A Department of Ecology Report

A Comparison of Two Analytical Methods for Measuring Mercury in Fish Tissue

Abstract

In 2005, Washington State Department of Ecology staff at the Manchester Environmental Laboratory adopted new methodology for determining mercury in fish tissue. Laboratory duplicates were analyzed using U.S. Environmental Protection Agency (EPA) Method 245.5 and Method 245.6 to determine if laboratory methods affected analytical results.

A regression analysis of the laboratory duplicates found Method 245.5 to report mercury concentrations 25-38% lower than Method 245.6, depending on the magnitude of concentration. The cause of the difference in measurements is likely due to the different digestion processes applied prior to measurement.

A correctional equation was developed to estimate mercury concentrations between methods.

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Publication No. 07-03-041
September 2007

Publication Information

This report is available on the Department of Ecology's website at
www.ecy.wa.gov/biblio/0703041.html

Ecology's Study Tracker Code for this project is 06-501-03.

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Introduction

In 2005, Washington State Department of Ecology (Ecology) staff at the Manchester Environmental Laboratory adopted new methodology for determining mercury in fish tissue. Previously, Manchester Laboratory used EPA Method 245.5 *Determination of Mercury in Sediments by Cold Vapor Atomic Absorption Spectrometry* before adopting Method 245.6 *Determination of Mercury in Tissues by Cold Vapor Atomic Absorption Spectrometry*. The latest EPA revisions to both methods appear in the *Metals Manual of 1991* (EPA 1991).

As a quality assurance (QA) measure, Ecology conducted a study to evaluate measurement differences that may exist between the two methods. This report details the findings of the study.

Both methods measure total mercury (organic and inorganic) using conventional cold vapor atomic absorption after reducing all mercury to elemental mercury. Significant differences between the two methods include sample digestion and oxidation. Method 245.5 digests tissue in aqua regia (hydrochloric and nitric acids) for 2 minutes at 95ºC, and then oxidizes with potassium permanganate for 30 minutes at 95ºC. Method 245.6 digests tissue with sulfuric and nitric acid at 58ºC followed by overnight oxidation with potassium permanganate and potassium persulfate at room temperature.

Methods

Ninety-one samples were analyzed using both methods. The analyses were done in succession in the same manner as a lab duplicate. Samples were obtained from the Washington State Toxics Monitoring Program 2005 (WSTMP) study and the Mercury Trends in Fish Tissues 2005 (Mercury Trends) study.

To quantify differences between analytical methods, graphical techniques are used along with a regression approach. QA data from the re-analysis study were also reviewed to evaluate discrepancies between the methods.

Results

Graphical Techniques

The laboratory duplicates were plotted (245.5 v 245.6) on a scatterplot to examine how closely the points fell around the line of equality (y = x, or slope of 1). Results are presented in Figure 1.

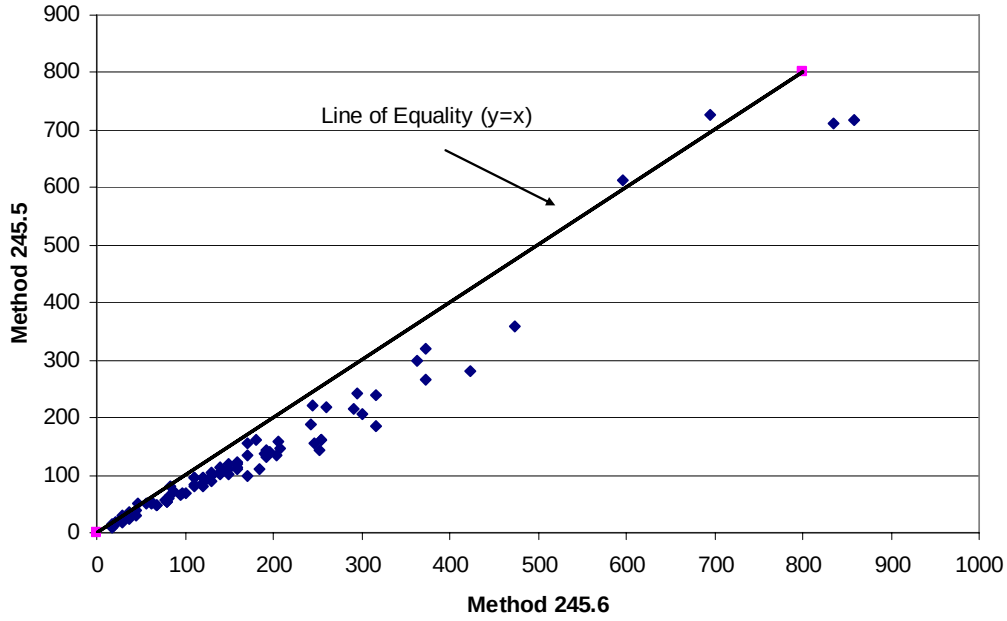

Figure 1. Mercury Concentrations (ppb) Measured by Method 245.5 and 245.6.

