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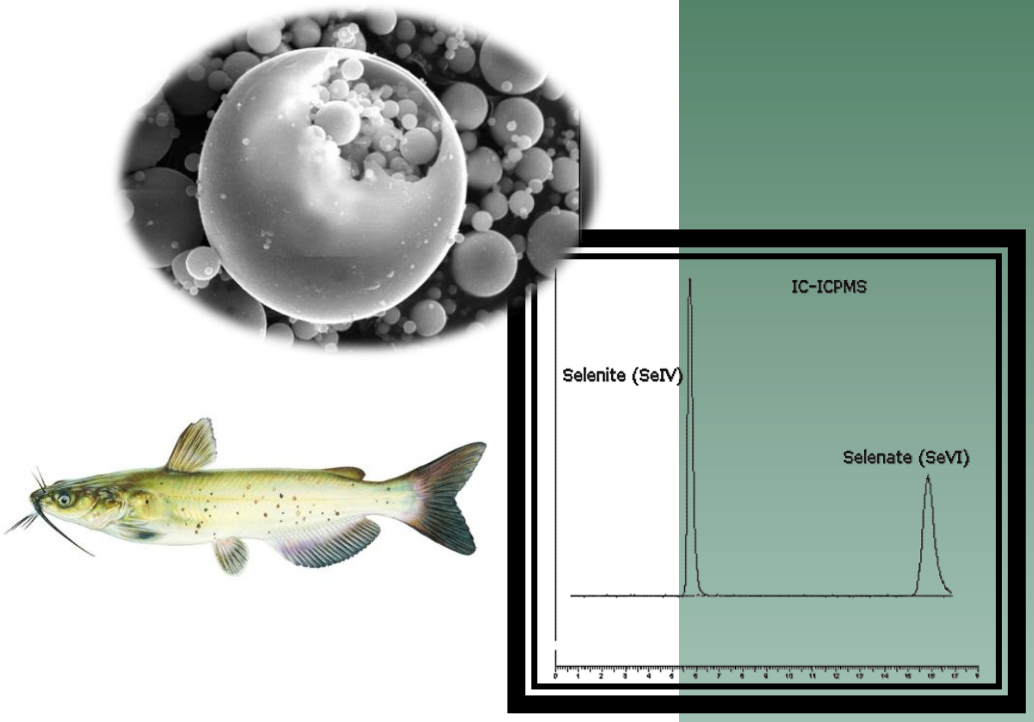
OAK RIDGE National Laboratory

INTERLABORATORY COMPARISON FOR DIGESTION METHODS, ANALYTICAL METHODS, AND HOLDING TIMES FOR THE ANALYSIS OF TRACE ELEMENTS IN BIOLOGICAL SAMPLES FOR THE KINGSTON ASH RECOVERY PROJECT



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Interlaboratory Comparison for Digestion Methods, Analytical Methods, and Holding Times for the Analysis of Trace Elements in Biological Samples for the Kingston Ash Recovery Project

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1. INTRODUCTION

In December 2008, an ash dike at the Tennessee Valley Authority (TVA) Kingston Fossil Plant (KIF) ruptured, releasing over 1 billion gallons of coal ash into the Emory and Clinch Rivers. Coal ash may contain several contaminants of concern; of these, selenium (Se), arsenic (As), and mercury (Hg) have been highlighted in this work because of their toxicity and potential to bioaccumulate in aquatic food chains (Reash et al. 2006, Chapman et al. 2010). To assess the potential impact of the spilled coal ash on humans and the environment, a comprehensive monitoring program was established at the Kingston site, for which resident aquatic organisms (among other sample media) are collected to determine contaminant exposure and evaluate the risk to humans and wildlife.

Initial biological monitoring began in January-March 2009, shortly after the spill. Various organizations [e.g., TVA, Oak Ridge National Laboratory (ORNL), Tennessee Department of Environment and Conservation (TDEC), Tennessee Wildlife Resources Agency (TWRA)] worked together to collect fish near the spill site as well as from reference locations. Fifty six fish (largemouth bass, channel catfish, blue catfish and spotted bass) were collected as part of this initial effort. Fish were filleted, and fillets were homogenized and split samples from these fillet homogenates were sent to four different contract laboratories for metals analysis, named Laboratory A, B, X, and Y in this study.

Notable quantitative differences were observed between split sample metals results reported by the different laboratories, but these differences were not consistent between laboratories or even within individual metals (Fig.1) and were beyond differences typically reported in literature for interlaboratory comparisons in biota samples (Wagemann & Armstrong 1988). Of particular concern were the differences between the laboratories in reported concentrations for Se, As, and Hg, the primary contaminants of concern in coal ash. Resolving the issue of interlaboratory analytical differences is of great importance to both ORNL and TVA because of the significance of these data to serve as a baseline for future environmental evaluations and ecological risk assessments.

The apparent quantitative differences for the reported metals results between the contract laboratories were included as a topic during on-site system audits of Lab A and Lab B, which were conducted in early 2010. Elevated detection limits and other technical discrepancies made it impossible to evaluate the results from Labs X and Y in the context of an interlaboratory comparison, so the results from Labs X and Y were not included in the present study. Results from Labs X and Y were not considered reportable in the Kingston Ash Recovery Project Database, and no further samples were sent to these labs.

The audits of Lab A and B revealed differences in the way these laboratories were preparing samples, analyzing samples and reporting results.

- Lab B prepared highly concentrated sample mass to digestion volume digestates in an attempt to achieve lower sample detection limits. Analysis was by

Inductively Coupled Plasma-Atomic Emission Spectrometry (EPA Method SW846 6010 or method 6010) (U.S. E.P.A. 2007a). Lab A prepared the tissue sample digestates using the more conventional sample mass to digestion volume ratio according to EPA method SW846 3050B (U.S. EPA 1996) followed by analysis by Inductively Coupled Plasma Mass Spectrometry (ICP-MS; EPA method SW846 6020A or method 6020) (U.S. EPA 2007).

- Lab B reported results down to previously derived method detection limits (MDLs), while Lab A reported results to their limit of quantitation (LOQ), resulting in many analytes being initially reported as non-detected at the higher LOQ reporting limits.

- Lab B took a separate aliquot of tissue from each sample and digested and analyzed this aliquot for total mercury (Hg_T) using cold vapor atomic absorption spectroscopy by EPA method 7471A, the conventional method for Hg analysis. Lab A, on the other hand, digested the entire aliquot of tissue by EPA method SW846-3050B (U.S. EPA 1996) and analyzed for Hg_T along with all other metals by ICP-MS (method 6020). Consequently, results from the different labs could not be directly compared.

To address and understand how these preparation, analysis, and reporting issues may have individually or collectively contributed to the notable differences between Lab A and Lab B's reported metals concentrations, ORNL, TVA, and Environmental Standards, Inc. (Environmental Standards), TVA's QA oversight and data management contractor for the KIF project, designed a round robin study in which four independent analytical laboratories participated. Each of the participating laboratories was requested to digest and analyze split homogenate fish tissue samples at different mass to digestion volume ratios and by EPA methods 6010 and 6020. The purposes of the round robin study were to evaluate:

1) the two different mass to digestion volume ratios that had previously been used for samples in this study,
2) the two different analytical methods for the various analytes of interest,
3) the abilities of the different analytical laboratories to achieve desired detection limits, and
4) the analyte recoveries for positive control samples (a NIST tissue standard reference material).

Results from this round robin study help clarify the uncertainties associated with different historical datasets on the TVA-KIF bioaccumulation project, thus help guide decisions on use of these datasets in evaluating temporal and spatial bioaccumulation trends for human health and ecological risk assessments. These results also inform decisions for future monitoring efforts on digestion procedures, analytical methods, and analytical laboratories that optimize accurate and precise results for coal ash contaminants of concern in biological samples. In addition, where only one dataset exists and the data are

recognized to be potentially compromised, these results provide a basis for treatment and analysis of these data that will improve their usefulness for various purposes.

2. METHODS

Fillets from four catfish collected in 2009 at Emory River Mile 0.9 (ERM 0.9), just downstream of the ash spill, and one carp collected from the KIF stilling pond were used to create most of the fillet homogenate samples for the round robin study. The KIF stilling pond is the last pond in the plant's system of ponds used for settling ash prior to discharge of process water back to the river system. As part of a treatment system, this pond is not considered to be "waters of the state," but it contains reproducing populations of a few species of fish which are exposed to much higher concentrations of coal ash contaminants than are fish in waters of the state. Homogenate samples representing each of these fish fillets were originally submitted to Labs A and B in 2009 for analysis; those historical analytical results reside in both TVA's and ORNL's project databases.

New aliquots of these fillet sample homogenates were prepared in July 2010 for use in the round robin study from frozen archives held at ORNL. Each aliquot was assigned a new, unique sample identifier (Table 1). Aliquots of a NIST certified reference material (NIST 1947- Lake Michigan Fish Tissue) were also included and were similarly assigned unique (but "blind") sample identifiers. No information as to the nature and provenance of the samples was provided to the laboratories. The original sample identifiers and morphological data for these fish tissues, as well as the "blind" round robin sample identifiers that were assigned are shown in Table 1.

