Appendix A: Standard Operating Procedures

SOP	Title
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3	Bulk X-Ray Fluorescence (XRF)
4	Electron Microprobe Analysis (EMPA)
5	Differential XRD
6	Synchrotron-Based X-Ray Diffraction, Bulk X-Ray Absorption (XAS), and μ-X-Ray Absorption Spectroscopy (μ-XAS) / μ-X-Ray Fluorescence Spectroscopy (μ-XRF) / μ-X-Ray Diffraction (μ-XRD):
7	Particle Size Analysis
8	Water Extraction (ASTM, 2004)
9	Simulated Gastric Fluid (SGF) Extraction
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Date: May 2009 **SOP No. # 1**

Title: *Bulk Soil Processing for Laboratory Studies*

Associated Investigator: Dr. Nicholas Basta

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SYNOPSIS:
This protocol details the processing of bulk soil samples into homogenous <250 µm sub-samples for distribution to the various investigators for further analysis.

Bulk Soil Processing for Laboratory Studies

1.0 Scope of Method

1.1 This method provides soil processing procedures that ensures and maintains homogeneity of field collected soils (<2mm) within and across storage containers. This is necessary for comparable experimental results across laboratories for the same soil. This method utilizes an electric cement mixer retrofit with a steel drum cone and No. 10 mesh screen. This allows for homogenous mixing followed by continuous mixing while sieving in order to prevent gravimetric settling out of fines. The drum cone also reduces the amount of dust emitted during this process.

2.0 Definitions

2.1 homogeneity: Analyte homogeneity within a soil matrix is achieved when analyte variation between test portions of the sample are not significantly different at $p<0.1$

2.2 < 2mm: The size fraction of soil that passes through a No. 10 mesh screen.

2.3 <250 um: The size fraction of soil that passes through a No. 60 mesh screen.

2.4 ICP: Inductively coupled plasma-atomic emission spectrometry.

3.0 Equipment and Supplies

3.1 Electric cement mixer capable of mixing up to 250 kg retro fit with a steel drum cone and No. 10 mesh screen (see photo).

3.2 220 watt power source.

3.3 At least sixteen 5 gal buckets

3.4 Drying oven

3.5 Benchtop reciprocal shaker capable of 280 osc/minute

3.6 No. 60 mesh sieve (12 inch), lid, and catch pan.

4.0 Homogenization and < 2mm Sieving Procedure

4.1 Oven Dry soil at 60°C.

4.1 Remove Drum cone and screen from mixer put soil to be homogenized into mixer.

4.2 Attach screen (2mm) to mixer opening, followed by the drum cone.

4.3 Attach drum cone support cables to mixer and tighten turnbuckles and close slide gate on the drum cone.

4.4 Rotate mixer to approximately 20° above horizontal and turn on. Allow mixer to homogenize soil for two hours.

4.5 After two hours, rotate mixer to approximately 45° below horizontal, place bucket below drum cone outlet and open slide gate. To eliminate gravimetric settling of the fine fraction, fill pre-labeled (sample name and <2mm) buckets with mixer on and screen in place.

5.0 <250 µm Sieving Procedure.

5.1 Place 500g (± 50g) of soil into # 60 sieve attached to catch pan and place lid atop the sieve/catch pan stack.

5.2 Secure sieve/catch pan stack to benchtop reciprocal shaker.

5.3 Shake sieve/catch pan stack at 280 osc/minute for 20 minutes.

5.4 The soil collected in the catch pan is the <250 µm size fraction. Pour into appropriately labeled (sample name and <250 µm) tubs.

5.5 Repeat procedure until desired amount of <250 µm is obtained.

6.0 Homogeneity Evaluation
Adapted from McClure, 2001.

6.1 Sampling procedure

6.1a Divide homogenized material into at least 16 containers.

6.1b Randomly select a sample size of c = 8 containers.

6.1c Randomly obtain n = 3 test portions (sub-samples) from each container.

6.1d Analyze the n x c = 24 samples by USEPA Method 3051a (SOP follows) followed by ICP analysis (SOP 12, Inductively Coupled Plasma (ICP) Spectrometry Analysis) for total arsenic.

6.2 Evaluate Within Container Variance

6.2a Calculate the CochransCochran's test statistic C_0 by dividing the largest within container variance (s_H^2) by the sum of all the within container variances ($\sum s_i^2$).

$$C_0 = s_H^2 / \sum s_i^2$$

6.2b Compare the calculated C_0 to the test statistic $C_{.05,c,(n-1)} = 0.52$. If $C_0 > 0.52$, the hypothesis that within-container variances are homogenous is rejected.

6.3 Across Container Variance

6.3a Use a one-way ANOVA to test across container variation to test the hypothesis:

$$H_0: \sigma^2_c = 0$$

At $p<0.1$

7.0 Corrective Action

7.1 If either within container or across container homogeneity tests fail, perform homogeneity evaluation (5.0) a second time.

7.2 If within container or across container homogeneity tests fail a second time, repeat homogenization procedure (4.0) and homogeneity evaluation (5.0).

8.0 Storage ,Preservation, and Shipment Use of Processed Soil

8.1 Homogenized soil should be stored at room temperature in 4 liter plastic or glass containers with screw top lids. No sample preservation is necessary for As contaminated soils after homogenization. Before use, the soil containers should be completely inverted 10 to 20 times to thoroughly remix soil and eliminating non-homogeneity due to settling during storage.

8.2 Package samples to be shipped to other laboratories in plastic containers and send certified mail via UPS priority next day air with return receipt. Include a chain of custody in the package.

9.0 QA/QC CHECKS

Complete the following table to summarize QA/QC checks.

Matrix	Measurement	QA/QC Check[1]	Frequency	Acceptance Criteria	Corrective Action
Soil	Within container variance	Cochran's test statistic C_0	Every soil	$C_0 < 0.52$	Repeat 5.0, if fails again, repeat homogenization procedure (4.0 and 5.0)
Soil	Across container variance	One-way Anova	Every soil	P <0.1	Repeat 5.0, if fails again, repeat homogenization procedure (4.0 and 5.0)

[1]Include all QA/QC checks (experimental and analytical, as applicable) for accuracy, precision, detection limits, mass balance, *etc*. (*e.g.*, matrix spikes, lab control samples, blanks, replicates, surrogates)

10.0 References

10.1 McClure, R.D. 2001. A statistical model to evaluate analyte homogeneity for a material. Journal of AOAC International. 84:947-954

SOP 1a: 3051a Microwave Assisted Acid Digestion of Sediments, Sludges, and Soils

1.0 Scope of Method

1.1 This method is a microwave-assisted extraction using aqua regia and HNO_3. This method is more aggressive in dissolving the sample matrix than methods using conventional heating with nitric acid (HNO_3), or alternatively, nitric acid and hydrochloric acid (HCl), according to EPA Methods 200.2 and 3050. However, because Method 3051a does not accomplish total decomposition of the sample, the extracted analyte concentrations may not reflect the total content in samples where the analytes are occluded in recalcitrant mineral phases. This method is applicable to the microwave-assisted acid extraction/dissolution‡ of sediments, sludges, and soils, for the following elements: Aluminum (Al)*, Antimony (Sb)*, Arsenic (As), Barium (Ba)*, Beryllium (Be)*, Boron (B), Cadmium (Cd), Calcium (Ca), Chromium (Cr)*, Cobalt (Co), Copper (Cu), Iron (Fe)*, Lead (Pb), Magnesium (Mg)*,

Manganese (Mn), Molybdenum (Mo), Nickel (Ni), Potassium (K), Selenium (Se), Silver (Ag)*, Sodium (Na), Strontium (Sr), Thallium (Tl), Vanadium (V)*, Zinc (Zn).
*Indicates elements which typically require the addition of HCl to achieve equivalent results with EPA Method 3050, as noted in reference 3.
This method is intended to provide a rapid multi-element acid extraction or dissolution prior to analysis. Many types of samples will be dissolved by this method. A few refractory sample matrix compounds, such as quartz, silicates, titanium dioxide, alumina, and other oxides may not be dissolved and in some cases may sequester target analyte elements. These bound elements are considered non-mobile in the environment and are excluded from most aqueous transport mechanisms of pollution.

2.0 Definitions

2.1 Laboratory Control Sample: The laboratory control used for the microwave digestion is a standard reference material (SRM) or certified reference material (CRM) that goes through the same extraction/preparation procedure as the samples. The analyte composition of the laboratory control sample is certified by acid dissolution method 3051a, 3050, or equivalent.

2.2 Duplicate Samples: A duplicate test involves splitting a sample two sub-samples and processing each through the same sample preparation procedure in order to determine the precision of the method.

2.3 Pre-digestion Spike: A duplicate sample is spiked prior to digestion in order to provide information about the effect of the sample matrix on the digestion and/or measurement methodology.

2.4 Preparation Blank: The Preparation Blank is a sample that contains only the reagents used in the extraction procedure. The preparation blanks is processed through the same preparation procedures as the samples and therefore gives an indication of any contamination picked up during the sample preparation process.

2.5 Serial Dilution: A serial dilution consists of a comparison of the results of a sample and another aliquot diluted by a known factor.

2.6 ICP-AES: Inductively Coupled Plasma-Atomic Emission Spectrometry.

2.7 ICP-HG-AES: ICP-AES with sample introduction using automated hydride generation

2.8 ICP-MS: Inductively Coupled Plasma-Mass Spectrometry.

3.0 Equipment and Supplies

3.1 MARS 1600 watt microwave (CEM corporation, Mathews, NC).

Note: The microwave power output test, power calibration, and temperature probe calibration should be performed according to manufactures specifications every six months.

3.2 Trace metal grade nitric acid.

3.3 Trace metal grade hydrochloric acid.

3.4 ≥18 MΩ deionized water (DI).

3.5 50ml volumetric flasks

3.6 Parafilm

4.0 Procedure

Review SOP for handling acids (attached) prior to beginning the procedure.

4.1 Weigh a well-mixed sample to the nearest 0.001 g into an acid washed Teflon vessel equipped with a controlled pressure relief mechanism.

4.2 Add 9.0 ± 0.1 mL concentrated nitric acid and 3.0 ± 0.1 mL concentrated hydrochloric acid to the vessel in a fume hood.

4.2a The addition of concentrated hydrochloric acid to the nitric acid is appropriate for the stabilization of certain analytes, such as Ag, Ba, and Sb and high concentrations of Fe and Al in solution.

4.3 Seal the vessel according to the manufacturer's directions. Properly place the vessel in the microwave system according to the manufacturer's recommended specifications.

4.4 Enable the appropriate 3051 method in the MARS unit software as determined by the number of samples and project requirements. Note: The 3051_40 express method does not adhere to the 4 minute ramp requirement of the USEPA 3051 method.

4.5 Once the digests have cooled, remove from the microwave and wholly transfer into labeled 50ml volumetrics that have been acid washed following the *Dish Washing SOP* and triple rinsed with ≥18 MΩ DI water immediately prior to transfer.

4.6 Bring samples to volume, cover with parafilm and mix thoroughly by inversion. Bring to volume and mix thoroughly again after samples have cooled.

4.7 Syringe filter samples into labeled falcon tubes using dry acid washed syringes and nylon 0.45um nylon syringe filters.

4.8 Analyze samples by ICP (SOP 12).

5.0 Quality Control

5.1 Laboratory Control Sample: The laboratory control sample must fall within ± 20% of the known value or within the 95% prediction interval of the certified value. The laboratory control sample must be run with each batch of microwave digestions.

5.2 Sample Duplicates: The relative percent difference (RPD) must be no more than 20%. One sample duplicate must be run with every microwave batch.

$$RPD = 100 \times \frac{|S - D|}{\text{Avg. } (S,D)}$$

5.3 Pre-digestion Spike: Spike recoveries must fall within the limits of 75-125%. At least one spike analyses (matrix spikes) shall be performed on each group of samples of a similar matrix type.

Final Spike concentration	mg/L spike solution	uL spike prior to digest
As - 400 mg/kg	1000	200

5.4 Preparation Blank: If any analyte concentration is above the detection limit, in the preparation blank, the lowest concentration of the analyte reported in associated samples must be ≥ 10 times the preparation blank concentration. A preparation blank must be performed with each batch of microwave digests.

5.5 QA/QC Checks

Matrix	Measurement	QA/QC Check[1]	Frequency	Acceptance Criteria	Corrective Action
Soil	Digestion Recovery/Method Accuracy	CRM	1 every microwave batch	±20%	Re-digest entire microwave batch.
Soil	Method Precision	Duplicate Analysis	1 every microwave batch	RPD < 20%	Re-digest entire microwave batch.
Soil	Matrix affect	Pre-digestion spike	At least 1 with each group of samples of similar matrix type.	±20%	Dilute extract used in ICP analysis and re-analyze.
Solution	Contamination	Preparation Blank	1 every microwave batch.	Below MDL or analyte concentration of samples > 10x blank concentration.	Determine source of contamination and re-analyze samples.

6.0 Reporting

6.1 If the QC limits are not met for any element or sample, the effect on the data set will be evaluated by the project manager and analyst.

7.0 References

7.1 United States Environmental Protection Agency. Method 3051A. Microwave assisted acid digestion of sediments, sludges, soils, and oils. In SW-846; U.S. EPA: Washington, DC, 1998.

7.2 United States Environmental Protection Agency. Method 6010C. Inductively Coupled Plasma-Atomic Emission Spectrometry. In SW-846; U.S. EPA: Washington, DC, 2007.

7.3 United States Environmental Protection Agency. Document number ILM04.0b. Contract Laboratory Program Statement of work for inorganic analysis, multi-media, multi-concentration. U.S. EPA: Washington, DC.

Date: May 2009 **SOP No. # 2**

Title: *Bulk X-Ray Diffraction (XRD)*

Associated Investigator: Dr. Dennis Eberl

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SYNOPSIS: This method is used to identify and quantify mineral concentrations, as weight percents, in soils, sediments, and rocks.

Quantitative Mineralogy of Rocks, Sediments and Soils,
Determined by X-ray Diffraction (XRD)

Scope and Application

This method is used to identify and quantify mineral concentrations, as weight percents, in soils, sediments and rocks.

Summary of Method

Mineral concentrations are determined by X-ray Diffraction (XRD). Samples are ground with a corundum internal standard, and the powder diffraction pattern of a randomly oriented sample is measured from 5 to 65 degrees 2 theta. The quantitative mineralogy is then determined using RockJock software (Eberl, 2003) which uses whole pattern fitting of the measured XRD pattern with a wide range of measured mineral standards to quantify the sample mineralogy.

Sample Preparation

Sample preparation is critical in quantitative XRD, and is discussed in detail in Eberl (2003) and summarized here. Samples are air dried, and then disaggregated by hand in a motor and pestle. Large samples (>~50 g) are then sub-sampled with a splitter, and smaller samples are split by cone and quartering. The subsample is ground to <250 microns. One gram of sample is combined with 0.0.2500 g of corundum (American Elements synthetic α-Al_2O_3, 3.5μm particle size) as an internal standard, which yields a finally sample 20% by weight corundum. The sample is then ground in a McCrone micronizing mill for 5 minutes with 4 ml methanol (Fisher brand, reagent grade), using yttrium doped zirconium beads. This yields a sample with ~20μm mean grain size with a narrow grain size distribution. The methanol slurry is then dried at 60 C in a polystyrene disposable beaker. The dried sample is ground for 5 minutes in Spex mixer with three acrylic balls to homogenize the sample. Then 0.625 ml of Vertrel is added and the sample is shaken for an additional 10 minutes. The sample is then sieved through a 500 micron mesh, and a side packed powder mount is prepared for XRD analysis. This procedure yields randomly oriented samples, including samples with high clay and mica concentrations, as indicated by comparison of measured XRD patterns to theoretical XRD patterns, and to samples prepared by spray drying.

XRD analysis

XRD patterns are collected using a Siemens D500 spectrometer, using Cu-K_α radiation source at 40 kV and 30mA, a Siemens graphite monochrometer, and a scintillation detector. Diffraction patterns are collected from 5 to 65 degrees 2 theta with a step size of 0.02 degrees and a counting time of 2 seconds per step.

Data Reduction and calculation of Mineral Concentrations

Mineralogy is determined using RockJock, a computer program that determines quantitative mineralogy in powdered samples by comparing the integrated X-ray diffraction intensities of individual minerals in complex mixtures to the intensities of an internal standard, and is described in detail in Eberl (2003) and http://pubs.usgs.gov/of/2003/of03-078). The X-ray data of randomly oriented sample preparations are entered into the RockJock program. Minerals likely to be present in the sample are chosen from a list of mineral standards, and the calculation is begun. The program then automatically fits the sum of stored XRD patterns of pure standard minerals (the calculated pattern) to the measured pattern by varying the fraction of each mineral standard pattern, using the Solver function in Microsoft Excel to minimize a degree of fit parameter between the calculated and measured pattern. The calculation analyzes the pattern (usually 19 to 64.5 degrees two-theta) to find integrated intensities for the minerals. Integrated intensities for each mineral then are determined from the proportion of each mineral standard pattern required to give the best fit. These integrated intensities then are compared to the integrated intensity of the internal standard, and the weight percentages of the minerals are calculated. The results are presented as a list of minerals with their corresponding weight percent.

Error Analysis

Artificial mixtures of quartz and kaolinite with a wide range of other mineral types yields a 2 sigma relative accuracy for the mineral determinations of ±3.6 % for quartz (n=42) and ±4.1% for kaolinite (n=64). The accuracy and precision of the mineral analysis is sample dependent, but this result is fairly typical and 2 sigma relative errors are usually <±6%. The major sample dependent sources of error are that prominent XRD peaks of minerals in a mixture may overlap, and that uncertainties in decomposing the mineral XRD patterns can enhance uncertainty and detection levels of mineral concentrations. Individual XRD peaks of minerals containing solid solutions may also shift in position and intensity with natural variations in mineral composition. However, because whole pattern fitting is used in RockJock, the interference between peaks, and shifts in the position of individual peaks, have a minimal effect on the overall pattern fitting, and the subsequent calculation of mineral abundances. To some extent, the quality of each sample analysis can be quality checked: a) because each mineral is analyzed independently, and the sum of the analysis should approach 100 percent, and b) by evaluating the goodness of fit between the calculated and measured XRD spectra, which should fall below 0.1.

Date: May 2009 **SOP No. # 3**

Title: ***X-ray Fluorescence (XRF)***

Associated Investigator: Dr. Charles Alpers

Total Pages 2

SYNOPSIS:
This technique determines the abundance of the major elements in geologic materials and selected trace elements at concentrations generally >1 to 100 ppm.

Determination of Elemental Abundances in Solid Samples by X-ray Fluorescence (XRF)

Scope and Application

The technique determines the abundance of the major elements in geologic materials (Si, Al, Fe, Mg, Ca, K, Na, Ti, Mn and P) and selected trace elements at concentration generally > 1 to 100 ppm (As, Ba, Co, Cr, Cu, Ga, Ge, Mo, Nb, Ni , Pb, Rb, Sr, Ta, Th, V, W, and Zr).

Summary of Method

Samples are fused before XRF analysis with lithium borate to form a glass bead, which homogenizes the sample, and reduces internal interferences. Sample are then analyzed with a Siemens model SRS 300AS wave length dispersive X-ray fluorescence spectrometer.

Sample Preparation

Samples are fired at 925 degrees Celsius for 45 minutes in porcelain crucibles. Then a sample mass of 0.8 g is fused at 1050 Celsius with 8 g of flux (high purity Premier Lab Supply GF-65-5I flux; 66% Lithium Tetraborate, 34% Li Metaborate, 0.5% LI) in a platinum crucible (95% Pt, 5% Au) using a Phoenix model VFD4000 fuser, and poured into a platinum mold (95% Pt, 5% Au) to form a glass bead for analysis.

Sample Analysis

Samples are analyzed using on a Siemens model SRS 300AS wave-length dispersive X-ray fluorescence spectrometer, with a Siemens 72 position autosampler, a Rh tube, LiF 200, LiF 220, PET and OVO55 analytical crystals, flow-counter and scintillation detector, and Siemens Spectra 3000 software. Standard curves are constructed using 12 USGS standards (ref) representing a wide range of composition in rocks and sediments. Additional calibration standards were prepared with USGS standards spiked with trace elements to extend the analytical range of trace element analysis. Elemental interferences (matrix effects) are calculated, and automatically corrected using the Spectra 3000 software.

Error Analysis for XRF

Oxide or element	Relative error (% of amount present; 2 sigma; 95% confidence level)	Lower limit of calibration curves (wt % for oxides, ppm for others)	Upper limit of calibration curves (wt % for oxides, ppm for others)
SiO2	1.67	47.32	68.62
Al2O3	3.21	11.60	18.97
Fe2O3	2.06	4.32	12.17
MgO	3.86	0.97	9.66
CaO	2.13	1.34	14.99
K2O	3.09	0.03	5.57
Na2O	10.66	0.49	5.35
TiO2	6.24	0.47	2.70
MnO	0.00	0.04	0.18
P2O5	14.06	0.05	0.63
As	64.40	0.10	218.28
Ba	9.83	6.98	1472.72
Bi	76.84	0.00	100.94
Co	28.21	6.67	158.78
Cr	11.63	3.23	477.52
Cu	29.61	29.29	233.56
Ga	20.19	15.96	122.35
Ge	39.67	0.00	102.11
Mo	16.20	0.50	269.65
Nb	24.15	0.60	127.27
Ni	18.85	5.86	263.64
Pb	38.84	2.59	166.77
Rb	11.03	0.25	354.17
Sr	3.66	96.19	845.31
Ta	36.02	0.04	100.67
Th	74.84	0.03	205.65
V	8.97	53.58	412.29
W	46.24	0.00	103.84
Zr	21.29	15.46	631.15

Date: May 2009 **SOP No. # 4**

Title: *Electron Microprobe Analysis (EMPA)*

Associated Investigator: Dr. Charles Alpers

Total Pages 8

SYNOPSIS: Describes how to prepare polished sections with rock fragments and the examination of the polished sections using a petrographic microscope.

Standard Operating Procedures for the Collection of Electron Microprobe Analysis (EMPA) Data

SOP# USGS-CAWSC-1
July 20, 2009
Revision 002

U.S. Geological Survey
California Water Science Center
Sacramento. CA

Prepared by: Charles N. Alpers

Table of Contents

a. Scope and Applicability

(modified from http://probelab.geo.umn.edu/electron_microprobe.html)

Electron microprobe analysis (EMPA), is an analytical technique that is used to establish the composition of small areas on solid specimens. An electron beam is focused on the surface of a specimen using a series of electromagnetic lenses, and these energetic electrons produce characteristic X-rays within a small volume (typically between one and nine cubic micrometers)

of the specimen. The characteristic X-rays are detected at particular wavelengths, and their intensities are measured to determine concentrations. All elements (except hydrogen, helium, and lithium) can be detected because each element has a specific set of X-rays that it emits. This analytical technique has a high spatial resolution and sensitivity, and individual analyses are reasonably short, requiring only a minute or two in most cases. Additionally, the electron microprobe can function like a scanning electron microscope (SEM) and obtain highly magnified images of a sample.

For the arsenic bioavailability project, the focus will be on identification characterization of sulfide, carbonate, and oxide minerals, with emphasis on the distribution of iron and arsenic. Specific minerals to be analyzed include arsenian pyrite [$Fe(S,As)_2$], arsenopyrite (FeAsS), ferroan dolomite a[$(Mg,Fe)(CO_3)_2$], ankerite [$Ca(Fe,Mg)(CO_3)_2$], and siderite-magnesite [$(Fe,Mg)CO_3$], goethite [FeO(OH)], and other hydrous ferric oxide minerals.

b. Summary of Method (briefly summarizing the procedure)

(modified from http://probelab.geo.umn.edu/electron_microprobe.html)

An element has a unique set of allowed transitions that produce photons with characteristic energies and wavelengths. When such transitions involve inner electron shells, the emitted photon falls into the X-ray range of the electromagnetic spectrum. This constitutes the basis of electron microprobe analysis: characteristic X-rays are identified using their unique energies or wavelengths to ascertain the composition of the sample. A beam of high-energy electrons that bombards the sample enables such electron transitions.

Electron microprobes contain an electron optical column, which produces the electron beam and controls its diameter when focused on a sample. At the top is an electron "gun" comprised of a tungsten wire bent into a v-shape and heated with an electric current to about 2700 Kelvins, which frees electrons from the apex of the wire. Because electrons are negatively charged, they are accelerated by an electrical potential, between 5 and 30 kV, toward a sample. As the electrons are accelerated, a pair of electromagnetic lenses focuses the electrons like a convergent lens focuses light. One lens restricts the number of electrons that pass down the column (the beam current) while another electromagnetic lens focuses the beam on the sample and controls its diameter. These lenses and a set of apertures can focus the beam to a diameter of 0.1 micrometers or less.