Eighty-nine of 91 points were distributed on the 245.6 side of the line of equality. The average relative percent difference (RPD) between samples found concentrations measured by Method 245.5 to be 27.11% less than Method 245.6.

The graphical representation of the data reveals possible outliers in the data sets. The Grubbs' Outlier test was conducted for each data set to determine if outliers existed:

$$z_i = \frac{\left| x_i - \bar{x} \right|}{s}$$

The critical z value for a sample size of 91 is 3.35 ($P < 0.05$). The four highest values in the Method 245.5 data set and the two highest values in the Method 245.6 data set exceeded the critical value. The four outliers were excluded from additional analysis due to lack of data with measurements over 500 ppb. Figure 2 displays the plotted results excluding the outliers.

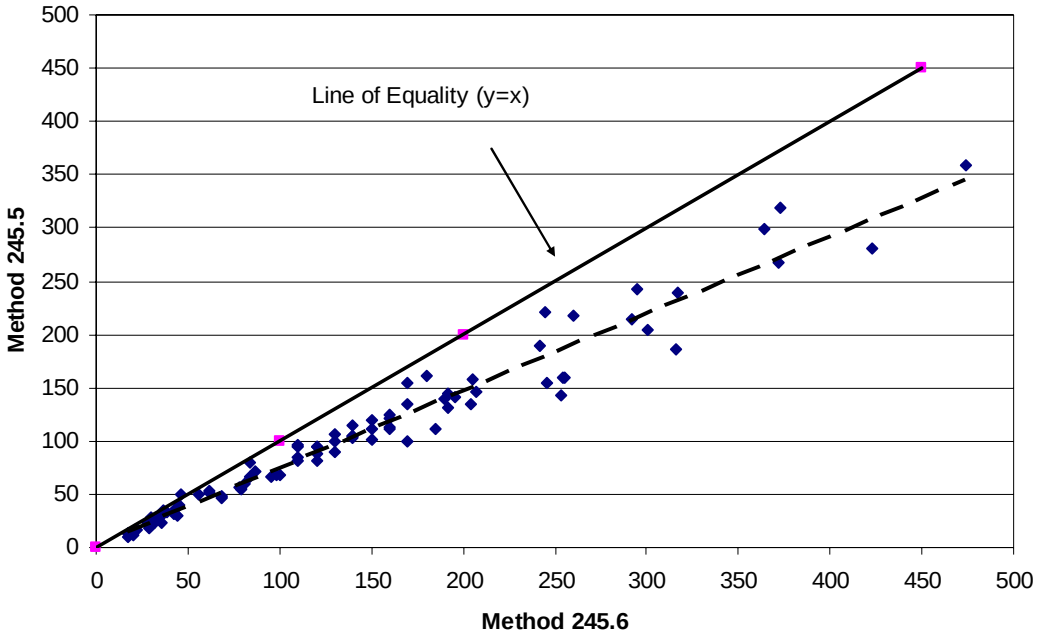

Figure 2. Mercury Concentrations (ppb) Measured by Methods 245.5 and 245.6 Excluding Outliers.

Figure 2 displays increasing deviations from the line of equality as the magnitude of measurement increases. This relationship is better visualized in Figure 3 by plotting the difference between methods on the y-axis and the average of the methods on the x-axis.

