

Technical Memorandum

Date: February 22, 2018

To: Nelson Walter, Amec Foster Wheeler, Portland, ME

From: Khalil Abusaba, Amec Foster Wheeler, Oakland, CA
Karen Merritt, Amec Foster Wheeler, Portland, ME

Ref: Penobscot River Phase III Engineering Study

Re: Analytical Methods Comparisons

EXECUTIVE SUMMARY

In 2016, Amec Foster Wheeler Environment & Infrastructure, Inc. (Amec Foster Wheeler) performed an analytical methods comparison study to evaluate the various procedures available for obtaining accurate, defensible mercury and methyl mercury results for sediment samples collected from the Penobscot River Estuary. The study involved performing an array of digestion and extraction procedures combined with measuring total organic carbon (TOC), total mercury, and methyl mercury content to determine which method(s) yielded the highest and the most consistent total mercury and methyl mercury results. This study showed that:

- For analysis of total mercury in sediment, hot *aqua regia* digestion and thermal decomposition appear to be the most effective methods for achieving high, consistent analytical recovery in sediment samples having substantial amounts of wood waste.
- Wood waste is highly heterogeneous, which necessitates either homogenizing samples or analyzing samples in triplicate in certain situations.
- Extraction of sediments and wood waste with methanolic potassium hydroxide (KOH) yields higher methyl mercury results than extraction with methylene chloride.
- Extraction of sediments with methanolic KOH generally yields lower methyl mercury results than analysis following distillation.

Based on the findings of this study, the sediment samples that were collected during 2016 for the Penobscot River Estuary Phase III Engineering Study and analyzed for total mercury were re-analyzed using hot *aqua regia* digestion. In brief, additional recommendations that are being followed for 2017 sampling, analysis, and database management are as follows:

- Upload re-analyzed results and flag originally reported results as unusable in the database.
- Perform total mercury analysis using hot *aqua regia* digestion or thermal decomposition for 2017 sediment sample analysis.
- Account for sediment sample heterogeneity in certain portions of the estuary when designing monitoring programs.
- Account for use of different analytical methods and sample heterogeneity when evaluating sediment data for trends.

Recommendations are presented in further detail in the Technical Memorandum below.

This study also shows that methanolic KOH extraction yields methyl mercury results that are approximately double the results obtained by methylene chloride extraction and are lower than, but more similar to, results obtained by distillation. Distillation results obtained in the Phase II Study were also approximately double the results that were obtained by methylene chloride extraction. Therefore, methanolic KOH is a more suitable chemical extraction for Penobscot Estuary sediments than methylene chloride because, similar to distillation, the results following methanolic KOH extraction are close to twice the concentration of the methylene chloride extraction results. Because the methanolic KOH extraction method is more widely available in commercial laboratories than the distillation method and so may be more feasibly applied across current and future monitoring programs, methyl mercury analyses in sediment using methanolic KOH extraction is suggested.

BACKGROUND AND PURPOSE

The purpose of this study was to address two questions related to analysis of sediments and sediment/wood waste mixtures from the Penobscot River Estuary in order to evaluate the influence of wood waste on analytical results:

- Are there digestion procedures available that result in better recovery of total mercury in samples having large amounts of wood waste?
- Are there extraction or distillation procedures available that result in better recovery of methyl mercury in samples having large amounts of wood waste?

These two questions came up when observations made during the spring, summer and fall of 2016 indicated wood waste was evident in the estuary in greater amounts than previously believed. The Phase II Study had focused on characterizing the mineral sediments and therefore had not fully evaluated the contribution of wood waste to the total mercury burden in the estuary. The Phase II Study performed some method comparisons for total mercury analysis, but not on samples with large amounts of wood debris, as evidenced by relatively low TOC concentrations in the samples analyzed—1.4 to 4.6 percent (%) for 2006 data, and 1.0 to 7.1% for 2007 data (**Figure 1**). TOC data were not available for 2009 analyses (**Figure 1**).

There are three accepted techniques for separating methyl mercury from a sediment sample for analysis, as defined in U.S. Environmental Protection Agency (EPA) Method 1630. Two of the techniques rely on chemical extraction (often referred to simply as extraction) with either methylene chloride or methanolic KOH. The third technique, which was used by some participants in the Phase II Study Group, relies on distillation.

The Phase II Study compared methyl mercury analyses by methylene chloride extraction vs. distillation; the study indicated that distillation yields methyl mercury concentrations that are approximately double the results obtained using methylene chloride extraction. Although the reason for this disparity between techniques was not determined, the Phase II Study group decided that distillation should be the method of choice for methyl mercury analysis in Penobscot River Estuary sediments.

During the first year of the Penobscot River Estuary Phase III Engineering Study, Amec Foster Wheeler also performed methyl mercury analysis using methylene chloride extraction. Consistent with advice from Phase II Study panelists, Amec Foster Wheeler also determined that an additional methods comparison would be necessary to improve understanding of how

the sample matrix and analytical method details affect results for total mercury and methyl mercury analyses. Eurofins Frontier Geosciences (Eurofins) was subcontracted for laboratory services and performed a comparison study to assess methyl mercury results obtained by methylene chloride extraction vs. methanolic KOH extraction, and how sediment containing large amounts of wood waste was affected by the extraction methods. Likewise, a comparison was undertaken of methyl mercury results obtained by methanolic KOH extraction vs. results obtained by distillation. For this comparison, Eurofins performed the analysis following methanolic KOH extraction and Flett Research Ltd. (Flett) performed the analysis following distillation.

Concurrently, WO 04A-020 was scoped and authorized to quantify the proportion of wood waste present in estuary sediments, and to separate wood waste from mineral sediment for more quantitative comparisons between the two solid phases. Under this work order, additional total mercury analytical inter-laboratory comparisons, as well as methyl mercury analytical comparisons, were performed to supplement the inter-laboratory comparison data collected under WO 04A-010. Techniques for homogenizing samples containing wood waste were also developed and implemented to address the heterogeneity introduced by having millimeter-sized chunks of wood often present in sediments collected from the estuary.

Analysis of lignin oxidation products was also scoped under WO 04A-010 to assess organic carbon quality in Penobscot estuary sediment. This analysis was undertaken as proof of concept for quantifying the contribution of legacy wood waste vs. contemporary vascular plant matter in the overall organic carbon budget of estuary sediments. Results of the lignin biomarker analysis are presented in the Analysis of Lignin Oxidation Products in Sediment Technical Memorandum (Amec 2018).

APPROACH

Six sediment samples collected by Amec Foster Wheeler in June 2016 were selected for inter-laboratory comparison and lignin biomarker analysis. The samples were collected from Bucksport, Hampden, and Gross Point, and spanned a range of TOC concentrations from 3 to 51%. The sample with 51% TOC was a sediment/wood waste mixture with a substantial fraction of visible wood waste present. Sample locations, total mercury results, and TOC concentrations are summarized in **Attachment 1**.

These six samples were homogenized with an industrial blender by Eurofins, and subsampled for distribution to participating laboratories. Three subsamples (designated as 1st Rep, 2nd Rep, and 3rd Rep) were distributed to each laboratory for analysis using the following EPA methods:

- Total mercury by Method 1631 using cold *aqua regia* (Eurofins)
- Total mercury by Method 1631 using warm 70% nitric/30% sulfuric acid (Eurofins)
- Total mercury by Method 7474 using hot *aqua regia* (Alpha Analytical Laboratories, Inc. [Alpha])
- Total mercury by Method 7473 using thermal decomposition (Flett)

Total mercury analysis by Method 1631 using cold *aqua regia* is an accepted protocol for sediment samples in large-scale ambient monitoring programs. Warm nitric/sulfuric acid is an option recommended in Method 1631 for samples with organic tissues. Method 7474 is a lower-cost alternative and the recommended method for samples containing organic tissues (e.g.,

sediments mixed with wood waste). Flett's analysis of total mercury by Method 7473 was the main method relied upon for analysis of total mercury in sediments during the Phase II Study.

After reviewing initial results from WO 04A-010, Eurofins was directed to re-analyze the six comparison samples described above by Method 1631, substituting a hot *aqua regia* digestion for cold *aqua regia* digestion. This direction was provided in response to differences between results from Eurofins compared to results from the other participating laboratories. Analyses conducted following this modification are identified in this technical memorandum as following Method 1631 using hot *aqua regia*.

A second set of samples was collected in the fall of 2016 under WO 04A-020 for additional mobile sediment characterization. A subset of those samples was selected for inter-laboratory and/or analytical methods comparison.

For total mercury, comparison was by the following EPA methods and laboratories:

- Method 1631 using cold *aqua regia* (Eurofins);
- Method 7474 using hot *aqua regia* (Alpha);
- Method 7473 using thermal decomposition (Flett); and
Method 1631 using hot *aqua regia* (Eurofins) (**Attachment 2**).

For methyl mercury, comparison was by the following EPA method:

- Method 1630 using methylene chloride (Eurofins) and
Method 1630 using methanolic KOH (Eurofins) (**Attachment 3**).

A third set of samples was collected in the summer of 2017 under WO 04A-070 for additional sediment characterization. A subset of those samples was selected for an inter-laboratory analytical methods comparison for methyl mercury by the following EPA method:

- Method 1630 using methanolic KOH (Eurofins); and
- Method 1630 using distillation (Flett).

For total mercury and methyl mercury, these analyses provided additional comparison data from readily-available commercial laboratories in comparison to the laboratory (Flett) that was principally employed in the Phase II Study. Comparison of the different extraction methods for methyl mercury evaluated an alternative to the methods used by the Phase II Study group in which methylene chloride extraction was initially employed and then replaced with the distillation method following determination of a two-fold difference in results between these two different methods. Based on recommendation by Eurofins, and as supported by research validating the application of methanolic KOH extraction to the analysis of methyl mercury in tissue samples (e.g., Carrasco and Vassileva 2005), this alternative was explored as a readily-available commercial method that may yield better analytical recovery for sediment samples enriched in organic content.

Samples from WO 04A-020 selected for the comparison had considerable amounts of wood waste. Sieve separations were performed on samples collected under WO 04A-020, and along with measurement of TOC and percent solids, this physical separation technique provided a means to evaluate the specific contribution of wood waste to analytical heterogeneity.

Samples from WO 04A-070 selected for the comparison had varying amounts of wood waste; these samples were selected to assess method recovery differences across a range of sediment organic matter content.

FINDINGS

Total Mercury Method Comparisons

As shown in **Figure 2**, for five out of the six samples initially evaluated under WO 04A-010, digestion by cold *aqua regia* (using Method 1631) yields total mercury results that are significantly lower than results when samples are analyzed by thermal decomposition (Method 7473). Significant difference as shown in **Figure 2** is based on the absence of overlap in the 95% confidence intervals for analyses conducted on replicate (n = 3) samples. As noted above, Method 7473 was the main method employed during the Phase II Study for total mercury in sediments.