Consequently, electron microprobe analysis is considered a spot analytical technique, which means compositional information is collected from only a small volume, not the entire sample. The beam electrons interact with a volume usually between one and nine cubic micrometers (1E-18 to 9E-18 cubic meters). This volume is known as the interaction volume of the electrons. The small interaction volume of EMPA permits a researcher to collect highly localized compositional data and to examine specimens too small to be studied with other analytical techniques. In addition, it allows for the determination of the chemical variability over the surface of a sample. Consequently, EMPA is well-suited to study specimens composed of mixed phases that one wishes to resolve and analyze in situ, leaving the contextual relationships of the phases unaltered and visible.

Within an interaction volume, numerous electron-specimen interactions occur, such as X-ray production. Characteristic X-rays, which are produced when electrons "fall" from an outer energy level to one of the inner ones, possess wavelengths and energies specific to the elements from which they are emitted. Because inner levels are ordinarily filled, an electron must be removed in order to create a vacancy. When a sample is bombarded by an electron beam, a beam electron can knock an atom's orbital electron from its shell. This process is called inner-shell ionization since the atom is left with a positive charge. It remains ionized for a short period, around 1E-14 second, before one of the outer-shell electrons jumps down to fill this vacancy. Since an electron that falls into the vacated position must lose some of its energy, a characteristic X-ray is emitted. Therefore, on exposure to a high-energy beam of electrons, every element -- except for H, He, and Li -- emits a distinctive set of characteristic X-rays that can, in turn, be detected by the spectrometers.

Secondary electrons are a result of the inner-shell ionization, the same process that produces characteristic X-rays. A secondary electron is the electron liberated from its energy level by a beam electron. It is a former orbital electron that, once freed, leaves a vacancy into which an electron from a higher energy level falls as it radiates a characteristic X-ray. These electrons have low energies, so only those created within a few nanometers of the sample surface can escape. Therefore, secondary electrons are very sensitive to surface topography and can be utilized to acquire images of a sample similar to those collected by SEMs. In fact, electron microprobes are very similar to SEMs; each can be used as simple versions of the other.

Unlike secondary electrons, backscattered electrons are not produced in a sample. They are beam electrons that have been scattered back toward the surface of the sample. When backscattered electrons re-emerge from the surface, they are collected by detectors. These electrons have energies greater than secondary electrons, so they are less sensitive to topography. Instead, the backscattered electrons are influenced by the atomic numbers of the elements in the interaction volume. In heavier elements, many electrons are backscattered as a result of a single deflection, and the electrons retain much of their original energies. In lighter elements, a backscattered electron is more likely to suffer small deflections and lose more energy before it re-emerges. This effect is used to produce images, called backscattered electron images, that show some compositional information: the images exhibit bright areas where the atomic number is high and dark areas where the mean atomic number is low. The compositional variations apparent in backscattered electron images indicate differences in mean atomic number; elements cannot be identified without characteristic X-rays.

X-rays have characteristics of both particles and waves and can be described, and therefore detected, in terms of their energies or wavelengths. An electron microprobe is equipped with an energy-dispersive (ED) spectrometer, which electronically sorts and measures X-rays with respect to their energies. Electron microprobes also have several wavelength-dispersive (WD) spectrometers, which use diffraction to sort X-rays by their wavelengths. When white light passes through a prism or diffraction grating, it divides into its constituent colors, each of which has its own wavelength. The same phenomenon occurs in WD spectrometers; the X-rays are dispersed with respect to their wavelengths by a crystal. In a particular arrangement of the sample, the crystal, and the detector, the atomic lattice of the crystal reflects just one wavelength of the incoming X-rays toward the detector. Consequently, a WD spectrometer is "tuned" to a

single wavelength at a time, which means it can better resolve X-rays and obtain more accurate measurements.

An ED spectrometer works best for simple qualitative analyses in many cases because it can rapidly record the full spectrum of interest. Within seconds, the X-ray spectrum collected by an ED spectrometer can reveal the major elements in a specimen and their relative concentrations, although the error in these measurements is rather large. Further, close X-ray peaks usually have ambiguous identifications, requiring use of a WD spectrometer, and ED spectrometers are not sensitive enough to reveal X-ray peaks from minor and trace elements. Peaks are more distinct from the background in a WDS spectrum, resulting in a better accuracy and minimum detection limits that are at least ten times lower. In addition, due to the higher resolution of WD spectrometers, there is little ambiguity in peak identification. Hence, WD spectrometers are better adapted to quantitative analysis than ED spectrometers.

Quantitative analysis is essentially a comparative method. It entails the measurement of the characteristic X-rays from a sample and a set of standards analyzed under the same conditions, and correction factors for various effects are calculated by the computer. The accuracy depends largely on the similarity of the standards and the specimen. For quantitative analysis, accuracy approaching ±1 percent (relative) is attainable for major elements. It is usually worse for trace and light elements or when significant differences exist between the compositions of the standard and the sample. The precision depends on counting statistics, particularly the number of X-ray counts from the standard and sample, and the reproducibility of the WD spectrometer mechanisms. The minimum obtainable precision is about 0.5 percent, although it is higher for elements at trace concentrations.

The detection limits differ for each element and are affected by the overall composition of a sample and the analytical conditions. For most elements, the detection limits for WD spectrometers is between 30 and 300 parts per million (ppm). It must be noted, however, that these detection limits for EMPA are misleading, particularly when compared to a bulk analytical technique. The microprobe can detect an amount of material a hundred thousand times smaller than that which can be detected with neutron activation analysis, a prevalent bulk analytical technique. As a result, EMPA is well-suited to the analysis of heterogeneous specimens.

c. Definitions (identifying any acronyms, abbreviations, or specialized terms used)

ED – energy dispersive
EMPA – electron microprobe analysis
kV - kilovolts
mA- milliamperes
ppm – parts per million
SEM – scanning electron microscope
WD – wavelength dispersive

d. Health & Safety Warnings (indicating operations that could result in personal injury or loss of life and explaining what will happen if the procedure is not followed or is followed incorrectly; listed here and at the critical steps in the procedure),

Some components of the EMPA system include high voltage electronics. Operators untrained in the maintenance of the electronics should not access these parts of the system. Maintenance requirements such as changing the filament should be done only by trained personnel.

e. Cautions (indicating activities that could result in equipment damage, degradation of sample, or possible invalidation of results; listed here and at the critical steps in the procedure)

Samples must be solid. Liquid or "sticky" samples may not be suitable for analysis because of the requirement the high vacuum requirements of the sample chamber.

f. Interferences (describing any component of the process that may interfere with the accuracy of the final product)

g. Personnel Qualifications/Responsibilities (denoting the minimal experience the user should have to complete the task satisfactorily, and citing any applicable requirements, like certification or "inherently governmental function")

Operator must be trained with regard to operation of equipment including start-up and shut-down procedures so as not to damage the equipment. Experience with calculation of mineral formulas from weight percentage data is necessary for proper interpretation of data.

h. Equipment and Supplies (listing and specifying, where necessary, equipment, materials, reagents, chemical standards, and biological specimens)

Epoxy
Glass slides, 1-inch diameter and 1x2 in. (2.5 x 5 cm)
Carbon rods for carbon coater

i. Procedure

1) Sample Collection and Preparation

Rock samples and rock fragments associated with soil samples are collected from field sites. Rock chips approximately 0.75 in thickness are prepared using a diamond saw. Small, disk-shaped chips approximately 2.5 cm in diameter and larger, rectangular chips approximately 2.5 x 5 cm in size may be prepared, depending on sample size and texture. Polished thick sections are prepared using standard petrographic techniques. Smaller rock and mineral fragments can be prepared for polishing by mounting in epoxy using small diameter (0.5 to 1.0 cm) cylindrical brass sleeves. Polishing is accomplished by using progressively finer grit sizes on a rotating lap. Water can be used as a lubricant for most materials. If water-soluble minerals are present in the sample, then an alternative lubricant may be used, such as mineral oil or kerosene.

Once polished samples are prepared, observations are made using a standard petrographic microscope in both in transmitted and reflected light with cross-polarizing capability to provide preliminary mineral identification. Micro-scale photography (using a camera mounted on the

microscope) is used to prepare maps of target mineral grains within samples to be analyzed by EMPA. A rapidograph pen should be used to circle portions of the sample intended for analysis. Connecting circled areas and marking circles with distinctive markings (e.g. numbers or distinct numbers of dashes) will aid navigation between areas to be analyzed.

Once samples are marked, carbon coating under vacuum is necessary to ensure conduction of the electron beam. This is accomplished using a carbon coater designed specifically for this purpose.

2) Instrument Calibration and Standardization

The JEOL JXA-8900 Superprobe at the USGS laboratory in Menlo Park, CA will be used. The instrument is calibrated routinely according to the manufacturer's specifications. Typical operating conditions are 15 kV (kilovolts) and 20 to 25 nA (nano-amperes). Natural benitoite, a mineral that fluoresces under the electron beam, is used to visualize the spot size of the electron beam. The focused beam is usually about 2 micrometers in diameter. An unfocused beam of about 20 micrometers in diameter can be used in some applications.

The instrument is standardized using a variety of standards including synthetic and natural compounds of known composition. Synthetic pyrite [FeS_2], arsenopyrite [FeAsS], and millerite [NiS] and cobaltite [CoS] will be used to calibrate for Fe, S, As, Ni, and Co in sulfide minerals. Natural calcite [$CaCO_3$], siderite [$FeCO_3$], magnesite [$MgCO_3$], rhodochrosite [$MnCO_3$], and strotianite [$SrCO_3$] will be used to calibrate for Ca, Mg, Fe, Mn, and Sr in carbonate minerals. During standardization, a minimum of three spots are analyzed on each standard with a counting time of 20 to 60 seconds.

3) Sample Analysis, Data Acquisition, Calculations & Data Reduction Requirements

After calibration and standardization, carbon-coated samples are introduced into the vacuum chamber. Photomicrographs are used to locate mineral grains of interest. Minerals of unknown composition are analyzed for 20 to 60 seconds. Counts are quantified in detectors tuned for the specific elements of interest. Counts on unknowns are compared with counts on standards. Automated correction procedures known as ZAF corrections are applied by the JEOL JXA-8900 software. Results are reported in weight percentage of each element of interest. Standardized normalization procedures are used to express results as mineral formulas.

In addition to quantitative spot analyses, maps of element concentration ("Area Analysis") can be constructed using either ED (8 elements) or WD (5 elements). The spatial resolution for individual pixels can be as small as 0.02 micrometers. The field of view can be as large as 90 square millimeters.

4) Troubleshooting

The total element composition of each spot analyzed is computed by the JEOL JXA-8900 software. In the case of minerals containing hydrogen, stoichiometry based on the standard mineral formula is assumed. If the sum of elements equals 98.5 to 101.5 percent, the analysis is considered acceptable. If the sum of elements is less than 98.5 percent, the operator will consider the presence of additional elements not analyzed. In ED mode, spectra will indicate any additional elements that may be present. If possible, standardization for additional elements will be done so that the entire composition can be quantified. Other reasons for low totals to be considered include mineral grains that are too small, considering the depth of penetration of the electron beam into the sample (usually about 10 to 20 micrometers).

5) Computer Hardware & Software

The JEOL JXA-8900 comes with a user-friendly Graphic User Interface (GUI). The standard computer interface is an HP Apollo 9000-series computer with a 20 inch color monitor.

j. Data and Records Management

All EMPA data will be stored in spreadsheet format. Data files include information on standardization and operating conditions. Backup copies will be stored at a remote location. Upon completion of the project, data will be archived according to USGS procedures.

k. QA/QC CHECKS

Complete the following table to summarize QA/QC checks.

Matrix	Measurement	QA/QC Check[1]	Frequency	Acceptance Criteria	Corrective Action
Sulfides	S, Fe, As, Ni, Co	Lab control samples – synthetic pyrite, arsenopyrite, millerite, cobaltite	3 or more spots, once per session	5% of expected amount based on mineral formula	Restandardize microprobe. If instrument is unstable, change filament
Carbonates	Ca, Mg, Fe, Mn, Sr	Lab control samples – natural calcite, magnesite, rhodocrosite, siderite, strontianite	3 ore more spots, once per session	5% of expected amount based on mineral formula	Restandardize microprobe. If instrument is unstable, change filament
Iron oxides	Fe, As, Mn, O	Lab control samples – hematite, goethite	3 or more spots, once per session	5% of expected amount based on mineral formula	Restandardize microprobe. If instrument is unstable, change filament
All minerals	All elements	Total equals 100 weight percent	All analyses	3% of expected amount, including estimates of non-measured elements such as hydrogen	Restandardize microprobe. If instrument is unstable, change filament
All minerals	All elements	Replicate analyses	3 or more spots within each mineral grain	If variability is greater than 5% of amount present, analyze 3 or more additional spots	Natural materials may be spatially homogeneous.

[1]Include all QA/QC checks (experimental and analytical, as applicable) for accuracy, precision, detection limits, mass balance, *etc*. (*e.g.*, matrix spikes, lab control samples, blanks, replicates, surrogates)

Date: May 2009 **SOP No. # 5**

Title: *Differential XRD*

Associated Investigator: Dr. Andrea Foster/ Dr. Dennis Eberl

Total Pages 4

SYNOPSIS:
This SOP describes the approach for differential X-ray diffraction analysis. This technique is still under development and will likely deviate from this SOP once work begins.

Standard Operating Procedure for Differential X-ray Diffraction (DXRD) Analysis

SOP # 5
June 1, 2009
Revision 001

USGS- Water Resources Discipline
X-ray Diffraction Laboratory
Boulder Colorado

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Scope and applicability

the purpose of differential x-ray diffraction analysis is to: (1) unambiguously identify low-abundance iron hydroxide mineral phases in complex heterogeneous samples, (2) to quantify their relative abundance with respect to other mineral phases present, and (3) to examine changes in the abundance or identity of iron hydroxide mineral phases as a function of sample treatment by one or more of the following procedures: (a) selected dissolution, (b) *in vitro* bioaccessibility, and (c) *in vivo* bioavailability.

Summary of Method

Samples are ground to a flour-like consistency along with a small amount of internal standard mineral (corundum, Al2O3), and mounted in holders for x-ray diffraction. Powder x-ray diffraction patterns are collected using a Siemens D500 diffractometer with CuKa (lambda = 1.54056), at 40 keV and 30 mA, CoKa, or monchromatized synchrotron radiation at 12705 eV. (3 GeV, 80-100 mA) and a diffracted beam monochromator. The patterns are aligned and normalized relative to one or more characteristic reflections of the internal standard to produce “standardized patterns”.

The standardized XRD pattern of the post-procedure sample is subtracted from the standardized XRD pattern of the pre-procedure sample to produce the differential XRD pattern containing only those phases that were removed during the procedure

Definitions

DXRD = differential x-ray diffraction
Cu Ka = x-ray energies specific to emission from Cu target
Co Ka = x-ray energies specific to emission from Co target
Monochromatized synchrotron radiation: highly collimated x-ray energy selected by diffraction from parallel silicon crystals.
mA = milliapmperage
keV = kiloelectron volts

Health and Safety Warnings

1. Working with x-rays may result in increased exposure to high energy x-radiation which has known mutagenic effects. All workers must be monitored via dosimeter for radiation exposure.
2. Working with arsenic-containing samples increases the risk of exposure to this element, which is a known carcinogen. Steps should be taken to minimize inhalation while grinding and preparing samples for analysis.
3. Working with chemicals used to prepare samples may increase the risk of exposure to caustic, corrosive, flammable, or toxic compounds.
4. Follow all listed safety procedures with regard to radiation exposure and chemical handling. Do not allow persons without proper training and radiation safety to collect x-ray diffraction data. Do not allow persons without proper training to manipulate hazardous chemicals and/or samples in the laboratory.

Cautions

Insure that the water cooling system is operational while the detector is running.

Proper preparation and mixing of sample with internal calibrant is critical for the successful subtraction of the before and after XRD patterns.

Interferences

Improper mixing of sample and internal standard and/or insufficient grinding of sample or internal standard could result in patterns where the relative intensity of peaks is shifted from that of an unoriented (random) sample; this could severely interfere with the analysis.

Prolonged sample storage under ambient conditions could result in undesired changes to the sample mineralogy.

Personnel qualifications and responsibilities

All personnel should be trained in radiation safety procedures and have assigned dosimeters. In addition all personnel should be explicitly trained in the use of the laboratory diffractometer and the synchrotron diffractometers. Personnel are responsible for minimizing the radiation exposure by following the safety interlock procedures.

Equipment and Supplies

XRD reflection geometry sample holders (low background)
Mortar and pestle
Acetone
reagent-grade water
laboratory x-ray diffractometer with Cu or Co x-ray source
computer with program for analyzing XRD data

Procedure

Weigh out 1.000 g of air-dried sample and 0.250 g of g-Al_2O_3. Grind in a McCrone mill for 5 minutes with methanol. Dry at 80 °C. Mix sample and standard in a small (25 mL) plastic vial by shaking with three plastic balls and a small amount of vertrel liquid for 10 minutes. Pass the resulting mixture through a 500 µm sieve, and side load aluminum sample holders.

Turn on x-ray diffractometer and cooling water system (for laboratory unit) allow to warm up for 5 minutes minimum; For synchrotron XRD patterns, follow procedures outlined in EGG Lab SOP #1 preparation.

Collect XRD pattern over the range 5-65 degrees 2 theta using CuKa, CoKa, or > 12000 eV using a diffracted beam monochromator, and 0.02 or 0.05 2 theta step, and a 5s or 20s count time per step.

Calibrate 2 theta position of collected patterns by shifting patterns relative to 2 theta peaks. (if necessary).

Calibrate intensity of collected patterns by normalizing relative to the most intense peak of the internal standard.

Subtract the calibrated pre-procedure sample from the calibrated post-procedure sample to obtain the DXRD pattern.

Compare the position and intensity of peaks to published values for iron hydroxide minerals for qualitative identification of the DXRD patterns.

Use a computer program (RockJock) to verify iron off the hydroxide mineral identity, and quantify the relative abundance of iron hydroxide phases in the DXRD patterns

Data and Records Management

1. A standardized electronic form will be completed for each XRD pattern, describing (a) the data collection date, data collection type (i.e., lab x-ray source, or synchrotron source, X-ray energy, voltage, and current). The form will be a record of all relevant details needed to reconstruct the manner in which the data were prepared, collected, and calibrated. Hardcopies of these forms will be printed and maintained in a binder, and electronic backup copies will be produced. Copies of the electronic version of the forms, the raw data, and the processed data will all be copied onto a dedicated external hard disk and stored offsite.
2. Summary reports of data collection events will be provided within approximately immediately on data collection These reports will list the samples and reference materials analyzed during that time, describe any technical difficulties encountered or any changes to the planned data collection protocols. The reports will also provide preliminary interpretations of the data, where possible.
3. Summary reports of data analysis will be provided. These reports will provide textual descriptions, figures and tabular summaries of the data analysis and error estimation suitable for publication.

Date: May 2009 **SOP No. # 6**

Title: *Synchrotron-Based X-Ray Diffraction, Bulk X-Ray Absorption (XAS), and µ-X-Ray Absorption Spectroscopy (µ-XAS) / µ-X-Ray Fluorescence Spectroscopy (µ-XRF) / µ-X-Ray Diffraction (µ-XRD):*

Associated Investigator: Dr. Andrea Foster

Total Pages 20

SYNOPSIS: These protocols describe the procedures that Dr. Andrea Foster will employ in order to quantify arsenic species and determine the mineralogy of iron (hydr)oxides in samples from mine-scarred lands.

Standard Operating Procedures for the Collection and Analysis of Synchrotron-based X-ray Diffraction (XRD) and X-ray Absorption (XAS) Spectroscopy Data

SOP# EGG-X-1
24 July 2009
Revision 003

Environmental Geochemistry and Geomicrobiology Laboratory
U.S. Geological Survey
Western Mineral and Environmental Science Center
Menlo Park, CA

Prepared by: Andrea Foster

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1. **Scope and Applicability** (describing the purpose of the process or procedure and any organization or regulatory requirements, as well as any limits to the use of the procedure), and any organization or regulatory requirements, as well as any limits to the use of the procedure),

   Naturally-occurring soil, sediment, mine tailings, and waste rock to be examined in this study are heterogeneous assemblages of crystalline minerals and poorly-to-non crystalline solid phases. Two complimentary techniques, X-ray diffraction (XRD) and X-ray absorption fine structure spectroscopy (XAFS) will be used at centimeter ("bulk) and micrometer ("μ") length scales to characterize these heterogeneous materials. XRD relies on long-range ordering of atoms to probe crystalline structure at a length scales ≥50 Å. XAFS spectroscopy probes the coordination environment of the selected element over length scales <10Å, and its theory and interpretation does not rely on any assumption of symmetry or periodicity.

   A third technique, x-ray fluorescence (XRF) will be used at the μmeter length scale in conjunction with XRD and XAFS to qualitatively determine the spatial distribution of elements within a sample. Application of this technique is necessary for identifying locations within a sample that are amenable to μ-XRD and μ-XAFS, but it has several other potential benefits to the project.

   Together these techniques will be used to: (1) characterize the solid-phase composition of the <250 μm fraction of naturally-occurring materials to be used in studies of relative bioavailability of arsenic (As); (2) quantify the relative abundance of crystalline and non-to poorly crystalline phases in the <250 μm fraction, with particular emphasis on iron-bearing phases; (3) to identify the As-bearing phases in the <250 μm fraction, and (4) to quantify the relative abundance of As among those phases.

   The first set of samples to be examined will be called "***scoping samples***"; these were collected from many sites at the Empire Mine. From this set, locations will be selected for re-sampling in larger volumes for use in *in vitro* bioaccessibility and *in vivo* bioavailability studies. "***Test samples***" will be those samples collected in large volume for use in *in vitro* bioaccessibility and *in vivo* bioavailability studies. The *scoping samples* will be used to develop and refine handling and processing methods to be used on the *test samples*, and as a result there final SOPs for *test samples* may differ in small ways from that of the *scoping samples*. There is also likely to be a greater uncertainty in the results of synchrotron-based analyses of the *scoping samples*.

2. **Summary of Methods**

   A. Samples and reference minerals/compounds are prepared for analysis by

      i. impact mill grinding and loading into 1 mm or 3 mm thick rectangular holders of aluminum or Teflon with kapton Windows (bulk XRD and bulk XAFS),

ii. immobilizing unground material in epoxy, slicing a 50-100 μm thick section, and polishing (μ-XRF, μ-XAFS, and μ-XRD), or

iii. sprinkling ground or unground material onto kapton tape (μ-XRF, μ-XAFS, and μ-XRD).

B. Data are collected by several means:

i. recording diffracted beams at a single energy with angular resolution (area diffraction);

ii. recording diffracted beams at a single energy without angular resolution (2-circle diffractometer);

iii. recording fluorescence intensity at a single energy while the sample is moved using a μ-sized x-ray beam (μ-XRF mapping);

iv. recording fluorescence intensity as energy is varied on an immobile sample with a μ-sized x-ray beam (μ-XAFS), and

v. recording fluorescence intensity as energy is varied on an immobile sample with a centimeter-sized x-ray beam (bulk XAFS).

C. Diffraction data are calibrated using a measured diffraction pattern of a known material collected during the same analytical period as the samples. XAFS data (spectra) are calibrated using a measured spectrum of a known material collected

i. simultaneously with the sample, or

ii. immediately preceding or following the sample.

D. Data will be processed and reduced according to previously published and widely accepted procedures. Diffraction data are analyzed by

i. matching the distribution and intensity of peaks in the diffraction pattern to known minerals, or

ii. using knowledge of the chemistry and mineralogy of the sample to reconstruct the whole pattern through a fitting procedure.

E. The methods of analysis of XAFS spectroscopic data will depend on the type and quality of the data collected. For low-quality data collected from samples low in As, analysis of the x-ray absorption near edge (XANES) structure will allow

i. determination of oxidation state and semi-quantitative assignment of mineral residence of the As, and

ii. a model-independent assessment of the relative differences among scoping and test samples (Principal Component Analysis).

F. For high-quality data collected from samples enriched in As, sample analysis will be as described above, but may also include constructing geometric models of As association with its host phases (i.e., calculation of the number, distance, and disorder of atoms within about 7 Å) through *ab initio* calculations of EXAFS structure.

3. **Definitions** (identifying any acronyms, abbreviations, or specialized terms used),

"**bulk" XRD, bulk XAFS:** data collected from thousands of grains simultaneously using centimeter-sized x-ray beams and 0.1-0.75 g of sample.

CCD or "area" detector: Charged Coupled Device

ICR: Incoming Count Rate

LC-LSA: Linear Combination, Least-Squares Analysis

PCA: Principal Component Analysis

Phase: a solid material exhibiting atomic order on a scale smaller than that measured by x-ray diffraction (i.e., < 50 angstroms)

Prepared sample (test, scoping, or reference): a sample that has been sieved to < 250 µm or has intrinsic particles less than 250 µm or has been ground to < 250 µm.