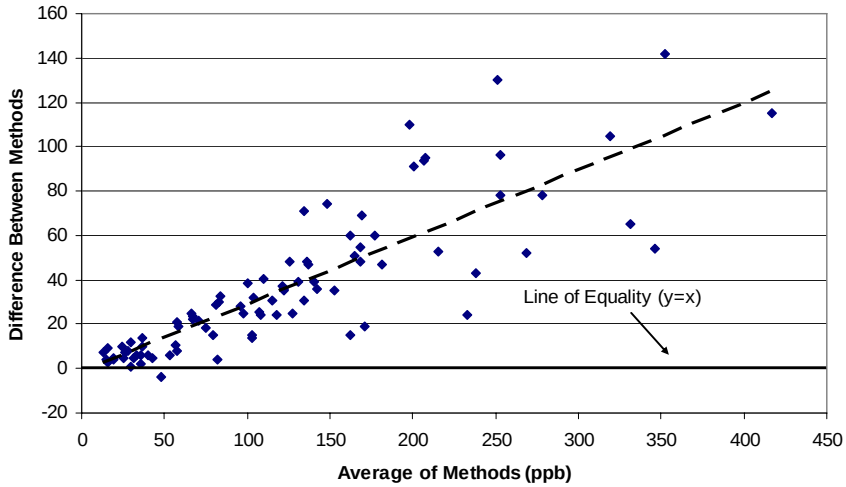

Figure 3. Difference Between Methods versus Average of Methods.

Additionally, the differences between methods (245.6-245.5) are not normally distributed (Figure 4).

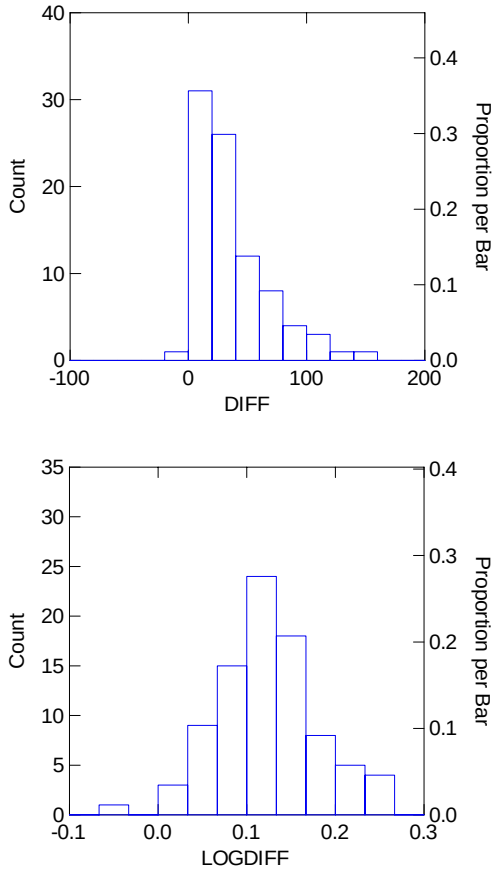

Figure 4. Distribution of Method Differences Before and After $\log_{10}$ Transformation.

To accurately calculate limits of agreement, the differences between the two methods must be normally distributed, and the mean and standard deviation must be the same throughout the range of measurements (Bland and Altman, 1999).

To normalize the data, results from both methods were $\log_{10}$ transformed. Figures 5 and 6 are the same plots used as Figures 2 and 3 with the $\log_{10}$ transformed data.

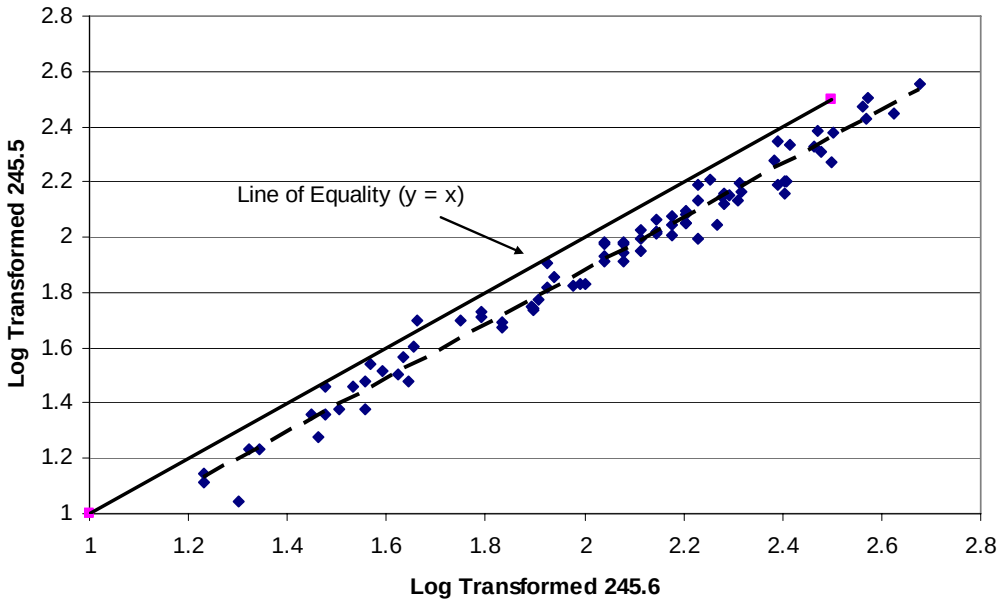

Figure 5. Log_{10} Concentration Method 245.5 versus Log_{10} Concentration Method 245.6.