For four out of the six samples, digestion by cold *aqua regia* yields mercury results that are significantly lower than results following digestion by either hot *aqua regia* or by warm nitric/sulfuric acid (Method 1631 alternative). In three out of four of the samples in which recovery was greater using warm nitric/sulfuric acid digestion than cold *aqua regia* digestion, recovery was still significantly lower than using thermal decomposition (Method 7473). Based on these data, it appears that (1) switching to the warm nitric/sulfuric alternative prescribed in Method 1631 may not be sufficiently aggressive for digesting the elevated organic content present in Penobscot Estuary sediments; and (2) matrix effects from elevated organic content in samples are less of a concern for thermal decomposition of samples than for acid extraction of samples.

For samples collected under WO 04A-020, **Figure 3** provides additional evidence of the impact of digestion method on mercury recovery. Following collection, these samples were processed by sieving to separate larger (>420 microns) wood waste chunks from mineral sediment. This approach was taken to examine the influence of wood waste on total mercury recovery. Sample fractions assessed in **Figure 3** consist of a bulk unsieved sample, wood chips retained on the sieve and material passing through the sieve.

As presented in **Figure 3**, Eurofins re-analyzed a sub-set of four samples that had been initially digested by cold *aqua regia*, this time following hot *aqua regia* digestion. One of these samples (from Frankfort Flats) was re-analyzed in triplicate. Results from this re-analysis are consistent with the general observation presented above that recovery of total mercury following cold *aqua regia* digestion tends to be lower than recovery following either hot *aqua regia* digestion or thermal decomposition.

While **Figure 3** does show variability in analytical results between different laboratories employing hot *aqua regia* digestion, the difference is not statistically significant when all samples are considered together (p = 0.054; paired t-test; see **Tables 1 and 2**). Reasons for variability between laboratories employing the same sample digestion procedure could include specific procedural details (e.g., Alpha heats samples with a hot block, whereas Eurofins uses a microwave heater), minor differences in the sequencing and volumes of reagent addition prescribed in each laboratory standard operating procedures, and differences in analytical methods. Eurofins followed a modified Method 1631 (employing hot *aqua regia* digestion) while Alpha followed Method 7474.

Data from **Figure 2** and **Figure 3** are combined in **Table 1** for statistical analysis. For samples analyzed in triplicate, **Table 1** includes the mean values presented in **Attachment 1. Table 2** summarizes the results of paired t-tests between different digestion/analytical techniques. As presented in **Table 2**, modifying Method 1631 to include a hot *aqua regia* digestion appears to generally improve analytical recovery relative to digestion under cold *aqua regia* and warm nitric/sulfuric acid regimes. This conclusion follows from a combination of results shown in **Table 2**; namely that analytical recovery following cold *aqua regia* digestion is significantly different than recovery following hot *aqua regia* digestion ($p < 0.05$, paired two-tailed t-test), but does not appear to be different than recovery following warm nitric/sulfuric acid digestion ($p = 0.26$). Moreover, results in **Table 2** suggest that analytical recovery following hot *aqua regia* digestion (either by Eurofins or by Alpha Labs) is not statistically different than recovery following thermal decomposition (Method 7473) ($p > 0.05$; paired two-tail t-tests).

Given the preliminary finding that Method 1631 with hot *aqua regia* digestion appears more effective than Method 1631 with cold *aqua regia* digestion, samples collected in 2016 for the Penobscot River Estuary Phase III Engineering Study and analyzed by cold *aqua regia* digestion were re-analyzed using hot *aqua regia* digestion. **Figure 4** presents results of the comparison of the sample results for the hot and cold *aqua regia* digestions performed by Eurofins.

The larger data set presented in **Figure 4** suggests that there is generally good agreement between results following hot *aqua regia* digestion and cold *aqua regia* digestion. This generally good agreement is indicated by the slope of the “best fit” comparison line between digestion techniques being close to a 1:1 slope, with the coefficient of determination (R^2) being close to 1.0. In the case of the data presented in **Figure 4**, $R^2 = 0.93$ for all data, including bulk (unsieved) samples, material retained on the #40 sieve, and material passing through the #40 sieve.

While the overall closeness of these data to the 1:1 line suggests that analytical recovery is generally consistent for these samples following both hot and cold *aqua regia* digestion, paired two-tail t-tests of separate fractions suggest that hot *aqua regia* digestion results in increased recovery relative to cold *aqua regia* digestion for the fraction retained on the #40 sieve ($p = 0.001$ [as compares to $\alpha = 0.05$]), but not on the fraction that passes through the sieve ($p = 0.49$). For bulk samples (i.e., sediment samples that have not been sieved to separate the coarser fraction), the difference between digestion techniques is statistically significant ($p = 0.01$), but less so than for the fraction retained on the sieve. As noted above, these differences between bulk samples and materials retained on a #40 sieve are small when considered in terms of methods comparisons and/or values of R^2 (**Figure 5**), but they are statistically significant at a standard level of evaluation ($\alpha = 0.05$). Because the sieved material (1) appears to be predominantly wood waste (as evidenced by visual inspection and measured TOC content) and (2) is characterized by elevated mercury concentrations (**Figures 4 and 5**), we believe that these data support our recommendation to employ Method 1631 with hot *aqua regia* digestion for future analyses.

In considering variability in analysis of replicate samples, the substantial heterogeneity evidenced by the error bars for bulk samples in **Figure 4** and **Figure 5** is a common occurrence in Penobscot River sediments. Sample heterogeneity is likely caused by the presence of wood waste with an elevated and variable mercury concentration intermixed with mineral sediment in which the mercury concentration is less variable between replicates. A second possible

contributor to analytical variability is the assumption of homogenous water content between subsamples subjected to different analyses.

Regarding the question of homogeneity and water content in samples, commercial laboratories correct wet-weight based analytical results to dry weight using total solids measurements. In practice, this correction means that at the time a sample is prepared for mercury and methyl mercury analysis, a laboratory like Eurofins will weigh three replicate sub-samples from the sample jar after homogenizing the sample by stirring. The first two wet samples are weighed and processed for analysis of mercury and methyl mercury, respectively. The third sample is weighed, dried, and weighed again to determine the percent of water present in the sample prior to drying. This percent solids measurement is then applied to the wet weights of the sub-samples taken for methyl mercury and mercury analysis, based on the assumption that the water content of a sample is relatively consistent.

Regarding sub-sampling and homogenization, there are also differences in analytical recovery of total mercury between laboratories (**Figures 6, 7, and 8**). For data presented in **Figure 6**, as an example, both Eurofins and Alpha used hot *aqua regia* to digest replicate samples; results from Alpha are ~ 20% higher than results from Eurofins, and ~ 8% higher than Flett, with much of the difference between laboratories appearing driven by the presence of coarse materials (i.e., the 'retained' fraction) with elevated mercury concentrations. As discussed above, reasons for the differences between laboratories can also potentially include specific procedural details, minor differences in the sequencing and volumes of reagent addition prescribed in each laboratory standard operating procedures, and differences in analytical method. However, the overall analytical results for all three laboratories agree within 25% (i.e., the slope on the regression lines in **Figures 6, 7, and 8** are less than 1.25), which is an accepted threshold for accuracy when performing quality control checks using spikes and reference materials.

The multiple replicate comparisons of mercury analysis performed by different laboratories for this study provides a chance to test the consistency of percent solids measurements between supposed replicate subsamples. **Figure 9** shows that while this assumption of consistent percent solids in replicate subsamples is generally valid, it is not always a correct assumption in Penobscot River sediments. That is, while percent solids in different replicates often agree, there are occasional large discrepancies between replicates analyzed at different laboratories. The magnitude and frequency of the occasional differences in percent solids measured for Penobscot River sediments also could contribute to the within-sample variability observed in the mercury analyses of these sediments.

Regarding sample variability, and of significance for the Penobscot River Estuary Phase III Engineering Study—including the development and implementation of future long-term monitoring plans for the Penobscot River—is the ability to achieve a reasonable balance between identifying analytical methods that can be employed consistently by multiple laboratories, while recognizing that sample heterogeneity, such as occurs in this system due to the presence of wood waste, will complicate the ability to achieve homogeneous sub-samples of field-collected material (which is required for inter-laboratory comparisons). For the purpose of the Penobscot River Estuary Phase III Engineering Study, the combination of these two factors (i.e., analytical variability and sample heterogeneity) results in the need to identify laboratories that are able to provide consistent data recovery for analysis of mercury as well as other analytes, such as methyl mercury.

Results of the analytical and inter-laboratory comparisons described above suggest that (1) hot *aqua regia* digestion and thermal decomposition appear to be the most effective methods for

achieving high and consistent analytical recovery of mercury in sediment samples having substantial amounts of wood waste; and (2) results from multiple laboratories employing a hot *aqua regia* digestion technique are generally consistent. For Eurofins, comparison to both Alpha (employing hot *aqua regia* digestion following Method 7474) and Flett (employing thermal decomposition following Method 7473) suggests agreement to within 20 - 30%, which is considered an accepted threshold for accuracy when performing quality control checks using spikes and/or reference materials.

Methyl Mercury Method Comparisons

Analysis of methyl mercury in coarse material yields significantly higher results when the samples are extracted by methanolic KOH compared to methylene chloride ($p < 0.0001$, **Figure 10**). The samples in **Figure 10** that are designated “coarse, not woody” come from eel traps retrieved from the Orland River and near Bucksport. They are designated “not woody” based on lower TOC concentrations (7.5% for the Orland River sample and 3.6% for the Bucksport sample). In contrast, samples designated “coarse, woody” have TOC concentrations ranging between 16% and 36%. In general, methyl mercury concentrations tend to increase with TOC, consistent with the findings of the Phase II Study. The average ratio of methyl mercury concentration determined by methanolic KOH extraction to concentration determined by methylene chloride extraction is 1.7 ± 0.4 (1 standard deviation).

The difference in methyl mercury concentration following distillation versus extraction with methanolic KOH was not significant ($p = 0.018$; paired two-tailed t-test; **Table 3**). The average ratio of methyl mercury concentration determined by distillation versus methanolic KOH extraction was 1.5 ± 0.6 (1 standard deviation). Only one sample extracted with methanolic KOH extraction resulted in higher results than distillation (ON-10-01-C-17_SED_01-03). If this sample is removed from linear regression of these data, $R^2 = 0.72$ for remainder of the data set (**Figure 11**). Further, if this sample is removed from statistical analysis, the difference in methyl mercury concentration following distillation versus extraction with methanolic KOH is significant ($p < 0.0001$; paired two-tailed t-test).

RECOMMENDATIONS

Because one goal of the Penobscot River Estuary Phase III Engineering Study is to evaluate the link between (1) reductions in sediment mercury and methyl mercury concentrations following remedy implementation and (2) resultant reductions in the tissue concentration of these chemicals in animals of concern (including lobster, diving ducks, and songbirds) that live within the Penobscot River estuary and/or adjacent marshes, data quality is an important factor in this evaluation. For this reason, the following recommendations are made based on the findings above.

Recommendation 1: Perform total mercury analysis in sediments using hot aqua regia digestion or thermal decomposition.

