



August 19, 2008

Randall Payne
Kentucky Geological Survey
14 Reilly Road
Frankfort, KY 40601
(502) 564-3410 x497

Re: Project Se in Eastern KY Se Study

Dear Mr. Payne,

Attached is the report associated with two (2) fish tissue samples submitted for selenium speciation and total selenium analyses. All samples were submitted on June 26, 2008. All samples were received on June 27, 2008 in a sealed cooler with a temperature of -0.5°C . Selenium speciation analysis was performed by ion chromatography inductively coupled plasma mass spectrometry (IC-ICP-MS) while total selenium analysis was performed via inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS). Any analytical issues associated with the analysis are addressed in the following report.

If you have any questions, please feel free to contact me at your convenience.

Sincerely,

A handwritten signature in black ink, appearing to read "Russell Gerads", written over a light blue horizontal line.

Russell Gerads
Vice President
Applied Speciation and Consulting, LLC

Applied Speciation and Consulting, LLC

Report Prepared for:

Randall Payne
Kentucky Geological Survey
14 Reilly Road
Frankfort, KY 40601

Project: Se in Eastern KY Se Study

August 19, 2008

1. Sample Reception

A total of two (2) fish tissue samples were submitted for selenite, selenate, selenocyanate, methylseleninic acid, selenomethionine, and total selenium analyses on June 26, 2008. All samples were received on June 27, 2008 in a sealed cooler with a temperature of -0.5°C.

The samples were received in a laminar flow clean hood void of trace metals contamination and ultra-violet radiation. Immediately upon reception all fish tissue samples were placed in a secure monitored freezer (maintained at a temperature of -30°C) until homogenization and sample preparation could be performed.

2. Sample Preparation

All sample preparation is performed in laminar flow clean hoods known to be free from trace metals contamination. All applied water for dilutions and sample preservatives are monitored for contamination to account for any biases associated with the sample results.

Selenium Speciation Analysis of Fish Tissue Samples by IC-ICP-MS Prior to analysis, each discrete sample was homogenized in its entirety. A subsample of the whole body homogenate and 40mL of 0.10 M KOH (v/v) was transferred to 50mL polypropylene centrifuge tubes. The slurry was placed on an inverted rotary shaker for 2 hours at 80rpm. The slurry was removed from the inverted shaker and centrifuged for 1 hour. The supernatant was decanted from the centrifuge tube, filtered (0.45um), and directly injected into sealed autosampler vials for analysis.

Total Selenium Quantification of Fish Tissue Samples by ICP-DRC-MS Prior to analysis, all fish tissue homogenates for total selenium were digested using a closed vessel HNO₃ bomb digestion. The digested samples were analyzed by inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS).

3. Sample Analysis

All sample analysis is precluded by a minimum of a five-point calibration curve spanning the entire concentration range of interest. Calibration curves are performed at the beginning of each analytical day. All calibration curves, associated with each species of interest, are standardized by linear regression resulting in a response factor. All sample results are **instrument blank corrected** to account for any operational biases associated with the analytical platform.

Prior to sample analysis, all calibration curves are verified using second source standards which are identified as initial calibration verification standards (ICV).

Ongoing instrument performance is identified by the analysis of continuing calibration verification standards (CCV) and continuing calibration blanks (CCB) at a minimal interval of every ten analytical runs.

Selenium Speciation Analysis by IC-ICP-MS All samples for selenite, selenate, selenocyanate, selenomethionine, and methylseleninic acid quantification were analyzed by ion chromatography inductively coupled plasma mass spectrometry (IC-ICP-MS). Aliquots of each sample are injected onto an anion exchange column and are mobilized by a basic (pH > 7) gradient. The eluting selenium species are then introduced into a radio frequency (RF) plasma where energy-transfer processes cause desolvation, atomization, and ionization. The ions are extracted from the plasma through a differentially-pumped vacuum interface and separated on the basis of their mass-to-charge ratio (m/z) by a mass spectrometer. A solid-state detector detects ions transmitted through the mass analyzer and the resulting current is processed by a data handling system.

Retention times for each eluting species are compared to known standards for species identification.

Total Selenium Quantification by ICP-DRC-MS All samples for total selenium quantification were analyzed by inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS). Aliquots of each sample are introduced into a radio frequency (RF) plasma where energy-transfer processes cause desolvation, atomization, and ionization. The ions are extracted from the plasma through a differentially-pumped vacuum interface and travel through a pressurized chamber (DRC) containing a specific reactive gas which preferentially reacts with interfering ions of the same target mass to charge ratios (m/z). A solid-state detector detects ions transmitted through the mass analyzer, on the basis of their mass-to-charge ratio (m/z), and the resulting current is processed by a data handling system.

4. Analytical Issues

The overall analyses went very well and no significant analytical issues were encountered. All quality control parameters associated with these samples were within acceptance limits with the following exceptions:

It should be noted that the estimated method detection limits (eMDLs) for all selenium species are generated from replicate analyses of the lowest standard in the calibration curve. Not all selenium species are present in preparation blanks; therefore, eMDL calculations based on preparation blanks are artificially biased low.