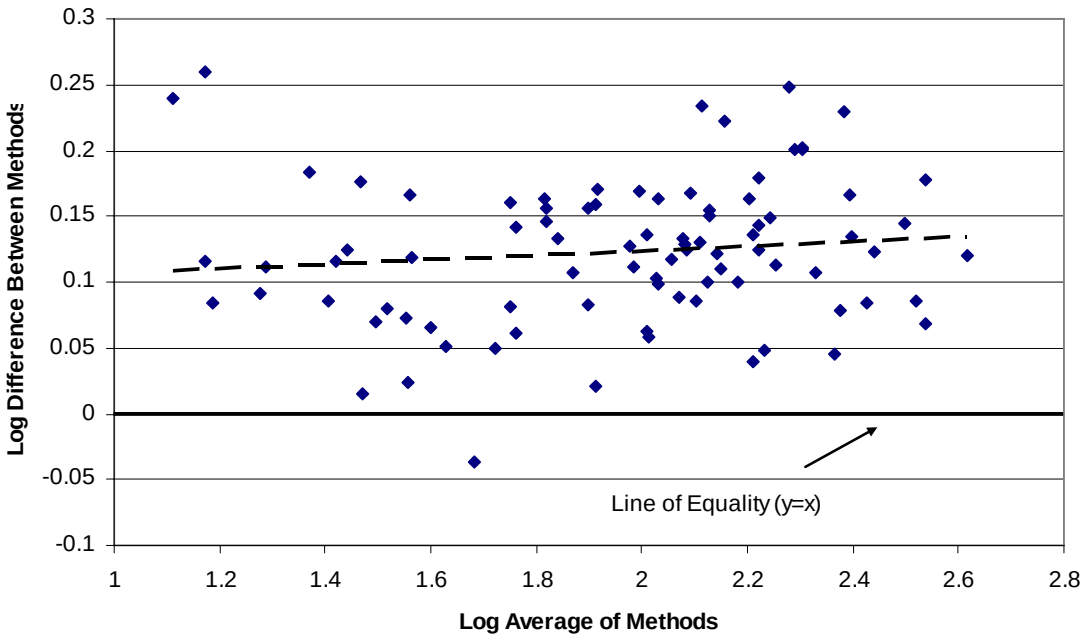

Figure 6. Difference Between Log_{10} of Methods versus Average of Log_{10} Methods.

With independence of between-method differences and size of measurement, the methods may be compared very simply by analyzing the individual 245.6 – 245.5 differences. The mean of these differences will be the relative bias, and their standard deviation is the estimate of error.

The mean logarithm difference ($\log_{10}$ 245.6 – $\log_{10}$ 245.5) is 0.1231, and the standard deviation is 0.0557. Since the differences are distributed normally, 95% of the differences between methods lay within the mean ± 1.96 standard deviations. For the methods comparison, log transformed upper and lower limits of agreement equal 0.2323 and 0.0139, respectively (Figure 7).