Four contract labs were invited to participate in this study. Two of these labs, Lab A and B, had been included from the baseline fish study, and two other labs, named Lab C and D for the purposes of this study, were included for comparison. Each lab received 21 fish fillet homogenate samples (Fig. 2) which included:

- low Se content tissues (four catfish from ERM 0.9 with one duplicate),
- high Se content tissues (one carp from the KIF stilling pond), and
- a certified fish fillet standard (NIST 1947).

The laboratories were instructed to digest the Round Robin tissue samples at two different digestion ratios, replicating the ratios used for the baseline samples at Lab A and B. Aliquots of each tissue sample were weighed to masses of 1 g and 5 g, and each lab was instructed to digest and dilute the 1 g samples to a final volume of 100 ml and the 5 g samples to a final volume of 50 ml. For each tissue type and each analytical method (method 6010 and 6020), the 1:100 digestion ratio was analyzed twice, yielding duplicate results for that treatment. Appendix A contains the instructions issued to the participating laboratories. Table 1 shows the "sample decoder" for the digestion ratios for each sample.

These digestates were then analyzed by EPA methods 6010 and 6020. Mercury was not included as an analyte in this Round Robin study, because labs other than Lab A do not analyze Hg by EPA Method SW846-6020A, but this element will be the focus of a future study (See Appendix B).

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3. RESULTS

3.1 Standard reference material

Table 2 presents the certified values for trace metals in the NIST standard reference material (SRM). Of these trace elements, only six (arsenic (As), copper (Cu), iron (Fe), manganese (Mn), selenium (Se), and zinc (Zn)) were analyzed as part of this study. Figure 3 shows the results reported by each of the participating analytical laboratories for the SRM sample for these six trace elements by each of the study digestion ratios and analytical methods. Elements which were above the participating analytical laboratory's reporting limits are shown with green bars, while estimated values are shown in blue, and reported not-detected results are in grey. The horizontal lines bound the 95% confidence intervals around the certified concentrations for this SRM.

Of the six elements that had certified values in the SRM, Se and As were of most concern for this project. As can be seen in Fig 3, for arsenic, Lab A was the only laboratory that reported results within the 95% confidence interval, with recoveries of over 90% of the certified values for both digestion ratios and analytical methods. While the Lab A 5:50 digestion ratio samples analyzed by method 6010 appeared to produce an As measurement closest to the certified value range, the 1:100 digestion ratio samples analyzed by method 6010 also produced results within this range for one of the two replicates in this study. Lab C reported As values that were considerably lower than the certified value, especially for the 5:50 digestion ratio samples analyzed by method 6010. Two of the Lab C results that came closer to the certified range for As were flagged as estimated values. Lab B had consistently low recoveries for As, and actually reported a not-detected result for As with the 1:100 digestion ratio analyzed by method 6010. Lab D reported not-detected results for As across all treatments, at a much higher detection limit than the other laboratories.

?Lab B reported Se values for method 6010 that were more than twice the certified values by either digestion ratio. Lab B results for Se using method 6020 were much closer to the certified range of values, with the 5:50 digestion ratio falling within the certified range, and the 1:100 digestion ratio falling just slightly over the certified range for one of the two replicate samples. Both Lab A and Lab C reported higher concentrations than the certified range for the 1:100 digestion ratio by method 6010, though not as high as the concentrations reported by Lab D. All other treatments produced concentrations within the certified range for Se at Lab C. The concentrations reported by Lab A using the 1:100 digestion ratio by method 6020 were within the certified range, and were just below the certified range for the 5:50 digestion ratio by method 6020. Lab D reported a not-detected result for Se at the 1:100 digestion ratio by method 6010, and a concentration slightly higher than the certified range for the 5:50 digestion ratio by this analytical method. Both digestion ratios at Lab D resulted in Se concentrations within the certified SRM range by method 6020.

For Cu, Lab A consistently reported concentrations slightly higher than the certified SRM range across all treatments. Lab B reported Cu values slightly lower than the certified range using method 6010, but within or slightly above the certified range using method 6020. Lab C reported concentrations that were significantly higher than the certified range using both analytical methods at the 1:100 digestion ratio, but the Cu concentrations were within the range of certified values using both analytical methods at the 5:50 digestion ratio. Lab D reported a non-detect for Cu at the 1:100 digestion ratio by method 6010, but the concentrations for all other treatments fell slightly above or within the certified SRM range.

For Fe, patterns were similar to Cu: Lab A reported values slightly higher than the certified SRM range across all treatments. Lab B reported Fe values slightly lower than the certified range using method 6010 (with a non-detect at the 1:100 digestion ratio), but within the certified range slightly above for both digestion ratios using method 6020. Lab C reported concentrations that were significantly higher than the SRM certified range for the 1:100 digestion ratio using both analytical methods, but reported concentrations within the certified SRM range for both analytical methods at the 5:50 digestion ratio. Lab D reported values that were slightly above the certified Fe values for both analytical methods at the 1:100 digestion ratio, and values that were slightly below the certified range of values at the 5:50 digestion ratio for both analytical methods.

For Mn, Lab A reported concentrations slightly higher than the certified SRM range across all treatments. Lab B reported a not-detected result for Mn at the 1:100 digestion ratio using method 6010, and concentrations within the range or slightly above or below the range for all other treatments. Lab C reported Mn values that were slightly higher than the certified SRM range across all treatments, but the values were significantly higher at the 1:100 digestion ratio for both analytical methods, and the values were flagged as estimated for both digestion ratios using method 6010. Lab D reported not-detected Mn results for all treatments except the 5:50 digestion ratio using method 6020, and reported a value that was slightly higher than the certified SRM range with this treatment.

For Zn, Lab A reported concentrations that were consistently higher than the certified SRM range across all treatments. Lab B reported elevated concentrations comparable to those reported by Lab A for the 1:100 digestion ratios using both analytical methods, but reported concentrations that were within or slightly above the certified SRM range for both analytical methods using the 5:50 digestion ratio. Lab C reported Zn concentrations that were higher than the certified SRM range for all treatments except at the 1:100 digestion ratio using method 6020. Lab D reported Zn concentrations comparable to the other three participating laboratories and slightly higher than the certified SRM range, except for the 1:100 digestion ratio by method 6020, for which a not-detected result was reported for Zn.

3.2 Comparison of Round Robin results with project database results

While the tissue homogenate samples used in this study were originally analyzed by Labs A and B within holding times for the analytes of concern (except mercury), the re-analysis of these samples for the Round Robin study exceeded the holding times for all analytes. Recommended holding times for metal analysis is 6 months (US EPA 2000); the samples in the Round Robin study were analyzed between 9-17 months after collection. Because the Round Robin study created a second set of data for samples that were analyzed by Labs A and B, this provides an opportunity to evaluate, for each analyte, digestion ratio, and analytical method, whether the exceeded regulatory holding time had any apparent effects on the analytical results reported for these samples.

Presented in Table 3 are the analytical results from the original analysis of the tissue samples, as well as the re-analysis of those same tissue samples during the Round Robin study. Note that while the Round Robin study included two digestion ratios and two analytical methods, the only results that are included in the evaluation presented on Table 3 are those that correspond to the methods used in the original analysis (method SW846 6010 at a digestion ratio of 5 g tissue to 50 ml at Lab B and SW846 6020 at a digestion ratio of 1 g tissue to 100 ml at Lab A). Table 3 also shows the relative percent difference (RPD) between the original analysis and the Round Robin study analysis. Analytes which were below the laboratory's detection limits were excluded from the RPD evaluation.

As shown in Table 3, the results from the Round Robin study are comparable to the original analysis of the same samples. There were generally no statistically significant differences ($p > 0.01$) between the original analysis and the Round Robin analysis. The only significant difference observed was for lead at Lab A, but this difference appears to be due to one outlier result for sample 15019 in the original analysis. Although results were not significantly different between the two sets of analyses, results tended to be slightly higher for the Round Robin analyses than for the original analyses. Of the 15 detected analytes reported by Lab B, 11 were reported at higher concentrations for the Round Robin study than for Lab B's original analysis. Of the 13 detected analytes reported by Lab A, eight(?) analytes were reported at higher concentrations for the Round Robin study than for Lab A's original analysis. These trends are largely consistent with those found during a 2011 study comparing the analytical results from a re-analysis of 53 fish tissue samples two years after collection (Iannuzzi & Jones 2011). These collective findings appear to support increasing the holding times for metals in tissue samples and will be the subject of a future publication.