SCA: Single Channel Analyzers

Scoping sample: 1 kg or smaller-sized grab samples collected in April 2009 from the Empire mine whose analysis will be used to guide selection of sites where large quantities of sample will be collected for extensive study.

Synchrotron: a pseudo-circular photoelectron accelerator.

Test sample: several kg-sized samples to be collected in summer 2009 from the Empire mine which will be split among the team investigators for *in vitro*, *in vivo*, and chemical characterization studies.

TT: Target Transformation

XRD: a long-range (≥50 Å) probe of atomic structure whose theory and interpretation relies on an assumption of periodic arrangement of atoms (i.e., crystallinity)

μ-**XRD, μ-XAFS:** data collected from individual grains or from micro- to nano-sized mixtures of grains.

XAFS: a short range (< 10 Å) probe of atomic structure whose theory and interpretation ***does not*** rely on an assumption of periodic arrangement of atoms (i.e., crystallinity)

4. **Health and Safety Warnings**:

A. Work at synchrotron facilities results in increased exposure to high energy x-radiation which has known mutagenic effects. However, the increased exposure is within allowable lifetime exposure limits and is less than in some other processions such as airline pilot and airline in-flight crew.

B. Working with As-containing samples increases the risk of exposure to this element, which is a known carcinogen.

C. Working with chemicals used to prepare samples may increase the risk of exposure to caustic, corrosive, flammable, or toxic compounds.

D. Follow all listed safety procedures with regard to radiation exposure and chemical handling. Do not allow persons without proper training and radiation safety to collect spectroscopic data. Do not allow persons without proper training to manipulate hazardous chemicals and/or samples in the laboratory.

5. **Cautions** (indicating activities that could result in equipment damage, degradation of sample, or possible invalidation of results; listed here and at the critical steps in the procedure)

A. XRD:

To avoid damage to the area detector (bulk XRD) or CCD detector (μ-XRD):

i. Place the lead shield on the detector when aligning the table (against I1) and when aligning the sample (against Ibeamstop);

ii. Do not allow exposure of x-rays to the detector for long periods of time, and do not routinely saturate the detector with overflow counts above 80,000 cps;

iii. Move the area detector to the far position from the sample (position = 300) when aligning the beamstop, changing the beamstop, or making major changes to sample geometry.

To avoid invalidation of the analytical run, during each data collection period, blank samples must be run (mounting substrate or windows only; no sample) under the exact conditions under which the samples were analyzed. During each data collection period, a suitable external calibration standard (LaB6) must be run. The parameters of beam energy, sample-detector distance, beam size, omega, and pixel resolution of the area detector should be invariant for each sample run, and should be recorded for calibration purposes.

B. XAFS: Calibration standards should be run along with every sample; for samples in which the x-ray beam is totally absorbed. Calibration standards should be run just prior or just after the unknown sample. The x-ray energy should be re-checked via the calibration standard after each beam fill, so that samples are very close to ideal calibration throughout the run. The germanium (Ge) detector is a valuable and sensitive instrument. It should be capped when filling the detector with liquid nitrogen, when making gross alignment of the horizontal sample position, and when adjusting machinery in the sample hutch. Care should be taken to maintain the total incoming count rate (ICR) on the detector below 150,000 cps or 30% deadtime, whichever is higher under normal operation. When collecting a deadtime curve for correction, ICR should reach no higher than approximately 400,000 cps. At the beginning of each analytical period, a deadtime curve should be collected at the energy of each element to be analyzed for later correction of XAFS data.

6. Interferences (describing any component of the process that may interfere with the accuracy of the final product*)*

A. Bulk XRD*:*

i. Samples should be of a thickness such that 20-40% of the beam is absorbed; the ideal thickness of a sample is usually obtained through trial and error.

ii. Inadequate grinding of the sample can lead to the predominance of the diffracted signal by large, single crystallites; this can interfere with the detection of less intense diffraction peaks, but can also be removed during the signal processing step.

B. Bulk XAFS:

i. Inadequate grinding can introduce non-random noise in the data arising from x-ray scattering off large crystallites.

ii. High iron (Fe) samples can give rise to excessive Fe fluorescence, dominating the ICR signal when collecting data at the As edge. If using the Ge detector, the use of soller slits plus layers of aluminum (Al) foil to diminish the Fe fluorescence and maximize the ratio of As fluorescence counts in the single channel analyzers (SCA) to the ICR counts is reccommended.

iii. Interactions of the x-ray beam with the sample may give rise to beam-induced oxidation state transformations of arsenic; this process can usually be diminished by analyzing samples at liquid nitrogen temperature or lower, and is most often observed in wet rather than dry samples.

C. **μ-XRD*:*** Intermixed materials exhibiting particle size smaller than that of the x-ray beam (i.e., < a few μm) may give a diffraction pattern characteristic of multiple phases.

D. **μ-XAFS:** Beam-induced oxidation state changes such as those described under **Bulk XAF (**above) sometimes occur with microbeam samples, even on dry samples. Analyzing samples under a jet of cooled, dry nitrogen (cryojet) may diminish this process.

7. Personnel Qualifications/Responsibilities (denoting the minimal experience the user should have to complete the task satisfactorily, and citing any applicable requirements, like certification or "inherently governmental function")

Persons collecting and analyzing synchrotron XRD/XRF/XAFS data for this project should have several years experience with the techniques to be used, **or** be trained by an individual with such experience during this project. Such training involves

A. One-on-one guidance through the procedures required for the collection and analysis of the data, and

B. Informal certification by Dr. C. Kim or Dr. A. Foster that personnel collecting these data are qualified to do so.

8. Equipment and Supplies (listing and specifying, where necessary, equipment, materials, reagents, chemical standards, and biological specimens**)**

- kapton tape
- scotch magic tape
- wig-l-bug impact grinder with beads and 2-ml screw top centrifuge tubes
- boron nitride
- agate mortar and pestle
- acetone
- teflon sample holders (3 mm thickness)
- aluminum sample holders (1 mm thickness)

9. Procedure (identifying all pertinent steps, in order, and the materials needed to accomplish the procedure such as:

A. Sample preparation

Scoping Samples: Small (< 1 kg) grab samples will be homogenized and split for several types of analyses: one split will be maintained near field-moist conditions, and another will be air dried; some air dried samples will be split, with one fraction hand-sieved to < 250 µm, and the other left unsieved. Both splits will be pulverized in an impact mill (mini bead beater, Biospec Products) to further reduce grain sizes to approximately 5-10 µm. Disposable, 2 millimeter capacity microcentrifuge tubes with 1/8" diameter Plexiglas ball pestle will be used in the bead mill. Subsequent to this process the samples will be called "*prepared scoping samples*."

***Test Samples*:** Large (~50 kg) samples collected by backhoe will be homogenized in a cement mixer dedicated to that purpose (see Basta *in vitro* bioaccessibility SOP), air dried under laminar flow hood, and sieved to < 250 µm on a shaker table designed for that purpose. Foster will receive a split of the < 250 µm, air-dried sample. The split will be processed by impact mill in the manner described above for preliminary samples. Subsequent to this process the samples will be called "*prepared test samples*."

Reference Samples: These materials are natural or synthetic minerals or phases for which the atomic structure and/or coordination environment of arsenic and/or iron is well known. They will serve as both qualitative and quantitative sample comparators, depending on the type of analysis being performed. Reference samples will be pulverized in an impact mill in the manner described above for preliminary and test samples. The pulverized material will be used in subsequent analyses as described below. Subsequent to this process the minerals and compounds will be called "*prepared reference samples*."

i. Bulk XAFS and Bulk XRD

a. XRD: BL 11-3 (area diffraction)

Prepared scoping, test, and reference samples will be either

(1) Loaded into lexan capillaries

(2) Loaded into rectangular Al holders or round Al gaskets with lexan windows, or

(3) Smeared onto matte ("frosted") "scotch" tape.

Preliminary samples will be tested by several of the methods to determine the ideal scenario.

b. XRD: BL 2-1 (angle-resolved diffraction)

Prepared scoping, test, and reference samples will be either:

(1) Loaded into capillaries as described in 9.A.iv.(1), or

(2) Packed into silicon or aluminum flat plate sample holders.

Data will be collected in theta-2θ scanning mode, in reflection geometry, with a photomultiplier tube as the post-sample detector.

ii. XAFS

Prepared reference samples containing > 1% As and/or Fe will be diluted in an appropriate amount of boron nitride, such that the total absorption from all atoms in the sample is less than 2.5 absorption lengths (≈ **1/□,** or the distance over which x-ray intensity decreases by roughly 37%), and the partial absorption due to arsenic is approximately 1 absorption length **(□□x** = 1, where x = sample thickness); using knowledge of chemical formulae and tabulated x-ray absorption cross-sections. An example calculation can be found in Kelly et al (2008).

Prepared samples and reference samples containing < 1% As and/or Fe will be packed into 30mm x 3mm x 3-mm (width x height x thickness) Teflon (Dupont) cells with kapton windows.

iii. μ-XAFS and μ-XRD BL 2-3

a. Tape mounts of prepared reference samples will be prepared as described in 1.a.i (2)

b. Both tape mounts of prepared samples and **<100 μm** slices of epoxy-mounted sections will be used for analysis. Epoxy-mounted sections will be prepared by suspending the <250 μm, non-pulverized sample in a low temperature epoxy and cutting slices to an approximate thickness of 50 μm as described in Walker et al (2005).

B. Data collection

i. Bulk XRD: BL11-3

Stanford Synchrotron Research Laboratory beamline 11-3 will be used for rapid screening of sample mineralogy from prepared scoping and test samples, for evaluation of the efficacy of selective iron (hydr)oxide mineral dissolution techniques, and for evaluation of the presence of nanoparticulate iron (hydr)oxide particles. It employs a Si(311), side deflecting, bent-cube root monochomator to select the beam energy and a rhodium-coated, vertically-focusing mirror to exclude beam harmonics. Diffraction patterns are collected without angular (Q-space) resolution using a large area detector (MAR345 Imaging Plate - 345 mm diameter: read-out time ~60-90 sec). The specifications for this beamline at 12735 eV are:

a. resolution dE/E = 5 x10^{-4} eV;

b. spot size: 0.15 x 0.15 mm)

Data will be collected under consistent resolution, spot size, energy conditions, and sample-detector distance. Additional fixed parameters will be ω (sample tilt) at 3.45 degrees and the beamstop position at 40 mm.

The sample (scoping, test, or reference) will be positioned to ensure that the beam upon the sample prior to data collection. Exposure times will be varied to maximize counts while minimizing saturation of the detector pixels. Scanning the sample while collecting diffraction data will be tested with scoping samples to determine the method of data collection resulting in the least saturation and highest signal to nose ratio. The number of sample scans will be increased to compensate for the lower exposure times, when necessary.

A blank sample (tape without powder, Al cell with lexan windos, and/or capillary without sample) will be collected under all the conditions (e.g. count times and rocking degrees) used for samples. It is necessary to subtract the integrated background pattern from the integrated sample pattern prior to further analysis (integration described below in data analysis).

Powder XRD data will be collected for reference sample LaB_6. And this material will be used to calibrate d-spacings at a fixed energy.

Powder XRD data will be collected for scoping and test samples as described.

ii. Bulk XRD: BL 2-1

A Si(111) monochromator will be used to collect XRD patterns will be collected from prepared sample mounts using no-background (quartz or silica?) plates with 150 μ (?) wells. Patterns will be collected in standard

reflection (flat plate) geometry, which is free of sample thickness effects using a Bicron NaI (photomultiplier) detector equipped with Soller slits at an incident energy of =11.25 keV (lambda = Å). Data will be collected in theta-2theta mode with 0.02 degree 2 theta steps and 2.5 second effective count time. A rocking motion will be applied to the samples to reduce preferred orientation effects while collecting data.

iii. Bulk XAFS

Generalities about the typical setup for collection of bulk XAFS are presented in Kelly et al (2008) and will not be repeated here. Collection of XAFS spectra will follow the ***HALO*** Principles: harmonics, alignment, linearity, offsets:

a. ***Harmonics*****:** X-ray beam harmonics will be reduced to insignificance by use of a harmonic rejection mirror ***or*** by detuning the crystal monochromator by appropriate amounts as discussed in Kelly et al (2008).

b. ***Alignment*****:** Standard alignment procedures will be employed to maintain beam at appropriate intensities for good counting statistics through all gas ionization chambers, upon the sample, and upon the solid-state detectors as discussed in Kelly et al (2008).

c. ***Linearity*****:** Gas ionization chambers will be operated in their linear performance region (between 0.5 and 5 volts) and solid state detectors will be operated below 30% deadtime. A deadtime curve will be collected over the energy range specific to each element during each period of analytical beamtime in order to correct for non-linear detector response.

d. ***Offsets*****:** After alignment of sample and check of detector linearity, offsets will be collected to measure the "dark current" or spurious counts recorded by the solid-state detector when no beam is present. Offsets will be collected prior to the start of each sample run (usually multiple scans within a single run)

e. ***Calibration*****:** Energy calibration for arsenic spectra will be achieved by simultaneous measurement of the absorption spectrum of a calibrant material (elemental gold, elemental arsenic, or sodium arsenate) with each XAFS spectrum (for As), or by measuring the near-edge absorption spectrum of the calibrant prior or subsequent to measurement of the sample (necessary in the case of Fe, where attenuation of the x-ray beam by water vapor in air does not allow simultaneous collection of a calibrant). The spectrum will be used as described in data reduction section below.

f. The parameters of XAFS scans will be consistent among samples, although the energy range over which data is collected may vary. The following table summarizes the data collection parameters to be employed in this study:

XAFS region	Step size	Count time
Pre-edge	7 eV	1
Edge (XANES)	0.2 eV	1
Post-edge (EXAFS)	0.05 k ($Å^{-1}$)	**3-10 x 10^2**

iv. **Reference samples**: XAFS spectra will be collected at the As K and Fe K edges from standard reference spectra out to k =16 for arsenic and k = 14 for iron. Reference samples containing approximately 1% of As or iron will be collected in transmission mode (sample oriented perpendicular to x-ray beam; absorption measured by gas ionization chambers before and after the sample), whereas XAFS spectra of more dilute reference samples will be collected in fluorescence mode (sample oriented at 45 degrees to the x-ray beam), fluorescence a gas ionization Stearn-Heald-type detector or a solid-state Ge detector.

v. **Scoping and test samples:** The length of the XAFS spectra collected for these samples will depend on the concentration of arsenic they contain. For samples containing greater than approximately 500 ppm As, full EXAFS spectra out to k =12 will be collected. For samples less than this value, a partial EXAFS spectra out to k = 7 will be collected.

vi. **Determination of the number of scans needed:** For EXAFS spectra, the determination of the number of scans needed will be determined for each sample by averaging successive scans as they are collected, measuring the peak-to-peak noise of the data between 9 and 11 $Å^{-1}$, and calculating the RMS noise of the data as peak-to-peak noise divided by 2* sqrt of 2. RMS noise ≤ 0.1% of the total signal is desirable. Alternatively, RMS may be calculated from individual scans by measuring the amplitude of FT between 15 and 20 Å as described in Newville et al (1999).

vii. **μ-XRF:** Element-specific maps of approximately 1-3 mm^2 area will be collected from test and reference samples before and after Fe-hydroxide-specific extractions and before and after and in-vitro gastrointestinal extractions. Data will be collected for the following elements: As, Fe, Co, Mn, Zn, Cu, Cr, Ni, and Ca.

viii. **μ-XAFS and μ-XRD:** Based on the geochemical associations of elements determined from μ-XRF mapping, areas differing in element association with arsenic will be selected for investigation using μ-XAFS and μ-XRD. Several areas in a single map exhibiting similar properties of interest will

be selected for analysis. Multiple identical observations of arsenic speciation will be required for the analyst to draw conclusions about arsenic speciation and micro-mineralogy in the sample.

Simultaneous collection of a calibration standard is not feasible with the μ-XAS geometry; calibrations are performed once or twice a day only. Determination of the number of spectra to be collected for averaging will be calculated as described in 2.c.iii.

Calibration of μ-XRD data consists of collecting a pattern from a reference material such as Al2O3 or LaB6 as described in 2a and 2b.

C. Data Reduction

i. Bulk XRD: BL11-2, μXRD

Area diffraction patterns of blanks and samples will be converted to integrated line patterns in q-space using the freeware program "Area Diffraction Machine" (Lande, 2008) or an equivalent program:

The area pattern of LaB_6 material is used to fit an equation which has as varying parameters the x- and y-center point of the sample, and the distance from the detector. Fixed values are: energy, pixel length, pixel height and the known d-spacings (or Q-data) of LaB_6. The optimized parameters for x- and y-center and detector-sample distance are then used as fixed quantities in the integration of sample and blank area diffraction patterns.

Each sample and blank will be integrated using the following procedure:

a. Load area pattern in to program

b. Mask beamstop

c. Check cake; if lines are not completely vertical, re-do calibration

d. Use auto-integrate with 2-theta conversion option and high resolution (1800) lines option to generate an integrated line pattern (in 2 theta units) from the area pattern

e. Where multiple patterns were collected of the same sample, the integrated 2theta line patterns will be averaged using the program SixPak (Webb, 2009) or other suitable program.

f. Undulating background present in integrated sample patterns will be removed by subtracting the integrated pattern of a blank sample

collected under identical conditions. A blank sample is one in which the sole diffracting materal is the sample window (kapton, clear tape, or Lexan).

ii. Bulk XRD: BL 2-1

Undulating background present in integrated sample patterns will be removed by subtracting the integrated pattern of the corresponding blank. In this case, "correspond" means equivalent in composition, count time, and rocking degree to the sample.

iii. Bulk XAFS, μ-XAFS

a. XANES and EXAFS scans to be averaged will be loaded as a group into SixPak or Athena.

b. Deadtime correction will be applied to each spectrum: as described in George and Pickering (1992) and implemented in SIXPAK (Webb, 2007).

c. Energy calibration of each spectrum will be performed as described in Kelly (2008), using a calibration spectrum collected along with the sample or immediately prior to or after collection of a suite of spectra.

d. Further data reduction procedures, including pre-edge subtraction, normalization to single-atom, spline fitting and exafs extraction will be performed as described in Kelly et al (2008).

iv. μ-XRF: no further preparation of these data are required.

D. Data Analysis

Specific reflections (namely those relating to iron phases of interest) will be analyzed to assess particle size as described in the XRD protocol.

i. Bulk and μ-XAFS

The following remarks apply to the analysis of sets of both XANES and EXAFS spectra.

a. ***Identification of arsenic-bearing phases by Principal Component Analysis*** **(PCA)** *coupled to Target Transformation (TT)* will be performed as described in Kelly et al (2008). The following additional measures will be applied:

Reference compound uniqueness will be assessed by fitting the model compounds to each other, and a relative scale of difference determined. This scale will be used when ranking models by target transformation. Reference compounds whose target transform residuals are equal to or less than their fit residuals will be determined to be identical under this analysis, and it will be concluded that they cannot be discriminated.

b. ***Tests of the ability of PCA to accurately determine the number of As species present in a set of unknown XAFS*** spectra will be performed by performing the analysis on known mixtures of arsenic species. The affect of noise in the accuracy of determination will be determined by conducting the analysis on a spectral set two which increasing amounts of random noise is added.

c. ***Quantification of arsenic species by linear combination, least-squares fitting to standard reference spectra***

Reference materials chosen for this project will include well-characterized minerals, absorbed phases, and aqueous species that contain As in chemical forms that are considered relevant to this system with respect to mineralogy, chemical composition, and pH. Although ideal standards would exactly match all aspects of the chemical species in the sample, it is generally not possible to synthesize or purchase solid phases that matched the crystallinity (or lack thereof), impurities, and defect structures of phases that were formed or degraded under the unique weathering conditions of the soil. Due to the intrinsic similarity of chemically and structurally similar phases, less-than-perfect standards often provide fits within the resolving power of the technique.

d. ***LC-LSA (Linear-Combination-Least Squares Fits) fits to XANES spectra*** will be considered complete when the residual (data minus fit) is within 5% of the intrinsic RMS noise level of the processed spectrum. The noise level of processed (see data reduction section) XANES spectra will be assessed by measuring peak to peak divergence at the point of lowest spectral intensity (may vary from sample to sample). Fits with larger residuals from data without correspondingly larger RMS noise will be considered to indicate that one or more As species present in the sample was not present in the set of reference spectra used in the fits.

e. ***Fits to EXAFS spectra*** will be accepted when the residual error (data minus fit) is within 5% of the intrinsic noise level of the processed spectrum. Spectral noise will be as RMS noise as described in I,2.c.iii.

LC-LSA fits to both XANES and EXAFS spectra will proceed by iterative testing of the reference spectra determined to be likely candidates for arsenic species present in the sample by target transformation. After determination of the first best-match reference spectrum, that contribution is fixed, and the next-most abundant is sought by applying each reference spectrum again in a fit along with the first and seeking the minimum residual. Tests of significance of improvement of fit will be given by the the "F" test as described in WinXAS or SixPak (Webb, 2007).

f. ***Description of the molecular-level arsenic coordination environment in reference spectra of arsenic*** (i.e., adsorbed on goethite vs. co-precipitated in ferrihydrite) will be determined by fitting the experimental XAFS spectrum with a theoretical spectrum generated from a model of the coordination environment using *ab-initio*-derived phase and amplitude functions of key absorber-backscatterer pairs (i.e., As-O, As-Fe), as described in Kelly *et a.l* (2008). The program Feff (version 6.0 or higher) will be used to generate the phase and amplitude functions.

g. ***μ XRF, XAFS, and XRD***: Correlations among elements in an XRF map will be examined in side-by-side single element maps, and in dual-and tri-color multi element maps. Element-element correlations will be quantitatively examined by plotting one against the other. procedures for the analysis of μXAFS are analogous to those described in 4b and the analysis of μXRD is analogous to 4a.

10. Data and Records Management (e.g., identifying any calculations to be performed, forms to be used, reports to be written)

A. A standardized electronic form will be completed for each type of data, describing the data collection date, beamline, and beamline-specific conditions (energy, mono-chromator crystal, etc). The form will specify the name and number of **SANS** used in averaging, and all relevant details needed to reconstruct generation of processed data ready for analysis from the raw data. Hard copies of these forms, will be printed and maintained in a binder, and electronic backup copies will be produced. The forms, the raw data, and the processed data will all be copied onto a dedicated external hard disk and stored offsite.

B. Summary reports of data collection, events will be provided within approximately 2 weeks of the experimental beamtime. These reports will list the samples and reference materials analyzed during that time, describe any technical difficulties encountered or any changes to the planned data collection protocols. The beamtime reports will also provide preliminary interpretations of the data, where possible.

C. Summary reports of data analysis will be provided annually and in coordination with two public workshops. These reports will provide textual descriptions, figures and tabular summaries of the data analysis and error estimation suitable for publication.

QA/QC CHECKS

Both the spectroscopic (bulk and spatially-resolved) and quantitative XRD methods are not quantitative for the element or mineral phase, but rather are quantitative for *relative* abundance of the elemental species (spectroscopy) or mineral phases (diffraction). As such, method blanks are irrelevant. Matrix spikes, lab control samples, and replicates are very important, however.

Elemental x-ray maps generated by synchrotron-based techniques will not be interpreted quantitatively.

Since spatially-resolved XAFS and XRD data are by nature heterogeneous on the micron scale, it is not possible to assess precision of the speciation or mineral quantification analyses (i.e., replicates of natural samples are impossible to find)

Complete the following table to summarize QA/QC checks.