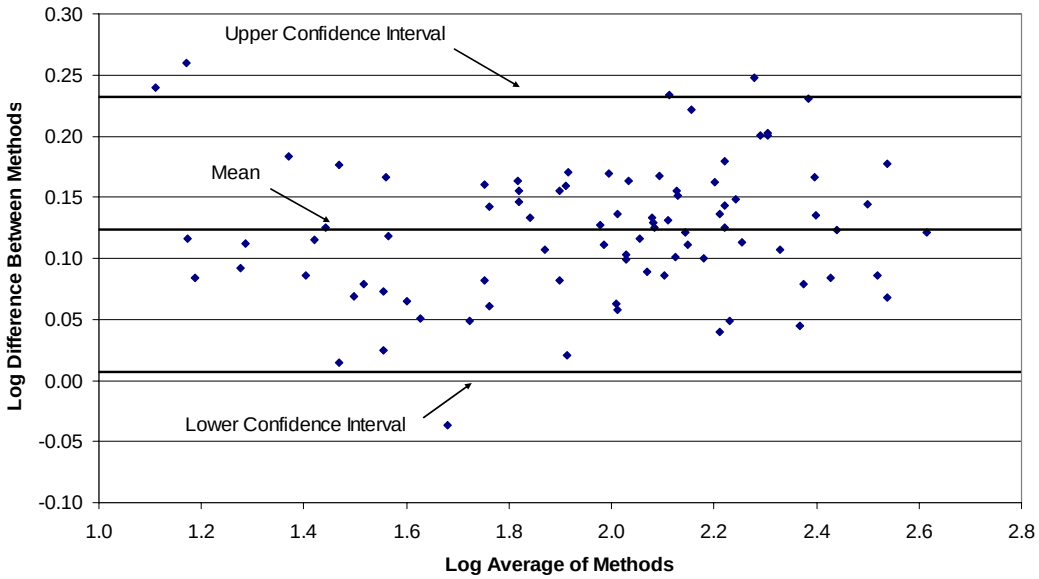

Figure 7. Log_{10} Difference plotted with Log_{10} Average.

The log transformed limits of agreement along with the mean can be back-transformed to give limits and mean for the ratio of actual measurements. The mean difference between 245.6 and 245.5 was 1.3278 with 95% limits of agreement as 1.0326 and 1.7074. Thus Method 245.6 exceeds Method 245.5 by between 3.26% and 70.74%, with an average value of 32.78% (Figure 8).

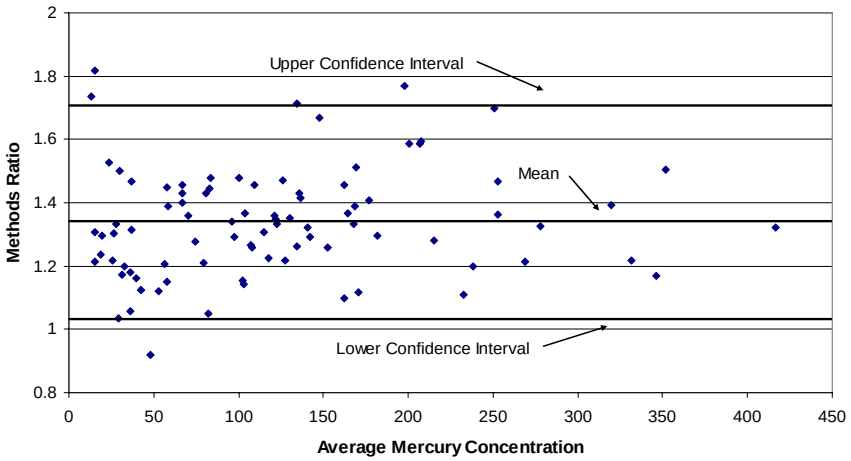

Figure 8. Methods Ratio versus Average Mercury Concentration.

Regression Approach

While $\log_{10}$ transformation of the data helped normalize the residuals, Figure 9 displays a slight increase in the trendline as concentrations increase. The approach discussed above calculates a percentage difference based off the average methods ratio that can be applied to concentrations. Since the slope does not equal zero, low concentrations of mercury would be overestimated at 32.78% and large concentrations (400<x<500 ppb) would be underestimated.