A direct comparison of reported results generated by different methods and by different laboratories was often confounded by the fact that many of the analytes were below the respective laboratory's detection (or reporting) limits in these samples. Quantitative comparisons were possible for 10 of the 18 analytes studied (calcium (Ca), chromium

(Cr), iron (Fe; Round Robin only), manganese (Mn), magnesium (Mg), lead (Pb; Round Robin only), sodium (Na), nickel (Ni), and potassium (K)). Of these, only Mg and K concentrations were reported significantly higher by Lab A than by Lab B. There is no significant difference ($p > 0.05$) between laboratories for selenium when all five samples in this study are considered. However, this is because the results from the stilling pond fish (#13734) had much higher results than the other four fish samples. Removing this one sample reveals highly significant differences ($p << 0.01$) in results for selenium between the laboratories, with the reported Se concentrations from Lab B being higher than those concentrations reported by Lab A, consistent with the findings in the original analysis of the fish used for the baseline study in 2009 (Fig. 1). That the one sample with high Se concentrations could mask such a significant difference in the Se results of the four other samples in this study suggests that the difference between the laboratories, digestion ratios and/or analytical methods appears to apply only to samples that have low concentrations of Se.

4. DISCUSSION AND CONCLUSIONS

Because Se and As are the contaminants of concern in coal ash, the focus of this study was to evaluate the sample mass to digestate volume ratios and analytical methods for these elements, while still producing acceptable results for the other trace elements. The SRM evaluation (Section 3.1) included in this study provided the most unequivocal evidence for how well the different sample mass to digest volume ratios and analytical methods performed for each of the elements considered. Essentially, without the use of the certified SRM, analytical results reported by the participating study laboratories for the sample mass to digest volume ratios and analytical methods could only be compared with respect to how similar or different they were from each other without indication as to how close the results are to some "true" value. In this way, the SRM evaluation provided a means for evaluating accuracy, while comparing results for the different replicates in this study provided a means for evaluating the precision of the labs and methods.

Across the participating study laboratories, method 6010 produced elevated results for Se in the SRM, while method 6020 produced more accurate results (Fig. 1). When the discovery of an apparent bias between labs A and B was first noted, it was originally hypothesized that the high Se bias observed in the samples at Lab B could be due to incomplete digestion of the tissue samples at the 5:50 digestion ratio since carbon emits in the same spectral window as Se and therefore can generate a false positive or high bias for Se (Machat et al. 2002). However, the reported Se results for the 1:100 digestion ratio by method 6010 are higher than the 5:50 digestion ratio by method 6010 across the participating study laboratories, suggesting that the bias may not necessarily be due to incomplete digestion or carbon interference. Regardless, on the basis of this study it appears that at any digestion ratio, method 6020 would be the preferable analytical method to use for optimal Se results. In addition, on the basis of this study it appears that Lab B results for Se by method 6010 are biased high compared to the other participating study laboratories, suggesting that either the ICP unit, its configuration, the reagents, or something else being used by Lab B may have a positive Se interference, or there are Se contamination issues. If this is true, the Se data generated by Lab B using method 6010 for baseline and historical samples (prior to December 2010) for this project may be questionable for use.

While all participating study laboratories performed well for Se using method 6020, the only laboratory that reported acceptable results for As across all treatments was Lab A. Based on the performance of the different mass to volume digestion ratios and analytical methods across four laboratories for Se and As in this study, ORNL and TVA made the decision in December 2010 that future tissue samples for the TVA KIF project would be prepared and analyzed by Lab A by method 6020 for all metals with the exception of mercury (see Appendix B). For samples that were analyzed prior to December 2010, the consensus of the Study Team was that the "official" tissue sample dataset to be used and retained in the project database would be the data generated by Lab A (except as specified in Appendix B). It was further concluded that the tissue data generated by Lab B would be verified, validated, and retained in the project database, but those data would

be qualified as "non-reportable" data. One caveat to these Study Team conclusions was for a small subset of tissues samples collected during the spring of 2009. For those samples, the only metals data available for tissue samples were generated by Lab B and no frozen archived homogenates exist for those samples. For this subset, the Lab B data are being retained in the project database as reportable, but the Se data for these samples will flagged with a qualifier to denote that the Se concentrations are known to be biased high and a quantitative correction factor should be applied as described in Appendix C.

While the results for the 5:50 mass to digestion volume ratio were largely comparable to those for the 1:100 digestion ratio, the decision was made to use the conventional 1:100 dilution since the more concentrated dilution ratio is not actually part of any of the applicable US EPA digestion methods.

The collective analytical results from this study and the baseline reanalysis study (Iannuzzi & Jones 2011) suggest that although the currently published holding time for metals in biological tissue samples is 6 months, metal concentrations in frozen (or freeze dried) biological tissues are quite stable for longer periods – perhaps at least two years. The tissue samples in this study were reanalyzed over a year after initial collection, and concentrations of most metals were not statistically different from the original analysis. One of the factors that affects the metals concentrations reported in frozen tissue samples over time is the potential for changes in percent moisture of the frozen tissue samples. The percent moisture of frozen tissue samples is more likely to change over time than are the concentrations of the metal and metalloid analytes. Iannuzzi & Jones (2011) report a decrease in moisture content of 0.7%, and an increase in the concentrations of several analytes after one year of storage for the baseline fish samples for the Kingston Ash Recovery project. The increase in metal concentrations was not fully explained by the difference in moisture content in their study, as dry weight concentrations were also higher in the samples that were held for a year (Ianuzzi & Jones 2011). Regardless of the storage time of the frozen tissue samples, it is recommended that the percent moisture of the tissue samples be measured at the same time when aliquots of tissue are removed for the digestion of metals regardless of wet or dry basis metals reporting.

In addition to providing insight on the mass to digestion volume ratios and analytical methods and holding times, the Round Robin Study highlighted the importance of the proper determination and use of reporting and detection limits, and the need for appropriate decision-making rules for considering not-detected or censored data. For example, in the original analyses of the baseline fish tissue samples at Lab A the reporting limits for many elements were not low enough to be toxicologically meaningful for biota samples. For most of these elements in the baseline analysis, results were reported as "estimated" (below the reporting limit or above the detection limit), even though the concentrations were well above Lab A's method detection limits.

The foregoing highlights the difficulty of data interpretation at or near a calculated detection limit and of attempting to utilize near-detect data without understanding the limitations of the chemical measurement process. For most commercial laboratories, method detection limits are determined on an annual basis per operating device and also

per matrix such as water, biota, high concentration acid leach of sediment and so forth. Coupled with this, data users often need to compare measured values, especially in biota, to calculated risk-based limits which may not be technically achievable (TDEC 2008). The method detection limit (MDL), as defined in EPA regulations is roughly three times the standard deviation of a low-concentration sample passed through the entire sample preparation process. The limit of quantitation (LOQ) is defined, also in EPA rules, as "three to five times" the MDL. The primary limitation of the MDL is that it does not take into account any blank contribution to the signal. Additionally, since the MDL is only determined once per year, the commercial analytical laboratory is aware that instrument response may deteriorate over the course of the year, rendering the previously-determined MDL lower than is actually achievable. Most commercial laboratories avoid this limitation by running a low-concentration standard with each analytical run at or about the LOQ. This is the "reporting limit" or RL. Unless the customer requests otherwise, the laboratory then reports detect/non-detect against the RL rather than the annually-determined MDL since detection at the RL is actually verified with each analytical run. On multi-analyte devices, the RL standard is usually constructed from commercially available standard mixes and the RL standard may in fact be higher than the true LOQ for convenience in mixing.

When comparing work at several laboratories, as in the initial work with these fish samples, one encounters different reporting conventions with MDL and RL as well as differing presented capabilities (i.e., one lab's instrumentation cannot match the low detection limit at another). For this study, MDL reporting was requested from each participating laboratory. Nevertheless, the four labs had differing capabilities for the elements being measured, leading to what some call "censored" data. That is, one laboratory reports a clear detect on an element and one or more of the others cannot match that detection limit and reports a "less than" number. On the other hand, one cannot eliminate the possibility that one laboratory might have an interference which raises (or lowers) the observed signal. In the case of ICP and ICP/MS, the selection of gases used in the devices to support the plasma and in the collision cell as well as the multitude of complex machine settings available in these methods of analysis may lead to such interferences.

The most common method for treating censored data during risk assessment is to use half the detection limit (U.S. E.P.A. 1998, Helsel 2005), but this routinely yields overly conservative results. In the case of As at Lab D, for example (Fig. 3), using half the reporting limit still yields much higher results than the concentrations in the samples measured at the other labs. For a screening level ecological risk assessment, this conservative method may be appropriate, presenting a "worst-case" scenario. However, if potential unacceptable human health or ecological risks are identified, it may be necessary to develop a more refined method for treating censored data.