Matrix	Measurement	QA/QC Check[1]	Frequency	Acceptance Criteria	Corrective Action
Lab-synthesized iron oxyhydroxides mixed in a series of proportions (10-90%; 1, 2, and 3 component mixtures)	Iron species quantification by least-squares fits to bulk or spatially-resolved ***XANES*** and *EXAFS* spectra	Accuracy	1	Species abundances determined in fits should be ± 10% of the known values	Reprepare and reanalyze to assure that error is not due to weighing or combining samples. If no, then upward correct acceptance criteria to account for non-uniqueness
Laboratory-synthesized and naturally-occurring solid phase arsenic species mixed in a series of proportions (10-90%; 1, 2, and 3 component mixtures)	Arsenic species quantification by least-squares fits to bulk or spatially-resolved ***XANES*** and *EXAFS* spectra	Accuracy	1	Species abundances determined in fits should be ± 10% of the known values	Reprepare and reanalyze to assure that error is not due to weighing or combining samples. If no, then upward correct acceptance criteria to account for non-uniqueness
Natural iron-rich sediment from Empire Mine study area	***Bulk*** Arsenic and Iron XAFS spectra will be collected from replicate sample splits and analyzed separately	Precision	1 in 10 samples	Species abundances determined in replicates should agree within 10% of each other	3rd replicate sample taken and analyzed; if results still do not agree, 2 more replicates analyzed and heterogeneity assessment determined using all replicates.
Natural iron-rich sediment from Empire Mine study area	Synchrotron-based XRD	Precision	1 in 10 samples	Species abundances	3rd replicate sample taken and analyzed;

	patterns will be collected from replicate sample splits and analyzed separately.			determined from in replicates should agree within 10% of each other	if results still do not agree, 2 more replicates analyzed and heterogeneity assessment determined using all replicates.
Laboratory-synthesized iron oxyhydroxides	Synchrotron-based XRD patterns will be collected from several points-of-interest on a single sample	Precision and accuracy	2 samples	XRD should be able to unequivocally identify d-spacings characteristic for the iron oxyhydroxide replicate analyses should retrieve the same d-spacings, to the 0.1 angstrom level	Determine if the d-spacings identified are not unique to a particular iron oxide, and collect new patterns of the same material that span a range of diffraction space that does contain unique d-spacings;

[1]Include all QA/QC checks (experimental and analytical, as applicable) for accuracy, precision, detection limits, mass balance, *etc*. (***e.g.***, matrix spikes, lab control samples, blanks, replicates, surrogates)

Method Sensitivity/Reporting Limits

Matrix	**Measurement**	**detection limit[1]**	**speciation minimum reporting level[2]**	**Fit Uniqueness[3]**
Natural iron-rich sediment from Empire Mine study area	Arsenic or Iron species quantification by least-squares fits to bulk or spatially-resolved ***XANES*** spectra	10 mg/kg	≥ 10%, where the sum of arsenic or iron species are ≤ 100%	Expressed as percentage, will be equivalent to 100 – [$\chi(2^{nd}$ best fit) – χ(best fit)]
Natural iron-rich sediment from Empire Mine study area	Arsenic or iron species quantification by least-squares fits to bulk or spatially-resolved ***EXAFS*** spectra	500 mg/kg	≥ 10%, where the sum of arsenic or iron species are ≤ 100%	Expressed as percentage, will be equivalent to 100 – [$\chi(2^{nd}$ best fit) – χ(best fit)]
Natural iron-rich sediment from Empire Mine study area	Iron or arsenic species identification by fitting EXAFS data ***using theoretical phase and amplitude functions***	1000 mg/kg	n/a; this procedure is only for identification, not quantification of relative abundance.	Expressed as percentage, will be equivalent to 100 – [$\chi(2^{nd}$ best model fit) – χ(best model fit)]
Natural iron-rich	Mineral quantification and identification using differential xrd	See QA/QC for XRD	See QA/QC for XRD	See QA/QC for XRD

sediment from Empire Mine study area	and quantitative xrd			

[1] level below which oxidation state cannot be determined with sufficient certainty

[2] level below which minor species cannot be differentiated from dominant species with sufficient certainty

3 this quantity expresses the level of uniqueness (a measure of confidence) of the species quantification as determined by the percent difference between the goodness-of-fit (Chi, χ) values between the best and second-best fits.

11. Cited Works

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Date: May 2009 **SOP No. # 7**

Title: *Particle Size Analysis*

Associated Investigator: Dr. Christopher Kim

Total Pages 2

SYNOPSIS: This SOP describes the methods for determining particle size of samples.

BET Surface Area Analyzer

Start Date: 2-7-07
Last Updated: 3-19-09
Author: Reyn Ono
Reviewed/Edited by: Mike Saenz, Stefanie Miller, Kim Wilson, James Dale, Nicole Leslie, James Akau

Summary: Preparation and using the BET Surface Area Analyzer.

Materials/Reagents/Equipment	Vendor
Liquid Nitrogen	
BET sample template	
BET surface area analyzer and outgasser	
Balance, sample funnel(s)	
BET sample holders, caps, and samples	

EH&S (Environmental Health and Safety)

Wear lab coat, cryo gloves, and goggles at all times when handling liquid nitrogen.

Procedure

Note: Record all data on a copy of the BET sample template, located on PC in Shared Files→ BET→ BET sample template.

1. Air dry sample in the hood if wet or moist.
2. Calculate the optimal amount of sample that will be analyzed. Use previous samples to estimate. Weigh out this calculated amount of air-dried sample. With natural samples, assume about 1 to 3 g, the amount to fill up the bottom of the smaller size sample holder.
3. Use gloves at all times, as finer oils can add weight to the sample holders, and label the sample holders and rubber caps so they do not get switched during analysis. It is extremely important that the caps stay with the same sample holders throughout this process.
4. Weigh sample holder and rubber cap.
5. Using the white sample funnel, add the sample to the sample holder. Tap edges of sample holder so the majority of the sample falls to the bottom of the sample holder.
6. Cap the sample holder with the appropriately marked cap.
7. Weigh the capped sample holder with sample. Subtract this weight from the weight of the sample holder and cap to obtain the weight of the sample. Record the weight of the sample.
8. The sample must now be degassed. Turn on the Outgasser. Be sure the Helium gas tank is turned on and the regulator is properly set (13 psi).
9. **Check pressure of helium and nitrogen tanks, if it is below 500 psi order more gas. It is important to have one tank full at all times to serve as a reserve, SO**

THERE SHOULD NEVER BE ANY EMPTY GAS TANKS. Call Muhammed at ext. 6064 to order gas tanks.

10. Set the temperature to 90°C.
11. Remove rubber cap and place sample holder in heater slot.
12. Turn on the appropriate airflow switch and adjust the airflow so the airflow meter reads about half way.
13. Place the air tube in the sample holder and allow degassing for about 12 hours.
14. Stop airflow and turn off degasser. Remove sample holder from Prep station, quickly cap sample holder, and allow sample holder to cool to room temperature.
15. Weigh the capped sample holder. Determine and record the exact weight of the sample.
16. Turn on the BET SA analyzer and allow it to warm up for 30 min. During this time:
    a. Fill small Dewars with liquid nitrogen, following all safety protocols.
    b. Open the main gas valve on the nitrogen and helium tanks (the silver knob). **NOTE:** if pressure is below 500 psi order more gas.
17. Press "Sample ID." The sample information screen will appear.
18. Enter the sample ID, your initials as the operator, and the exact degassed sample weight. The profile should be set at BET and max temperature should be at 350. Press "OK" when finished.
19. Go to "Setup," "Edit Profile," enter UNKNOWN for "Sample Category," and then press "OK" when done. Return to the home screen.
20. You are now ready to analyze the sample. Detailed instructions and pictures for analysis can be found in the product manual starting on p.7-30. Be sure to use cryo gloves when handling the liquid nitrogen. Also, place a stir rod in your sample holder right before screwing it in for analysis; this minimizes the amount of space the gas has to fill, minimizing the amount of time the analysis takes and reducing gas usage. Wipe the stir rod before and after usage with a kimwipe.
21. Once analysis is complete, record the information displayed on the screen to the appropriate form whose location appears under the Notes section of this protocol.
22. **Transfer the information back to the computer version and print out 2 copies to tape into the BET notebook.**
23. Shut down the BET SA 3100.
24. Close the gas valves on both the nitrogen and helium tanks.
25. Make sure to clean the sample holders when finished:
    a) Pour samples back into appropriate archive jars.
    b) Rinse once with tap water and 3 times with DI.
    c) Put in acid bath by themselves or with other small glassware (that are not likely to break them if knocked against them, i.e. stirring rods) for 24 hours. (clean caps by rinsing once with tap and 3 times with DI and letting air dry—do not put in acid bath).
    d) Rinse 3 times with DI and 1-2 times with ethanol to aid in evaporation.
    e) If some sample still remains inside of the sample holder, fill the bottom portion with 70% HNO_3 overnight (observe all safety precautions when handling).
    e) Put sample holders in a beaker with paper towel lining and place in oven at 90°C in Rm. 35 for thorough drying
    f) Cap sample holders once dry to avoid dust collecting.

Date: May 2009 **SOP No. # 8**

Title: *Water Extraction (ASTM, 2004)*

Associated Investigator: Dr. Christopher Kim

Total Pages 2

SYNOPSIS: Water Extraction Protocol

Water Extraction Protocol

Start Date: 10-14-06
Last Updated: : 7/29/09
Author: Stefanie Miller
Reviewed/Edited by: Eric Sugihara, Suzie Shdo

Summary of Water Extraction Protocol

Water extraction tests are conducted on samples in triplicate, with selected size fractions plus 1 blank. The suspensions are composed of a 1:4 solid to liquid ratio (3g:12mL), with deionized water previously adjusted to pH 5.50 using concentrated nitric acid. The solutions are rotated at 8 rpm for 18 hours at room temperature, centrifuged for 20 minutes at 3000 RPM, and then filtered through 0.45 µm filters. Post-extraction conductivity and pH are measured. Solutions are then acidified with concentrated nitric acid to pH < 2.00.

Materials/Reagents/Equipment	Vendor
Lab coat, gloves, goggles, respirator, 15-mL falcon tubes, marker, sample, spatula, laboratory balance	
Deionized water, pH meter, notebook, pen, Ultrex nitric acid, pipette tips and pipette	
Rotator, centrifuge	
Syringe filters of pore size 0.45um, syringes	

EH&S (Environmental Health and Safety)

Always wear a lab coat, gloves, and goggles during this experiment. Always wear a respirator when working with the dry sample.

Procedure

1. Place 3g (+/- .005g) of each selected size fraction of sample into a labeled 15-mL falcon tube. Do triplicates of each size fraction, and label tubes with "sample name_size fraction_w1, 2, or 3." In a beaker (large enough to hold total amount of water needed), acidify deionized water to pH 5.5 with Ultrex nitric acid and add 12mL of the acidified deionized water to the each tube (1:4 solid to liquid ratio).

2. Place tubes (to include one blank of acidified water) on the rotator for 18 hours. Record the start time and pulled time.

3. Centrifuge solutions for 20 minutes at 3000rpm.
4. Decant each tube's supernatant into a syringe with a filter of pore size 0.45um. Push the solution through the syringe filter into a new falcon tube with a

matching label for the sample (label these falcon tubes prior to pulling the rotating samples for efficiency).

5. Measure the pH of each solution with a pH meter and record. Also measure the conductivity of each solution and record. Acidify each solution with Ultrex nitric acid in 20-uL increments to pH < 2. Following acidification, measure the pH and conductivity of each solution and record.

6. Store the solutions in a 4°C refrigerator for future analysis.

7. Create an excel data sheet and record all the data electronically. Locate the water extraction data sheet template: on the PC desktop, open the "Shared Files" folder → "Extractions" → "Water Leach" → "Water Data Sheet." In the excel spreadsheet, input each individual sample ID as it reads on the label of the falcon tube. For each sample, input the mass, initial pH, start and pulled times, post-extraction pH and conductivity, and the 2nd pH and conductivity readings after acidification. Within the "Water Leach" folder, create a new folder with the sample name. Save the excel water data sheet as "Water Data Sheet_sample ID" within this new folder. Print out two copies and tape into the notebook.

8. Analysis of the water leachates includes ICP-MS or ICP-OES analysis and is done by the National Water Quality Laboratory, Denver Federal Center, Denver CO 80225-0585. Contact number: 303-236-3000.

QA/QC CHECKS

Complete the following table to summarize QA/QC checks.

Matrix	Measurement	QA/QC Check[1]	Frequency	Acceptance Criteria	Corrective Action
Water	As Concentration	Reproducibility	Every Sample	5% standard deviation	Triplicates
Water	As Concentration	Precision	Every Sample	0.5 ug/L	Instrumental Precision
Water	As Concentration	Detection Limit	Every Sample	1 ug/L	Instrumental Limit
Water	As Concentration	Precision	Every Sample	5% standard deviation	Triplicates
Water	pH	Accuracy	Before Use	+/- .05 units	Calibration Standards
Solid	Mass	Accuracy	Once/ month	.005 mg.	Calibration Standards

[1]Include all QA/QC checks (experimental and analytical, as applicable) for accuracy, precision, detection limits, mass balance, *etc*. (*e.g.*, matrix spikes, lab control samples, blanks, replicates, surrogates)

ASTM Designation: D 3987-85, Standard Test Method for Shake Extraction of Solid Waste with Water

Date: May 2009 **SOP No. # 9**

Title: Simulated Gastric Fluid (SGF) Extraction

Associated Investigator: Dr. Christopher Kim

Total Pages 3

SYNOPSIS: This is an extraction procedure that stimulates the gut to determine how much arsenic is extracted in gastric fluid.

Simulated Gastric Fluid (SGF) Extraction Protocol

Start Date: 6-11-07
Last Updated: 7/29/09
Author: Stefanie Miller
Reviewed/Edited by: Eric Sugihara, Suzie Shdo

Summary of Simulated Gastric Fluid Extraction

Simulated gastric fluid extractions are conducted in triplicate, with selected size fractions (ingestible size fractions S6-S11) plus a blank for every 20 samples. The solutions are composed of a 1:100 solid to liquid ratio (1g:100mL), with simulated gastric fluid previously adjusted to pH 1.50 using concentrated nitric acid. The solutions are agitated for 1 hour at 90 RPM in a 37°C incubator and then filtered through 0.45 µm filters. Post-extraction conductivity and pH are measured.

Materials/Reagents/Equipment	Vendor
Agitating incubator, water bath	
SGF data sheet, 125-mL HDPE bottles	
Glycine, 2L volumetric flask	
Ultrex nitric acid (HNO_3), 100mL volumetric flasks	
50-mL falcon tubes, 0.45um filters	

EH&S (Environmental Health and Safety)

Unfiltered extraction fluid with sample in it and used filters must be disposed of as hazardous waste. Consolidate and label according to the established hazardous waste labeling system.

Procedure

1. Prepare simulated gastric fluid (SGF): measure out 1.9L deionized (DI) water, add 60.06g (+/- .005g) glycine, and bring solution volume to 2L (0.4M glycine).
2. Place SGF solution in 37 °C water bath until it reaches 37 °C.
3. Standardize the pH meter with pH 4.0 buffer maintained at 37 °C in water bath.
4. Add Ultrex concentrated nitric acid until SGF solution reaches pH 1.50 ± 0.05 and record in notebook (about 60mL).
5. Place 1.00 (+/- .005g) of each selected size fraction of sample (ingestible < S4) into a labeled 125-mL HDPE bottle (record actual mass in notebook). Do triplicates of each size fraction, and label bottles “sample name_size fraction_SGF1, 2, or 3.” Label the caps as well, as sometimes labels rub off the sides of the bottles during agitation.
6. Using a 100mL volumetric flask, transfer 100 ± 0.05mL SGF solution to each bottle (1:100 solid to liquid ratio). Include a blank with just the SGF solution for every 20 samples. Always run a blank if you have less than 20 samples.
7. Place bottles, on their sides, in a bin that fits in the incubator (in HSC 35) and agitate at 190 RPM for 1 hour, keeping temperature at 37 ± 2 °C. Record

start time of rotation. Prior to pulling the bottles, label 50-mL falcon tubes with corresponding sample ID's for each sample and blank. Also attach the 0.45um filters to the syringes before pulling the bottles for efficiency. All samples must be filtered in 30 minutes or less (at least two KEG members should filter samples after the bottles are pulled).

8. Decant about 30mL of the SGF extracts into syringes and filter into respective 50mL falcon tubes, with matching labels. After all samples are filtered, record end time. If total time elapsed from start of rotation is greater than 1 hour 30 minutes, test must be repeated.
9. Measure pH and conductivity of remaining fluid in extraction bottle and record. If pH is not within 0.5 units of initial pH, test must be re-ran:
   a. If second test results in a decrease in pH of greater than 0.5 units, record this and filter extract for analysis.
   b. If second test results in an increase in pH of greater than 0.5 units, repeat test again and stop extraction at 5, 10, 15, and 30 minutes into extraction and adjust the post-extraction pH to 1.5, drop-wise using nitric acid.
10. Store solutions in 4°C refrigerator.
11. Create an excel data sheet and record all the data electronically. Locate the SGF extraction data sheet template: on the PC desktop, open the "Shared Files" folder → "Extractions" → "SGF Leach" → "SGF Data Sheet." In the excel spreadsheet, input each individual sample ID as it reads on the label of the falcon tube. For each sample, input the mass, initial pH, start and end filtered times, and post-extraction pH and conductivity. Within the "SGF Leach" folder, create a new folder with the sample name. Save the excel SGF data sheet as "SGF Data Sheet_sample ID" within this new folder. Print out two copies and tape into the notebook.
12. Analysis of the SGF leachates includes ICP-MS or ICP-OES analysis and is done National Water Quality Laboratory, Denver Federal Center, Denver CO 80225-0585. Contact number: 303-236-3000.

QA/QC CHECKS

Complete the following table to summarize QA/QC checks.

Matrix	Measurement	QA/QC Check1	Frequency	Acceptance Criteria	Corrective Action
SGF fluid	As Concentration	Reproducibility	Every Sample	5% standard deviation	Triplicates
SGF fluid	As Concentration	Precision	Every Sample	0.5 ug/L	Instrumental Precision
SGF fluid	As Concentration	Detection Limit	Every Sample	1 ug/L	Instrumental Limit
SGF fluid	As Concentration	0 ppm As	Every 20 Samples or less	Used for self correction	Blank
SGF fluid	pH	Accuracy	Before Use	+/- .05 units	Calibration Standards
Solid	Mass	Accuracy	Once/ month	.5 mg.	Calibration Standards

1Include all QA/QC checks (experimental and analytical, as applicable) for accuracy, precision, detection limits, mass balance, *etc*. (*e.g.*, matrix spikes, lab control samples, blanks, replicates, surrogates)

Drexler, John. University of Colorado Relative Bioavailability Leaching Procedure: Standard Operating Procedure. <http://www.colorado.edu/geolsci/legs/invitro1.html>

Date: May 2009 **SOP No. # 10**

Title: *Simulated Lung Fluid (SLF) Extraction*

Associated Investigator: Dr. Christopher Kim

Total Pages 3

SYNOPSIS: This extraction technique mimics the lung and aids in determining how much arsenic is extracted and made bioavailable in the lung.

Simulated Lung Fluid (SLF) Extraction Protocol

Start Date: 5-8-07
Last Updated: 7-29-09
Author: Helen Mortera and Nathalie Petersen
Reviewed/Edited by: Eric Sugihara, Stefanie Miller, Suzie Shdo

Summary of Simulated Lung Fluid Extraction

Simulated lung fluid extractions are conducted in triplicate, with the S11 size fraction plus 2 blanks. The solutions are composed of a 1:20 solid to liquid ratio (1g:20mL), with simulated lung fluid previously adjusted to pH 7.19 by bubbling 5%CO_2 in O_2. The solutions are agitated for six days in an incubator at 37ºC at 90 RPM. The pH and conductivity are monitored daily and pH is held at 7.19 by adding concentrated nitric acid. On day 6, samples are centrifuged at 3000 RPM for 15 minutes. Samples are then filtered through 0.45 µm filters and acidified to pH < 2. Final pH and conductivity are measured and recorded.

Materials/Reagents/Equipment	**Vendor**
Sodium chloride – NaCl →6.431g	Fisher
Sodium bicarbonate – $NaHCO_3$ →2.600g	Fisher
Calcium acetate – $Ca(C_2H_3O_2)_2$ →0.401g	Fisher
Calcium chloride – $CaCl_2$ →0.370g	Fisher
Magnesium acetate – $Mg(C_2H_3O_2)_2$ →0.2130g	Fisher
Magnesium chloride – $MgCl_2$ →0.2029g	Fisher
Potassium dihydrogen phosphate – KH_2PO_4 →0.271g	Fisher
Dipotassium sulfate – K_2SO_4 →0.1704g	Fisher
Citric acid – $C_6H_8O_7$ →0.0700g	Fisher
Albumin (fraction 5) →0.0200g	Fisher
Benzalkonium chloride (solid) →0.0506g	Sigma
Ultrex nitric acid	Fisher
0.1 M nitric acid	Sigma-Aldrich
1 liter plastic bottle with screw cap	Cynmar
10-50ml Falcon tubes	Fisher
0.45 um cellulose filters	Fisher
60 mL syringes	Cole-Parmer
Agitating incubator	
Tank of 5% CO_2 in O_2	

EH&S (Environmental Health and Safety)

Gloves should be worn at all times. A respirator should be worn when handling the soil samples and benzalkonium chloride. Unfiltered extraction fluid with sample in it and used filters must be

disposed of as hazardous waste. Consolidate and label according to the established hazardous waste labeling system.

Procedure

1. Rinse 1L bottle with ethanol, rinse with DI water, then air dry.

2. Fill bottle with 1L DI Water; add salts listed above in order sequentially (for concentrations in volume different from 1L, refer to Twining et al., "Risk ranking of bioaccessible metals from fly ash dissolved in simulated lung and gut fluids", found in Endnote-Converted archive on PC, or in hard copy paper archive).

3. Mix on vortex mixer.

4. Refrigerate in between uses and any remaining SLF.

5. Measure pH of SLF and record.

6. Acidify to pH 7.19 by bubbling 5%CO_2 in O_2 into SLF solution and record.

7. Label 5 falcon tubes 1-5. Add 20mL SLF solution to each tube. Add 1.00g (+/- .005g) S11 soil sample to tubes 1-3 (S11 sample triplicate). Tubes 4-5 are blanks.

8. Vortex all tubes and secure them with tape, laying flat in a plastic container. Incubate/agitate at 37ºC at 90 rpm.

9. Record pH and conductivity daily for 6 days. Then adjust samples to pH 7.19 using 0.1M HNO_3 (diluted from ULTREX conc. nitric acid) and record amount added to each tube. Do not regas. Return tubes to incubator and resume agitation.

10. On day 6, remove tubes from incubator, record pH and conductivity. Then adjust samples to pH 7.19 and centrifuge at 3000 rpm for 15 minutes.

11. Filter leachates into new tubes labeled 1A-5A through 60ml syringes with 0.45µm filters.

12. Acidify each leachate to pH < 2 using Ultrex concentrated (70%) HNO_3 and record the amount added. Also record final pH and conductivity. Store solutions in a 4°C refrigerator for future ICP-MS or ICP-OES analysis at the National Water Quality Laboratory, Denver Federal Center, Denver CO 80225-0585. Contact number: 303-236-3000.

13. See USGS leachate shipping protocol for preparation and shipping.

14. Create an excel data sheet and record all the data electronically. Locate the SLF extraction data sheet template: on the PC desktop, open the "Shared Files" folder → "Extractions" → "SLF Leach" → "SLF Data Sheet." In the excel spreadsheet, input each individual sample ID as it reads on the label of the falcon tube. For each sample, input the mass, initial pH, daily pH and conductivity, final acidified pH and conductivity, and start time, and end time. Within the "SLF Leach" folder, create a new folder with the sample name. Save the excel SLF data sheet as "SLF Data Sheet_sample ID" within this new folder. Print out two copies and tape into the notebook.

QA/QC CHECKS

Complete the following table to summarize QA/QC checks.

Matrix	Measurement	QA/QC Check[1]	Frequency	Acceptance Criteria	Corrective Action
SLF fluid	As Concentration	Reproducibility	Every Sample	5% standard deviation	Triplicates
SLF fluid	As Concentration	Precision	Every Sample	0.5 ug/L	Instrumental Precision
SLF fluid	As Concentration	Detection Limit	Every Sample	1 ug/L	Instrumental Limit
SLF fluid	As Concentration	0 ppm As	Every 20 Samples or less	Used for self correction	Blank
SLF fluid	pH	Accuracy	Before Use	+/- .05 units	Calibration Standards
Solid	Mass	Accuracy	Once/ month	.5 mg.	Calibration Standards

[1]Include all QA/QC checks (experimental and analytical, as applicable) for accuracy, precision, detection limits, mass balance, *etc*.
(*e.g.*, matrix spikes, lab control samples, blanks, replicates, surrogates)

Twining, J., McGlinn, P., Loi, E., Smith, K., and Giere, R. (2005) Risk Ranking of Bioaccessible Metals from Fly Ash Dissolved in Simulated Lung and Gut Fluids. Environmental Science and Technology 39(19): 7749-7756.

Date: May 2009 **SOP No. # 11**

Title: *In Vitro Gastrointestinal Method*

Associated Investigator: Dr. Nicholas Basta

Total Pages 5

SYNOPSIS: A detailed extraction method that serves as a surrogate for gastrointestinal system to determine how much arsenic is extracted and made bioaccessable after consumption.

Title: OSU *In Vitro* Gastrointestinal Method for Determination of the Bioaccessiblity of Select Metals and Metalloids in Soil and Geomedia.

Summary: This SOP describes an *in vitro* gastrointestinal method (OSU IVG) to determine the bioaccessible contaminant content in soil or contaminated geomedia. The OSU IVG method simulates human gastrointestinal tract conditions. The measured percent bioaccessible Pb, As, and Cd has been shown to be correlated with *in vivo* bioavailability data determined from dosing trials using immature swine.