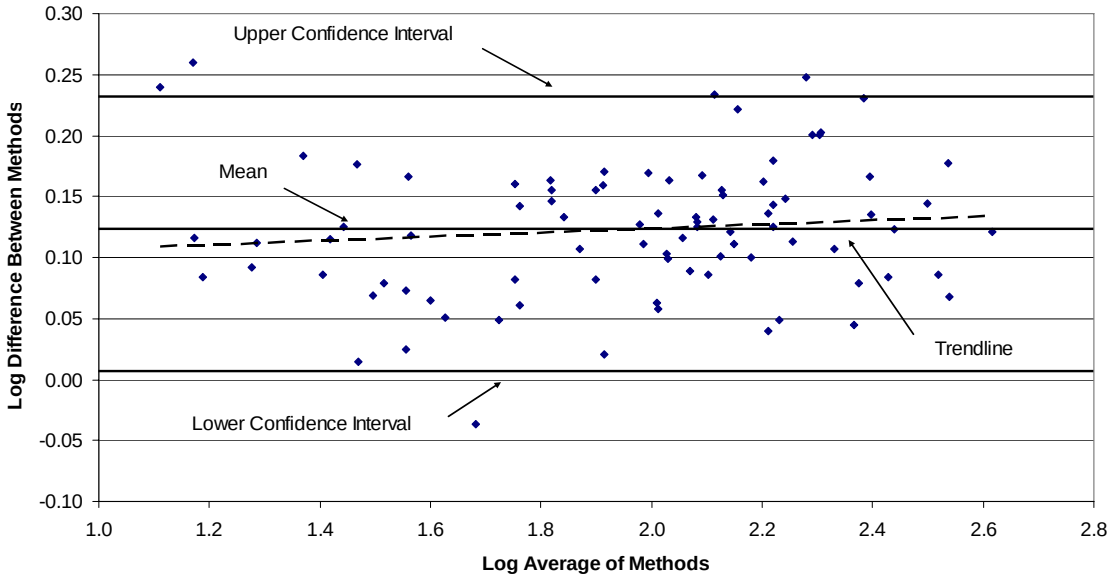

Figure 9. Log_{10} Differences Plotted with Trendline.

To estimate method bias based on level of concentration, the least squares regression equation can be used (Figure 10, Equations 1 and 2).

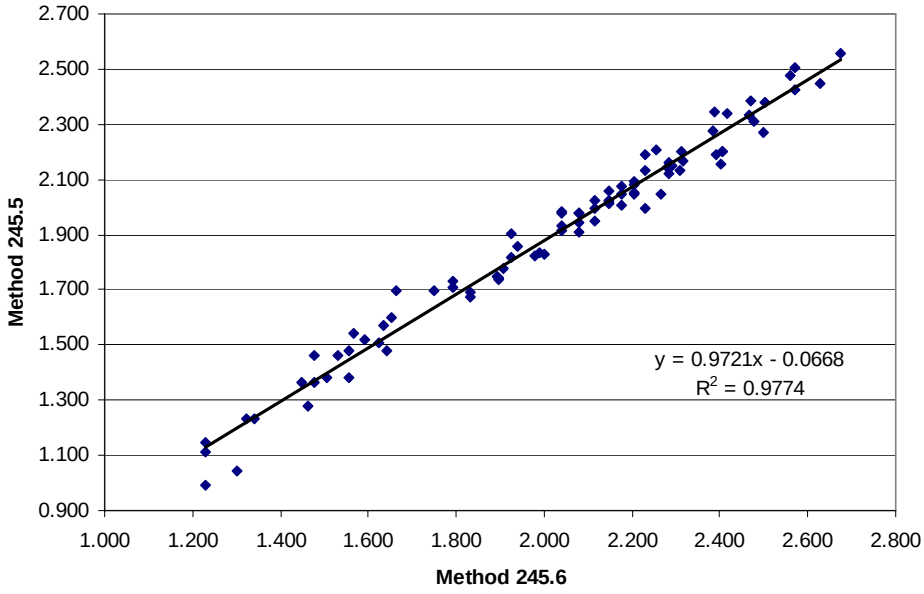

Figure 10. Plotted Pairs of the Log_{10} Transformed Concentrations.

$$1.\ (245.5Hg) = 10^{0.9721(245.6Hg)-0.0668}$$

or

$$2.\ (245.6Hg) = 10^{((245.5Hg)+0.0668)/0.9721}$$

Using this approach, Method 245.5 is approximately 25% less than Method 245.6 at the lowest concentrations, and 38% lower than Method 245.6 at the highest concentrations.

Quality Assurance/ Quality Control

Ecology staff use a variety of tests and standards to evaluate the quality of analytical data. Laboratory Matrix Spikes, Laboratory Control Samples, and Standard Reference Materials measure accuracy and bias of the tests.

Laboratory Matrix Spikes (LMXs)

LMXs are conducted by adding a known amount of SRM to a tissue sample and measuring percent recovered. Figure 11 plots the recoveries of matrix spike duplicates (LMX1 and LMX2) during the method comparison study. Eight of the 12 LMX recoveries for Method 245.5 were below 80% with an average recovery of 85%. No LMX recoveries were below 80% for Method 245.6. Only two of 22 LMX recoveries were below 90% for Method 245.6 with an average of 95%.

