in the given matrix at a concentration at or near the detection limit. (99% confidence level, 6 degrees of freedom). Client results are flagged below the ML.
Estimated Uncertainty: The estimated uncertainty of this method has preliminarily been determined to be ± 22 % at 0.5 ng/L (95 % confidence).
Quality assurance material: There is no certified reference material for methyl mercury in water. On each day when analyses are done, two reference materials are analyzed. The ongoing precision reference (MeOPR, 1000ng/L) is a 1/100 dilution of a lab standard "Y" solution, which was originally prepared in this lab from solid MeHgCl dissolved in isopropanol, diluted, and preserved with 0.05% acetic acid and 0.2% HCl. Recoveries must be with 77-123% of the expected value. The second material (Alfa, 200 ng MeHg/L) is a purchased standard. Mean recovery must be with 80-111% of the expected value.
Matrix effects: Recovery of methyl mercury spikes added to samples should be within 71-125% of the expected value, with RPD between duplicate spikes <24%. . Spike Matrix additions are done at a rate of once for every 10 samples.
Precision: The relative percent difference (RPD) between duplicate analyses must be <20%.

Appendix D. Analytical methods for mercury and methyl mercury in sediments used by Battelle Marine Sciences Laboratory and Flett Research Ltd.

Mercury in Sediments:

Battelle Marine Sciences Laboratory.
Analytical Method: All samples stipulated for total mercury were analyzed for total mercury by EPA Method 7473 (Thermal Decomposition, Amalgamation, and Cold Vapor Atomic Spectrophotometry). Samples were analyzed within the EPA holding time of 180 days.
Detection Limit: 3.2 ng/g
Quality Assurance material: The standard reference material (SRM) was IAEA-405. The criteria for recovery of total Hg from the SRM was 80-120%.
Precision: The relative percent difference between analytical duplicates must be $\leq$ 25%.
Matrix effects: Known quantities of total mercury are added to selected samples, and the recovery of this spike is quantified. Recoveries must be with +/- 20% of the expected value.

Flett Research Ltd.
Analytical Method: Total Mercury in Sediment, Soil, and Peat by Digestion, Purge and Trap, and CVAFS, adaptation of EPA Method 1631e, FR internal method # T00130, version 3.
Detection Limit: e.g. MDL = 2.4ng/g The method detection limit (MDL) is calculated to be the concentration equivalent to approximately three times the standard deviation of replicate measurements of the analyte in method blanks. (99% confidence level, 6 degrees of freedom) This limit assumes a 100 mg sample size. Lower detection limits are possible if greater sample weights are used.
Estimated Uncertainty: Preliminary determination: ± 18 % @ 95 % confidence at a concentration level of 40-100 ng/g; ± 32 % @ 95 % confidence at a concentration level of < 15 ng/g.
Reference Material: On each day when analyses are done, a certified reference material (Mess-2, 92ng Hg/g, from the National Research Council) is analyzed and compared to the certified concentration (the expected concentration). The total mercury concentration obtained must be within 80-110% of the expected value.
Precision: The relative percent difference (RPD) between duplicate analyses must be <20%.
Matrix effects: Known quantities of total mercury are added to selected samples. The recovery of this added mercury must be 71-125% of the expected value.

Methyl Mercury in Sediments:

Battelle Marine Sciences Laboratory
Methyl Mercury in Sediment: In 2006, all samples stipulated for methylmercury were extracted by the method of Bloom et al, 1997 for methyl mercury. Samples were analyzed by a modification of EPA Method 1630. Methylmercury in the extracted sample was ethylated and then purged onto carbon traps as a means of pre-concentration and interference removal. The ethylated methylmercury was thermally desorbed into a fluorescence cell. Fluorescence (peak area) is proportional to the quantity of methylmercury collected, which is quantified using an average response factor as a function of the quantity of sample purged. Samples were analyzed within the EPA holding time of 180 days. In 2007, Battelle began

also using the distillation method for methylmercury in sediments. In this method, sulfuric acid and KCl are added to thawed sediments, and distillation is carried out at 125 C in a Teflon still. The distillate is ethylated, and the ethyl methyl mercury is collected by purge and trap.
Detection Limit (both methods): 0.016-0.017 ng MeHg/gdw.
Reference Material (both methods): The standard reference material was IAEA-405. The criteria for recovery was 65-135%.
Precision: The relative percent difference (RPD) between analytical duplicates must be ≤ 35%.
Matrix effects: Known quantities of methyl mercury are added to selected samples, and the recovery of this methyl mercury should be with 65-135% of the expected values.