Current Author: Dr. Nicholas Basta Professor of Soil and Environmental Chemistry	**Signature:**	**Date:**
Quality Assurance Officer: Shane Whitacre Soil Environmental Chemistry Laboratory Technician	**Signature:**	**Date:**

1.0 Scope of Method

1.1 Incidental soil ingestion is an important exposure pathway for assessing public health risks associated with contaminated soils (Dudka and Miller, 1999; Chaney and Ryan, 2004). The bioavailability of Pb, As, and Cd in soils can be determined by conducting dosing trials using animal models. Immature swine have been successfully used as an animal model for the gastrointestinal (GI) function of children (Casteel et al., 2001; Chaney and Ryan, 2004; Weis and LaVelle, 1991). However, conducting *in vivo* animal trials is lengthy and expensive.

To overcome the difficulty and expense associated with *in vivo* trials, research effort has been directed toward the development of *in vitro* methods to simulate human gastrointestinal conditions. Several of these methods have been reviewed (Oomen et al., 2002; Rodriguez et al., 1999; Ruby et al., 1999). The OSU IVG is a rapid, inexpensive and reliable screening tool for determining the potential bioavailability (i.e., bioaccessible) of soil contaminants including As (Basta et al., 2007; Rodriguez et al., 1999), Cd (Schroder et al., 2003), and Pb (Schroder et al., 2004). The OSU IVG method simulates important parameters of the human GI tract under fasting conditions. The amount of contaminant extracted by the OSU IVG is assumed to be available for absorption across the intestinal membrane (i.e., bioaccessible) and incorporation into systemic circulation (Ruby et al., 1999). Contaminant bioaccessiblity is expressed as a percentage of the total contaminant content of the test sample.

2.0 Definitions

2.1 OSU IVG: The Ohio State University *In Vitro* Gastrointestinal Method

2.2 Bioaccessible: Amount of contaminant extracted by OSU IVG and potentially available for absorption across the intestinal membrane

2.3 Gastric Phase: simulated conditions for the stomach environment.

2.4 Intestinal Phase: simulated conditions for the small intestine (e.g., duodenum) environment.

2.5 ICP-AES: Inductively Coupled Plasma-Atomic Emission Spectroscopy

2.6 ICP-HG-AES: ICP-AES with sample introduction using automated hydride generation

3.0 Equipment and Supplies

3.1 A hot water bath with temperature control of 37± 2 ºC is used throughout the extraction to maintain samples at body temperature. The water bath should be deep enough to ensure 2/3 of the extraction vessel (e.g., beaker) is surrounded with warm water. The extraction is done in open vessels with constant stirring (plastic paddles, 100 rpm). Solution pH is monitored and adjusted throughout the procedure.

3.2 All reagent chemicals are stored as recommended by the distributor.

3.3 Reagent solutions are made fresh daily.

4.0 Sample Preparation

4.1 Contaminated soil or geomedia are oven dried 80ºC. Highly aggregated samples are tumbled by placing the sample in a ceramic jar on a rotary mill for up to 6 h. Samples are sieved to < 250 µm, the particle size assumed to adhere to hands. Dried (< 250 µm) sample is stored at room temperature in covered plasticware. Sample homogeneity is assured through mixing prior to extraction.

5.0 Procedure

5.1 For the **gastric phase**, 150 mL of gastric solution (0.10 M ACS grade NaCl and 1% porcine pepsin, Sigma Aldrich, St. Louis, MO, Cat. No. P7000) is heated in an open extraction vessel, in a 37º C hot water bath. When the solution reaches 37º C, the pH is adjusted to 1.8 ± 0.1 using 6 M trace metal grade HCl followed by addition of the soil or geomedia (1 g, < 250 µm). The sample is thoroughly mixed with the solution, using a paddle stirrer (100 rpm) to maintain a homogenous suspension. The pH is continuously monitored and adjusted to 1.8 ± 0.1 for 1 h. After 1 h, 10 mL of gastric solution is removed for analysis. The extract is immediately centrifuged (11,160 g for 15 min) and then filtered (0.45 µm). Filtered extracts are refrigerated (4º C) for preservation prior to analysis. Contaminant extracted during the gastric phase is expressed as gastric extractable bioaccessible (GE BA).

5.2 For the **intestinal phase** the pH of the remaining solution is adjusted to 6.1 ± 0.1 using dropwise additions of a saturated Na_2CO_3 solution followed by the addition of 0.563 g of porcine bile extract (Cat. No. B8631) and 0.563 g of porcine pancreatin (Cat. No. P1750 Sigma Aldrich, St. Louis, MO). The pH is continuously monitored and adjusted to 6.1 ± 0.1. After 2 h of mixing, 10 mL of intestinal solution is collected for analysis. The extract is immediately centrifuged (11,160 g for 15 min) and then filtered (0.45 µm). Filtered extracts are refrigerated (4º C) for preservation prior to analysis. Contaminant extracted during the intestinal phase is expressed as intestinal extractable bioaccessible (IE BA).

5.3 A minimum of three replicates analyses of samples are preformed to determine bioaccessible contaminants by OSU IVG.

5.4 Extracts are analyzed using (ICP-AES) or ICP-HG-AES for any or all of the following elements; Al, As, Ca, Cd, Cr, Cu, Fe, Mg, Mn, P, Pb, Zn.

6.0 Quality Control

6.1 Calibration standards, check standards, and dilutions are prepared in 0.1 M ACS grade NaCl, and 0.5 M trace metal grade HCl matrix.

6.2 A blank and a laboratory control sample are included with each batch of in vitro sample extractions for quality control.

6.3 Other quality Assurance procedures for determination by ICP are described in the attached “OSU Soil Environmental Chemistry Laboratory QA/QC Protocol for Measurement of Analytes by ICP (SOP 12).

6.4 QA/QC Checks

Complete the following table to summarize QA/QC checks.

Matrix	Measurement	QA/QC Check[1]	Frequency	Acceptance Criteria	Corrective Action
Solution	Contamination	Method blank	1 with every batch of in vitros.	Below MDL or analyte concentration of samples > 10x blank concentration.	Re-extract entire batch of in vitros.
Soil	Accuracy	Laboratory Control Sample	1 every batch of in vitros	± 20%	Re-extract entire batch of in vitros.

[1]Include all QA/QC checks (experimental and analytical, as applicable) for accuracy, precision, detection limits, mass balance, *etc*. (*e.g.*, matrix spikes, lab control samples, blanks, replicates, surrogates)

7.0 Data Calculation and Assessment

7.1 **Bioaccessible contaminant** is calculated as the percentage of the total contaminant (or metal of interest) content extracted during the *in vitro* gastric or intestinal phase as shown in the following equation.

$$\% \text{ Bioaccessible} = [\text{OSU IVG extractable } (mg/kg^{-1})]/[\text{total contaminant } (mg/kg^{-1})] * 100$$

7.2 Total contaminant content is determined using USEPA Method 3051A, a standard analysis for soil metal content via microwave digestion using aqua regia, (3:1 v/v trace metal grade HNO_3:HCl, U.S. EPA, 1998), or an equivalent method with subsequent analysis by ICP-AES.

8.0 References

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Date: May 2009 **SOP No. # 12**

Title: *Inductively Coupled Plasma (ICP) Spectrometry analysis*

Asssociated Investigator: Dr. Nicholas Basta

Total Pages 6

SYNOPSIS: Outline of the *in vivo* bioavailability study procedures

Inductively Coupled Plasma (ICP) Spectrometry analysis

1.0 Scope

Inductively coupled plasma-atomic emission spectrometry may be used to determine the following trace elements in solution; Aluminum (Al), Antimony (Sb), Arsenic (As), Barium (Ba), Beryllium (Be), Boron (B), Cadmium (Cd), Calcium (Ca), Chromium (Cr), Cobalt (Co), Copper (Cu), Iron (Fe), Lead (Pb), Magnesium (Mg), Manganese (Mn), Molybdenum (Mo), Nickel (Ni), Potassium (K), Selenium (Se), Silver (Ag), Sodium (Na), Sulfur (S) Strontium (Sr), Thallium (Tl), Vanadium (V)*, Zinc (Zn.

2.0 Definitions

2.1 Matrix Spike: A duplicate sample is spiked prior in order to provide information about the effect of the sample matrix on the sample preparation and/or measurement methodology.

2.2 Serial Dilution: A serial dilution consists of a comparison of the results of a sample and another aliquot diluted by a known factor.

2.3 Laboratory Control Sample: The laboratory control samples is a certified QC standard (or dilution) for ICP analysis. The laboratory control sample is SPEX CertiPrep Group LPC standard 1, Fisher Cat. No. LPC-1-100N

2.4 ICP-AES: Inductively Coupled Plasma-Atomic Emission Spectrometry.

2.5 ICP-HG-AES: ICP-AES with sample introduction using automated hydride generation

3.0 Instrumentation and Facilities

3.1 ICP-AES and ICP-HG-AES analysis are carried out on a Varian Vista-MPX ICP-OES (Varian Inc., Walnut Creek, CA) at the Soil Environmental Chemistry Lab, The Ohio State University, Dr. Nick Basta, Director.

3.2 Determination by ICP-MS is conducted at the Trace Element Research Laboratory (TERL), The Ohio State University, Dr. John Olesic, Director.

4.0 Materials and Supplies

4.1 Automated Vapor Generator (VGA-77).

4.2 Single element ICP grade standards (SPEX CertiPrep Group, Metuchen, NJ, Assurance ICP Standards).

4.3 Laboratory control sample, SPEX CertiPrep Group LPC standard 1, Fisher Cat. No. LPC-1-100N.

4.4 Varian tuning solution, Varian part no. 190005800.

4.5 Trace metal grade HCl.

4.6 Potassium iodide, molecular biology grade.

4.7 $NaBH_4$, ACS grade.

5.0 Instrumental Analysis

5.1 Detection Limits

5.1a Method detection limits (MDL) are calculated for specific methods and consequent conditions of that method developed for analysis on ICP. The method detection limit is determined by multiplying by 3.143 the standard deviation of seven replicate analyses of standard solutions at 2-5x the instrument detection limit (IDL).

5.1b Instrument Detection Limits (IDL) were determined by three times the standard deviation of the signal of 10 blanks solutions.

5.1c Project Required Detection Limits for As: 1ug/L is the required detection limit for the project. Measurements above 20ug/L will be made by ICP-AES. Measurements below 20ug/L will be made by ICP-HG-AES.

6.0 ICP-AES Procedure

6.1 Visually inspect ICP torch for residue build up as well as peristaltic pump tubing for wear. If either the torch or the peristaltic pump tubing is in need of replacement, replace and perform subsequent alignments (for torch replacement) according to instruction provided in ICP Expert (v. 2.0) software help.

6.2 Ignite ICP torch using Varian ICP Expert (v. 2.0) software 45 minutes prior to instrument tuning, calibration, and sample analysis.

6.3 Calibrate detector while pumping DI water to the spray chamber. Store detector calibration in dark current folder.

6.4 Calibrate wavelength while pumping Varian tuning solution (Varian part no. 190005800) diluted by a factor of 10.

6.5 Instrument optimization for As:

6.5a Open 01Neboptimize method and open instrument configuration window.

6.5b Set power to 1.2 KW.

6.5c Set plasma flow to 15 L/min.

6.5d Set Auxiliary flow to 2.25 L/min.

6.5e Adjust nebulizer flow to obtain maximum signal for As 188.980.

6.6f Transfer optimized instrument parameters to method set up for analysis.

7.0 As(V) + As(III) ICP-HG-AES Procedure

7.1 Matrix match standards to samples and prepare both in 1M trace metal grade HCl and 1% w/v potassium iodide (KI) and allow As(V) to reduce to As (III) for at least 1 hour at room temperature.

7.2 Visually inspect ICP torch for residue build up as well as peristaltic pump tubing for wear. If either the torch or the peristaltic pump tubing is in need of replacement, replace and perform subsequent alignments (for torch replacement) according to instruction provided in ICP Expert (v. 2.0) software help menu.

7.3 Set up VGA-77 designated for use with KI according to operation manual.

7.3a Reductant container: 0.6% $NaBH_4$, 0.5% NaOH.

7.3b Acid container; Concentrated (12M) trace metal grade HCl.

7.4 Ignite ICP torch using Varian ICP Expert (v. 2.0) software 45 minutes prior to instrument tuning, calibration, and sample analysis.

7.5 Calibrate detector while pumping DI water to the spray chamber. Store detector calibration in dark current folder.

7.6 Instrument optimization for As:

7.6a Open 01Neboptimize method and open instrument configuration window.

7.6b Set power to 0.8 KW.

7.6c Set plasma flow to 10.5 L/min.

7.6d Set Auxiliary flow to 2.25 L/min.

7.6e Adjust nebulizer flow to obtain maximum signal for As 188.980.

7.6f Transfer optimized instrument parameters to method set up for analysis.

8.0 **Analysis**

8.1 Stock standards are prepared using certified ICP grade standards (SPEX CertiPrep Group, Metuchen, NJ, Assurance ICP Standards). Calibration standards are prepared daily by serial dilution from at least two independent stock standards. The dilutions should be done into a matrix comparable to the samples.

8.2 Instruments shall be calibrated daily and each time the instrument is set up. Calibrate the instrument according to instrument manufacturer's recommended procedures. At least four standards shall be used for ICP calibration. One of the standards shall be a blank. Linear calibration must meet the criteria of: $r^2 = 0.995$, and calculated concentrations from the regression within 10% for each standard in the calibration.

8.3 Initial calibration verification (ICV) is performed using the laboratory control sample (SPEX CertiPrep Group LPC standard 1, Fisher Cat. No. LPC-1-100N) run immediately after instrument calibration. Standards must fall within ± 10% for ICP-AES/MS and 15% for ICP-HG-AES of certified value.

8.4 Continuing calibration verification (CCV) is a dilution of the ICV QC standard and is run after every ten samples. Standards must fall within ± 10% for ICP-AES/MS and 15% for ICP-HG-AES of certified value.

8.5 Initial calibration blank (ICB) is a calibration blank run just prior to the first sample. The calibration blank must fall below the method detection limit (MDL) detection limit. If the calibration blank is above the MDL, the problem should be fixed and instrument re-calibrated.

8.6 Continuing calibration blank (CCB) is a calibration blank run after every ten samples with the CCV. The calibration blank must fall below the MDL. If a calibration blank is above the detection limit, the instrument must be recalibrated and the previous samples to the last CCB re-run.

8.7 Limit of quantitation (LOQ) is a check standard used to verify linearity at the MDL for ICP analysis. The LOQ standards at a concentration equal to the MDL are analyzed at the beginning and end of each sample analysis and at a frequency of not greater than 20 analytical samples. Standards must fall within ± 20% for ICP-AES and 30% for ICP-HG-AES.

8.8 A linear range verification (LRV) check standard shall be analyzed for each wavelength concentrations that exceed the highest calibration standard by more

than 20%. The standard shall be analyzed during the analytical run. The analytically determined concentration of this standard shall be within 10% of the true value. This concentration is the upper limit of the ICP linear range beyond which results cannot be reported without dilution of the analytical sample.

8.9 Potential interferences (ICP-AES) are determined by calibration of all potential lines used for analysis followed by the analysis of single element standards as samples containing 10 to 500mg/L. Interferences were identified as a signal greater than the IDL on any line other than the element in the standard. The single element standards investigated included; Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, Sb, Se, Tl, V, Zn.

9.0 Quality Control

9.1 Matrix Spike: Spike recoveries must fall within the limits of 75-125%. At least one spike analyses (matrix spikes) shall be performed on each group of samples of a similar matrix type.

9.2 Serial Dilution: The % difference for the serial dilution tests must be no more than 10%. At least one serial dilution should be performed on each group of samples with similar matrix.

$$\%\text{Difference} = 100 * [\text{initial}] - \frac{([\text{diluted}] * \text{DilutionFactor})}{[\text{initial}]}$$

9.3 Laboratory Control Sample: The QC standard is run after every ten samples. Standards must fall within ± 10% for ICP-AES/MS and 15% for ICP-HG-AES of certified value.

9.4 QA/QC CHECKS

Complete the following table to summarize QA/QC checks.

Matrix	Measurement	QA/QC Check[1]	Frequency	Acceptance Criteria	Corrective Action
Solution	Calibration	ICV/LCS	After calibration but before samples.	±10% ICP-AES, ±15% ICP-HG-AES.	Stop analysis, determine and correct problem, and recalibrate.
Solution	Calibration	CCV/LCS	Every 10 samples	±10% ICP-AES, ±15% ICP-HG-AES.	Stop analysis, determine and correct problem, and recalibrate. Report only values prior to the last good CCV.
Solution	MDL	LOQ check	After calibration but before samples and every 20 samples.	±20% ICP-AES, ± 30% ICP-HG-AES.	Stop analysis, determine and correct problem, and recalibrate. Report only values prior to the last good LOQ check.
Solution	Instrument Drift/ Sample Carryover	ICB	After calibration but before samples.	Below MDL.	Stop analysis, determine and correct problem, and recalibrate.
Solution	Instrument Drift/ Sample Carryover	CCB	Every 10 samples.	Below MDL.	Stop analysis, determine and correct problem, and recalibrate. Report only values prior to the last good CCB..
Solution	Linear Range	LRV	Once per analytical run if analyte concentration in the	±10% ICP-AES, ±15% ICP-HG-AES.	If LRV fails, samples with analyte concentrations above the highest calibration standard, must be

			samples is more than 20% greater than highest calibration standard		diluted and re-analyzed.
Solution	Matrix affects	Matrix spike	At least one per group of samples with similar matrix type.	±25% ICP-AES and ICP-HG-AES.	If Matrix spike fails: 1st) Dilute sample, perform matrix spike on diluted sample. If spike still fails or analyte is below MDL then, 2nd) Use internal standard to correct for matrix affect and perform matrix spike using internal correction. If matrix spike still fails then, 3rd) Use standard additions to analyze samples.
Solution	Matrix affects	Serial Dilution	At least one per group of samples with similar matrix type.	% difference ± 10%	If serial dilution fails: 1st) Dilute sample, perform serial dilution on diluted sample. If serial dilution still fails or analyte is below MDL then, 2nd) Use internal standard to correct for matrix affect and perform serial dilution using internal correction. If serial dilution still fails then, 3rd) Use standard additions to analyze samples.

[1]Include all QA/QC checks (experimental and analytical, as applicable) for accuracy, precision, detection limits, mass balance, *etc*. (*e.g.*, matrix spikes, lab control samples, blanks, replicates, surrogates)

10.0 Reporting

10.1 If the QC limits are not met for any element or sample, the effect on the data set will be evaluated by the project manager and analyst.

11.0 References

11.1 United States Environmental Protection Agency. Method 6010C. Inductively Coupled Plasma-Atomic Emission Spectrometry. In SW-846; U.S. EPA: Washington, DC, 2007.

11.2 United States Environmental Protection Agency. Method 6020A. Inductively Coupled Plasma-Atomic Mass Spectrometry. In SW-846; U.S. EPA: Washington, DC, 2007.

11.3 United States Environmental Protection Agency. Document number ILM04.0b. Contract Laboratory Program Statement of work for inorganic analysis, multi-media, multi-concentration. U.S. EPA: Washington, DC.

Date: May 2009 **SOP No. # 13**

Title: *In Vivo* Bioavailability Testing

Total Pages 27

SYNOPSIS: Outline of the *in vivo* bioavailability study procedures

Study Design for Arsenic Bioavailability Testing

Group	Number of Animals	Material Administered	Dose of Arsenic	
			ug/day (a)	ug/kg-day (b)
1	3	Control	0	0
2	3	NaAs	600	50
3	3	NaAs	900	100
4	3	Soil #1	TBD	70
5	3	Soil #1	TBD	140
6	3	G&P Material #1	TBD	70
7	3	G&P Material #1	TBD	140

(a) Administered doses of arsenic (ug/day) will be held constant during the study, even though body weight of the test animals is increasing. This approach is selected because the absorption and urinary excretion of arsenic is not believed to be dose dependent, at least in the dose range investigated in this study.
(b) The dose level shown (ug/kg-day) is the expected average dose based on the expected average body weight during the exposure interval (10 days).

(c) The dose of the test materials will be chosen when the analytical data for total arsenic concentration of the soil and G&P material selected for the study has been determined.

Male pigs weighing 10-12 kg will be housed individually in metabolism cages. All doses will be delivered daily for 10 days in a low-arsenic vehicle. Urine samples are to be collected over 48 hours on days 6-7, and 9-10 into plastic containers. Animal weights will be recorded and doses and feed adjusted on days -1, 2 and every third day thereafter until study termination.

Animals will be fed and dosed according to the regular daily schedule outlined in the Project Notebook. Water will be provided by nipple waterer ad libitum concurrent with feeding to allow sufficient intake throughout the study.

A. PURCHASE OF SUPPLIES AND PREPARATION OF REAGENTS

1.0 INTRODUCTION

Various disposable supplies and laboratory reagents are needed to perform the investigations that comprise this study. This SOP details the preparation of laboratory solutions and a protocol to follow to ensure that the supplies and reagents are not significantly contaminated with arsenic.

2.0 SUPPLIES AND EQUIPMENT

A variety of items are required to perform the investigations including:

- Feed containers
- Blue top 15 mL Falcon® centrifuge tubes
- Whirlpac® plastic bags
- Micropipette tips
- Volumetric pipettes
- Volumetric flask
- 250 ml plastic bottles
- 2.5 gallon plastic containers

Prior to employing such items in an investigation, one or more samples of each item or each batch, as appropriate, must be tested to ensure that the equipment will not contribute significant arsenic contamination.

3.0 STOCK REAGENTS

Purchase only the highest purity reagents available, with special attention to the level of arsenic contamination. Store all stock reagent bottles in a locked room. Never place any object into a stock reagent bottle. Rather, pour portions of the chemical into separate clean, arsenic-free containers, as needed. Never return any material to a stock reagent bottle.

4.0 STOCK SOLUTIONS

Prepare fresh stock solutions for each new study. Label each prepared solution with the following information:

- Reagent name
- Composition
- Date prepared
- Expiration date
- Initials of preparer

Store all stock solutions in a secure cabinet. If any visible sign of precipitation or microorganism growth is detected in stock solution, discard all of that stock solution and prepare fresh. Never place a pipette or any other object in a stock solution bottle. Rather, pour stock solution into a clean tube as needed. Never return unused solution to the stock bottle.

4.1 Sodium Arsenate Solutions

Stock Solution A

Weight 41.6 grams of sodium arsenate (Na_2HAsO_4 * $7H_2O$) and dissolve in distilled water to a final volume of 1 liter. (Volumes may be adjusted as necessary to prepare amount of reagent needed for study). The concentration of arsenic in this solution is 10 ug/uL.

Stock Solution B and C

Prepare Stock Solutions B and C by diluting Stock Solution A as follows:

> Stock Solution B = 20 mL of Stock Solution A + distilled water to a final volume of 100 mL (1:5 dilution). The concentration of arsenic in this solution is 2 ug/uL.
>
> Stock Solution C = 10 mL of Stock Solution A + distilled water to a final volume of 100 mL (1:10 dilution). The concentration of arsenic in this solution is 1 ug/uL.

IV Solution

This Stock Solution is used for intravenous dosage or preparation of arsenic standards. Prepare the IV Stock Solution by diluting Stock Solution B as follows:

> IV Solution = 1 mL of Stock Solution B + distilled water to a final volume of 100 mL. The concentration of arsenic in this solution is 0.02 ug/uL or 20 ug/mL.

The formula for CDC matrix modifier is 0.2% v/v nitric acid, 0.5% v/v Triton X-100, and 0.2% w/v ammonium phosphate. Prepare this solution as follows, scaling as appropriate for the amount needed.

Dilute 2.0 mL ultrapure concentrated nitric acid and 5.0 mL Triton X-100 in about 750 mL ultrapure water in an acid-cleaned 1000 mL volumetric flask. Weigh out and add 2.0 g dibasic ammonium phosphate, washing the material into the flask with ultrapure water from a wash bottle. Add a clean magnetic stirring bar and stir until the Triton X-100 has dissolved. Remove the stirring bar and bring the solution to volume with ultrapure water. Store at room temperature. Check the solution for contamination before use by submitting an aliquot to the laboratory for arsenic determination.

The 853 ppm lanthanum solution used in the preparation of bone samples for analysis is prepared as needed by mixing one volume of a 1706 ppm stock solution with one volume of double distilled water. The 1706 ppm stock solution is prepared by dissolving 2.0 gm of lanthanum oxide in approximately 250 mL double distilled water, adding 160 mL of nitric acid and bringing the volume to 1.00 L with double distilled water.