The hot *aqua regia* digestion standard operating procedure (SOP) developed and validated by Eurofins should be applied to all sediment samples from the Penobscot River Estuary that are to be analyzed for total mercury. The digestion SOP used by Alpha will be compared to the Eurofins SOP to ensure the two laboratories are aligned in their specific method details. Although matrix interference in analysis of total mercury is most significant when wood waste is present and/or when TOC exceeds 10%, it cannot be known ahead of time whether substantial amounts of interfering material are present in a sample. Analysis of total mercury following

thermal decomposition using Method 7473 is an acceptable alternative to hot *aqua regia* digestion.

Recommendation 2: Perform methyl mercury analysis in sediments using chemical extraction by methanolic KOH.

Discussions with Eurofins indicate that chemical extraction is generally preferred over distillation by commercial laboratories. The apparatus and techniques necessary for distillation are specialized and labor intensive, and therefore not readily available from most commercial laboratories. With the goal of identifying a robust analytical method that is also commercially available, future work in the Penobscot River Estuary performed by commercial laboratories should rely on chemical extraction by methanolic KOH rather than distillation or chemical extraction by methylene chloride.

Recommendation 3: Account for sediment sample heterogeneity in certain portions of the estuary when designing monitoring programs.

Samples having substantial amounts of wood waste are highly heterogeneous, leading to the introduction of subsampling error as a potential source of uncertainty. There are three options for dealing with this heterogeneity and resultant analytical uncertainty, depending on the goals of the monitoring:

- Ground-freeze heterogeneous samples following the SOP developed by Eurofins. This option is essential for methods comparison. It is also potentially useful for delineation studies if the delineation does not rely on methyl mercury analysis. Grinding samples introduces an uncertainty as to whether methyl mercury is lost as redox conditions change within the sample.
- Analyze heterogeneous samples in triplicate. This option would be applicable to grab samples collected with a ponar-style sampler, but could be more difficult to implement in thin core sections where sample volume is limiting. The areas where this approach seems most relevant are in both subtidal and intertidal zones in the reaches between Orrington and the southern tip of Verona Island because sediment in these areas contains significant wood waste. Triplicate sampling is not as important outside these areas, or in wetland areas, because there is generally less wood waste present and therefore less assumed sample heterogeneity.
- Design the monitoring program to address spatial heterogeneity. This option is relevant in delineating affected areas and conducting “hill-topping” exercises to determine how much material needs to be removed to reach a sediment concentration goal. Because it is important to address spatial heterogeneity in delineation, and decisions to proceed with sediment removal would generally not rely on only a single data point for delineating an area, characterizing a site via several cores or grab samples may sufficiently address the heterogeneity of the material sampled.

Relatedly, sediment heterogeneity can have substantial effects on trend analysis. None of the three options discussed above can be applied post-hoc to historic data. Samples in the historic database that were collected from subtidal and intertidal areas between Orrington and the southern tip of Verona Island likely contained wood waste in varying and unknown proportions. As noted in the findings above, the presence of wood waste is likely a significant contributor to high variability in replicate analyses for total mercury. Findings of trend analyses

based on sediment samples should be carefully vetted for the potential impact of heterogeneity creating apparent outliers in trend analysis.

Recommendation 4: Account for multiple methods used when evaluating sediment data for trends.

An additional challenge to trends analysis is the variety of methods used for total mercury, methyl mercury, TOC, and organic content analyses. Some of the disparities between method-specific results can be resolved with adjustment factors such as transparently multiplying methyl mercury results generated by methylene chloride extraction by a correction factor of 2 to allow comparison with results generated by KOH extraction or distillation. This approach to data adjustment is consistent with both the recommendation made by the Phase II Study Group and the data presented in this Technical Memorandum regarding increased recovery of methyl mercury from organic-rich sediment using KOH extraction. Other disparities, such as the selection of appropriate adjustment factors for total mercury analysis are more difficult, because the disparity between methods is not consistent across all samples, and appears most significant for samples having higher proportions of wood waste. While higher sediment organic content and a higher percentage of the organic content as wood waste likely impact recovery of methyl mercury as well, concentration trends analysis for methyl mercury also requires taking of analytical feasibility and commercial laboratory availability into account.

REFERENCE CITED:

Amec Foster Wheeler (Amec). 2018. Analysis of Lignin Oxidation Products in Sediment Technical Memorandum. Penobscot River Phase III - Engineering Study, Penobscot River, Maine. February 2018.

Carrasco, L. and Vassileva. E. 2014. Determination of methyl mercury in marine biota samples: Method Validation. Talanta. 122: 106-114.

Attachment(s): Tables
Figures
Attachment 1
Attachment 2
Attachment 3



TABLES

TABLE 1
Compiled Total Mercury Inter-Laboratory Comparison Data for Paired T-testing¹

Penobscot River Phase III Engineering Study
 Penobscot River Estuary, Maine

Sample Description	Total Mercury (ng/g) ²					Replicate % Difference ³	Average RPD (%)
	Eurofins Cold AR	Eurofins Warm Nitric/Sulfuric	Alpha Hot AR	Flett Thermal Decomposition	Eurofins Hot AR		
Bucksport - 2	715	842	988	921	1,000	22.3	15.4
Bucksport - 3	888	891	1,074	989	901	10.3	
Bucksport - 21	197	235	279	289	324	31.6	
Gross Point	210	250	288	278	274	24.4	
Hampden	1,290	1,197	1,283	1,333	1,220	3.2	
Verona East Sieved	1,720		2,010	1,710	1,710	-0.6	
Verona East Un-Sieved	1,730		1,860	2,080	1,890	16.8	
Bucksport-1 Wood Chips	641	978	934	1,083	950	40.8	22.4
Verona East Wood Chips	2,050		2,610	2,330	2,360	12.0	
Frankfort Flats Wood Chips	1,143		1,493	1,333	1,277	14.3	

Notes:

1. Data presented in this table represent either laboratory-reported results or mean values calculated from three replicate analyses.
2. Data as dry weight determination
3. Eurofins Cold AR vs Flett Thermal Decomposition

Prepared by: KPA 7/12/2017

Checked by: DK 7/13/2017

Abbreviations:

Alpha = Alpha Analytical Laboratories, Inc.

AR = *aqua regia*

Eurofins = Eurofins Frontier Geosciences

Flett = Flett Research Ltd.

ng/g = nanograms per gram

RPD = relative percent difference

TABLE 2
Paired T-test Summary of Inter-Laboratory Methods Comparison¹

Penobscot River Phase III Engineering Study
 Penobscot River Estuary, Maine

Analytical Method	Eurofins Cold AR	Eurofins Warm Nitric/Sulfuric	Alpha Hot AR	Flett Thermal Decomposition	Eurofins Hot AR
Eurofins Cold AR	--	0.26	0.002	0.004	0.01
Eurofins Wam Nitric/Sulfuric	--	--	0.073	0.003	0.151
Alpha Hot AR	--	--	--	0.399	0.054
Flett Thermal Decomp	--	--	--	--	0.140

Notes:

1. Results in **bold** are statistically significant at $\alpha = 0.05$

Abbreviations:

-- = No test value; reason may be a test of same (i.e., Eurofins Cold AR vs. Eurofins Cold AR) or a duplicate test (i.e., Eurofins Cold AR vs. Alpha Hot AR is assessed; Alpha Hot AR vs. Eurofins Cold AR would constitute the same test)

Alpha = Alpha Analytical Laboratories, Inc.

AR = *aqua regia*

Eurofins = Eurofins Frontier Geosciences

Flett = Flett Research Ltd.

Prepared by: KPA 7/12/2017

Checked by: DK 7/13/2017

TABLE 3
Comparison of Extraction Methods for Methyl Mercury Analysis by EPA Method 1630

Penobscot River Phase III Engineering Study
 Penobscot River Estuary, Maine

Location	Sample ID	Sample Depth (ft)	Methyl Mercury (ng/g)				TOC		RPD
			KOH Extraction ¹		Distillation ²		%		
			Result	Qual	Result	Qual	Result	Qual	
FF-08-02-G	FF-08-02-G-17_SED_00-01	0 - 0.1	15.8		20.2		5.2		24%
FF-08-02-G	FF-08-02-G-17_SED_01-03	0.1 - 0.3	12.2		15.7		4.9		25%
MM-04-01-F	MM-04-01-F-17_SED_00-01	0 - 0.1	6.4		17.7		6.3		94%
MM-04-01-F	MM-04-01-F-17_SED_01-03	0.1 - 0.3	12.6		15		6.8		17%
ON-10-01-C	ON-10-01-C-17_SED_00-01	0 - 0.1	23.9		32.7		7.3		31%
ON-10-01-C	ON-10-01-C-17_SED_01-03	0.1 - 0.3	37.4		19		5.9		65%
ON-18-01-F	ON-18-01-F-17_SED_00-01	0 - 0.1	10.2		14.6		4.0		35%
ON-18-01-F	ON-18-01-F-17_SED_01-03	0.1 - 0.3	11.6		19.7		5.6		52%
VN-01-01-B	VN-01-01-B-17_SED_00-01	0 - 0.1	7.3		20.2		6.2		94%
VN-01-01-B	VN-01-01-B-17_SED_01-03	0.1 - 0.3	14.4		16.9		5.2		16%
VN-04-02-C	VN-04-02-C-17_SED_00-01	0 - 0.1	5.9		7.55		1.6		25%
VN-04-02-C	VN-04-02-C-17_SED_01-03	0.1 - 0.3	1.4	J	1.29		1.7		8%
VN-08-01-E	VN-08-01-E-17_SED_00-01	0 - 0.1	18		23.1		5.6		25%
VN-08-01-E	VN-08-01-E-17_SED_01-03	0.1 - 0.3	11.2		13.3		5.1		17%
VW-02-01-E	VW-02-01-E-17_SED_00-01	0 - 0.1	3.7	J	4.25		1.8		14%
VW-02-01-E	VW-02-01-E-17_SED_01-03	0.1 - 0.3	2.6	J	2.63		2.2		1%
VW-14-01-F	VW-14-01-F-17_SED_00-01	0 - 0.1	9.4	J	21.4		4.7		78%
VW-14-01-F	VW-14-01-F-17_SED_01-03	0.1 - 0.3	14.5		21		5.4		37%
WP-06-02-F	WP-06-02-F-17_SED_00-01	0 - 0.1	8.4		10.9		6.4		26%
WP-06-02-F	WP-06-02-F-17_SED_01-03	0.1 - 0.3	6.2		11.3		6.4		58%

Notes:

1. Eurofins Frontier Geosciences
2. Flett Research Ltd.

Data qualifiers:

J = Reported value is an estimate.