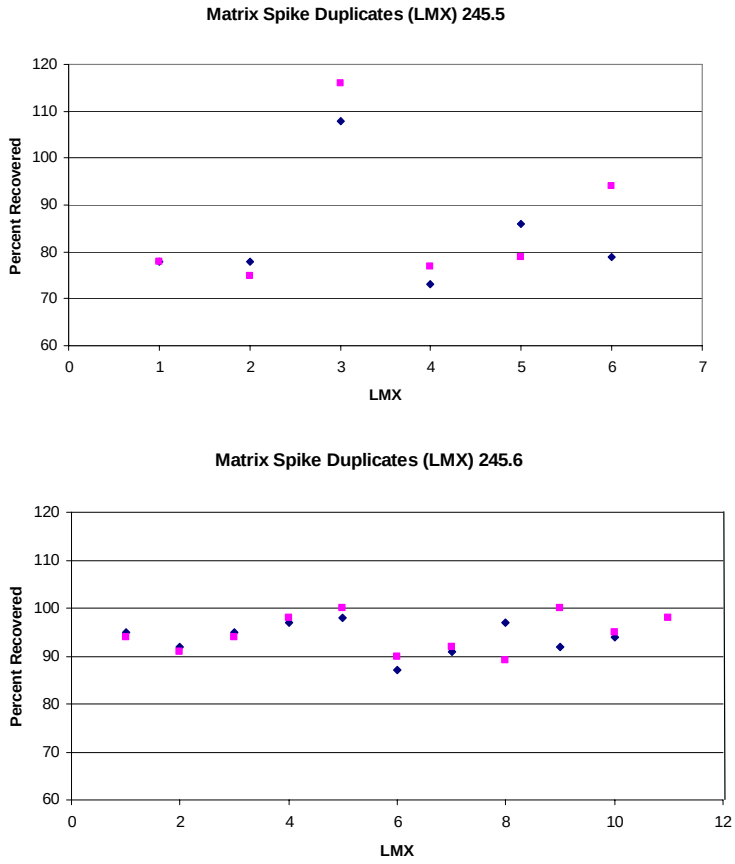

Figure 11. Matrix Spike Duplicates with Methods 245.5 and 245.6.

Laboratory Control Samples (LCSs) and Standard Reference Materials (SRMs)

LCSs are SRMs are measured in the same manner as LMXs. The only difference is the media in which the SRM is added. LCSs are prepared by adding a known amount of SRM to de-ionized water and then measuring percent recovered. SRM tests are conducted by adding a known amount of SRM to Ottawa Sand, a standards testing material, and then measuring percent recovered. Thirty-six SRM and LCS tests were conducted between the two methods. Both methods had excellent recovery rates for the two tests 100% ± 5% (Appendix).

Discussion

Review of the plotted paired data and regression analysis reveal mercury measurements using Method 245.5 are lower than measurements made by Method 245.6. The explanation for the differences can be attributed to the different digestion processes between methods.

One possible explanation is digestion via aqua regia (245.5) does not completely solubilize the sample, and a portion of mercury, bound in tissue, was left unmeasured.

A second possible explanation for the incongruous measurements is the heating process applied during sample digestion. Method 245.5 digests the sample for 2 minutes at 95ºC in a biochemical oxygen demand (BOD) bottle before the sample is oxidized, reduced, and measured. Method 245.6 digests the sample at 58ºC before oxidation, reduction, and measurement. Mercury is highly volatile in many forms, and rapid heating can cause decreasing sorption from the sample due to increased thermal motion (Schluter, 2000). The rapid heating of the sample during digestion could partially explain measurement differences.

LCSs and SRM tests were recovered very close to 100% using both methods, while LMXs were recovered at much more accurate rates by Method 245.6. The discrepancy between recovery rates when compared to LMX are probably a reflection of the nature of the media in which they are tested. Recovery rates were very high when digestion processes (i.e., SRM and LCS) were eliminated.

Conclusions and Recommendations

The following conclusions and recommendation are made as a result of this study:

- In direct comparisons of laboratory duplicates, Method 245.5 measured at least 25% less mercury than Method 245.6.
- The cause of the large difference in measurements is most likely due to the different digestion processes prior to measurement.
- We recommend applying the regression equation discussed above to all Method 245.5 mercury measurements for the purpose of trends monitoring.

References

Bland, J.M and D.G Altman, 1999. Measuring Agreement in Method Comparison Studies. Statistical Methods in Medical Research. Vol. 8:135-160.

EPA, 1991. Metals Manual of 1991. U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, OH.