4.2 Sodium Arsenate Check Standards

The following amounts of the IV Stock Solution will need to be diluted with control animals urine to 60 mL in a 100 mL graduated cylinder to achieve the required check standards:

Arsenic Low (10 ug As/L urine)

AsLow = 30 uL of IV Stock Solution + urine from control animals to a final volume of 60 mL. Mix well.

Arsenic Medium (30 ug As/L urine)

AsMed = 90 uL of IV Stock Solution + urine from control animals to a final volume of 60 mL. Mix well.

Arsenic High (100 ug As/L urine)

AsHigh = 300 uL of IV Stock Solution + urine from control animals to a final volume of 60 mL. Mix well.

B. ANIMAL PURCHASE, IDENTIFICATION, AND ASSIGNMENT TO GROUPS

1.0 ANIMAL PURCHASE

All animals used in these investigations will be purchased from the Pig Improvement Corporation (PIC) facility located near Monroe City, Missouri. All animals will be intact males of the genetically defined Line 26. The number of animals purchased should be approximately 10% more than the number called for in the study plan. The body weights and/or ages of the animals purchased should all be as uniform as possible. The target body weight at time of purchase is about 7-8 kg.

2.0 ANIMAL IDENTIFICATION

Immediately upon receipt of the animals, each animal will be given an ear tag which contains a permanent and unique identification number. This number will consist of the investigation number followed by a two-digit identification number.

3.0 PRE-INVESTIGATION HANDLING

The ear tag number of each animal will be entered into the logbook on the page designed for recording pre-investigation observations. Animals will be fed and watered as detailed elsewhere, and the body weight of each animal will be recorded prior to dosing during the holding period. Any animals that do not appear healthy or are not growing at the same rate as the other animals will be excluded from the investigation. Of the remaining animals, the heaviest and the lightest will be excluded in an alternate fashion until the number remaining is equal to the number called for in the study protocol. Each of these animals is then assigned to a dose/treatment group at random, as detailed below.

4.0 RANDOM ASSIGNMENT TO TREATMENT GROUPS

a. Prepare a list of the animals in ear tag order.

b. Use a computer to generate a series of random numbers, assigning these numbers in turn to each animal in the list.

c. Sort the animals sequentially based on the random numbers.

d. Assign the first four animals to group 1, the next 4 to group 2, etc.

e. Sort animals sequentially within assigned groups.

This randomization procedure is general and can be used to assign Sample Numbers or any other items that requires a random approach.

C. ANIMAL WEIGHING, FEEDING AND WATERING

1.0 FEEDING

All investigations performed in this study call for animals to be provided with 100% of their daily food requirements. This is achieved by supplying each animal with food equivalent to 4% of its body weight each day. Since the animals are expected to grow significantly (0.3 to 0.8 kg/day) over the investigation period, the food portions must be constantly adjusted upward over time.

1.1 Food Supply

The feed used in these experiments will provide 100% of the recommended dietary requirements of swine. The feed will be analyzed prior to usage to confirm low arsenic concentrations. The dietary composition will be reviewed by a swine nutritionist.

1.2 Weighing Schedule

Each animal must be weighed once every three days of the investigation, normally beginning on day -1. These weights will be used to calculate the appropriate amount of feed to give during the

following three days (see below). Animals will also be weighed on the day of sacrifice. All body weights will be recorded in the laboratory log book to the nearest 0.1 kg.

1.3 Calculation of Food Portions

Food portions administered in these experiments will be based on the **mean body weight** (MBW) of all animals on study. Further, the mean body weight used will be adjusted to account for the gain in body weight expected to occur over the next two days following weighing, such that the mean body weight used is the estimated weight on day 2 of the 3-day period. This adjustment (based on the growth rates observed in EPA Phase I experiments) simply requires adding 1 kg to the mean body weight measured.

An example calculation is shown below.

Mean Body Weight (day -1) 8.3 kg (measure)

Estimated MBW on day +1 9.3 kg (MBW + 1 kg)

$$Portion\,(g) = (1/2)(0.05)(9.3\,kg)(1000\,\frac{g}{kg}) = 232.5\,g$$

Calculation of food portion to be given twice each day on days 0, 1, and 2:

Weigh feed portions (accurate to within ± 5% of the target) into disposable paper containers. The total number of portions needed is equal to six times the number of animals on paper study (two portions per day for each of three days). Provide one portion to each animal twice each day according to the time schedule specified in the experimental protocol.

Table 1 summarizes the growth rates observed during Phase I, and illustrates the range of food portions that may be required.

Feed Analysis

To ensure the feed delivered actually contains low arsenic levels, two random samples (each about 5 g) from each batch of food provided by the supplier will be placed in separate 15 mL Falcon® centrifuge tubes and sent to the laboratory for arsenic analysis prior to beginning each investigation.

2.0 WATERING

Water Supply

The protocol for all investigations performed during this study calls for animals to be provided with drinking water ad libidum. The source of the drinking water will be the municipal drinking water system, and drinking water will be provided to each cage via a pipe and nozzle which is activated by the animal. Laboratory technicians will check each day to ensure that all water delivery nozzles are functioning properly.

Water Analysis

To ensure the drinking water delivered to the animals is not significantly contaminated with arsenic, one sample (about 5-10 mL) will be drawn at random from a drinking water nozzle and placed in 15 mL Falcon® tube for shipment to the analytical laboratory for arsenic analysis. This process will be repeated approximately once each week during the investigation.

TABLE 1: SUMMARY OF SWINE GROWTH CURVES OBSERVED

Study Day	Mean Body Weight (kg)	Food Portion (g) (twice/day)
-7	8.8	220
0	10.7	268
5	12.8	320
9	14.8	370
13	17.1	428
17	19.6	490
21	22.6	565
25	25.7	643

D. ANIMAL HEALTH EVALUATION

1.0 Swine chosen for each investigation will be monitored throughout the investigation to identify any evidence of disease. This monitoring program will consist of the following elements:

1.1 Daily observation by the PI or designated assistant, with consultation as needed by a board-certified food-animal clinician. Observations for each animal will be recorded daily on a health-status chart attached to the cage of each animal. Observations will be generally akin to the "SOAP" (subjective, objective, analysis, plan) process. If any interventive steps are taken for an animal (e.g., administration of antibiotics), this action shall also be recorded on the chart for that animal.

1.2 Any pig that dies during the study period will have a thorough post-mortem examination conducted to determine the cause of death. The post-mortem examination will include gross and histologic examinations and any ancillary tests, such as microbiology, deemed appropriate by the veterinary pathologist. All observations and findings will be recorded.

1.3 Medical records from the swine producer and the producer's veterinarian, including documentation of health status, will be available if needed to assess overall swine herd health, history of vaccinations, etc.

E. COLLECTION, PREPARATION, AND SHIPMENT OF TEST MATERIALS

1.0 SAMPLE SELECTION

The primary reason for testing site-specific samples in this study is to obtain information that will improve the accuracy of exposure and risk calculations. Therefore, it is expected that site samples will be selected to be representative of materials which are of current or potential future human health concern. Samples may either be from discrete locations, or may be composites from an area. Samples may either be relatively pure mineral or physical forms, or may be mixtures that are typical of mixtures found on site. It is recommended that the input of all concerned parties be considered before final sample selection.

2.0 SAMPLE COLLECTION

2.1 Target Concentration

The concentration of arsenic in the material submitted must be high enough to provoke a measurable response in the animal test system (if the arsenic is bioavailable). The most convenient concentration is 200 to 2,000 ppm, but higher or lower concentrations are acceptable.

However, no sample less than 200 ppm should be submitted without first discussing with the investigative group.

2.2 Amount Required

The target amount of material required is about 1 kg. Higher amounts may be needed if the concentration value is near the low end of the acceptable concentration range

3.0 SAMPLE COLLECTION AND PREPARATION

Samples will be collected according to the protocols developed for the VB-I70 Intensive Sampling QAPP (Attachment 1 to Appendix A).

4.0 SAMPLE LABELING

Each sample of test material must be labeled with the following information:

- Site name

- Sample description
- Sample collection date
- Initials of person collecting sample
- Arsenic concentration value (mg/kg)

6.0 SAMPLE SHIPPING

All packages will be opened by the PI or authorized staff following chain-of-custody procedures. Receipt of all samples or test chemicals will be recorded. Chain-of-custody forms will remain attached to all test samples.

7.0 Storage of Test Materials.

All test samples will be retained in their original shipment containers and stored in a secure room that is locked at all times except when it is being used for preparation of doses or samples, or other operations associated with performance of these investigations. Access to the locked room will be restricted to the PI or dedicated staff authorized by the PI.

F. PREPARATION AND ADMINISTRATION OF DOSES

1.0 INTRODUCTION

Administered doses of arsenic (ug/day) will be held constant during the study, even though body weight of the test animals is increasing.

2.0 ORAL EXPOSURE

2.1 Sodium Arsenate

Oral exposure to sodium arsenate is achieved by placing a small volume of sodium arsenate stock solution into a depression in a 5 g mass of moistened feed ("doughball"). After the stock solution has permeated into the doughball and no free liquid remains, the depression is filled by squeezing the dough ball in on itself, and the doughball is administered to the animal by hand feeding.

All animals in each dose group will receive the same volume of sodium arsenate stock solution, based on the mean body weight of all animals in the group. (The precise dose to each animal will subsequently be calculated from the individual measured body weights). Calculate the volume of stock solution to place in the dough balls of each dose group (twice each day) using

$$Vol = 0.5\left(\frac{MBW \bullet Dose}{Conc}\right)$$

the following equation:
where:

Vol = Volume of stock solution (uL)
MBW = Mean body weight (kg), adjusted as detailed in SOP 4
Dose = Target dose for the group (ug/kg-d)
Conc = Concentration of stock solution (ug/uL)

Three different stock solutions of sodium arsenate will be used as described in SOP 2. Choose Stock Solution A (10 ug/uL), Stock Solution B (2 ug/uL) or Stock Solution C (1 ug/uL) so that the volume of liquid added to the dough ball is at least 20 uL and not more than 100 uL.

EXAMPLE CALCULATIONS

	Example 1	Example 2	Example 3
Mean Body Weight (kg)	9.7	14.3	15.8
Target Dose (ug/kg)	25	50	125
Volume of A (uL)	12.1	35.8	98.8
Volume of B (uL)	60.6	178.8	493.8
Volume of C (uL)	121.3	357.5	987.6
Solution Selected	B (60.6 uL)	A (35.8 uL)	A (98.8 uL)

All volumes must be measured with an accurate adjustable micropipette using disposable plastic tips.

2.2 Soil Samples

Administration in "Doughballs"

Oral exposure to arsenic in test soil or mine waste is achieved by placing the required mass of the test material into a depression in a mass of moistened feed ("doughball"). The size of this doughball should be approximately 5 g (± 1 g). The depression is then filled by squeezing the doughball in on itself, trapping the test material in the center. Typically, all of the required mass of soil for each dose can be placed into a singe doughball. If the mass of soil required is too large to encapsulate into a single doughball, the mass of soil shall be divided into approximately equal portions and placed in the minimum number of doughballs reqired to contain the soil.

Calculation of Soil Mass

All animals in each dose group will receive the same mass of test material, based on the mean body weight of all animals within the dose group. (The precise dose to each animal will subsequently be calculated from the individual measured body weights). Calculate the mass of test material to administer to each dose group (twice each day) using the following equation:

$$Mass = 1/2\left(\frac{MBW \bullet Dose}{Conc}\right)(1000\ \mu g/mg)$$

where:

Mass = Mass of test material (mg)
MBW = Mean body weight (kg)

Dose = Target dose for the group (ug/kg-d)
Conc = Concentration of arsenic in the test material (ug/g or ppm)

A few example calculations are shown below. The calculations for each group on each day must be recorded.

	Example 1	Example 2	Example 3
Mean Body Weight (kg)	9.7	14.3	15.8
Target Dose (ug/kg)	25	50	125
Concentration As in soil (ppm)	500	1000	3000
Soil mass per dose (mg)	242.5	357.5	329.7

Soil doses must be weighed with a precision of at least ± 5%.

2.3 Soil Mixing Prior to Weighing

It is expected that the bulk soil sample will be non-homogeneous with respect to particle size, and the concentration and form of arsenic is expected to vary as a function of particle size. Therefore, it is important that the soil be well-mixed prior to removal of the dose aliquots. This is achieved by placing the bottle containing the bulk soil sample on a roller operating at low speed for about 30 minutes. After rolling, the bottle should be further mixed by inverting five times. It is important that vigorous methods of mixing not be used, since this might lead to a redistribution of the particle size distribution.

2.4 Oral Dose Verification Samples

At least one extra dough balls (or sets of doughballs, if more than one doughball is required to administer the soil) should be prepared for each dose "batch" (a "batch" is a group of doughballs, sufficient for three days administration to a particular set of animals). After all doughballs in the batch are prepared, select one at random, and place both in a Whirl-Pac plastic bag labelled with the appropriate "self-reading" sample identification number, as described in Section 1.2 of SOP 10. The adhesive sample label should be affixed to the plastic bag and covered with clear packaging tape to avoid loss of the labels during storage. [NOTE -- If it is determined that the tape/label method does not work, assigned sample labels will be written on the plastic bags with an indelible ink marker.] Store (archive) all potential dose verification samples in the freezer until the end of the study.

G. DATA RECORDING AND REPORTING

1.0 INTRODUCTION

Data that must be recorded and reported for each animal over the course of each study includes:

- Measured body weights
- Doses administered
- Samples collected
- Exceptions or variances from the planned protocol

For each investigation performed during these experiments, a log book will be provided for entry of these data items on the schedule required.

2.0 DAILY LOG RECORDING PROCEDURE

There will be one log book for each investigation, and this log book will contain one or more pages per day (as needed), beginning on the day the animals arrive at the facility. Each page will list the animals on test down the left side, and will provide space to record any data items scheduled for collection on that day. On days when biological samples are scheduled for collection, the logbook will specify the sample identification number to be attached to each sample after collection.

All data must be recorded daily on the appropriate page of the log book. All data must be recorded at the time each value is measured or observed. All entries must be in ink (preferably black). Take care to ensure entries are readily legible. In the event that a data entry error is made, the incorrect entry must be crossed out with a single line through the value and the correct value entered in an adjacent location. This change must be initialed and dated by the person making the change. If another person is present, have that person initial the change as well. An explanatory note giving a brief reason for the change must also be provided. The log book should also be used to record any problems, errors, exceptions or variances from the intended protocol, along with any explanatory notes or other comments.

3.0 DATA REVIEW AND SUBMITTAL

At the completion of data entry for a given day, the log book will be provided to the principle investigator or designated co-investigator for review and signature.

H. SAMPLE LABELING AND ASSIGNMENT OF SAMPLE NUMBERS

1.0 DECIPHERABLE LABELS

All samples collected during this study will be assigned a unique label that can be readily deciphered. The nomenclature for labels assigned to biological samples and dose verification samples are detailed below.

1.1 Biological Samples

Each biological sample (blood, tissue, bone) label will have 5 elements, as follows:

PHASE. All labels will begin with a two letter code to indicate from which study the sample is derived (e.g., XX-).

INVESTIGATION NUMBER. The next element of the label will be the investigation number for a given phase. Investigations will be numbered sequentially using Arabic numerals. The number of each investigation will also be clearly indicated in the study protocol.

ANIMAL NUMBER. The next element of each label is the unique identification number assigned to each animal at the start of each investigation (see Section B).

TREATMENT DAY. The next element of the label is the day of the investigation on which the sample was collected. Day zero is the first day of dosing/treatment. Samples collected before dosing should be identified with the label "-" (e.g., -7 means 7 days before exposure/treatment begins).

SAMPLE TYPE. The final element of the sample label is the sample type, using the following codes: U = urine and P = feces. Other codes can be used as needed if other sample types are prepared or collected.

Thus, a sample labeled "XX-3 317-4-U" would be a sample of urine collected from animal 317 on the 4th day of investigation 3 in Phase XX. Likewise, a sample labeled "XX-2 283-15-P" would be a sample of feces collected from animal 283 on the 15th day of investigation 2 in Phase XX.

1.2 Dose Verification Samples

Two extra dough balls will be prepared for each three-day batch for each dose group for possible use as dose verification samples. Both extra doughballs from each group should be placed in individual plastic bags, and assigned labels using a code system similar to that above. However, substitute the group/treatment number for the animal number. Also, since the doughballs are prepared in batches adequate for three days, give the range of days rather than a discrete day. Thus, a bag labeled "XX-4 Grp5-6to8-DV1" would identify the first dose verification sample for Group 5 on days 6, 7, and 8 of Investigation 4 in Phase XX.

2.0 SAMPLE IDENTIFICATION NUMBERS

Analysis of all samples sent to the laboratory is blind, except for identification of the sample matrix. Therefore, each sample is assigned a non-decipherable sample number. As an added level of protection against error, the archive sample (e.g. whole blood, tissue sample) that is used to prepare a sample for analysis will be labeled with the same sample number that is assigned to the prepared sample, except the suffix "-AS" ("archive sample") will be included.

3.0 SELF-ADHESIVE LABELS

As noted above, all sample labels and sample numbers will be pre-assigned and listed in the laboratory notebook. A hypothetical example of such a label sheet is shown in Figure 1. As shown, the labels are arranged from top to bottom in order of cage number. This is because sample collection will proceed in this order.

FIGURE 1
EXAMPLE LABEL SHEET

US EPA REGION 8 SWINE BIOAVAILABILITY STUDY -- PHASE XX
INVESTIGATION 3, DAY 5

Cage No	Pig No.	Sample Label	Sample Number		
			Archive	ESD	Splits
1	337	XX-3 337-5-U	8-930567-AS	8-930567	--
2	318	XX-3 318-5-U	8-930594-AS	8-930594	8-930567CDC 8-930567PRP
3	355	XX-3 355-5-U	8-930561-AS	8-930561	--
4	314	XX-3 314-5-U	8-930577-AS	8-930577	--
5	311	XX-3 311-5-U	8-930582-AS	8-930582	8-930582CDC 8-930582PRP
6	305	XX-3 305-5-U	8-930575-AS	8-930575	--
etc					
etc					

I. PREPARATION OF SAMPLES FOR ANALYSIS

Samples of water and feed will be prepared for shipment to the analytical laboratory as follows.

1. Water:

    a. Random samples of drinking water (approximately one per week) will be placed in 15 mL Falcon® tubes and shipped unprocessed to the analytical laboratory.

    b. Random samples (approximately one per week) of the double distilled water used to prepare samples and reagents will be placed in 15 mL Falcon® tubes and shipped unprocessed to the analytical laboratory.

2. Feed:

    a. 0.50 gram sub-samples of feed will be placed into a Teflon container and 5 ml of 70% nitric acid added. The digest will be brought to a 25 mL volume with double distilled water.

b. Two gram portions of feed not utilized for analysis will be stored at ca. -10°C for potential future reanalysis.

3. Dose Verification Samples:

As described in SOP 4, two extra "doughballs" will be prepared for each dose group for each three-day dosing period. Doughballs selected for analysis will be prepared and analyzed.

J. CHAIN OF CUSTODY FORMS AND PROCEDURES

All samples collected during this study must be accounted for and traceable from the time of collection through analysis. A chain of custody (COC) form is created at the time that samples are originally collected, and this form must accompany the samples during each step of the preparation and analysis sequence.

1.0 Custody of Samples at the Animal Testing Facility

Collection of Primary Samples

Each day that a group of samples is collected, a chain of custody form should be filled out to accompany those samples. These forms will be pre-printed prior to each investigation, based on the detailed protocol for that investigation. Each form will contain a unique identification number. These forms contain the following information:

- The Phase and Investigation Number
- A list of the sample numbers on all samples collected
- The date each sample was collected
- The type of sample (blood, liver, kidney, bone, water, etc.)

An example form is shown in Figure 1. The person responsible for collecting the samples should carefully review the pre-printed sheet to ensure that the samples collected match the list on the COC form. Any incorrect entries should be changed by drawing a single line through the entry and entering the correct information adjacent. All entries and changes must be made in ink, dated, and initialed. When all entries are correct, the sheet should be signed and dated.

Transfer to the Laboratory Technician

Most primary samples (blood, tissue, bone) require preparation before being sent to the analytical laboratory. When the samples are transferred from the person who collected the samples to the person responsible for preparing the samples, this transfer should be recorded on the COC sheet. The technician who receives the samples is responsible for checking to ensure that all samples on the COC form are actually provided and are in good condition. Any exceptions should be noted on the form.

Security of Primary and Prepared Samples

The PI is responsible for ensuring that all primary and analytical samples generated at the animal facility are maintained in a secure location and that no one has access to the samples except the PI or staff authorized by the PI

2.0 Transfer of Samples from the Animal Facility to Other Locations

Whenever samples are sent from the animal facility to the analytical laboratory or any other location, a careful record of this transfer must be kept. The person who sends the package is responsible for ensuring that the contents of the package and the COC forms are in agreement. All samples and the accompanying COC forms must be securely enclosed in a shipping container, and this container must be sealed with an EPA custody seal. The EPA custody seal should be over-wrapped with clean packing tape to ensure the seal is not broken accidentally during shipment. A copy of all COC forms sent to offsite locations is maintained by the facility.

Whenever a shipment of samples is being sent to the analytical laboratory, the person sending the package should call ahead to notify the contract Laboratory of the time which samples will be shipped and the expected arrival date.

3.0 Receipt and Custody of Samples at the Analytical Laboratory

Responsibilities

The analytical laboratory will have a designated Sample Custodian who is responsible for insuring compliance with the provisions of this SOP. The lab will also designate an Alternate Sample Custodian. Any laboratory employee may receive samples, provided they follow the provisions of this SOP.

Sample Receipt

Samples are received from two main sources. These are direct delivery (hand carried) from the sampling team or delivered by a third party carrier (e.g. Federal Express).

> For hand carried samples, the sampling team member will deliver the samples and chain-of-custody documents to the receiving lab employee. The sampler will remain during the opening and inspection process.
>
> When third party delivery occurs, the shipping container is received and secured until opening. It is customary for the shipper to require a signed receipt form. A copy of this receipt is included in the custody record which becomes part of the final data package.

Opening the shipping container and inspecting the contents.

WARNING: It is possible for sample spillage, leaking containers or sample adhering to containers to pose health problems. The receiver must determine the proper level of personal protection required. A lab coat, gloves and goggles represent a minimum protective level for all persons present. Additional protection may include the use of a respirator or fume hood while opening and inspecting the shipment. The room selected for sample receipt must contain a fume hood and allow for easy containment and clean-up in case of spills.

Before opening the shipping container, it should be inspected for signs of damage. Note the condition of custody seals and open the shipping container. If third party shipping was used, the chain-of-custody (COC) forms should be located inside the container. The condition of the container and custody seals are now noted on the COC forms.

Remove each container in the shipment and check for damage, spillage or leakage. Spillage from a broken or leaking sample should be treated with an appropriate absorbent. Dispose of the absorbent and any broken containers according to Hazardous Waste Management procedures.

Cross-reference the sample tag with the sample identification on the COC forms. Any discrepancies including missing or mis-labeled samples, spillage or broken containers should be noted on the COC. It may be possible to identify samples with missing or illegible tags through some other characteristic. This should be carefully recorded on a separate document. This document will become part of the case narrative which accompanies the final report. Any discrepancies should also be verbally reported to the site project officer. Samples which can not be uniquely identified will not be analyzed, unless so directed by the analytical lab manager.

Samples are grouped by type of matrix and placed in secondary containers appropriate to the sample size and type. Each sample is checked for accuracy of its sample tag/label vs. entries on the COC form. The date and time of receipt are entered in the proper boxes of the COC form. The receiver then signs the box "Received at Laboratory by:". Any additional comments are entered at this time in the comments section of the form.

Sample Storage

In some cases, the sample receiver may distribute the required samples (or fractions) to the appropriate analytical section for immediate analysis. In all other cases, the samples are segregated by parameter and locked in cold storage.

Controlled sample access is required during their storage period. Samples are deemed to be in custody during their residence at the laboratory.

Sample log-in and assignment of analyses

After securing the samples, the sample receiver gives the chain-of-custody forms, Lab Service Requests and sampler's notes to the Laboratory Information Management System (LIMS) manager. He/she then enters relevant sample information into the LIMS. The manager then makes copies of the COC forms for each analytical group involved and highlights the parameters relevant to each group. These copies are then distributed to the analysts. The originals go into the site project file.

Sample analysis

When the analyst is ready to begin sample processing, he or she obtains the appropriate key from the sample custodian. After removal of samples, the cooler is immediately locked and the key returned to the sample custodian. After sample extraction or analysis, unused portions are returned to the the locked refrigerated storage area.

Sample disposal

Sample remainders are kept in the locked refrigerated storage for a minimum of three years after the final report is sent to the data user and approved by the Quality Assurance Office (QAU). At any time after this three years, the Sample Custodian in consultation with the data user may designate the samples for disposal.