Flett Research
Analytical Method: Methyl Mercury in sediment by distillation, ethylation, purge and trap, and CVAFS, adaptation of EPA Method 1630, FR internal method #M10140, Version 3. Sulfuric acid and KCl are added to thawed sediment, and distillation is carried out at 147 C in a Teflon still. The distillate is ethylated, and the ethyl methyl mercury is collected by purge and trap.
Detection Limit: MDL = 0.02 ng/g based on 7 replicates of analytical blanks (99% confidence level).
Estimated Uncertainty: ± 18.3 % @ 95 % confidence at a concentration level of 0.1-50 ng/g
Reference material: On each day when analyses are done, a certified reference material (IAEA 405, 5.49 ng MeHg/g ±0.53, from the International Atomic Energy Agency) is analyzed and compared to the certified concentration (the expected concentration). The values obtained should be within 67-133% of the expected value.
Precision: The relative percent difference between analytical duplicates should be < 30 %.
Matrix effects: Known quantities of methyl mercury are added to selected samples, and the recovery of this methyl mercury should be with 65-135% of the expected values.

Appendix E. Analytical methods for mercury and methyl mercury in tissues used by Battelle Marine Sciences Laboratory and Flett Research Ltd.

Total Mercury in tissues:

Flett Research Ltd.
Analytical Method: EPA Method 7473 CVAA, using automated DMA-80, and EPA method 1631e after a nitric/sulfuric acid digestion of the tissue.
Detection Limit: MDL = 2.0 ng/g based on sets of 7 replicates of analytical blanks (99% confidence level, 6 degrees of freedom). This limit assumes a 200 mg wet sample size. Lower detection limits are possible if greater sample weights are used.
Estimated Uncertainty: At the 95 % confidence level, uncertainty has been preliminarily estimated at ± 12 % for fish muscle, 17.3 % for liver tissue, 27.8 % for fatty tissue, and 20.7 % for plant tissue.
Precision: The relative per cent difference between duplicate analyses must be less than 20%.
Reference Material: On each day when analyses are done, a certified reference material (DORM-2, 4640 ng/g, or DORM-3, 409 ng/g) is analyzed and compared to the certified concentration (the expected concentration). The mean recovery result must be within 80-110% of the expected concentration.
Recovery efficiency: In addition to determining the recovery efficiency of a certified reference material, known additions of Hg are made to selected samples, and the recovery efficiencies of these additions ("spike matrix additions") are determined. These must be within an acceptable limit (71-125% of expected value).
Correction of sample results for recovery efficiency: The recovery efficiency of the standard reference material is used to adjust the sample results for this factor.

Battelle Marine Sciences Laboratory
Analytical Method: Total mercury in liver and muscle was analyzed by EPA Method 7473 CVAA. Total mercury in blood and feathers was analyzed by EPA Method 1631e CVAF.
Achieved Detection Limit: 3.07 ngTHg/g
Reference Material: DOLT-2, certified value = 2.14 +/- 0.28 ugTHg/gdw. One sample of this material is analyzed with each batch of 20 samples, or each day if fewer than 20 samples are run. The analytical results should be within +/- 20% of the certified value.
Precision: One to two samples are analyzed in duplicate with each batch of 20 samples. The relative percent difference between replicate samples should be within 25%.
Spike Matrix Recoveries: Two matrix spike duplicate pairs are done with each batch of 20 samples. Recovery of known spikes of total mercury to sample matrices should be within 80 to 120% of the added mercury.

Methyl Mercury in tissues:

Flett Research Ltd.
Analytical Method: M10120: Methyl Mercury in biological tissue by KOH digestion, ethylation, purge and trap, and CVAFS (Version 3). From the digestion step forward, the method is similar to methyl mercury in water (EPA 1630).
Detection Limit: MDL = 0.11 ng/g. The MDL was determined based on 7 replicates of analytical blanks (99% confidence level).
Estimated Uncertainty: The estimated uncertainty (95% CI) of this method has preliminarily been determined to be + 68% at a concentration level of 0.1 ng/g and 22.4% at a concentration level of 4470 ng/g, based on 7 measurements.
Precision: The relative percent difference (RPD) between analytical duplicates should be less than 30%.
Reference Material: On each day when analyses are done, a certified reference material (DORM-2, 4640 ng/g) is analyzed and compared to the certified concentration (the expected concentration). The analytical result should be within 78-113% of the expected reference value.
Recovery efficiency: In addition to determining the recovery efficiency of a certified reference material, known additions of MeHg are made to selected samples, and the recovery efficiencies of these additions are determined. The recovery efficiency should be with 65-135% of the expected value.
Correction of sample results for recovery efficiency: The recovery efficiency of the standard reference material is used to adjust the sample results for this factor.

Battelle Marine Sciences Laboratory
Analytical Method: Digestion in 25% KOH in methanol (liver and muscle) followed by a modification of EPA method 1630.
Achieved Detection Limit: 1.03-1.17 ng/g.
Precision: One to two samples are analyzed in duplicate with each batch of 20 samples. The relative percent difference (RPD) between duplicate analyses should be les than 35%.
Reference Material: DOLT-2, certified value = 0.693 +/- 0.053 ug MeHg/gdw. The analytical results should be within +/- 35% of this value.
Recovery efficiency: Two spike matrix duplicate pairs are done with each batch of 20 samples. The recovery of known spikes of reference material to samples (spike matrix recoveries) should be within 65-135% of the expected value.