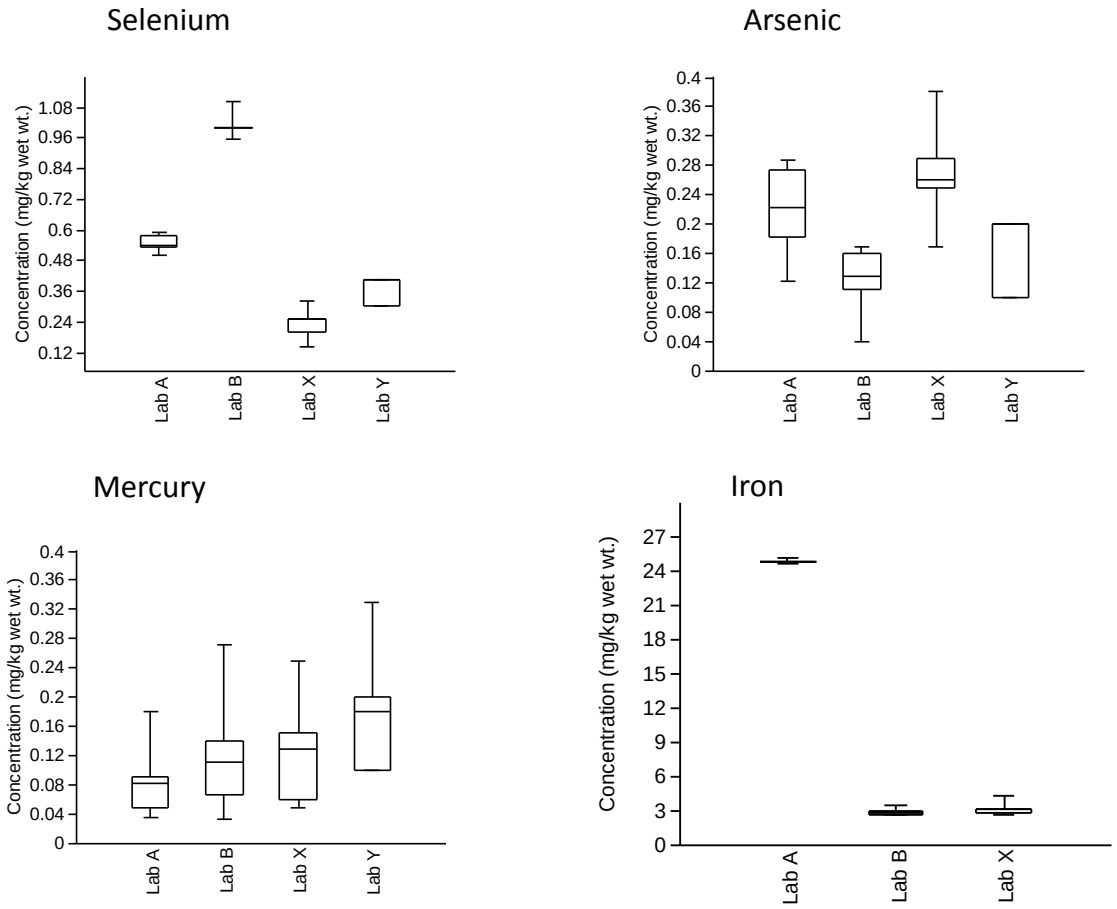

Figure 1. Comparison of analytical results for selenium, arsenic, mercury, and iron fillet concentrations in largemouth bass collected in 2009 from the Clinch River (CRM 9.5). Box plots show the spread in the data for 5 fish tissue samples collected. The plots shown in this figure are a small subset of the data collected as part of the baseline study, and were selected as examples to demonstrate the variability between the analytical results obtained from the different laboratories, and to highlight that this variability was not consistent among elements.

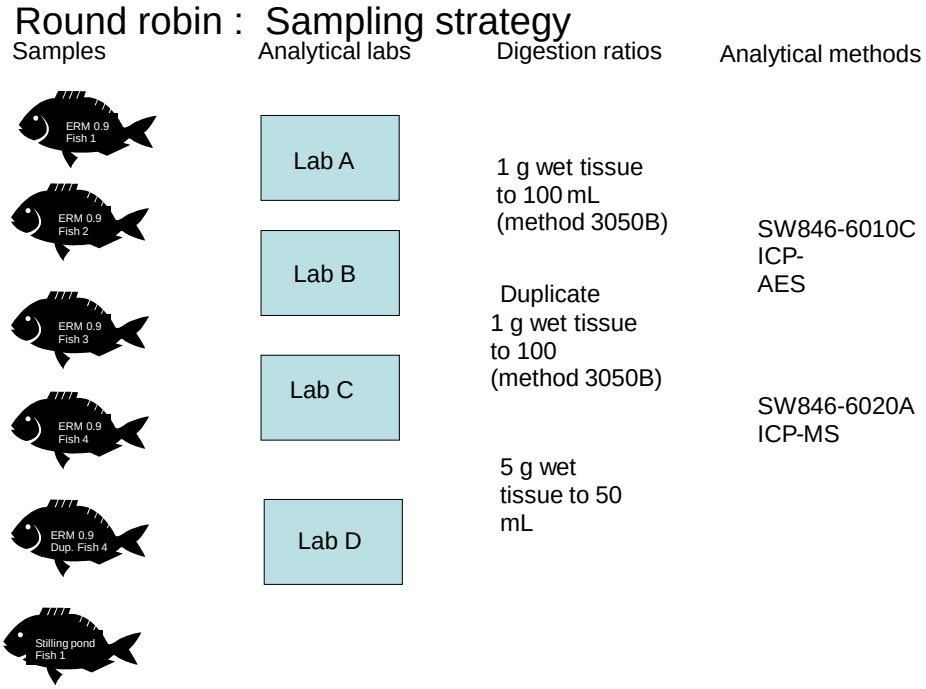

Figure 2. Study design for the interlaboratory comparison.

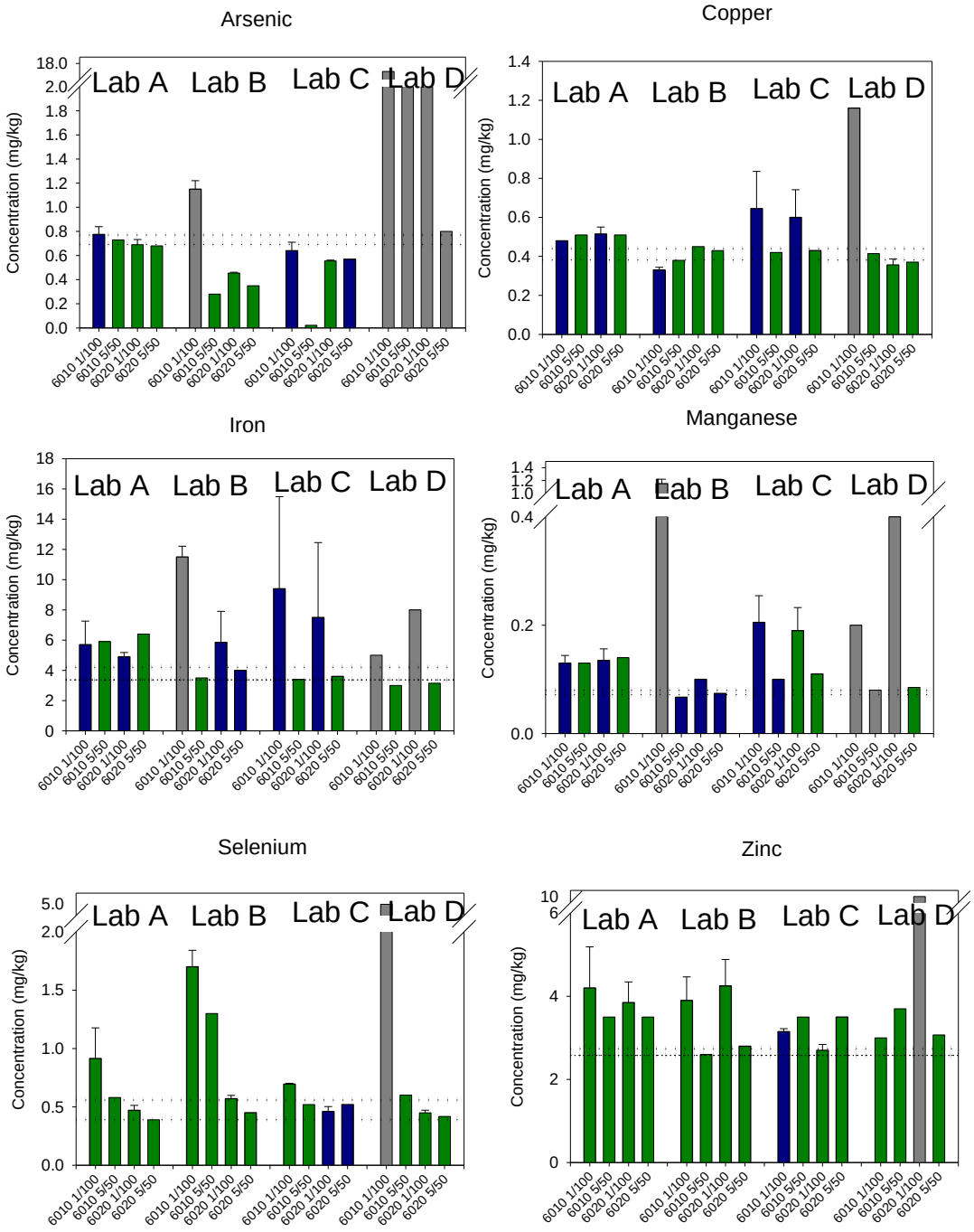

Figure 3. Analytical results for standard reference material (NIST 1947, Lake Michigan Fish Tissue) samples. The different analytical method and digestion ratio treatments are shown on the x-axes. Green bars represent detected concentrations, blue values represent estimated values, and grey bars represent the method detection limit (MDL) for analytes which were below detection limits (i.e., "not-detected" results, or those results qualified with "U"). Dotted horizontal lines bound the 95th percentile of certified concentrations for NIST 1947. Note breaks in the y-axes for all analytes except iron and copper.