ft = feet

MeHg = methyl mercury

ng/g = nanograms per gram

TOC = total organic carbon; analyzed by Lloyd Kahn

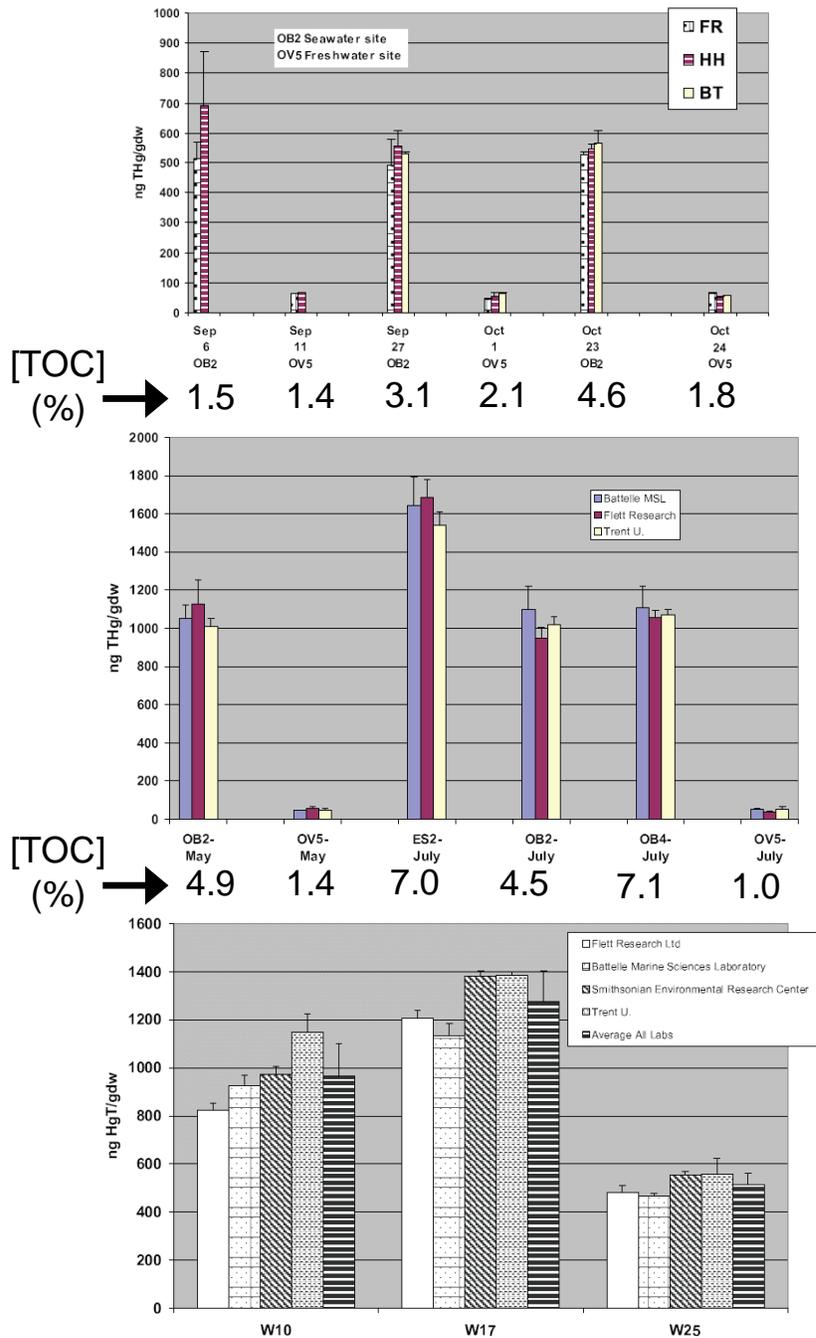
% = percent

RPD = relative percent difference

Prepared By: KMS 1/19/2018

Checked By: KAM 1/19/2018

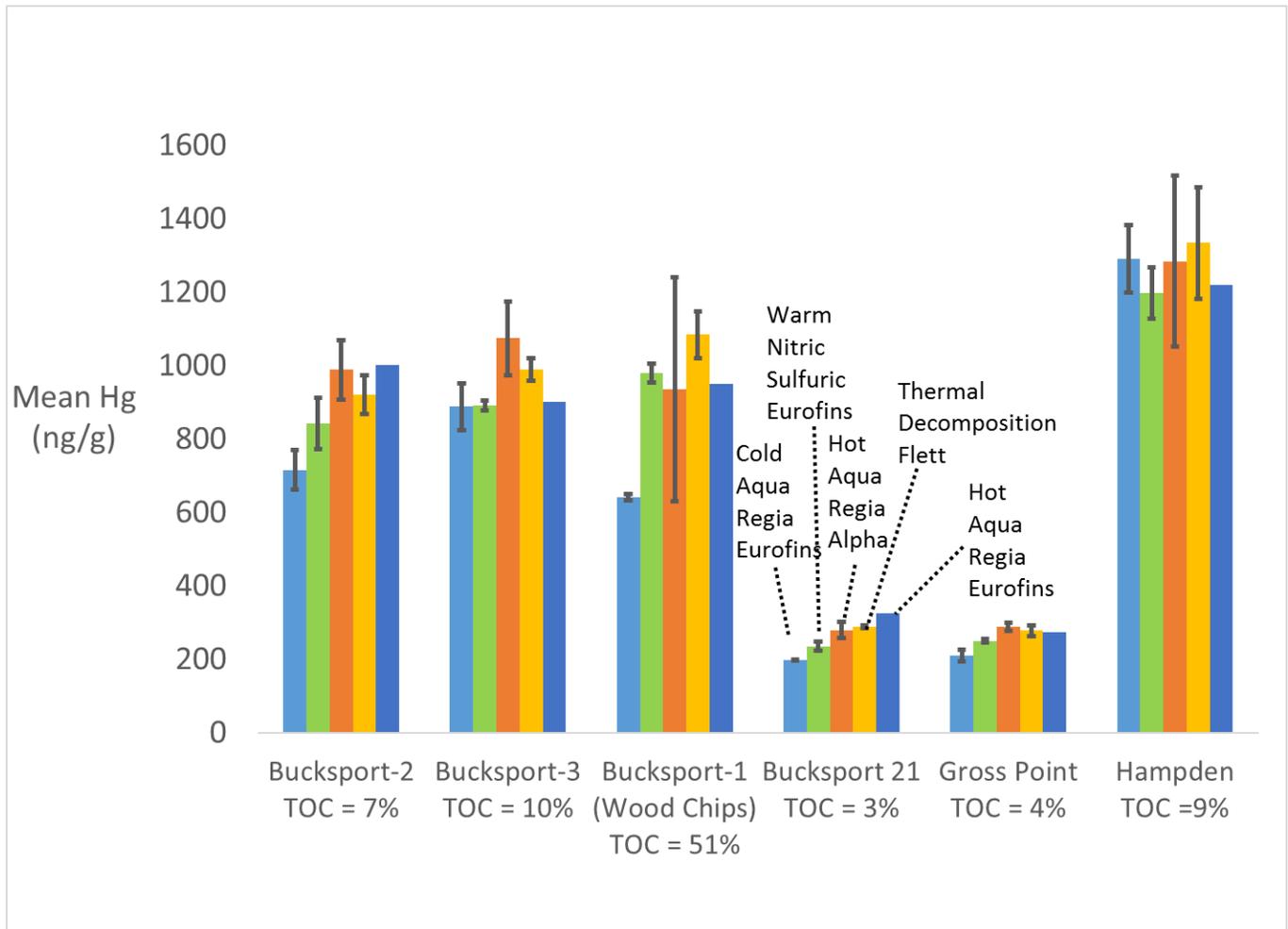
FIGURES



Comparisons were performed for total mercury on samples collected in 2006 (top), 2007 (middle), and 2009 (bottom). The top two charts have been amended by Amec Foster Wheeler to indicate the reported TOC concentration in each sample. TOC data were not available for 2009 samples. [prepared by KPA/checked by KAM]

Figure 1
Total Mercury Results from Initial Phase II Study Methods Comparison

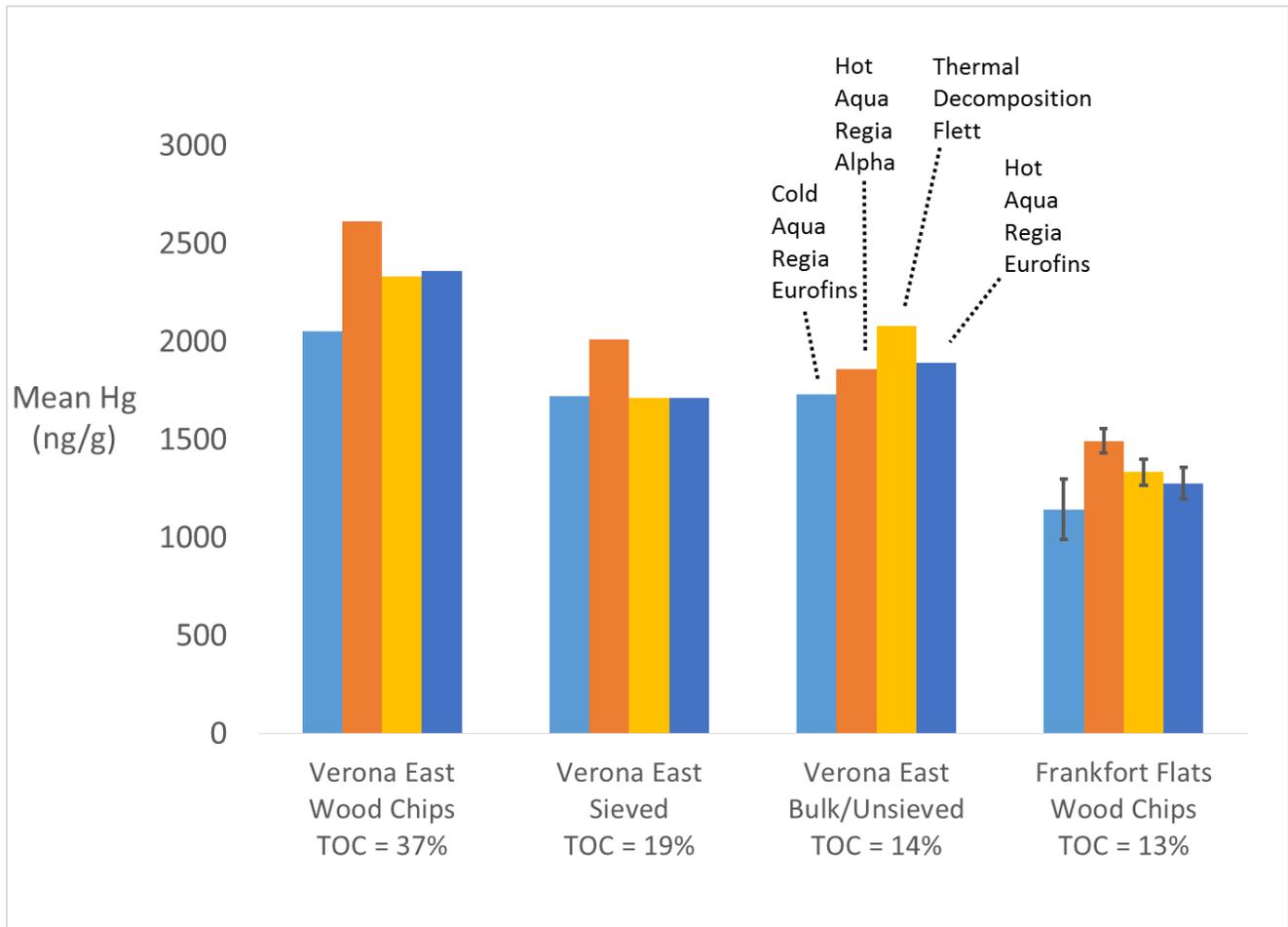
WO 4A-010 Analytical Methods Comparison
Penobscot River Estuary Phase III Engineering Study