The eMDL for methylseleninic acid and selenomethionine are referenced from the average eMDL for selenite, selenate, and selenocyanate. The source of methylseleninic acid contains selenite which would alter all facets of the speciation analysis associated with selenite quantification. Due to the limited sources of methylseleninic acid a second source verification standard could not be attained; therefore, all reported results should be considered estimates.

The interaction of selenoproteins, seleno-amino acids, and other organo-selenium compounds with the applied extraction procedure is unknown; therefore, concentrations for selenium species other than selenite, selenate, and selenocyanate should be viewed as reference values only.

The methylseleninic acid recoveries associated with the matrix spikes and matrix spike duplicates were low. The methylseleninic acid recoveries for the laboratory control samples were within acceptance limits signifying that the low matrix spike recoveries are attributed to matrix interference.

If you have any questions or concerns regarding this report, please feel free to contact me.

Sincerely,



Russell Gerads
Vice President
Applied Speciation and Consulting, LLC

Total Selenium and Selenium Speciation Results for Kentucky Geological Survey
Project Name: Se in Eastern KY Coal Field
Contact: Randall Payne

Date: August 19, 2008
Report Generated by: Russell Gerads
Applied Speciation and Consulting, LLC

Sample Results

Sample ID	Description	Sampling Date	Se(IV)	Se(VI)	SeCN	MeSe(IV)	SeMe	Other Se Species (n)	Total Se
Hypenelium Nigfaeans	Big Creek	6/26/08	ND (<0.036)	ND (<0.021)	0.107	ND (<0.033)	ND (<0.033)	0.132 (3)	1.28
Somatalus atromacklatus	Big Creek	6/26/80	ND (<0.036)	ND (<0.021)	0.117	ND (<0.033)	0.071	ND (<0.038)	0.67

All results reflect the applied dilution and are reported in mg/kg on an as received basis

ND = Not detected at the applied dilution

SeCN = Selenocyanate

MeSe(IV) = Methylseleninic acid

SeMe = Selenomethionine

(n) = Number of other selenium species identified

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Quality Control Summary - Preparation Blank Summary

Analyte (mg/kg)	PBT1	PBT2	PBT3	PBT4	Mean	StdDev	eMDL*
Se(IV)	0.000	0.000	0.000	0.000	0.000	0.000	0.036
Se(VI)	0.000	0.000	0.000	0.000	0.000	0.000	0.021
SeCN	0.000	0.000	0.000	0.000	0.000	0.000	0.040
MeSe(IV)	0.000	0.000	0.000	0.000	0.000	0.000	0.033
SeMe	0.000	0.000	0.000	0.000	0.000	0.000	0.033
Total Se	0.068	0.061	0.050	0.040	0.053	0.015	0.038

eMDL = Estimated Method Detection Limit

*Please see narrative regarding eMDL calculations

MeSe(IV) = Methylseleninic acid

SeMe = Selenomethionine

Quality Control Summary - Certified Reference Materials

Analyte (mg/kg)	CRM	True Value	Result	Recovery
Se(IV)	LCS	88.96	84.45	94.9
Se(VI)	LCS	80.72	78.33	97.0
SeCN	LCS	73.20	68.35	93.4
MeSe(IV)	LCS	51.76	58.36	112.8
SeMe	LCS	74.56	79.95	107.2
Total Se	DOLT-4	8.300	8.913	107.4

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Quality Control Summary - Matrix Duplicates

Analyte (mg/kg)	Sample ID	Description	Rep 1	Rep 2	Mean	RPD
Se(IV)	Somatalus atromacklatus	Big Creek	ND (<0.036)	ND (<0.036)	NC	NC
Se(VI)	Somatalus atromacklatus	Big Creek	ND (<0.021)	0.027	NC	NC
SeCN	Somatalus atromacklatus	Big Creek	0.117	0.119	0.118	1.7
MeSe(IV)	Somatalus atromacklatus	Big Creek	ND (<0.033)	ND (<0.033)	NC	NC
SeMe	Somatalus atromacklatus	Big Creek	0.071	0.064	0.067	11.2
Total Se	Somatalus atromacklatus	Big Creek	0.670	0.688	0.679	2.7

ND = Not detected at the applied dilution

NC = Value was not calculated due to one or more concentrations below the eMDL

*Concentration is within 10x the eMDL

Quality Control Summary - Matrix Spike/ Matrix Spike Duplicate

Analyte (mg/kg)	Sample ID	Description	Spike Conc	MS Result	Recovery	Spike		Recovery	RPD
						Conc	MSD Result		
Se(IV)	Somatalus atromacklatus	Big Creek	78.41	74.85	95.5	80.16	75.67	94.4	1.1
Se(VI)	Somatalus atromacklatus	Big Creek	71.14	67.03	94.2	72.73	70.59	97.1	5.2
SeCN	Somatalus atromacklatus	Big Creek	64.52	61.74	95.5	65.96	65.10	98.5	5.3
MeSe(IV)	Somatalus atromacklatus	Big Creek	47.61	28.189	59.2*	47.61	30.18	63.4*	6.8
SeMe	Somatalus atromacklatus	Big Creek	68.58	72.73	105.9	65.96	70.43	106.7	3.2
Total Se	Somatalus atromacklatus	Big Creek	9.764	11.58	111.7	8.710	10.35	111.1	11.2