Table 1. Sample "decoder" for Round Robin study.

Original Sample ID	Sample date	Site	Species	Length	Weight	Sex	Sample type	Lab A	Lab B	Lab C	Lab D	Dilution ratio (g tissue/mL digestion acid)
									Lab Sample ID			
								201	301	501	601	5/50
								207	307	507	607	1/100
15018	10/6/2009	ERM 0.9	Channel catfish	50.6	1115.6	F	Regular	213	313	513	613	1/100
								202	302	502	602	5/50
								208	308	508	608	1/100
15019	10/6/2009	ERM 0.9	Channel catfish	54.5	1377.3	F	Regular	214	314	514	614	1/100
								203	303	503	603	5/50
								209	309	509	609	1/100
15019	10/6/2009	ERM 0.9	Channel catfish	54.5	1377.3	F	Replicate	215	315	515	615	1/100
								204	304	504	604	5/50
								210	310	510	610	1/100
15020	10/6/2009	ERM 0.9	Channel catfish	45.7	819	F	Regular	216	316	516	616	1/100
								205	305	505	605	5/50
								211	311	511	611	1/100
15021	10/6/2009	ERM 0.9	Channel catfish	47.7	839.3	M	Regular	217	317	517	617	1/100
								206	306	506	606	5/50
								212	312	512	612	1/100
13734	4/8/2009	KFP 0.1	Common carp	45.3	1060	F	Regular	218	318	518	618	1/100
								200	300	500	600	5/50
NIST standard		Lake	Lake					219	319	519	619	1/100
1947	NA	Michigan	trout	NA	NA	NA	Reference	220	320	520	620	1/100

Table 2. Certified trace element concentrations in NIST 1947 Lake Michigan fish tissue.

Element	Mass fraction (wet mass basis) mg/kg
As	0.732 ± 0.039
Cu	0.411 ± 0.029
Fe	3.79 ± 0.42
Hg	0.254 ± 0.005
Mn	0.076 ± 004
Rb	4.51 ± 0.09
Se	0.475 ± 0.084
Zn	2.66 ± 0.08
Methylmercury	0.233 ± 0.010

Table 3. Comparison of results from original analysis and Round Robin analysis. Estimated values are shown in blue, while not-detected results are shown in red. Where values were not detected, the method detection limit (MDL) is reported. All results reported in this table from the Lab A are for method SW846-6020A at a digestion ratio of 1 g tissue to a final digest volume of 100 ml while results reported in this table from Lab B are for method SW846-6010C at a digestion ratio of 5 g tissue to a final digest volume of 50 ml. See text for more details. Percent difference is calculated as the percent difference between original and round robin results for each lab, such that positive results denote higher concentrations in the Round Robin study, and negative values denote lower concentrations in the Round Robin study. Non-detected results are not included in this evaluation, so that where all results are non-detects, it was not possible to calculate the percent difference between the original analysis and the Round Robin study, and "N.D." is reported (not determined).

		Analytical Results (mg/kg)				% difference	
		Lab A		Lab B		Lab A	Lab B
Analyte	Sample ID	original	RR	original	RR		
Aluminum	13734	12.4	2.2	3.2	0.67	-49.1	N.D.
	15018	3.5	2	1.5	2.2		
	15019	5.2	4.4	1.4	2.1		
	15020	3	1.6	1.4	2.1		
	15021	5	3.2	1.9	2.1		
	Average	**4.175**	**2.8**				
Arsenic	13734	0.13	0.17	0.15	0.16	23.5	6.3
	15018	0.016	0.035	0.038	0.041		
	15019	0.015	0.037	0.038	0.039		
	15020	0.014	0.034	0.038	0.038		
	15021	0.015	0.035	0.038	0.039		
	Average	**0.13**	**0.17**	**0.15**	**0.16**		
Calcium	13734	300	753	450	600	57.6	25.2
	15018	70.4	69.3	58	56		
	15019	64.3	375	54	89		
	15020	75.5	103	57	53		
	15021	64.3	54.3	68	120		
	Average	**114.9**	**270.92**	**137.4**	**183.6**		
Cobalt	13734	0.05	0.025	0.014	0.016	-69.9	12.5
	15018	0.012	0.0062	0.011	0.11		
	15019	0.0063	0.0065	0.011	0.1		
	15020	0.012	0.008	0.011	0.1		
	15021	0.014	0.0073	0.018	0.11		
	Average	**0.013**	**0.00765**	**0.014**	**0.016**		
Copper	13734	0.25	0.33	0.27	0.28	N.D.	5.9
	15018	0.24	0.28	0.19	0.23		
	15019	0.25	0.25	0.15	0.17		
	15020	0.46	0.29	0.19	0.18		
	15021	0.24	0.23	0.16	0.16		
	Average			**0.192**	**0.204**		

Table 3. (cont'd)

		Analytical Results (mg/kg)				% difference	
		Lab A		Lab B		Lab A	Lab B
Analyte	Sample ID	original	RR	original	RR		
Chromium	13734	0.23	0.21	0.23	0.2	31.9	5.8
	15018	0.2	0.4	0.16	0.23		
	15019	0.15	0.23	0.23	0.23		
	15020	0.13	0.15	0.15	0.13		
	15021	0.31	0.38	0.37	0.42		
	Average	**0.1975**	**0.29**	**0.228**	**0.242**		
Iron	13734	12.4	6.9	5.2	5.3	N.D.	8.4
	15018	12.4	6.3	2.8	3.4		
	15019	12	4.3	2.6	2.8		
	15020	11.3	3.6	2.2	2.3		
	15021	11.8	5	3.5	4		
	Average		**5.22**	**3.26**	**3.56**		
Lead	13734	0.05	0.012	0.031	0.045	-1211.4	39.6
	15018	0.084	0.0064	0.024	0.032		
	15019	1.3	0.025	0.012	0.03		
	15020	0.57	0.13	0.011	0.11		
	15021	0.32	0.012	0.017	0.032		
	Average	**0.5685**	**0.04335**	**0.021**	**0.03475**		
Magnesium	13734	235	269	210	190	4.4	-7.3
	15018	215	226	170	160		
	15019	235	242	180	180		
	15020	229	222	170	150		
	15021	188	194	150	140		
	Average	**220.4**	**230.6**	**176**	**164**		
Manganese	13734	0.25	0.53	0.27	0.37	35.8	18.7
	15018	0.19	0.21	0.11	0.12		
	15019	0.14	0.34	0.1	0.12		
	15020	0.22	0.24	0.15	0.14		
	15021	0.15	0.3	0.11	0.16		
	Average	**0.175**	**0.2725**	**0.148**	**0.182**		
Molybdenum	13734	0.5	0.012	0.007	0.1	N.D.	N.D.
	15018	0.011	0.013	0.011	0.11		
	15019	0.01	0.013	0.011	0.1		
	15020	0.0098	0.012	0.011	0.1		
	15021	0.01	0.012	0.011	0.11		
	Average						
Nickel	13734	0.05	0.097	0.055	0.079	13.1	-4.9
	15018	0.051	0.1	0.027	0.025		
	15019	0.048	0.055	0.029	0.031		
	15020	0.052	0.063	0.046	0.034		
	15021	0.095	0.065	0.1	0.076		
	Average	**0.0615**	**0.07075**	**0.0514**	**0.049**		

Table 3. (cont'd)