Error bars show 95% confidence intervals for total mercury for three replicate samples analyzed by cold *aqua regia* digestion (Eurofins), warm nitric/sulfuric acid digestion (Eurofins), hot *aqua regia* digestion (Alpha), and thermal decomposition (Flett). No error bars indicate single sample analysis of total mercury for samples analyzed by hot *aqua regia* digestion (Eurofins). Mercury concentrations as dry weight determination. [prepared by KPA/checked by KAM]

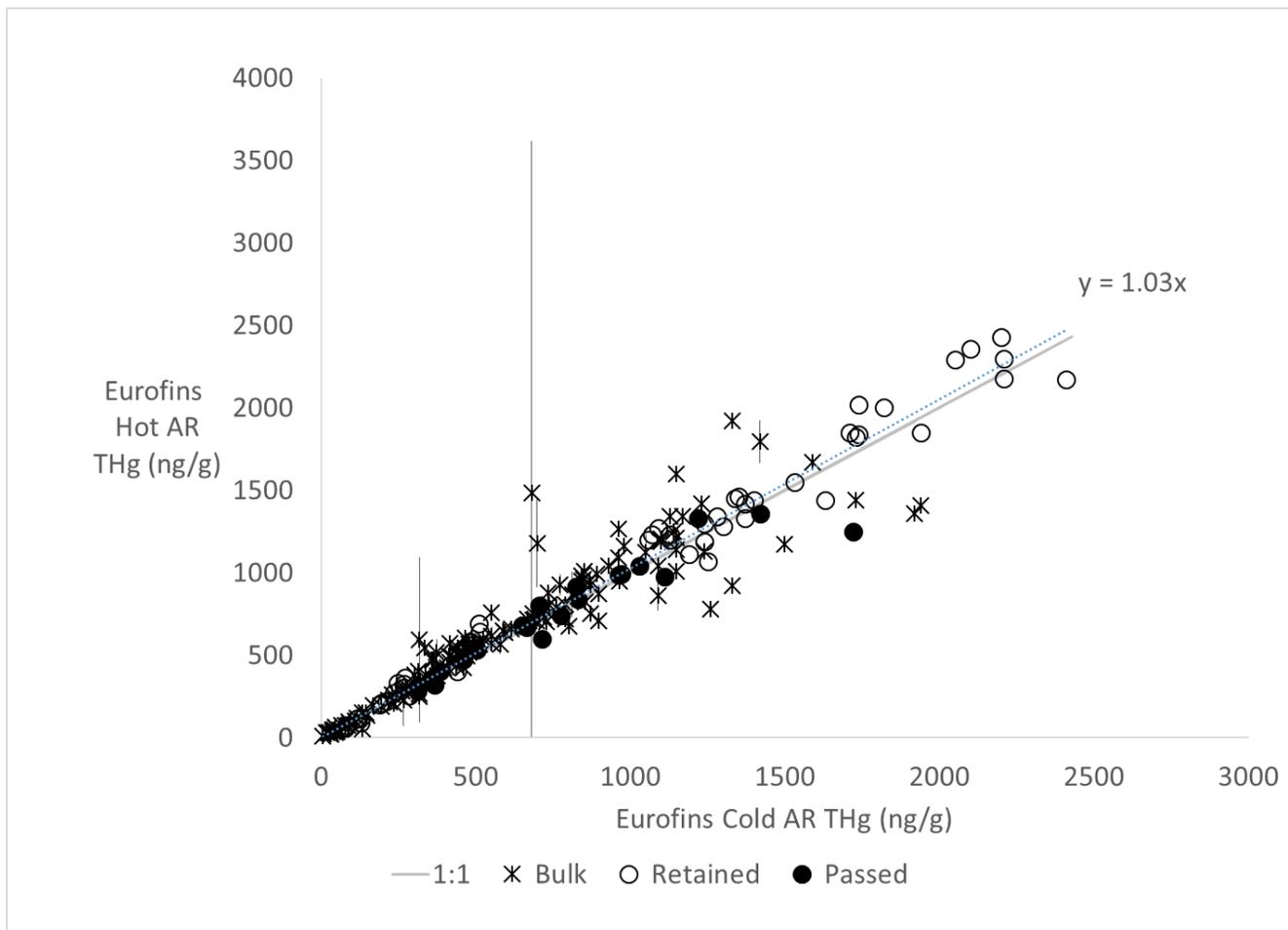
Figure 2
Comparison of Total Mercury Results by Different Analytical Methods

WO 4A-010 Analytical Methods Comparison
Penobscot River Estuary Phase III Engineering Study



Error bars show 95% confidence intervals of three replicate samples from Frankfort Flats. No error bars indicate single sample analysis for sample fractions (wood chips, sieved and bulk/unsieved) from Verona East. Mercury concentrations as dry weight determination. [prepared by KPA/checked by KAM]

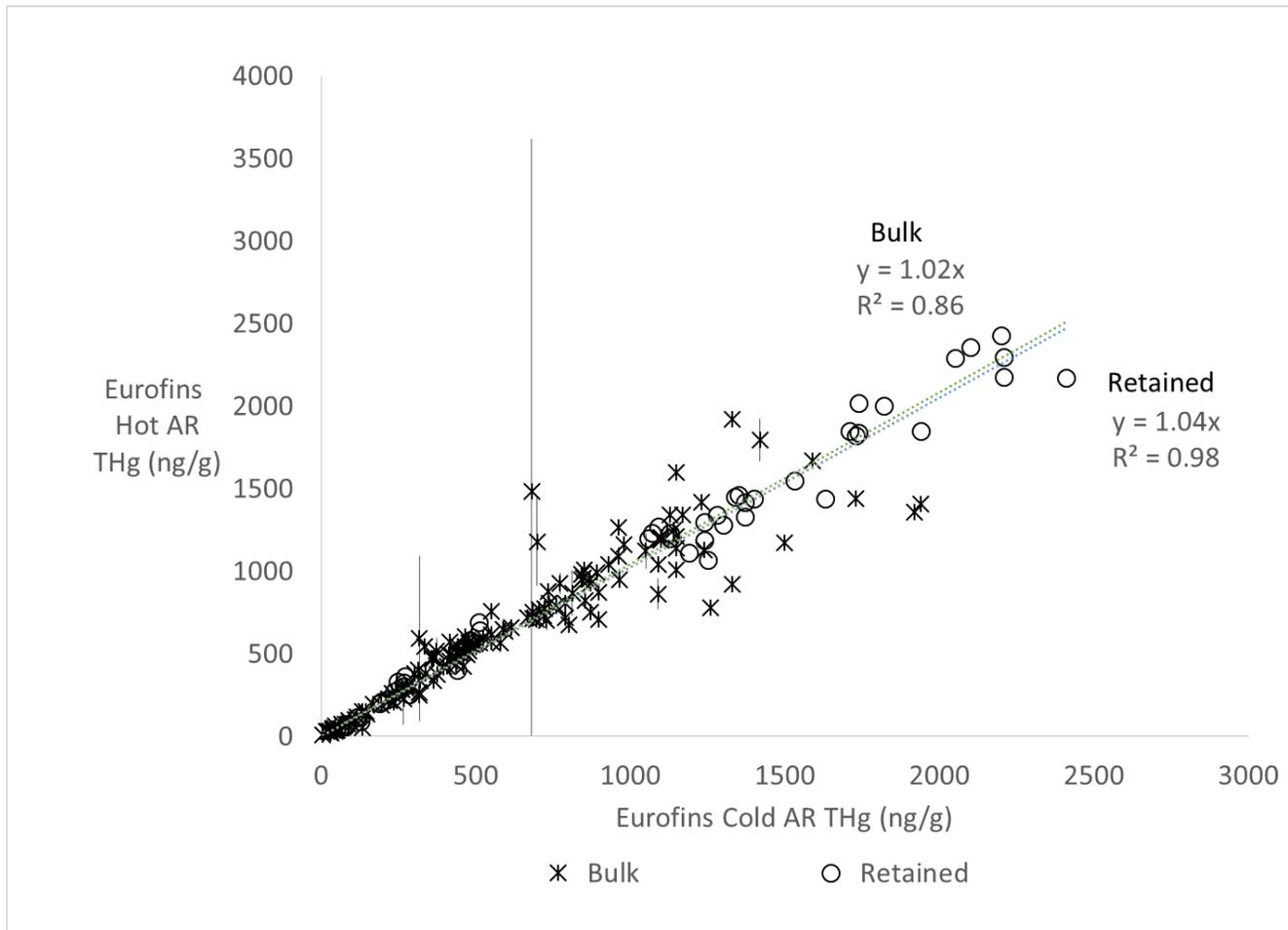
Figure 3
Additional Comparison of Total Mercury Results
WO 4A-010 Analytical Methods Comparison
Penobscot River Estuary Phase III Engineering Study



Error bars on samples characterized as 'Bulk' indicate 95% confidence of the mean for three replicate subsamples. 'Bulk' is defined as an unsieved sample; 'retained' is captured on a #40 sieve; 'passed' is passing through a #40 sieve. Mercury concentrations as dry weight determination. [prepared by KPA/checked by KAM]

Figure 4
Comparison of Mercury Results by Hot *Aqua Regia* vs. Cold *Aqua Regia* Digestion Performed by Eurofins