Transmittal of documents

When analyses are complete, the analytical results, letter of transmittal, chain-of-custody forms, LSRs and samplers notes will be sent to the data user. Copies of these documents and laboratory raw data will be kept in laboratory files for at least ten years.

FIGURE 1
CHAIN OF CUSTODY FORM **COC XXX**

ARSENIC RELATIVE BIOAVAILABILITY STUDY
Investigation 3, Day 7

Samples Collected By:
Signature Date

Index	Sample No.	Date	Matrix	Analytes	Remarks
1	8-931561	9/10/10	Urine	Arsenic	
2	8-931562	9/10/10	Urine	Arsenic	
3	8-931563	9/10/10	Urine	Arsenic	
4	8-931564	9/10/10	Urine	Arsenic	
5	8-931565	9/10/10	Urine	Arsenic	
6	8-931566	9/10/10	Urine	Arsenic	
7	8-931567	9/10/10	Urine	Arsenic	
8	8-931568	9/10/10	Urine	Arsenic	
9	8-931569	9/10/10	Urine	Arsenic	
10	8-931570	9/10/10	Urine	Arsenic	
11	8-931571	9/10/10	Urine	Arsenic	
12	8-931572	9/10/10	Urine	Arsenic	
13	8-931573	9/10/10	Urine	Arsenic	
14	8-931574	9/10/10	Urine	Arsenic	
15	8-931575	9/10/10	Urine	Arsenic	
16	8-931576	9/10/10	Urine	Arsenic	
17	8-931577	9/10/10	Urine	Arsenic	
18	8-931578	9/10/10	Urine	Arsenic	
19	8-931579	9/10/10	Urine	Arsenic	
20	8-931580	9/10/10	Urine	Arsenic	
21	8-931581	9/10/10	Urine	Arsenic	
22	8-931582	9/10/10	Urine	Arsenic	
23	8-931583	9/10/10	Urine	Arsenic	
24	8-931584	9/10/10	Urine	Arsenic	
25	8-931585	9/10/10	Urine	Arsenic	

TRANSFER OF CUSTODY RECORD

Transfer	Relinquished by: Signature Date/Time	Received by: Signature Date/Time
1		
2		
3		
4		

K. PREPARATION AND ANALYSIS OF DOSE VERIFICATION SAMPLES

1.0 INTRODUCTION

This SOP provides a detailed description of the methods to be used to digest and analyze dose verification samples (approximately 5 g portions of feed mixed with various amounts of either sodium arsenate or test material) from these investigations. The SOP addresses different sample preparation techniques that may be necessary to provide complete dissolution of the arsenic species present in materials added to the feed portions. This SOP is written as a guideline for an experienced chemist. Many routine details or techniques of sample preparation are not reiterated in this document. Minor method changes may be dictated by sample-type variations and should only be made by experienced laboratory personnel.

2.0 SAMPLE HANDLING AND PRESERVATION

Samples will be received as frozen "doughballs" contained in a plastic bag. Prior to preparation, samples should be kept in their shipping containers to maintain integrity. Samples should be kept frozen until analysis to avoid possible loss of analyte by sample flow.

3.0 APPARATUS AND MATERIALS

3.1 Equipment

The following equipment items are required for sample preparation:

- Muffle furnace, capable of maintaining temperatures in the range of 400 - 500 degrees Centigrade.
- Ceramic crucibles.
- Erlenmeyer flasks, 125 or 250 mL, with watch glasses or small funnels for covers.
- Reagent dispensers or pipettes to deliver reagents at volumes and accuracies discussed below.
- Hotplate with variable temperature controller.
- Miscellaneous laboratory materials and equipment including volumetric glassware, sample digestate containers, water wash bottles, reagent grade water source, and protective clothing and paraphernalia.

3.2 Reagents

The following reagents are required for sample preparation:

- Nitric Acid (HNO_3), concentrated (c. 60%).
- Hydrogen peroxide (H_2O_2), concentrated (c. 30%).
- Reagent grade water (DI water), to meet or exceed specified purity.
- Stock solutions of known, certified elemental concentrations for sample spike preparation.
- High purity cellulose fiber (filter and ashing aid material).

4.0 HANDLING AND DIGESTION PROCEDURES

4.1 Sample Transfer

Each swine feed portion ("doughball") is a unit sample. The entire sample must be digested to ensure complete metals recoveries. If the sample has been allowed to thaw during shipping or storage, care must be taken to ensure that all phases of the sample are quantitatively transferred to the digestion flask. Oil and moisture separations have been noted in thawed samples.

4.2 Sample Digestion

The method outlined below should yield a digestate amenable to GFAA or ICP analyses of arsenic and other common metals of environmental interest. Two digestion schemes are provided. One is followed for samples spiked with soluble arsenic salts and the other for samples which contain added soil or soil-like components. Some variations of reagent quantities, digestion times, and final dilution volumes may be required to produce a suitable analytical matrix. It is suggested that preliminary digestion trials of anticipated sample types be performed prior to actual analyses to evaluate the applicability of the general method.

1. About 20 mL of de-ionized (DI) water is added to the transferred and thawed sample in a 250 mL erlenmeyer flask. Twenty-five mL of concentrated nitric acid is added and the slurried sample is allowed to stand several hours or over-night at room temperature.

2. With a small glass funnel (or small watch-glass cover) in the neck of the flask, to provide reflux action, the sample is heated on a hotplate until the majority of the organic matrix is disrupted and digested. The digestate should have a pale yellow to clear appearance at that point.

3. The funnel is removed to allow the aqueous nitric acid phase to reduce to about 10 mL. The flask is removed from the hotplate and cooled.

4. Two mL of 30% hydrogen peroxide is added and the flask is returned to the hotplate to initiate the oxidation step. Additional aliquots of peroxide are added (up to 30 mL) as needed to complete the destruction of organic material.

5. After cooling, about 20 mL of DI water and 5 - 10 mL of nitric acid are added to each flask and the contents are heated to near boiling.

At this point, samples containing soil or other soil-like test material should be set aside and handled as described in steps 6A - 8A.

6. The cooled digestate is diluted to a suitable volume for analysis. A minimum volume of 500 mL is suggested to avoid potential solubility problems.

7. Filtration of the finished digestate prior to GFAA or ICP analysis is suggested.

If the sample contains soil or soil-like material (slag, waste rock, etc), follow steps 6A to 8A, below.

6A. The soil-spiked sample digestates from step 5 (above) are filtered through Whatman 40 (or other ashless equivalent) filter papers. The filtrate and water wash aliquots are diluted to a suitable final volume. The filter paper and residue is ashed in a porcelain crucible at a final maximum temperature of 450 degrees centigrade. Cellulose filter-aid is recommended to facilitate this step.

7A. The ash residue is transferred to a digestion flask. Five mL of water and 5 mL of concentrated nitric acid is added to the sample. The sample is heated on a hotplate to reduce the volume to 3 - 5 mL. Five mL of water and two mL of hydrogen peroxide are added to the cooled sample. The treated samples are returned to the hotplate to initiate the peroxide reaction. Subsequent portions of peroxide may be added if required.

8A. The cooled samples are diluted to final volume for analysis.

5.0 QUALITY CONTROL

Contamination from handling, glassware, or reagents is monitored by the examination of a DI water blank sample digested in the same fashion as the samples. As the entire sample is consumed during sample preparation, no duplicate or matrix spike samples are possible.

6.0 DOCUMENTATION AND DATA HANDLING

Swine feed samples will be identified throughout the preparation and analysis steps by the sample number.

The concentration results of analysis will be converted to total micrograms of arsenic per sample. In the case of soil-bearing samples, the reported value will be the sum of the masses of arsenic measured from each digestate part.

7.0 REFERENCES

Graphite Furnace AAS, A Source Book, Walter Slavin, 1984, Perkin-Elmer Corp., Norwalk CT 06856

Standard Operating Procedure: Perkin Elmer 5100 Graphite Furnace Atomic Absorption Spectrophotometer, ESAT SOP AI 02, 09/92.

L. COLLECTION AND PREPARATION OF URINE SAMPLES FOR ARSENIC ANALYSIS

1.0 INTRODUCTION

Several studies suggest that arsenic as well as lead may be absorbed less extensively from soils and mine wastes than from aqueous solutions. Because absorbed arsenic is excreted mainly (about 60-80%) in urine, an excellent endpoint for monitoring arsenic exposure is the arsenic concentration in a timed urine void. The ratios of the amount of arsenic excreted in a given time period to the amounts dosed can be used to estimate the amounts absorbed over the dose range given.

2.0 URINE COLLECTION

Animals will be quartered in metabolism cages to facilitate urine collections. These cages will have a fine-mesh screen placed between the coarse-mesh bottom of the cage above and the collecting pan below to prevent fecal contamination of urine samples. The V-shaped collecting pan will be sloped so that all urine will drain to the central site where a plastic catch-container will be placed. Cage flooring will be situated to prohibit drinking-water contamination.

Urine collection will begin at about 8:00 AM on the days specified in the protocol, and will end 48 hours later. During the 48-hour collection period, urine will be removed from the collection pans at least twice daily and stored in a separate container for each animal. Therefore at the end of the 48-hour period, all collected urine will be located in one container and can be addressed as to the total volume of urine collected.

2.1 SAMPLE PRESERVATION

The 48-hour urine volume samples will be mixed by swirling in the collection vessels and the volume measured by transfer into a graduated cylinder. Three 60 mL aliquots of urine will be retrieved from the 48-hour urine volume samples, placed in capped plastic urine storage bottles and acidified by addition of 0.6 mL of concentrated nitric acid. One bottle will be maintained in the refrigerator as the archive sample, while the second bottle will be sent to the laboratory for arsenic analysis.

M. QA/QC CHECKS

Complete the following table to summarize QA/QC checks.

Matrix	Measurement	QA/QC Check[1]	Frequency	Acceptance Criteria	Corrective Action
All	As	CCS	10 Samples	90-110%	Rerun/Reprep
All	As	CCB	10 Samples	Less than D.L.	Rerun/Reprep
All	As	Blanks	5%	Less than D.L.	Rerun/Reprep
All	As	Duplicates	10%	0-20%	Rerun/Reprep
All	As	Spikes	10%	80-120%	Rerun/Reprep
All	As	Ref. Samples	5%	80-120%	Rerun/Reprep

[1]Include all QA/QC checks (experimental and analytical, as applicable) for accuracy, precision, detection limits, mass balance, *etc*.
(*e.g.*, matrix spikes, lab control samples, blanks, replicates, surrogates)

Date: May 2009 **SOP No. # 14**

Title: *Standard Operating Procedures for Metals Determination by ICP-AES*

Associated Investigator: John Quinn**,** The Environmental Chemistry Laboratory, California Department of Toxic Substances Control

Total Pages 57

SYNOPSIS: The Environmental Chemistry Laboratory at Berkeley, California, follows SW-846 preparation method 3050B and determinative method 6010C for metals analyses in soils and solid matrices with the following modification:

Digestion of samples includes both sections 7.2 and 7.3 of method 3050B. Samples are digested with 1:1 Nitric acid, 30% Hydrogen Peroxide, and 1:1 Hydrochloric acid. The calibration and QC checks for method 6010C are summarized in the table below.

Methods 3050B and 6010C from Test America, Pleasanton are also included. Test America is ECL's subcontrator and it is likely that they will perform the analysis for DTSC.

Table 1: Calibration & QC Procedures for ICP Metals - Method SW6010C

Method	Applicable Parameter	QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action
SW6010C	ICP Metals	Choice of 1: Daily check using blank and one high standard, or 2:Blank and three nonzero standards.	Daily initial calibration prior to sample analysis	Linear regression correlation – coefficient r > 0.998	Correct problem, then repeat initial calibration
		Initial midpoint calibration verification (ICV) and low calibration verification(LLICV)	Daily after initial calibration	All analytes within +/- 10% of expected value; LLICV +/- 30% of expected value	Correct problem, then repeat initial calibration
		Calibration Blank	After every calibration verification and at the end of the analysis batch	No analytes detected > RL	Correct problem, then analyze calibration blank and previous 10 samples
		Continuing Calibration Verification (CCV) Standard Continuing low level calibration verification standard (LLCCV)	After every 10 samples and at the end of the analysis sequence	All analyte(s) within +/- 10% of expected value and RSD of replicate integrations <5%; LLCCV acceptance is at +/-30% of true value	Repeat calibration and reanalyze all samples since last successful calibration verification
		Interference check solution (ICS-AB)	At the beginning and end of an analytical run and every 8 hours	Within +/- 20% of expected value	Terminate analysis; correct problem; reanalyze ICS; reanalyze all affected samples
		Method blank	One per digestion batch	No analytes detected > RL	Correct problem, then redigest and analyze method blank and all samples in affected blank
		LCS for the analyte	One LCS per digestion batch	QC acceptance criteria for project or 80 to 120% recovery	Correct problem, then reprep and analyze the LCS and all samples in the affected batch
		MS/MSD	One MS/MSD per every 20 project samples per matrix	QC acceptance criteria for project or 70 to 130 % recovery 20% RPD	None
		Serial Dilution	One per digestion batch	1:5 dilution should agree within +/- 10% of the original determination	

INDUCTIVELY COUPLED PLASMA-ATOMIC EMISSION SPECTROMETRY

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required method use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique which a laboratory can use as a basic starting point for generating its own detailed Standard Operating Procedure (SOP), either for its own general use or for a specific project application. The performance data included in this method are for guidance purposes only, and are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

1.0 SCOPE AND APPLICATION

1.1 Inductively coupled plasma-atomic emission spectrometry (ICP-AES) may be used to determine trace elements in solution. With the exception of groundwater samples, all aqueous and solid matrices need acid digestion prior to analysis. Groundwater samples that were prefiltered and acidified will not need acid digestion. Samples which are not digested need either an internal standard or should be matrix-matched with the standards. If either option is used, instrument software should be programmed to correct for intensity differences of the internal standard between samples and standards. Refer to Chapter Three, "Inorganic Analytes," for a listing of digestion procedures that may be appropriate. The following analytes have been determined by this method:

Element	Symbol	CAS Number	Element	Symbol	CAS Number
Aluminum	Al	7429-90-5	Mercury	Hg	7439-97-6
Antimony	Sb	7440-36-0	Molybdenum	Mo	7439-98-7
Arsenic	As	7440-38-2	Nickel	Ni	7440-02-0
Barium	Ba	7440-39-3	Phosphorus	P	7723-14-0
Beryllium	Be	7440-41-7	Potassium	K	7440-09-7
Boron	B	7440-42-8	Selenium	Se	7782-49-2
Cadmium	Cd	7440-43-9	Silica	SiO_2	7631-86-9
Calcium	Ca	7440-70-2	Silver	Ag	7440-22-4
Chromium	Cr	7440-47-3	Sodium	Na	7440-23-5
Cobalt	Co	7440-48-4	Strotium	Sr	7440-24-6
Copper	Cu	7440-50-8	Thallium	TI	7440-28-0
Iron	Fe	7439-89-6	Tin	Sn	7440-31-5
Lead	Pb	7439-92-1	Titanium	Ti	7440-32-6
Lithium	Li	7439-93-2	Vanadium	V	7440-62-2

Element	Symbol	CAS Number	Element	Symbol	CAS Number
Magnesium	Mg	7439-95-4	Zinc	Zn	7440-66-6
Manganese	Mn	7439-96-5			

CAS Number: Chemical Abstract Service Registry Number.

1.2 Table 1 lists all of the elements for which this method was validated. The sensitivity and the optimum and linear ranges for each element will vary with the wavelength, spectrometer, matrix, and operating conditions. Table 1 lists the recommended analytical wavelengths and estimated instrumental detection limits (IDLs) for the elements in clean aqueous matrices with insignificant background interferences. Other elements and matrices may be analyzed by this method if appropriate performance at the concentrations of interest (see Sec. 9.0) is demonstrated.

1.3 Analysts should clearly understand the data quality objectives prior to analysis and must document and have on file the required initial demonstration performance data described in the following sections prior to using the method for analysis.

1.4 Prior to employing this method, analysts are advised to consult the each preparative method that may be employed in the overall analysis (e.g., a 3000 series method) for additional information on quality control procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives for the intended application.

1.5 Use of this method is restricted to use by, or under supervision of, spectroscopists appropriately experienced and trained in the correction of spectral, chemical, and physical interferences described in this method. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Prior to analysis, samples must be solubilized or digested using the appropriate sample preparation methods (see Chapter Three). When analyzing groundwater samples for dissolved constituents, acid digestion is not necessary if the samples are filtered and acid preserved prior to analysis (refer to Sec. 1.1).

2.2 This method describes multielemental determinations by ICP-AES using sequential or simultaneous optical systems and axial or radial viewing of the plasma. The instrument measures characteristic emission spectra by optical spectrometry. Samples are nebulized and the resulting aerosol is transported to the plasma torch. Element-specific emission spectra are produced by a radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer, and the intensities of the emission lines are monitored by photosensitive devices.

2.3 Background correction is required for trace element determination. Background emission must be measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used should be as free as possible from spectral interference and should reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result. The possibility of additional interferences identified in Sec. 4.0 should also be recognized and appropriate corrections made; tests for their presence are described in Secs. 9.6 and 9.7. Alternatively, users may choose multivariate calibration methods. In this case, point selections for background correction are superfluous since whole spectral regions are processed.

3.0 DEFINITIONS

Refer to Chapter One, Chapter Three, and the manufacturer's instructions for definitions that may be relevant to this procedure.

4.0 INTERFERENCES

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be necessary. Refer to each method to be used for specific guidance on quality control procedures and to Chapter Three for general guidance on the cleaning of glassware. Also refer to the preparative methods to be used for discussions on interferences.

4.2 Spectral interferences are caused by background emission from continuous or recombination phenomena, stray light from the line emission of high concentration elements, overlap of a spectral line from another element, or unresolved overlap of molecular band spectra.

4.2.1 Compensation for background emission and stray light can usually be conducted by subtracting the background emission determined by measurements adjacent to the analyte wavelength peak. Spectral scans of samples or single element solutions in the analyte regions may indicate when alternate wavelengths are desirable because of severe spectral interference. These scans will also show whether the most appropriate estimate of the background emission is provided by an interpolation from measurements on both sides of the wavelength peak or by measured emission on only one side. The locations selected for the measurement of background intensity will be determined by the complexity of the spectrum adjacent to the wavelength peak. The locations used for routine measurement must be free of off-line spectral interference (interelement or molecular) or adequately corrected to reflect the same change in background intensity as occurs at the wavelength peak. For multivariate methods using whole spectral regions, background scans should be included in the correction algorithm. Off-line spectral interferences are handled by including spectra on interfering species in the algorithm.

4.2.2 To determine the appropriate location for off-line background correction, the user must scan the area on either side adjacent to the wavelength and record the apparent emission intensity from all other method analytes. This spectral information must

be documented and kept on file. The location selected for background correction must be either free of off-line interelement spectral interference or a computer routine must be used for automatic correction on all determinations. If a wavelength other than the recommended wavelength is used, the analyst must determine and document both the overlapping and nearby spectral interference effects from all method analytes and common elements and provide for their automatic correction on all analyses. Tests to determine spectral interference must be done using analyte concentrations that will adequately describe the interference. Normally, 100 mg/L single-element solutions are sufficient. However, for analytes such as iron that may be found in the sample at high concentration, a more appropriate test would be to use a concentration near the upper limit of the analytical range (refer to Chapter Three).

4.2.3 Spectral overlaps may be avoided by using an alternate wavelength or can be compensated for by equations that correct for interelement contributions. Instruments that use equations for interelement correction require that the interfering elements be analyzed at the same time as the element of interest. When operative and uncorrected, interferences will produce false positive or positively biased determinations. More extensive information on interferant effects at various wavelengths and resolutions is available in reference wavelength tables and books. Users may apply interelement correction equations determined on their instruments with tested concentration ranges to compensate (off-line or on-line) for the effects of interfering elements. Some potential spectral interferences observed for the recommended wavelengths are given in Table 2. For multivariate calibration methods using whole spectral regions, spectral interferences are handled by including spectra of the interfering elements in the algorithm. The interferences listed are only those that occur between method analytes. Only interferences of a direct overlap nature are listed. These overlaps were observed with a single instrument having a working resolution of 0.035 nm.

4.2.4 When using interelement correction equations, the interference may be expressed as analyte concentration equivalents (i.e., false positive analyte concentrations) arising from 100 mg/L of the interference element. For example, if As is to be determined at 193.696 nm in a sample containing approximately 10 mg/L of Al, according to Table 2, 100 mg/L of Al will yield a false positive signal for an As level equivalent to approximately 1.3 mg/L. Therefore, the presence of 10 mg/L of Al will result in a false positive signal for As equivalent to approximately 0.13 mg/L. The user is cautioned that other instruments may exhibit somewhat different levels of interference than those shown in Table 2. These data are provided for guidance purposes only. The interference effects must be evaluated for each individual instrument, since the intensities will vary.

4.2.5 Interelement corrections will vary for the same emission line among instruments because of differences in resolution, as determined by the grating, the entrance and exit slit widths, and by the order of dispersion. Interelement corrections will also vary depending upon the choice of background correction points. Selecting a background correction point where an interfering emission line may appear should be avoided when practical. Interelement corrections that constitute a major portion of an emission signal may not yield accurate data. Users should continuously note that some samples may contain uncommon elements that could contribute spectral interferences.

4.2.6 The interference effects must be evaluated for each individual instrument, whether configured as a sequential or simultaneous instrument. For each instrument, intensities will vary not only with optical resolution but also with operating conditions (such as power, viewing height and argon flow rate). When using the recommended wavelengths, the analyst is required to determine and document for each wavelength the effect from referenced interferences (Table 2) as well as any other suspected

interferences that may be specific to the instrument or matrix. The analyst is encouraged to utilize a computer routine for automatic correction on all analyses.

4.2.7 Users of sequential instruments must verify the absence of spectral interference by scanning over a range of 0.5 nm centered on the wavelength of interest for several samples. The range for lead, for example, would be from 220.6 to 220.1 nm. This procedure must be repeated whenever a new matrix is to be analyzed and when a new calibration curve using different instrumental conditions is to be prepared. Samples that show an elevated background emission across the range may be background corrected by applying a correction factor equal to the emission adjacent to the line or at two points on either side of the line and interpolating between them. An alternate wavelength that does not exhibit a background shift or spectral overlap may also be used.

4.2.8 If the correction routine is operating properly, the determined apparent analyte(s) concentration from analysis of each interference solution should fall within a specific concentration range around the calibration blank. The concentration range is calculated by multiplying the concentration of the interfering element by the value of the correction factor being tested and dividing by 10. If after the subtraction of the calibration blank the apparent analyte concentration falls outside of this range, in either a positive or negative direction, a change in the correction factor of more than 10% should be suspected. The cause of the change should be determined and corrected and the correction factor updated. The interference check solutions should be analyzed more than once to confirm a change has occurred. Adequate rinse time between solutions and before analysis of the calibration blank will assist in the confirmation.

4.2.9 When interelement corrections are applied, their accuracy should be verified daily, by analyzing spectral interference check solutions. The correction factors or multivariate correction matrices tested on a daily basis must be within the 20% criteria for five consecutive days. All interelement spectral correction factors or multivariate correction matrices must be verified and updated every six months or when an instrumentation change occurs, such as one in the torch, nebulizer, injector, or plasma conditions. Standard solutions should be inspected to ensure that there is no contamination that may be perceived as a spectral interference.

4.2.10 When interelement corrections are <u>not</u> used, verification of absence of interferences is required.

4.2.10.1 One method to verify the absence of interferences is to use a computer software routine for comparing the determinative data to established limits for notifying the analyst when an interfering element is detected in the sample at a concentration that will produce either an apparent false positive concentration (i.e., greater than the analyte instrument detection limit), or a false negative analyte concentration (i.e., less than the lower control limit of the calibration blank defined for a 99% confidence interval).

4.2.10.2 Another way to verify the absence of interferences is to analyze an interference check solution which contains similar concentrations of the major components of the samples (>10 mg/L) on a continuing basis to verify the absence of effects at the wavelengths selected. These data must be kept on file with the sample analysis data. If the check solution confirms an operative interference that is $20% of the analyte concentration, the analyte must be determined using (1) analytical and background correction wavelengths (or spectral regions) free of the interference, (2) by an alternative wavelength, or (3) by another documented test procedure.

4.3 Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they must be reduced by diluting the sample, by using a peristaltic pump, by using an internal standard, or by using a high solids nebulizer. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, affecting aerosol flow rate and causing instrumental drift. The problem can be controlled by wetting the argon prior to nebulization, by using a tip washer, by using a high solids nebulizer, or by diluting the sample. Also, it has been reported that better control of the argon flow rate, especially to the nebulizer, improves instrument performance. This may be accomplished with the use of mass flow controllers. The test described in Sec. 9.9 will help determine if a physical interference is present.