		Analytical Results (mg/kg)				% difference	
		Lab A		Lab B		Lab A	Lab B
Analyte	Sample ID	original	RR	original	RR		
Potassium	13734	3410	3490	2400	2100	-3.6	-21.4
	15018	3610	3400	2800	2100		
	15019	3890	3800	2900	2400		
	15020	3600	3310	2300	1800		
	15021	3200	3100	2100	1900		
	Average	**3542**	**3420**	**2500**	**2060**		
Selenium	13734	6.2	7.2	6.4	7.2	13.0	16.9
	15018	0.43	0.52	0.81	1.2		
	15019	0.44	0.45	0.84	1.1		
	15020	0.36	0.37	0.76	0.96		
	15021	0.26	0.3	0.61	0.87		
	Average	**1.538**	**1.768**	**1.884**	**2.266**		
Silver	13734	0.025	0.0034	0.0083	0.1	N.D.	N.D.
	15018	0.003	0.0036	0.012	0.11		
	15019	0.0029	0.0038	0.012	0.1		
	15020	0.0027	0.0034	0.012	0.1		
	15021	0.0029	0.0036	0.039	0.11		
	Average						
Sodium	13734	381	370	400	350	-5.4	-19.6
	15018	455	409	440	320		
	15019	366	361	350	210		
	15020	271	250	270	220		
	15021	249	244	250	330		
	Average	**344.4**	**326.8**	**342**	**286**		
Vanadium	13734	0.099	0.2	0.12	0.18	N.D.	22.3
	15018	0.054	0.057	0.011	0.11		
	15019	0.053	0.061	0.011	0.1		
	15020	0.05	0.053	0.011	0.1		
	15021	0.052	0.056	0.03	0.013		
	Average			**0.075**	**0.0965**		
Zinc	13734	14	21.5	20	19	19.0	3.3
	15018	6.3	8.1	5.4	6.6		
	15019	6.1	6.5	4.3	5		
	15020	5.7	5.8	4.2	3.9		
	15021	6.2	5.4	3.9	4.6		
	Average	**7.66**	**9.46**	**7.56**	**7.82**		

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APPENDIX A

STATEMENT OF WORK FOR ANALYSIS OF METALS IN BIOLOGICAL TISSUE SAMPLES

Statement of Work for Analysis of Metals in Biological Tissue Samples

Project Description: Analysis of biota samples collected from TVA Kingston fly ash spill site for metals. Different digestion and analytical methods will be tested to evaluate potential differences in the sample weight to digest volume ratio and the analytical technique.

Vendor will provide analysis of metals on 21 frozen fish samples using EPA Method SW-846-6010C (Inductively Coupled Plasma-Atomic Emission Spectrometry) *and* SW-846-6020A (Inductively Coupled Plasma-Mass Spectrometry). Buyer will provide sample weights to vendor, vendor will digest **entire sample** as described in Table A1 with metal concentrations reported using the measured wet weight of the sample. Vendor will digest samples using EPA Method 3050B, as modified in Table A1. Vendor will also perform percent solids on each sample, to be reported separately. Chain of custody will be maintained and documented. Electronic delivery of results will be provided within 30 calendar days of receipt of samples. A detailed summary of specific analyses, digestion methods, and number of samples is given in Table A1.

Report results formally to:
Teresa Mathews
Oak Ridge National Laboratory
Environmental Sciences Division
P.O. Box 2008, Bldg. 1504
Oak Ridge, TN 37831-2008
Tel: (865) 241-9405
Fax: (865) 576-9938

and

Bill Rogers
Tennessee Valley Authority
Dr. William J. Rogers
Kingston Ash Recovery Operations
Environmental Trailer 3
1134 Swan Pond Road
Harriman, TN 37748
Tel: (865) 717-1627 (Kingston Ash)

Reporting limits: Metals to be analyzed are listed in Table A2, along with required reporting limits. Vendor will perform a low-level calibration check with analyte concentrations 1-3 times the Table A2 required reporting limits immediately before and immediately following project samples. All analytes in these low-level calibration checks must recover within 50-150%. **Results will be reported on a wet weight basis to the MDL**.

Data deliverable: Vendor will provide data in electronic format (results shall be submitted in an Excel spreadsheet format or comma delimited text file (ASCII), and quality assurance documentation and case narratives should be provided in pdf format) within 30 days of receipt of samples.

Quality assurance – Standard level IV QA package – blanks, reference materials, matrix spikes, and internal standards as per method requirements. QA summary will be included in narrative report of results.

Approximate start date: July 30, 2010.
End date: September 30, 2010.

All work must be completed and billed by the end of UT-Battelle fiscal year (9/30/2010).

Sample disposal: Lab disposal
Required archival: 3 months

Vendor Shipping Address:

Table A1. List of samples to be analyzed by EPA Method SW846-6010C **AND** SW846-6020A. Sample digestion should follow EPA Method 3050B in terms of reagents and procedures except sample masses and final volume of digests, which are listed below. Entire sample is to be re-weighed at laboratory, and ENTIRE sample is to be digested and results to be reported on a wet weight basis, using sample weights measured at lab.

Sample number	Wet wt. (g)*	Final volume
00	5.06	50
01	5.03	50
02	5.17	50
03	5.08	50
04	5.15	50
05	5.08	50
06	5.13	50
07	1.03	100
08	1.01	100
09	1.08	100
10	1.04	100
11	1.16	100
12	1.08	100
13	1.12	100
14	1.06	100
15	1.07	100
16	1.02	100
17	1.03	100
18	1.14	100
19	1.13	100
20	1.11	100

Table A2. Required reporting limits for all analytical methods and analytes.

Quantity	Matrix type	Analytical Method	Analyte	Cas. No.	Req. reporting limit (ug/g)	Turnaround time (calendar days)
21	biota	SW846-6010	Aluminum	7429-90-5	25	30
21	biota	SW846-6010	Antimony	7440-36-0	0.1	30
21	biota	SW846-6010	Arsenic	7440-38-2	0.1	30
21	biota	SW846-6010	Barium	7440-39-3	0.1	30
21	biota	SW846-6010	Beryllium	7440-41-7	0.05	30
21	biota	SW846-6010	Boron	7440-42-8	0.5	30
21	biota	SW846-6010	Cadmium	7440-43-9	0.05	30
21	biota	SW846-6010	Calcium	7440-70-2	100	30
21	biota	SW846-6010	Chromium	7440-47-3	0.1	30
21	biota	SW846-6010	Cobalt	7440-48-4	0.1	30
21	biota	SW846-6010	Copper	7440-50-8	0.5	30
21	biota	SW846-6010	Iron	7439-89-6	25	30
21	biota	SW846-6010	Lead	7439-92-1	0.1	30
21	biota	SW846-6010	Magnesium	7439-95-4	100	30
21	biota	SW846-6010	Manganese	7439-96-5	0.5	30
21	biota	SW846-6010	Molybdenum	7439-98-7	1	30
21	biota	SW846-6010	Nickel	7440-02-0	0.1	30
21	biota	SW846-6010	Potassium	7440-09-7	100	30
21	biota	SW846-6010	Selenium	7782-49-2	0.2	30
21	biota	SW846-6010	Silver	7440-22-4	0.05	30
21	biota	SW846-6010	Sodium	7440-23-5	100	30
21	biota	SW846-6010	Thallium	7440-28-0	0.1	30
21	biota	SW846-6010	Uranium	7440-61-1	0.05	30
21	biota	SW846-6010	Vanadium	7440-62-2	0.2	30
21	biota	SW846-6010	Zinc	7440-66-6	2	30
21	biota	SW846-6020	Aluminum	7429-90-5	25	30
21	biota	SW846-6020	Antimony	7440-36-0	0.1	30
21	biota	SW846-6020	Arsenic	7440-38-2	0.1	30
21	biota	SW846-6020	Barium	7440-39-3	0.1	30
21	biota	SW846-6020	Beryllium	7440-41-7	0.05	30
21	biota	SW846-6020	Boron	7440-42-8	0.5	30
21	biota	SW846-6020	Cadmium	7440-43-9	0.05	30
21	biota	SW846-6020	Calcium	7440-70-2	100	30
21	biota	SW846-6020	Chromium	7440-47-3	0.05	30
21	biota	SW846-6020	Cobalt	7440-48-4	0.1	30
21	biota	SW846-6020	Copper	7440-50-8	0.05	30
21	biota	SW846-6020	Iron	7439-89-6	25	30
21	biota	SW846-6020	Lead	7439-92-1	0.1	30
21	biota	SW846-6020	Magnesium	7439-95-4	100	30
21	biota	SW846-6020	Manganese	7439-96-5	0.5	30
21	biota	SW846-6020	Molybdenum	7439-98-7	0.05	30
21	biota	SW846-6020	Nickel	7440-02-0	0.05	30
21	biota	SW846-6020	Potassium	7440-09-7	100	30
21	biota	SW846-6020	Selenium	7782-49-2	0.1	30
21	biota	SW846-6020	Silver	7440-22-4	0.05	30
21	biota	SW846-6020	Sodium	7440-23-5	100	30
21	biota	SW846-6020	Thallium	7440-28-0	0.1	30
21	biota	SW846-6020	Uranium	7440-61-1	0.05	30
21	biota	SW846-6020	Vanadium	7440-62-2	0.2	30
21	biota	SW846-6020	Zinc	7440-66-6	0.05	30