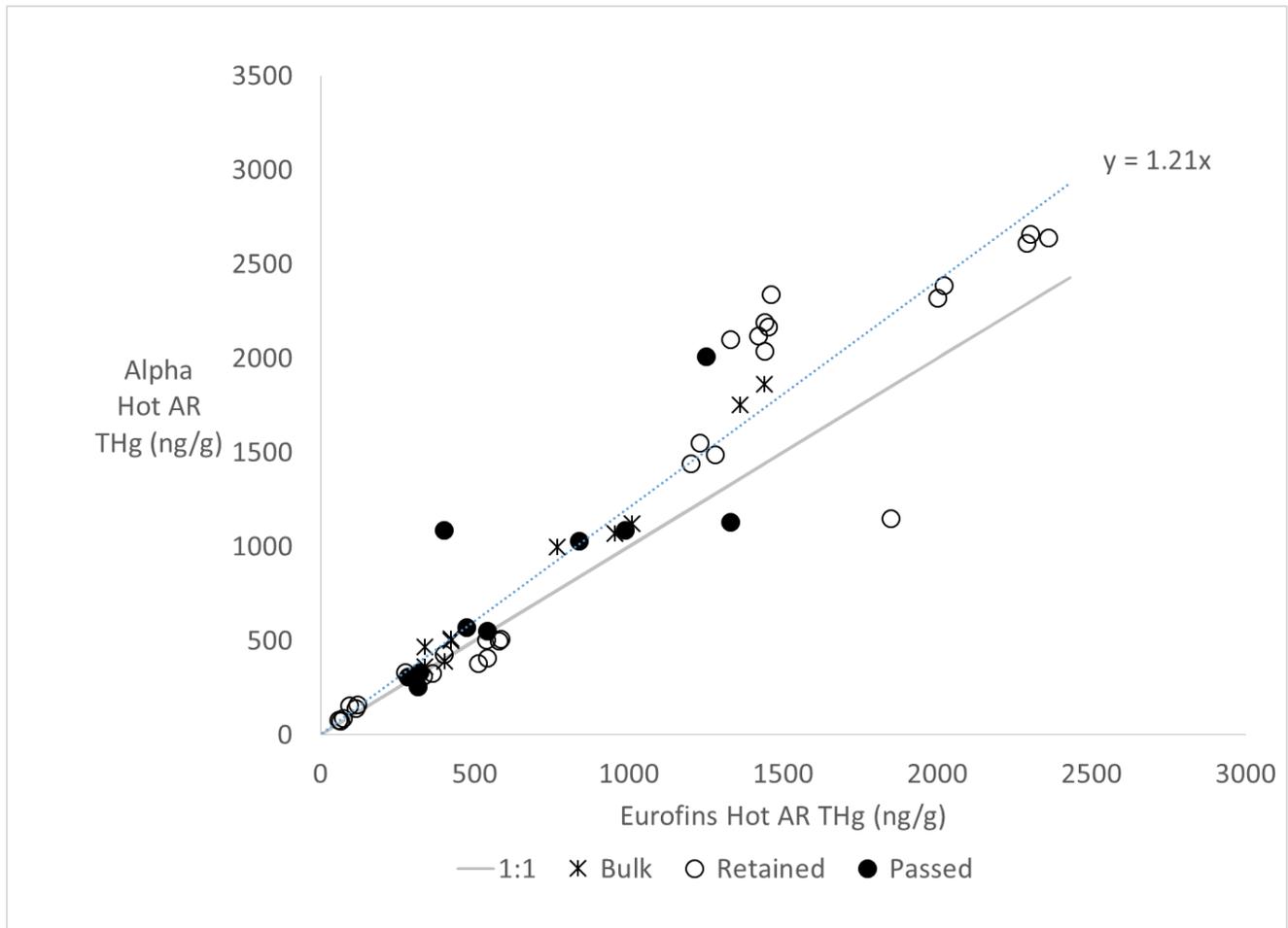
**WO 4A-010 Analytical Methods Comparison
 Penobscot River Estuary Phase III Engineering Study**



'Bulk' is defined as an unsieved sample; 'retained' is captured on a #40 sieve. Mercury concentrations as dry weight determination. [prepared by KPA/checked by KAM]

Figure 5
Further Comparison of Mercury Results by Hot *Aqua Regia* vs. Cold *Aqua Regia* Digestion Performed by Eurofins

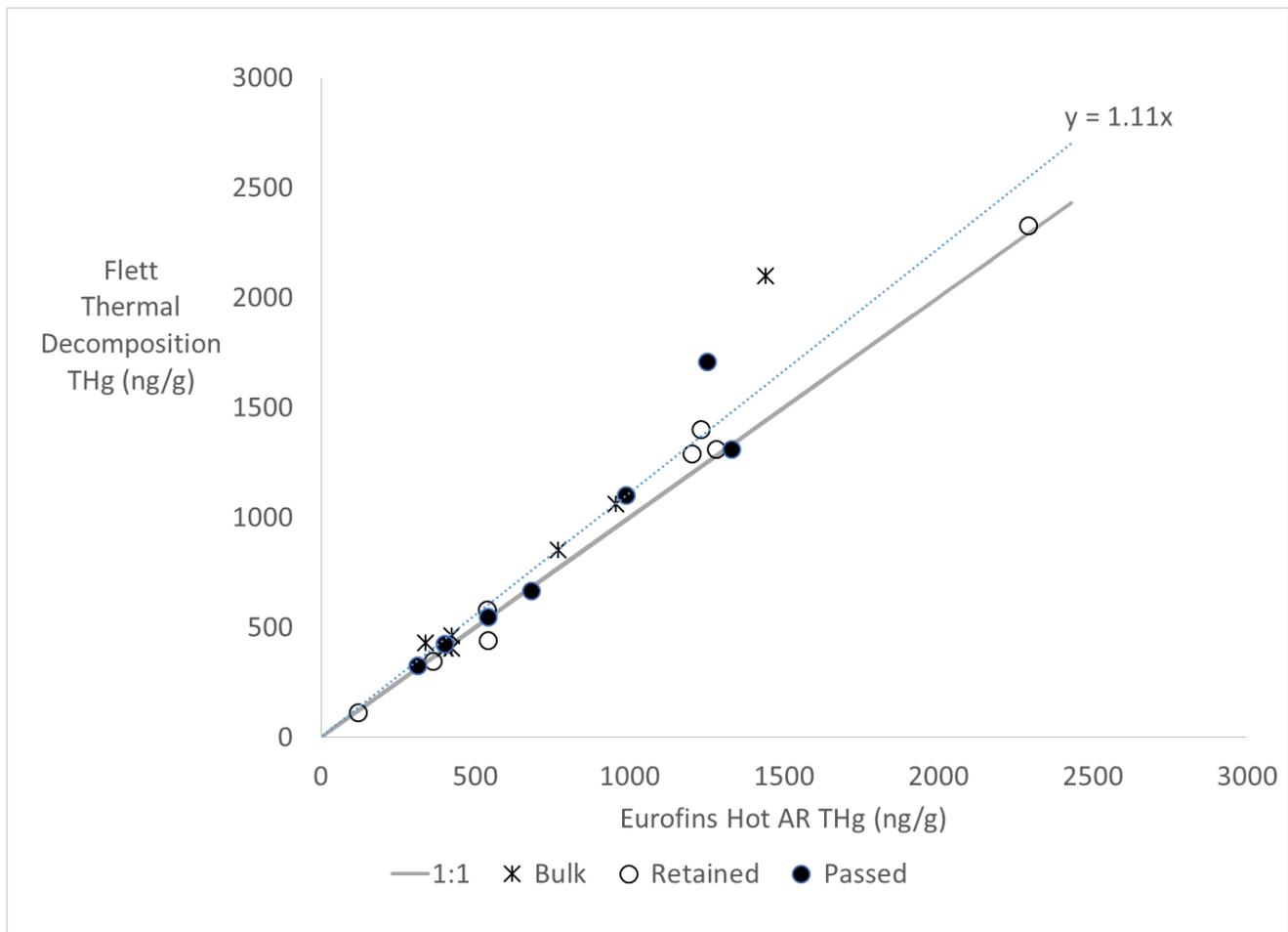
**WO 4A-010 Analytical Methods Comparison
 Penobscot River Estuary Phase III Engineering Study**



'Bulk' is defined as an unsieved sample; 'retained' is captured on a #40 sieve; 'passed' is passing through a #40 sieve. Mercury concentrations as dry weight determination. [prepared by KPA/checked by KAM]

Figure 6
Comparison of Mercury Results by Hot Aqua Regia Digestion
Performed by Alpha vs. Eurofins

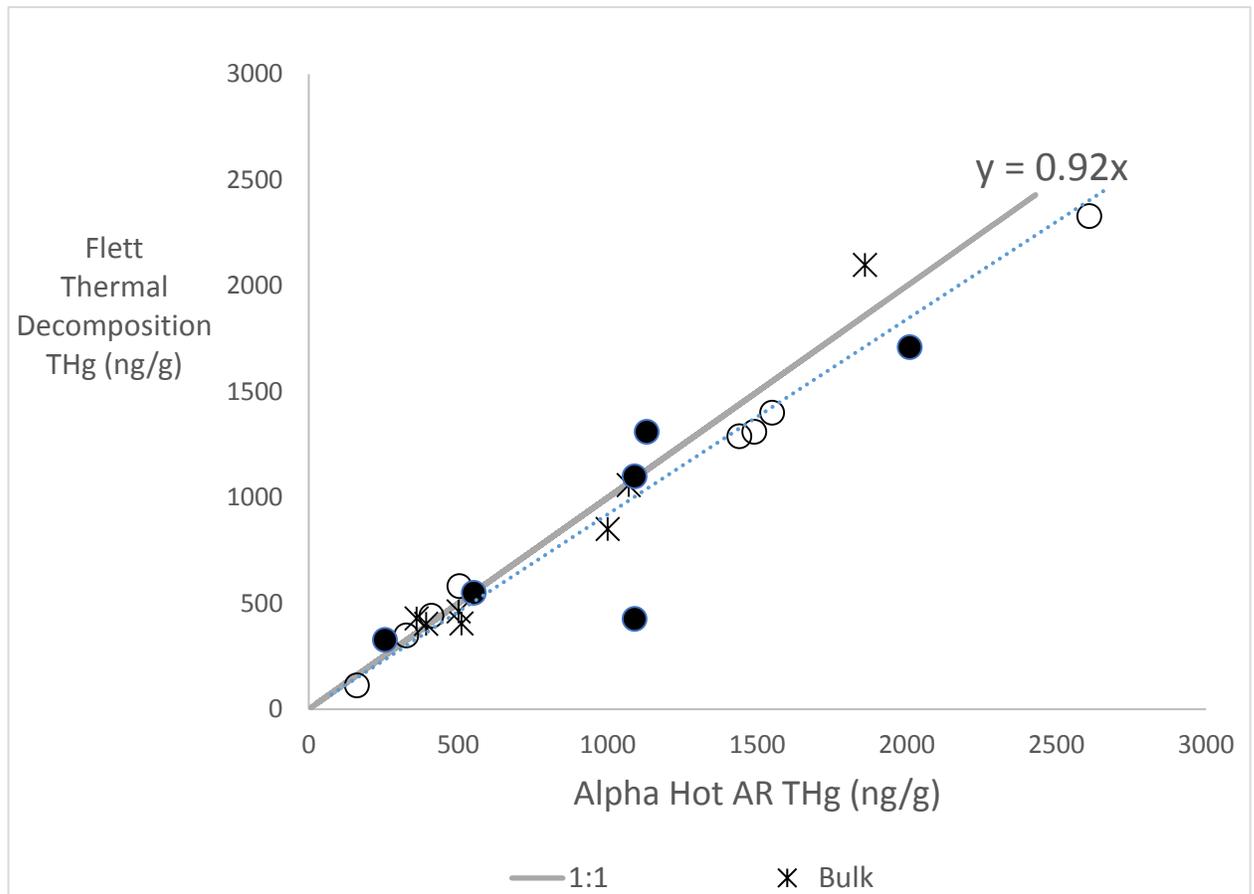
WO 4A-010 Analytical Methods Comparison
Penobscot River Estuary Phase III Engineering Study



'Bulk' is defined as an unsieved sample; 'retained' is captured on a #40 sieve; 'passed' is passing through a #40 sieve. Mercury concentrations as dry weight determination. [prepared by KPA/checked by KAM]