Schluter, K., 2000. Review: Evaporation of Mercury from Soils. An Integration and Synthesis of Current Knowledge. Environmental Geology. Vol. 39 No. 3-4.

Appendix. Analytical Results

Table A1. Laboratory Duplicate Results

Sample ID	EPA Method	
	245.6	245.5
5514715	17	9.8
6085051	17	14
6085056	17	13
6054764	20	11
5524725	21	17
6085054	22	17
5524724	28	23
6054766	29	19
5514701	30	29
6085053	30	23
6085055	32	24
6085037	34	29
6054763	36	24
6085034	36	30
6024740	37	35
6085039	39	33
6054768	42	32
5514716	43	37
6054761	44	30
5524730	45	40
6024741	46	50
6085035	56	50
6085036	62	53.9
6085038	62	51.4
6024749	68	49
6085057	68	47
6054758	78	55.7
5514705	79	55.2
6054771	79	54.3
6085058	81	59.6
5524723	84	65.7
6024745	84	80.1
6085059	87	72
5514704	95	66.4
6085050	98	67.9
6085052	100	67.6
5514709	110	82.1
6085001	110	85.1
6085030	110	96.2
6085033	110	95.2
6054752	120	87.7
6054762	120	81.2
5524728	120	94.7
6085005	120	95.5
5514700	130	106
6054769	130	89.3
6054753	130	99.4
5524720	140	105
6085003	140	104
6085004	140	103
6085031	140	115
6054767	150	102
6085000	150	111
6085032	150	119
5524727	160	124
5524717	160	121
6085006	160	112
6085008	160	113
5514703	170	135
6024743	170	155
6085002	170	99.3
6024751	180	161
6085007	185	111
5524731	190	139
6054770	192	144
6085011	192	132
6054754	196	141
6085009	204	135
5514706	205	158
5514710	207	147
5524721	242	189
5524729	245	221
6085010	246	155
6085015	253	143
6085019	254	160
6085018	255	160
5514702	260	217
6085014	292	214
6054760	295	243
6085012	301	205
6085017	316	186
6085013	317	239
6024747	364	299
6085016	372	267
6054759	373	319
6024748	423	281
5524722	474	359
6024738	596	613
6024746	696	726
5514708	834	713
5514707	859	718

Table A2. Laboratory Matrix Spikes.

Sample #	Method	ID	Result (%)	ID	Result (%)
6127073	EPA245.5	LMX1	78	LMX2	78
6127103		LMX1	78	LMX2	75
6087017		LMX1	108	LMX2	116
6087039		LMX1	73	LMX2	77
6127001		LMX1	86	LMX2	79
6127067		LMX1	79	LMX2	94
5514705	EPA245.6	LMX1	95	LMX2	94
5524717		LMX1	92	LMX2	91
6024749		LMX1	95	LMX2	94
6054752		LMX1	97	LMX2	98
6054771		LMX1	98	LMX2	100
6085017		LMX1	87	LMX2	90
6085025		LMX1	91	LMX2	92
6085040		LMX1	97	LMX2	100
6127000		LMX1	92	LMX2	95
6127066		LMX1	94	LMX2	98

Table A3. Standard Reference Material.

Sample ID	SRM	Method	Result (%)
ML06080H5	1946	EPA245.5	94
ML06095H2	1946		99
ML06096H6	1946		99
ML06096H7	1946		95
ML06096H8	1946		93
ML06121H3	1946		112
ML06121H4	1946		106
ML06121H5	1946		107
ML06067H3	DORM		97
ML06080H3	1946	EPA245.6	95
ML06086H3	1946		105
ML06089H2	1946		105
ML06096H2	1946		104
ML06096H3	1946		101
ML06096H4	1946		107
ML06121H6	1946		111
ML06121H7	1946		110
ML06121H8	1946		113
ML05362H2	DORM		104
ML06003H2	DORM		106
ML06024H2	DORM		76
ML06065H2	DORM		99

1946 – Lake Superior Fish Tissue
DORM – Dogfish Muscle

Table A4. Laboratory Control Samples.

Sample ID	Method	Result (%)
ML06067H2	EPA245.5	104
ML06080H4		98
ML06095H1		96
ML06096H5		101
ML06121H1		103
ML05362H1	EPA245.6	96
ML06003H1		102
ML06024H1		105
ML06065H1		102
ML06080H2		98
ML06086H2		98
ML06089H1		98
ML06096H1		96
ML06121H2		97