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APPENDIX B

MERCURY STUDY

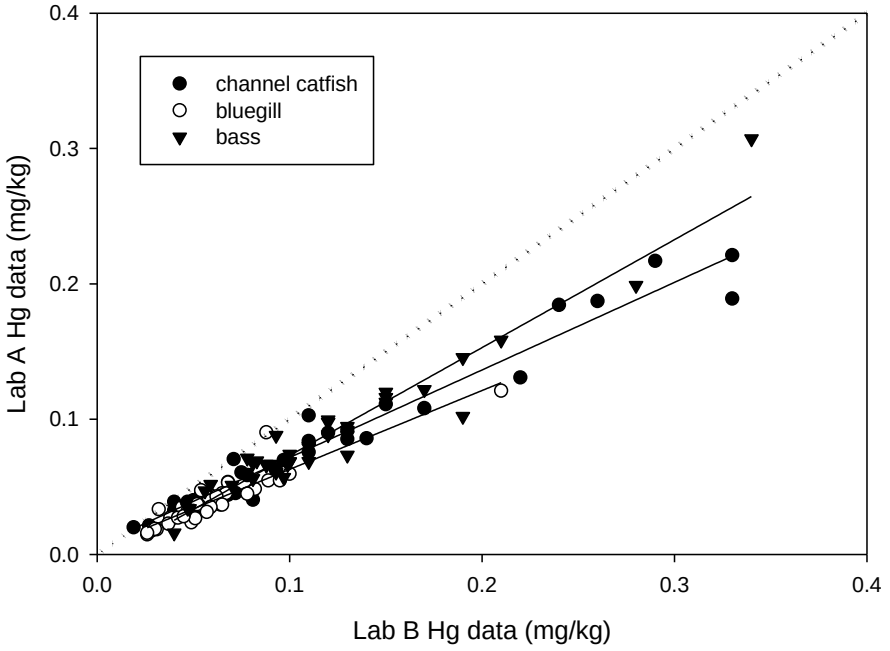
Appendix B. Mercury study

While mercury (Hg) is an important contaminant associated with coal combustion products and the coal cycle, it is also historically an important contaminant in the vicinity of TVA's Kingston Fossil Plant (KIF). Large amounts of elemental Hg were used in the 1950's and inadvertently released by the Department of Energy (DOE) facilities at Oak Ridge, which lies approximately 45 river miles upstream of the KIF. The waters, sediments, and fish downstream of the DOE facilities are contaminated with Hg. ORNL and more recently, TVA have been monitoring Hg and other contaminants in fish along this watershed for decades.

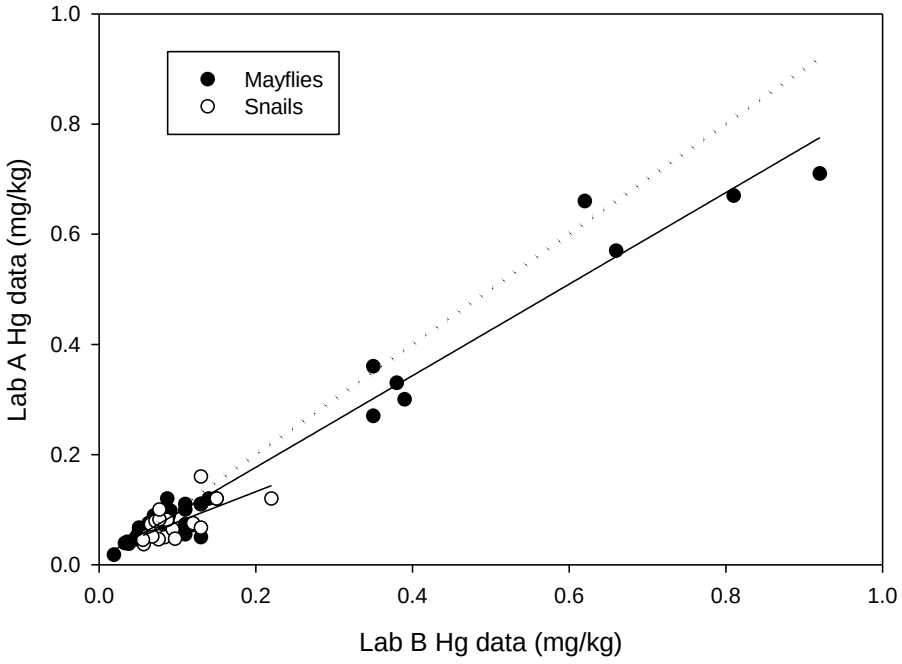
Sample preparation and analytical methods for Hg are different from that of other metals, in part because Hg can be volatile. The recommended methods for digesting tissue samples for Hg analysis involve gently heating samples in acid (without boiling) in closed vessels to avoid the loss of Hg vapor. This digestion method is different from the digestion method used for the other metals in this study, and therefore requires splitting each sample and performing two different digestion and analytical methods in order to obtain accurate measurements for Hg and the other metals. Mercury in samples is then most commonly measured by cold vapor atomic absorption (CVAA).

Indeed, for samples submitted prior to December 2010 for the TVA KIF project, Lab B split each sample in two and performed different digestions for Hg and other metals samples and then analyzed metals by method 6010 (ICP-AES) and Hg by method 7471A (CVAA). In contrast, Lab A performed only one digestion of samples in open vessels on a hot block (method 3050b) and analyzed samples for all metals including Hg by method 6020 (ICP-MS). Figure B-1 compares Hg results from Lab A and Lab B for fish fillet and invertebrate samples for which data sets from both labs are available. For all organisms considered, data generally fall below the line of perfect agreement, shown with a dotted line on both plots. The slopes of the regressions for each species are different from one another, and are significantly different from 1, suggesting that data from Lab A are consistently lower than data from Lab B. Indeed, Hg data from Lab A generally were lower than for all other labs (Fig. 1). The difference in Hg results was generally not the same between different tissue types, as witnessed by the different slopes in Fig. B-1.

While the Round Robin study did not consider Hg (because of the need for separate digestion and analytical methods), the decision was made to maintain as reportable all Hg data from Lab B in the project database, and all data from Lab B using method 6020 as non-reportable. As of December 2011, Lab A has installed a Direct Mercury Analyzer (DMA-80; Milestone), which does not require a digestion step as samples are directly combusted and analyzed by atomic absorption. All samples submitted to Lab A for the TVA KIF project as of December 2011 will be analyzed for Hg by method 7473 (U.S. E.P.A. 2007b) in addition to metals analysis by ICP-MS. Further, a subset of samples which was previously analyzed for Hg using ICP-MS is currently being pulled from the archive for analysis with the DMA-80. Once the data for these samples are available, an in-depth comparison of the different methods for Hg analysis will be conducted. Depending on the results of this study, all samples will be re-analyzed where enough sample remains or an appropriate correction factor will be determined to apply to all historical data from Pace that currently reside in the project database. This comparison of Hg data will be the subject of a future report.



A.



B.

Figure B-1. Comparison of Hg data for fish samples (A) and invertebrate samples (B) from Lab A and Lab B (mg/kg). Results for fish are from fillets and are reported on a wet weight basis, and results for invertebrates are on a dry weight basis. Results for snails are for soft tissues only. The line of perfect agreement is shown by a dotted line. Solid lines represent regressions for each of the species.

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APPENDIX C

CORRECTION FACTOR FOR BIASED SELENIUM DATA

Appendix C: Correction factor for biased Selenium data

While there was consensus among the Study Team to utilize the fish tissue data from Lab A as the official data set for the TVA KIF project, there are some tissue samples (fish liver and ovary tissue samples collected in spring 2009) for which the only available data were generated by Lab B using the 5:50 digestion ratio and method 6010. Because of the low masses of these samples, no archived tissue exists and it is not possible to reanalyze these samples. Because it is clear that the Se results reported by Lab B using method 6010 are biased high (Figs. 1 and 3), the results for these samples have been assigned a qualifier in the project database to indicate that they should be used with caution.

Figure C1 shows the reported Se results from the baseline fish tissue samples analyzed in 2009 by Lab A and B. In the top panel of Fig. 4, Se results from Labs A and B for fish collected from various sites in the Clinch and Emory Rivers are plotted against each other, while the bottom graph shows the reported Se results for fish collected from the KIF stilling pond. In both plots, the results fall along a straight line. In the top panel of Fig. 4, results are parallel to but well above the line of perfect agreement, shown by a dotted line in this graph. For this range of Se concentrations, the results reported by Lab B are not only consistently higher than those from Lab A, but they are also systematically higher (slope = 1.005), such that the difference in Se concentrations between the two labs is predictable. This predictable correction factor relationship for Se in tissue holds across sites and across species. For this range of Se concentrations, the results from Lab B are on average 0.42 $\pm$ 0.01 mg/kg higher than the results from Lab A (Fig. C1-A).