Figure 7
Comparison of Mercury Results by Thermal Decomposition by Flett vs. Hot *Aqua Regia* Digestion by Eurofins

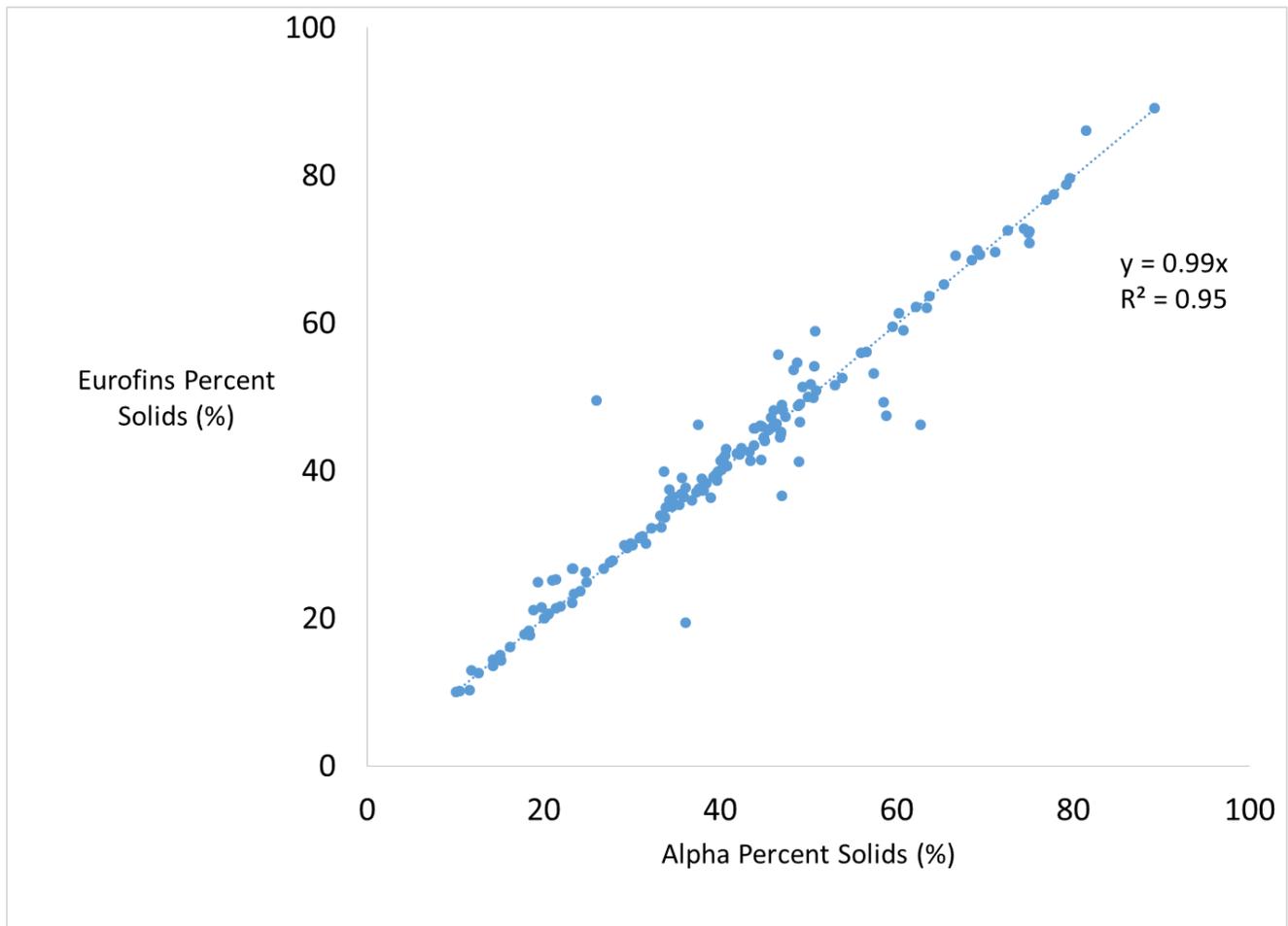
**WO 4A-010 Analytical Methods Comparison
 Penobscot River Estuary Phase III Engineering Study**



'Bulk' is defined as an unsieved sample; 'retained' is captured on a #40 sieve; 'passed' is passing through a #40 sieve. Mercury concentrations as dry weight determination. [prepared by KPA/checked by KAM]

Figure 8
Comparison of Mercury Results by Thermal Decomposition by Flett vs. Hot *Aqua Regia* Digestion by Alpha

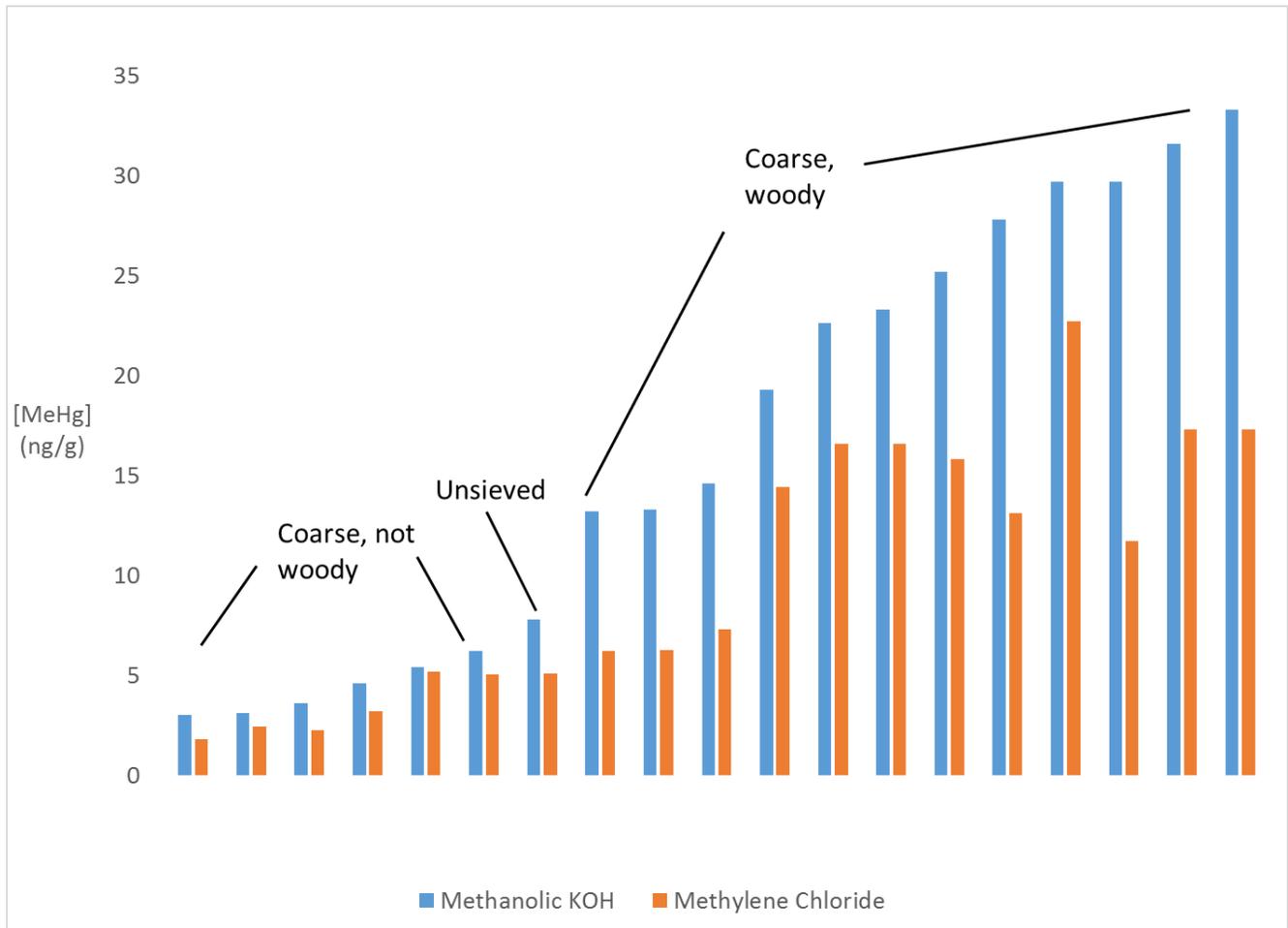
**WO 4A-010 Analytical Methods Comparison
Penobscot River Estuary Phase III Engineering Study**



[prepared by KPA/checked by KAM]

Figure 9
Comparison of Percent Solids Results by Eurofins vs. Alpha
on Replicate Subsamples from the Same Bulk Sediment Sample

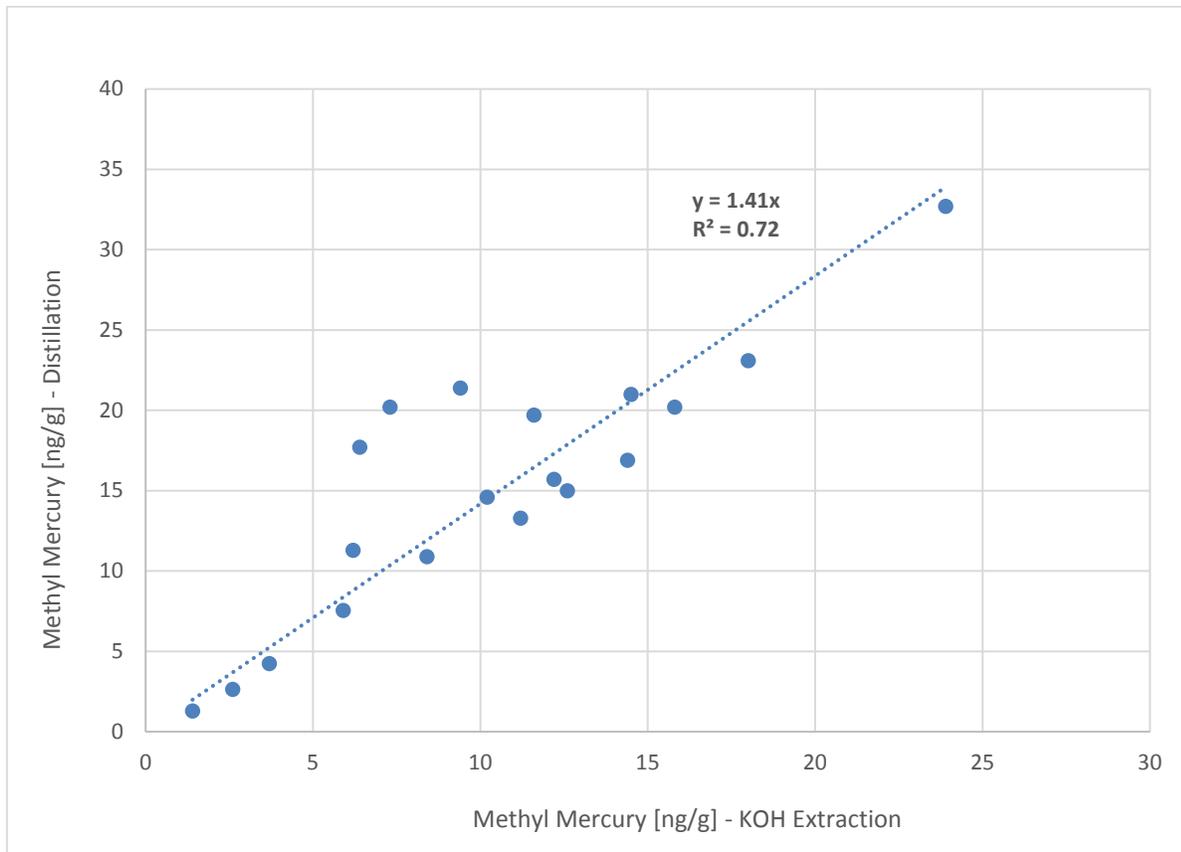
WO 4A-010 Analytical Methods Comparison
Penobscot River Estuary Phase III Engineering Study