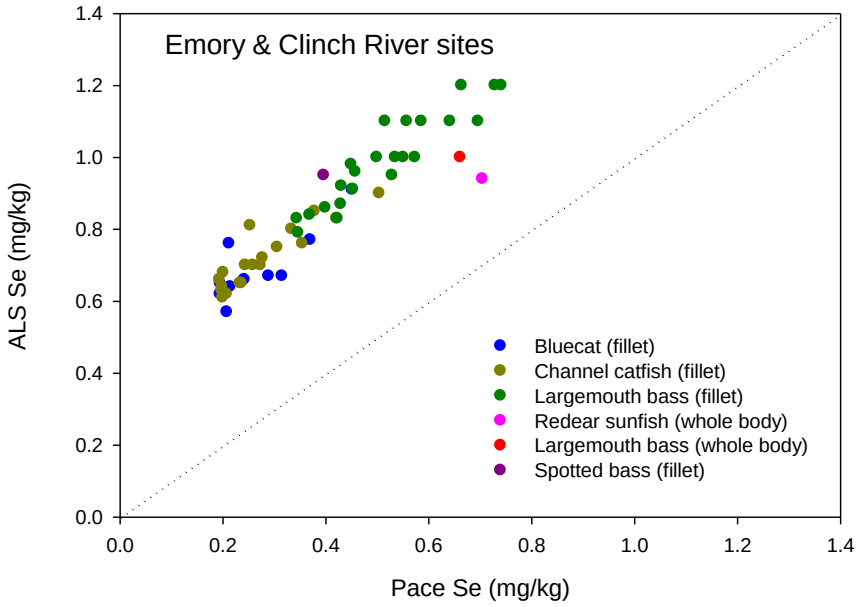
For the fish tissue samples collected from the KIF stilling pond where exposure to coal ash is greater than in the river systems, concentrations of Se in fish fillet are approximately an order of magnitude greater than for the fish tissue samples collected from the Clinch or Emory Rivers (Fig. C1-B). At these higher concentrations of Se, there is no apparent difference between the Se results reported by Labs A and B. Clearly, these data suggest that the relationship between the Se results of the two labs is concentration-dependent, and is not linear.

Interpreting the data presented in Fig. C-1, if Se concentrations in tissue samples reported by Lab B are between 0.6 – 1.2 mg/kg (wet wt.), a reasonable correction factor for these Se data would be a constant subtraction of 0.42 mg/kg. If Se concentrations in tissue samples reported by Lab B are above 3 mg/kg, no correction factor is necessary. Unfortunately, the majority of the Se concentrations reported by Lab B in the liver and ovary tissue samples from Spring, 2009, fall between 1.2 and 3 mg/kg Se. In this case, the most utilitarian approximation to relate the Lab A and B data and hence to derive a correction factor for Se would be the logarithmic model shown in Fig. C-2. This figure shows the relationship between the discrepancy between the Se results from both Lab A and Lab B and the reported results Se measured at Lab A, which have been demonstrated to represent the most accurate Se results based on Lab A's SRM data.

The Se correction factors discussed above were calculated based on data for fish fillets; however, based on the work performed it appears that a Se correction factor is also needed for liver and ovary tissue samples. Applying the fillet tissue-derived Se correction factors to liver and ovary tissue samples would assume that the difference in Se analytical results between Labs A and B is constant across these and other different tissue types. This is not likely to be a valid assumption. For example, the results for Se in fish fillets in Fig. C-1A fall on a straight line, approximately 0.42 mg/kg above the line of perfect

agreement. There are two data points (shown in red and pink) that fall slightly below this line (difference between Lab A and B < 0.42 mg/kg). These two data points are for whole body fish samples, rather than fillets. While these are only two data points, they suggest that different tissue types may indeed show a difference in their relationships between Lab A and B Se data. Nonetheless, this difference is not so great that the derived correction factor would be unreasonable.

A.



B

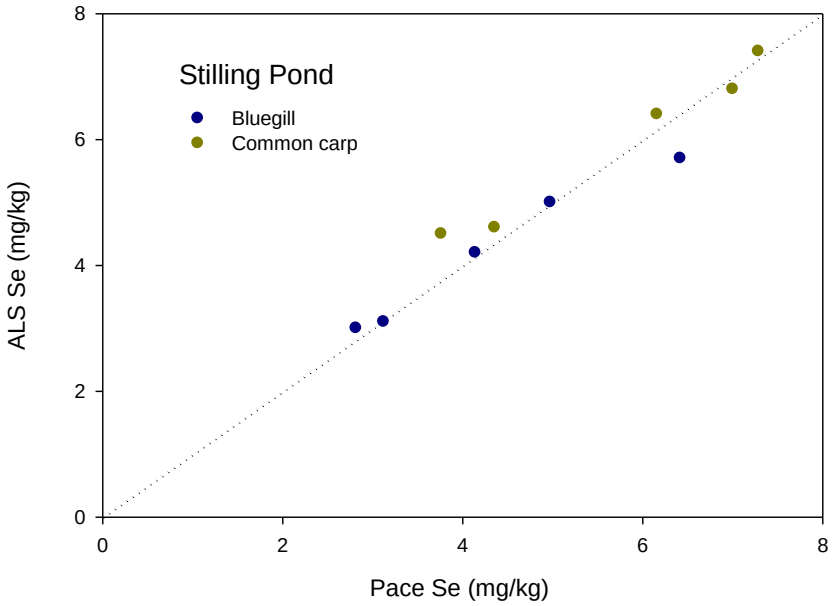


Fig. C-1. Comparison of the analytical results for selenium (mg/kg) from ALS and Pace for the fish samples used in the baseline study (2009) collected from A) various sites in the Emory and Clinch Rivers and B) from the stilling pond at the Kingston fossil plant. The dotted line represents the 1:1 line. Note the difference in scale between the two plots.

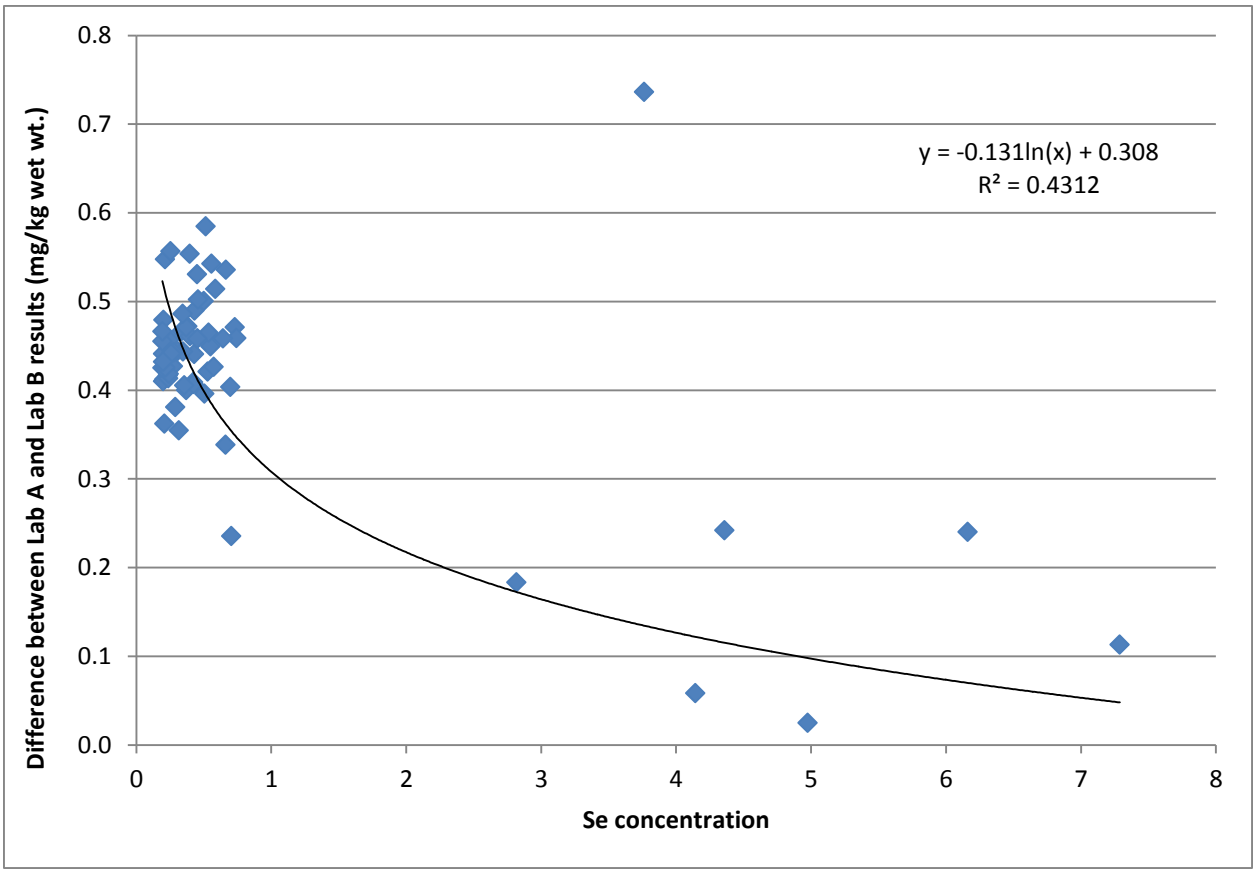


Fig. C-2. Relationship between the Se concentration in samples (mg/kg) and the discrepancy between Se results from ALS and Pace labs (mg/kg). Results are for the fish samples used in the baseline study (2009) collected from various sites in the Emory and Clinch Rivers as well as from the stilling pond at the Kingston fossil plant.