Methyl mercury concentrations as dry weight determination. [prepared by KPA/checked by KAM]

Figure 10
Methyl Mercury Concentration in Samples Following Extraction
by Methanolic KOH vs. Methylene Chloride

WO 4A-010 Analytical Methods Comparison
Penobscot River Estuary Phase III Engineering Study



Methyl mercury concentrations as dry weight determination [prepared by KAM/checked by KMS]

Figure 11
Comparison of Methyl Mercury Results by KOH Extraction
by Eurofins vs. Distillation by Flett

WO 4A-010 Analytical Methods Comparison
Penobscot River Estuary Phase III Engineering Study

ATTACHMENT 1

June 2016 Results from WO 04A-010 Methods Comparison

**ATTACHMENT 1
 JUNE 2016 RESULTS FROM WO 4A-010 METHODS COMPARISON**

Penobscot River Phase III Engineering Study
 Penobscot River Estuary, Maine

Location	Field Sample ID	Laboratory	TOC ² (%)	EPA Method	Total Mercury (ng/g) ¹						STDEV	Confidence	%RSD
					1st Rep	2nd Rep	3rd Rep	Min	Max	Mean			
Bucksport	T_Bu2_060916_SED_G	Eurofins	6.9	1631 Cold AR	740	660	745	660	745	715	47.7	54.0	6.7
Bucksport	T-Bu3_060916_SED_G	Eurofins	9.8		849	861	953	849	953	888	56.9	64.4	6.4
Bucksport	T-Bu1_061016_SED_G_WC	Eurofins	50.8		640	650	633	633	650	641	8.5	9.7	1.3
Bucksport	T_Bu21R_061016_SED	Eurofins	2.9		198	199	195	195	199	197	2.1	2.4	1.1
Gross Point	T_GP33H_060916_SED_C	Eurofins	4.1		226	200	204	200	226	210	14.0	15.8	6.7
Hampden	HA1_060916_SED_G	Eurofins	8.9		1,360	1,200	1,310	1,200	1,360	1,290	81.9	92.6	6.3
Bucksport	T_Bu2_060916_SED_G	Eurofins	6.9	1631 Warm Nitric/Sulfuric	888	771	867	771	888	842	62.4	70.6	7.4
Bucksport	T-Bu3_060916_SED_G	Eurofins	9.8		900	878	895	878	900	891	11.5	13.1	1.3
Bucksport	T-Bu1_061016_SED_G_WC	Eurofins	50.8		986	996	953	953	996	978	22.5	25.5	2.3
Bucksport	T_Bu21R_061016_SED	Eurofins	2.9		226	231	247	226	247	235	11.0	12.4	4.7
Gross Point	T_GP33H_060916_SED_C	Eurofins	4.1		254	246	249	246	254	250	4.0	4.6	1.6
Hampden	HA1_060916_SED_G	Eurofins	8.9		1,130	1,250	1,210	1,130	1,250	1,197	61.1	69.1	5.1
Bucksport	T_Bu2_060916_SED_G	Alpha	6.9	7474 Hot AR	985	918	1,060	918	1,060	988	71.0	80.4	7.2
Bucksport	T-Bu3_060916_SED_G	Alpha	9.8		1,080	1,160	983	983	1,160	1,074	88.6	100.3	8.3
Bucksport	T-Bu1_061016_SED_G_WC	Alpha	50.8		898	684	1,220	684	1,220	934	269.8	305.3	28.9
Bucksport	T_Bu21R_061016_SED	Alpha	2.9		293	288	256	256	293	279	20.1	22.7	7.2
Gross Point	T_GP33H_060916_SED_C	Alpha	4.1		280	299	284	280	299	288	10.0	11.3	3.5
Hampden	HA1_060916_SED_G	Alpha	8.9		1,440	1,360	1,050	1,050	1,440	1,283	206.0	233.1	16.1
Bucksport	T_Bu2_060916_SED_G	Flett	6.9	7473 Thermal Decomposition	896	892	974	892	974	921	46.2	52.3	5.0
Bucksport	T-Bu3_060916_SED_G	Flett	9.8		1,020	976	972	972	1,020	989	26.6	30.1	2.7
Bucksport	T-Bu1_061016_SED_G_WC	Flett	50.8		1,100	1,130	1,020	1,020	1,130	1,083	56.9	64.3	5.2
Bucksport	T_Bu21R_061016_SED	Flett	2.9		292	285	289	285	292	289	3.5	4.0	1.2
Gross Point	T_GP33H_060916_SED_C	Flett	4.1		273	268	292	268	292	278	12.7	14.3	4.6
Hampden	HA1_060916_SED_G	Flett	8.9		1,180	1,390	1,430	1,180	1,430	1,333	134.3	152.0	10.1
Bucksport	T_Bu2_060916_SED_G	Eurofins	6.9	1631 Hot AR	1,000	NA	NA	NA	NA	NA	NA	NA	NA
Bucksport	T-Bu3_060916_SED_G	Eurofins	9.8		901	NA	NA	NA	NA	NA	NA	NA	NA
Bucksport	T-Bu1_061016_SED_G_WC	Eurofins	50.8		950	NA	NA	NA	NA	NA	NA	NA	NA
Bucksport	T_Bu21R_061016_SED	Eurofins	2.9		324	NA	NA	NA	NA	NA	NA	NA	NA
Gross Point	T_GP33H_060916_SED_C	Eurofins	4.1		274	NA	NA	NA	NA	NA	NA	NA	NA
Hampden	HA1_060916_SED_G	Eurofins	8.9		1,220	NA	NA	NA	NA	NA	NA	NA	NA

Notes:

1. Data as dry weight determination
2. TOC data from Elemental Analysis as provided to Amec Foster Wheeler by Global Aquatic Research

Prepared By: KPA
 Checked By: KAM

Abbreviations:

Alpha = Alpha Analytical Laboratories, Inc.
 AR = *aqua regia*
 EPA = Environmental Protection Agency
 Eurofins = Eurofins Frontier Geosciences
 Flett = Flett Research Ltd.
 max = maximum
 min = minimum
 NA = not applicable
 ng/g = nanograms per gram
 RSD = relative standard deviation
 STDEV = standard deviation
 TOC = total organic carbon

ATTACHMENT 2

Fall 2016 Results from WO 04A-020 Methods Comparison

**ATTACHMENT 2
 FALL 2016 RESULTS FROM WO 04A-020 METHODS COMPARISON**

Penobscot River Phase III Engineering Study
 Penobscot River Estuary, Maine

Location	Field Sample ID	TOC (%)	Total Mercury (ng/g) ¹			
			EPA Method 1631 Cold AR (Eurofins)	EPA Method 7474 Hot AR (Alpha)	EPA Method 7473 Thermal Decomposition (Flett)	EPA Method 1631 Hot AR (Eurofins)
Verona East	VE_TRAP2+3_SIEVE_03112017_WCH_R1	37.1	2,050	2,610	2,330	2,360
Verona East	VE_TRAP2+3_SIEVE_03122017_SED	19.4	1,720	2,010	1,710	1,710
Verona East	VE_TRAP2+3_SIEVE_03112017_SED_PRE	13.8	1,730	1,860	2,080	1,890
Frankfort Flats	FF5152_SIEVE_03082017_WCH_R1	14.5	1,070	1,550	1,400	1,200
Frankfort Flats	FF5152_SIEVE_03082017_WCH_R3	12.8	1,300	1,490	1,310	1,340
Frankfort Flats	FF5152_SIEVE_03082017_WCH_R2	11.0	1,060	1,440	1,290	1,290

1. Data as dry weight determination

Abbreviations:

Alpha = Alpha Analytical Laboratories, Inc.

AR = *aqua regia*

EPA = Environmental Protection Agency

Eurofins = Eurofins Frontier Geosciences

Flett = Flett Research Ltd.

TOC = total organic carbon

Prepared By: KPA

Checked By: KAM

ATTACHMENT 3

Fall 2016 Results and WO 04A-020 Extraction Methods Comparison for Methyl Mercury

**ATTACHMENT 3
 FALL 2016 RESULTS AND WO 04A-020 EXTRACTION METHODS
 COMPARISON FOR METHYLMERCURY**

Penobscot River Phase III Engineering Study
 Penobscot River Estuary, Maine

Sample ID	Methylmercury (ng/g) ¹	
	EPA Method 1630 KOH/Methanol Extraction	EPA Method 1630 Methylene Chloride Extraction
OR_TRAP1+2_SIEVE_03092017_WCH_R1	3	1.79
OR_TRAP1+2_SIEVE_03092017_WCH_R3	3.1	2.42
OR_TRAP1+2_SIEVE_03092017_WCH_R2	3.6	2.24
BU_TRAP1+3_SIEVE_03112017_WCH_R3	4.6	3.22
BU_TRAP1+3_SIEVE_03112017_WCH_R2	5.4	5.2
BU_TRAP1+3_SIEVE_03112017_WCH_R1	6.2	5.06
BU50THRU52_02102017_SED_PRE	7.8	5.1
VN51THRU58_SIEVE_02082017_WCH-R2	13.2	6.24
VN51THRU58_SIEVE_02082017_WCH-R1	13.3	6.26
VN51THRU58_SIEVE_02082017_WCH-R3	14.6	7.29
FF_TRAP1+3_03112017_WCH_R3	19.3	14.4
BU50THRU52_02102017_WCH_R1	22.6	16.6
FF_TRAP1+3_03112017_WCH_R1	23.3	16.6
FF_TRAP1+3_03112017_WCH_R2	25.2	15.8
VE_TRAP2+3_SIEVE_03112017_WCH_R3	27.8	13.1
BU50THRU52_02102017_WCH_R3	29.7	22.7
VE_TRAP2+3_SIEVE_03112017_WCH_R1	29.7	11.7
VE_TRAP2+3_SIEVE_03112017_WCH_R2	31.6	17.3
BU50THRU52_02102017_WCH_R2	33.3	17.3

1. Data as dry weight determination

Abbreviations:

EPA = Environmental Protection Agency
 KOH = potassium hydroxide
 ng/g = nanograms per gram

Prepared By: KPA
 Checked By: KAM