4.4 Chemical interferences include molecular compound formation, ionization effects, and solute vaporization effects. Normally, these effects are not significant with the ICP technique, but if observed, can be minimized by careful selection of operating conditions (incident power, observation position, and so forth), by buffering of the sample, by matrix matching, and by standard addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element. The analyst is encouraged to review the information in all of Sec. 4.0 to deal with the majority of interferences likely to be encountered when using this method.

4.4.1 The method of standard additions (MSA) can be useful when certain interferences are encountered. Refer to Method 7000 for a more detailed discussion of the MSA.

4.4.2 An alternative to using the method of standard additions is to use the internal standard technique, which involves adding one or more elements that are both not found in the samples and verified to not cause an interelement spectral interference to the samples, standards, and blanks. Yttrium or scandium are often used. The concentration should be sufficient for optimum precision, but not so high as to alter the salt concentration of the matrix. The element intensity is used by the instrument as an internal standard to ratio the analyte intensity signals for both calibration and quantitation. This technique is very useful in overcoming matrix interferences, especially in high solids matrices.

4.5 Memory interferences result when analytes in a previous sample contribute to the signals measured in a new sample. Memory effects can result from sample deposition on the uptake tubing to the nebulizer and from the build up of sample material in the plasma torch and spray chamber. The site where these effects occur is dependent on the element and can be minimized by flushing the system with a rinse blank between samples. The possibility of memory interferences should be recognized within an analytical run and suitable rinse times should be used to reduce them. The rinse times necessary for a particular element must be estimated prior to analysis. This may be achieved by aspirating a standard containing elements at a concentration ten times the usual amount or at the top of the linear dynamic range. The aspiration time for this sample should be the same as a normal sample analysis period, followed by analysis of the rinse blank at designated intervals. Note the length of time necessary for reducing analyte signals to "equal to" or "less than" the lower limit of quantitation. Until the required rinse time is established, the rinse period should be at least 60 sec between samples and standards. If a memory interference is suspected, the sample must be reanalyzed after a rinse period of sufficient length. Alternate rinse times may be established by the analyst based upon the project-specific DQOs.

4.6 Users are advised that high salt concentrations can cause analyte signal suppressions and confuse interference tests. If the instrument does not display negative

values, fortify the interference check solution with the elements of interest at 0.5 to 1 mg/L and measure the added standard concentration accordingly. Concentrations should be within 20% of the true spiked concentration or dilution of the samples will be necessary. In the absence of a measurable analyte, overcorrection could go undetected if a negative value is reported as zero.

4.7 The dashes in Table 2 indicate that no measurable interferences were observed even at higher interferant concentrations. Generally, interferences were discernible if they produced peaks, or background shifts, corresponding to 2 to 5% of the peaks generated by the analyte concentrations.

4.8 The calibration blank (Sec. 7.5.1) may restrict the sensitivity of the quantitation limit or degrade the precision and accuracy of the analysis. Consult Chapter Three for recommended precautions and procedures necessary in reducing the magnitude and variability of the calibration blank.

5.0 SAFETY

5.1 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

5.2 Concentrated nitric and hydrochloric acids are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a hood and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection when working with these reagents. Hydrofluoric acid is a very toxic acid and penetrates the skin and tissues deeply if not treated immediately. Injury occurs in two stages; first, by hydration that induces tissue necrosis and then by penetration of fluoride ions deep into the tissue and by reaction with calcium. Boric acid and other complexing reagents and appropriate treatment agents should be administered immediately. Consult appropriate safety literature and have the appropriate treatment materials readily available prior to working with this acid. See Method 3052 for specific suggestions for handling hydrofluoric acid from a safety and an instrument standpoint.

5.3 Many metal salts are extremely toxic if inhaled or swallowed. Extreme care must be taken to ensure that samples and standards are handled properly and that all exhaust gases are properly vented. Wash hands thoroughly after handling.

5.4 The acidification of samples containing reactive materials may result in the release of toxic gases, such as cyanides or sulfides. For this reason, the acidification and digestion of samples should be performed in an approved fume hood.

6.0 EQUIPMENT AND SUPPLIES

6.1 Inductively coupled argon plasma emission spectrometer

6.1.1 Computer-controlled emission spectrometer with background correction.

6.1.2 Radio-frequency generator compliant with FCC regulations.

6.1.3 Optional mass flow controller for argon nebulizer gas supply.

6.1.4 Optional peristaltic pump.

6.1.5 Optional autosampler.

6.1.6 Argon gas supply -- high purity.

6.2 Volumetric flasks of suitable precision and accuracy.

6.3 Volumetric pipets of suitable precision and accuracy.

7.0 REAGENTS AND STANDARDS

7.1 Reagent- or trace metals-grade chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. If the purity of a reagent is in question, analyze for contamination. If the concentration of the contamination is less than the lower limit of quantitation, then the reagent is acceptable.

7.1.1 Hydrochloric acid (conc), HCl.

7.1.2 Hydrochloric acid HCl (1:1) -- Add 500 mL concentrated HCl to 400 mL water and dilute to 1 L in an appropriately- sized beaker.

7.1.3 Nitric acid (conc), HNO_3.

7.1.4 Nitric acid, HNO_3 (1:1) -- Add 500 mL concentrated HNO_3 to 400 mL water and dilute to 1 L in an appropriately-sized beaker.

7.2 Reagent water -- All references to water in the method refer to reagent water, unless otherwise specified. Reagent water must be free of interferences.

7.3 Standard stock solutions may be purchased or prepared from ultra-high purity grade chemicals or metals (99.99% pure or greater). With several exceptions specifically noted, all salts must be dried for 1 hr at 105 EC.

CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.

Typical stock solution preparation procedures follow. Concentrations are calculated based upon the weight of pure metal added, or with the use of the element fraction and the weight of the metal salt added.

NOTE: This section does not apply when analyzing samples prepared by Method 3040.

NOTE: The weight of the analyte is expressed to four significant figures for consistency with the weights below because rounding to two decimal places can contribute up to 4% error for some of the compounds.

For metals:

$$\text{Concentration (ppm)} = \frac{\text{weight (mg)}}{\text{volume (L)}}$$

For metal salts:

$$\text{Concentration (ppm)} = \frac{\text{weight (mg) x mole fraction}}{\text{volume (L)}}$$

7.3.1 Aluminum solution, stock, 1 mL = 1000 µg of Al

Dissolve 1.000 g of aluminum metal, accurately weighed to at least four significant figures, in an acid mixture of 4.0 mL of HCl (1:1) and 1.0 mL of concentrated $HN0_3$ in a beaker. Warm beaker slowly to dissolve the metal. When dissolution is complete, transfer solution quantitatively to a 1000-mL volumetric flask, add an additional 10.0 mL of HCl (1:1) and dilute to volume with reagent water.

7.3.2 Antimony solution, stock, 1 mL = 1000 µg of Sb

Dissolve 2.6673 g of $K(SbO)C_4H_4O_6$ (element fraction Sb = 0.3749), accurately weighed to at least four significant figures, in reagent water, add 10 mL of HCl (1:1), and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.3 Arsenic solution, stock, 1 mL = 1000 µg of As

Dissolve 1.3203 g of As_2O_3 (element fraction As = 0.7574), accurately weighed to at least four significant figures, in 100 mL of reagent water containing 0.4 g of NaOH. Acidify the solution with 2 mL of concentrated HNO_3 and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.4 Barium solution, stock, 1 mL = 1000 µg of Ba

Dissolve 1.5163 g of $BaCl_2$ (element fraction Ba = 0.6595), dried at 250 EC for 2 hr, accurately weighed to at least four significant figures, in 10 mL of reagent water with 1 mL of HCl (1:1). Add 10.0 mL of HCl (1:1) and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.5 Beryllium solution, stock, 1 mL = 1000 µg of Be

Do not dry. Dissolve 19.6463 g of $BeSO_4 4H_2O$ (element fraction Be = 0.0509), accurately weighed to at least four significant figures, in reagent water, add 10.0 mL of concentrated HNO_3, and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.6 Boron solution, stock, 1 mL = 1000 µg of B

Do not dry. Dissolve 5.716 g of anhydrous H_3BO_3 (B fraction = 0.1749), accurately weighed to at least four significant figures, in reagent water and dilute in a 1-L

volumetric flask with reagent water. Transfer immediately after mixing in a clean polytetrafluoroethylene (PTFE) bottle to minimize any leaching of boron from the glass container. The use of a non-glass volumetric flask is recommended to avoid boron contamination from glassware.

7.3.7 Cadmium solution, stock, 1 mL = 1000 µg of Cd

Dissolve 1.1423 g of CdO (element fraction Cd = 0.8754), accurately weighed to at least four significant figures, in a minimum amount of (1:1) HNO_3. Heat to increase the rate of dissolution. Add 10.0 mL of concentrated HNO_3 and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.8 Calcium solution, stock, 1 mL = 1000 µg of Ca

Suspend 2.4969 g of $CaCO_3$ (element Ca fraction = 0.4005), dried at 180 EC for 1 hr before weighing, accurately weighed to at least four significant figures, in reagent water and dissolve cautiously with a minimum amount of (1:1) HNO_3. Add 10.0 mL of concentrated HNO_3 and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.9 Chromium solution, stock, 1 mL = 1000 µg of Cr

Dissolve 1.9231 g of CrO_3 (element fraction Cr = 0.5200), accurately weighed to at least four significant figures, in reagent water. When dissolution is complete, acidify with 10 mL of concentrated HNO_3 and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.10 Cobalt solution, stock, 1 mL = 1000 µg of Co

Dissolve 1.000 g of cobalt metal, accurately weighed to at least four significant figures, in a minimum amount of (1:1) HNO_3. Add 10.0 mL of HCl (1:1) and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.11 Copper solution, stock, 1 mL = 1000 µg of Cu

Dissolve 1.2564 g of CuO (element fraction Cu = 0.7989), accurately weighed to at least four significant figures, in a minimum amount of (1:1) HNO_3. Add 10.0 mL of concentrated HNO_3 and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.12 Iron solution, stock, 1 mL = 1000 µg of Fe

Dissolve 1.4298 g of Fe_2O_3 (element fraction Fe = 0.6994), accurately weighed to at least four significant figures, in a warm mixture of 20 mL HCl (1:1) and 2 mL of concentrated HNO_3. Cool, add an additional 5.0 mL of concentrated HNO_3, and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.13 Lead solution, stock, 1 mL = 1000 µg of Pb

Dissolve 1.5985 g of $Pb(NO_3)_2$ (element fraction Pb = 0.6256), accurately weighed to at least four significant figures, in a minimum amount of (1:1) HNO_3. Add 10 mL (1:1) HNO_3 and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.14 Lithium solution, stock, 1 mL = 1000 µg of Li

Dissolve 5.3248 g of lithium carbonate (element fraction Li = 0.1878), accurately weighed to at least four significant figures, in a minimum amount of HCl (1:1) and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.15 Magnesium solution, stock, 1 mL = 1000 µg of Mg

Dissolve 1.6584 g of MgO (element fraction Mg = 0.6030), accurately weighed to at least four significant figures, in a minimum amount of (1:1) HNO_3. Add 10.0 mL of (1:1) concentrated HNO_3 and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.16 Manganese solution, stock, 1 mL = 1000 µg of Mn

Dissolve 1.00 g of manganese metal, accurately weighed to at least four significant figures, in acid mixture (10 mL of concentrated HCl and 1 mL of concentrated HNO_3) and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.17 Mercury solution, stock, 1 mL = 1000 µg of Hg

WARNING: Do not dry, **mercury is a highly toxic element**.

Dissolve 1.354 g of $HgCl_2$ (Hg fraction = 0.7388) in reagent water. Add 50.0 mL of concentrated HNO_3 and dilute to volume in 1000-mL volumetric flask with reagent water.

7.3.18 Molybdenum solution, stock, 1 mL = 1000 µg of Mo

Dissolve 1.7325 g of $(NH_4)_6Mo_7O_{24}·4H_2O$ (element fraction Mo = 0.5772), accurately weighed to at least four significant figures, in reagent water and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.19 Nickel solution, stock, 1 mL = 1000 µg of Ni

Dissolve 1.000 g of nickel metal, accurately weighed to at least four significant figures, in 10.0 mL of hot concentrated HNO_3, cool, and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.20 Phosphate solution, stock, 1 mL = 1000 µg of P

Dissolve 4.3937 g of anhydrous KH_2PO_4 (element fraction P = 0.2276), accurately weighed to at least four significant figures, in water. Dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.21 Potassium solution, stock, 1 mL = 1000 µg of K

Dissolve 1.9069 g of KCl (element fraction K = 0.5244) dried at 110 EC, accurately weighed to at least four significant figures, in reagent water, and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.22 Selenium solution, stock, 1 mL = 1000 µg of Se

Do not dry. Dissolve 1.6332 g of H_2SeO_3 (element fraction Se = 0.6123), accurately weighed to at least four significant figures, in reagent water and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.23 Silica solution, stock, 1 mL = 1000 µg SiO_2

Do not dry. Dissolve 2.964 g of NH_4SiF_6, accurately weighed to at least four significant figures, in 200 mL (1:20) HCl with heating at 85 EC to dissolve the solid. Let solution cool and dilute to volume in a 1000-mL volumetric flask with reagent water. Store in a PTFE container and protect from light.

7.3.24 Silver solution, stock, 1 mL = 1000 µg of Ag

Dissolve 1.5748 g of $AgNO_3$ (element fraction Ag = 0.6350), accurately weighed to at least four significant figures, in water and 10 mL of concentrated HNO_3. Dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.25 Sodium solution, stock, 1 mL = 1000 µg of Na

Dissolve 2.5419 g of NaCl (element fraction Na = 0.3934), accurately weighed to at least four significant figures, in reagent water. Add 10.0 mL of concentrated HNO_3 and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.26 Strontium solution, stock, 1 mL = 1000 µg of Sr

Dissolve 2.4154 g of strontium nitrate ($Sr(NO_3)_2$) (element fraction Sr = 0.4140), accurately weighed to at least four significant figures, in a 1000-mL flask containing 10 mL of concentrated HCl and 700 mL of reagent water. Dilute to volume with reagent water.

7.3.27 Thallium solution, stock, 1 mL = 1000 µg of Tl

Dissolve 1.3034 g of $TlNO_3$ (element fraction Tl = 0.7672), accurately weighed to at least four significant figures, in reagent water. Add 10.0 mL of concentrated HNO_3 and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.28 Tin solution, stock, 1 mL = 1000 µg of Sn

Dissolve 1.000 g of Sn shot, accurately weighed to at least 4 significant figures, in 200 mL of HCl (1:1) with heating to dissolve the metal. Let solution cool and dilute with HCl (1:1) in a 1000-mL volumetric flask.

7.3.29 Vanadium solution, stock, 1 mL = 1000 µg of V

Dissolve 2.2957 g of NH_4VO_3 (element fraction V = 0.4356), accurately weighed to at least four significant figures, in a minimum amount of concentrated HNO_3. Heat to dissolve the metal. Add 10.0 mL of concentrated HNO_3 and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.30 Zinc solution, stock, 1 mL = 1000 µg of Zn

Dissolve 1.2447 g of ZnO (element fraction Zn = 0.8034), accurately weighed to at least four significant figures, in a minimum amount of dilute HNO_3. Add 10.0 mL of concentrated HNO_3 and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.3.31 Yttrium solution, stock, 1 mL = 1000 µg of Y

Dissolve 4.3081 g of $Y(NO_3)_3$0$6H_2$0 (element fraction Y = 0.2321), accurately weighed to at least four significant figures, in a minimum amount of dilute HNO_3. Add 10.0 mL of concentrated HNO_3 and dilute to volume in a 1000-mL volumetric flask with reagent water.

7.4 Mixed calibration standard solutions

Prepare mixed calibration standard solutions (see Table 3) by combining appropriate volumes of the stock solutions above in volumetric flasks. Add the appropriate types and volumes of acids so that the standards are matrix-matched with the sample digestates. Prior to preparing the mixed standards, each stock solution should be analyzed separately to determine possible spectral interference or the presence of impurities. Care should be taken when preparing the mixed standards to ensure that the elements are compatible and stable together. Transfer the mixed standard solutions to FEP fluorocarbon or previously unused polyethylene or polypropylene bottles for storage. For all intermediate and working standards, especially low level standards (i.e., <1 ppm), stability must be demonstrated prior to use. Freshly-mixed standards should be prepared, as needed, with the realization that concentration can change with age. (Refer to Sec. 10.3.1 for guidance on determining the viability of standards.) Some typical calibration standard combinations are listed in Table 3.

NOTE: If the addition of silver to the recommended acid combination initially results in a precipitate, then add 15 mL of water and warm the flask until the solution clears. Cool and dilute to 100 mL with water. For this acid combination, the silver concentration should be limited to 2 mg/L. Silver is stable under these conditions in a water matrix for 30 days, if protected from the light. Higher concentrations of silver require additional HCl.

7.5 Blanks

Two types of blanks are required for the analysis of samples prepared by any method other than Method 3040. The calibration blank is used in establishing the analytical curve and the method blank is used to identify possible contamination resulting from either the reagents (acids) or the equipment used during sample processing including filtration.

7.5.1 The calibration blank is prepared by acidifying reagent water to the same concentrations of the acids found in the standards and samples. Prepare a sufficient quantity to flush the system between standards and samples. The calibration blank will also be used for all initial (ICB) and continuing calibration blank (CCB) determinations.

7.5.2 The method blank must contain all of the reagents in the same volumes as used in the processing of the samples. The method blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis (refer to Sec. 9.5).

7.6 The initial calibration verification (ICV) standard is prepared by the analyst (or a purchased second source reference material) by combining compatible elements from a

standard source different from that of the calibration standard, and at concentration near the midpoint of the calibration curve (see Sec. 10.3.3 for use). This standard may also be purchased.

7.7 The continuing calibration verification (CCV) standard should be prepared in the same acid matrix using the same standards used for calibration, at a concentration near the mid-point of the calibration curve (see Sec. 10.3.4 for use).

7.8 The interference check solution is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest, particularly those with known interferences at 0.5 to 1 mg/L. In the absence of measurable analyte, overcorrection could go undetected because a negative value could be reported as zero. If the particular instrument will display overcorrection as a negative number, this spiking procedure will not be necessary.

8.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

See the introductory material to Chapter Three, "Inorganic Analytes."

9.0 QUALITY CONTROL

9.1 Refer to Chapter One for additional guidance on quality assurance (QA) and quality control (QC) protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific criteria and those criteria given in Chapter One, and technique-specific QC criteria take precedence over the criteria in Chapter One. Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those that will implement the project and assess the results. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and quality control data should be maintained for reference or inspection.

9.2 Refer to the 3000 series method to be used (e.g., Method 3005, 3010, 3015, 3031, 3040, 3050, 3051, or 3052) for appropriate QC procedures to ensure the proper operation of the various sample preparation techniques.

9.3 Instrument detection limits (IDLs) are useful means to evaluate the instrument noise level and response changes over time for each analyte from a series of reagent blank analyses to obtain a calculated concentration. They are not to be confused with the lower limit of quantitation, nor should they be used in establishing this limit. It may be helpful to compare the calculated IDLs to the established lower limit of quantitation, however, it should be understood that the lower limit of quantitation needs to be verified according to the guidance in Sec. 10.0.

IDLs in µg/L can be estimated by calculating the average of the standard deviations of three runs on three non-consecutive days from the analysis of a reagent blank solution with seven consecutive measurements per day. Each measurement should be performed as though it were a separate analytical sample (i.e., each measurement must be followed by a rinse and/or any other procedure normally performed between the analysis of separate samples). IDLs should be determined at least every three months or at a project-specific designated frequency and kept with the instrument log book.

9.4 Initial demonstration of proficiency

Each laboratory must demonstrate initial proficiency with each sample preparation (a 3000 series method) and determinative method combination it utilizes by generating data of acceptable accuracy and precision for target analytes in a clean matrix. If an autosampler is used to perform sample dilutions, before using the autosampler to dilute samples, the laboratory should satisfy itself that those dilutions are of equivalent or better accuracy than is achieved by an experienced analyst performing manual dilutions. The laboratory must also repeat the demonstration of proficiency whenever new staff members are trained or significant changes in instrumentation are made.

9.5 Dilute and reanalyze samples that exceed the linear dynamic range or use an alternate, less sensitive calibration for which quality control data are already established.

9.6 For each batch of samples processed, at least one method blank must be carried throughout the entire sample preparation and analytical process. A method blank is prepared by using a volume or weight of reagent water at the volume or weight specified in the preparation method, and then carried through the appropriate steps of the analytical process. These steps may include, but are not limited to, prefiltering, digestion, dilution, filtering, and analysis. If the method blank does not contain target analytes at a level that interferes with the project-specific DQOs, then the method blank would be considered acceptable.

In the absence of project-specific DQOs, if the blank is less than 10% of the lower limit of quantitation check sample concentration, less than 10% of the regulatory limit, or less than 10% of the lowest sample concentration for each analyte in a given preparation batch, whichever is greater, then the method blank is considered acceptable. If the method blank cannot be considered acceptable, the method blank should be re-run once, and if still unacceptable, then all samples after the last acceptable method blank should be reprepared and reanalyzed along with the other appropriate batch QC samples. These blanks will be useful in determining if samples are being contaminated. If the method blank exceeds the criteria, but the samples are all either below the reporting level or below the applicable action level or other DQOs, then the sample data may be used despite the contamination of the method blank.

9.7 Laboratory control sample (LCS)

For each batch of samples processed, at least one LCS must be carried throughout the entire sample preparation and analytical process. The laboratory control samples should be spiked with each analyte of interest at the project-specific action level or, when lacking project-specific action levels, at approximately mid-point of the linear dynamic range. Acceptance criteria should either be defined in the project-specifc planning documents or set at a laboratory derived limit developed through the use of historical analyses. In the absence of project-specific or historical data generated criteria, this limit should be set at ± 20% of the spiked value. Acceptance limits derived from historical data should be no wider that ± 20%. If the laboratory control sample is not acceptable, then the laboratory control sample should be re-run once and, if still unacceptable, all samples after the last acceptable laboratory control sample should be reprepared and reanalyzed.

Concurrent analyses of standard reference materials (SRMs) containing known amounts of analytes in the media of interest are recommended and may be used as an LCS. For solid SRMs, 80 -120% accuracy may not be achievable and the manufacturer's established acceptance criterion should be used for soil SRMs.

9.8 Matrix spike, unspiked duplicate, or matrix spike duplicate (MS/Dup or MS/MSD)

Documenting the effect of the matrix, for a given preparation batch consisting of similar sample characteristics, should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch or as noted in the project-specific planning documents. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, laboratories should use a matrix spike and matrix spike duplicate pair.

For each batch of samples processed, at least one MS/Dup or MS/MSD sample set should be carried throughout the entire sample preparation and analytical process as described in Chapter One. MS/MSDs are intralaboratory split samples spiked with identical concentrations of each analyte of interest. The spiking occurs prior to sample preparation and analysis. An MS/Dup or MS/MSD is used to document the bias and precision of a method in a given sample matrix.

Refer to Chapter One for definitions of bias and precision, and for the proper data reduction protocols. MS/MSD samples should be spiked at the same level, and with the same spiking material, as the corresponding laboratory control sample that is at the project-specific action level or, when lacking project-specific action levels, at approximately mid-point of the linear dynamic range. Acceptance criteria should either be defined in the project-specifc planning documents or set at a laboratory-derived limit developed through the use of historical analyses per matrix type analyzed. In the absence of project-specific or historical data generated criteria, these limits should be set at ± 25% of the spiked value for accuracy and 20 relative percent difference (RPD) for precision. Acceptance limits derived from historical data should be no wider that ± 25% for accuracy and 20% for precision. Refer to Chapter One for additional guidance. If the bias and precision indicators are outside the laboratory control limits, if the percent recovery is less than 75% or greater than 125%, or if the relative percent difference is greater than 20%, then the interference test discussed in Sec. 9.9 should be conducted.

9.8.1 The relative percent difference between spiked matrix duplicate or unspiked duplicate determinations is to be calculated as follows:

$$RPD = \frac{*D_1 - D_2*}{\left(\frac{*D_1 + D_2*}{2}\right)} \times 100$$

where:

RPD = relative percent difference.
D_1 = first sample value.
D_2 = second sample value (spiked or unspiked duplicate).

9.8.2 The spiked sample or spiked duplicate sample recovery should be within ± 25% of the actual value, or within the documented historical acceptance limits for each matrix.

9.9 If less than acceptable accuracy and precision data are generated, additional quality control tests (Secs. 9.9.1 and 9.9.2) are recommended prior to reporting concentration data for the elements in this method. At a minimum, these tests should be performed with each batch of samples prepared/analyzed with corresponding unacceptable data quality results. These tests will then serve to ensure that neither positive nor negative interferences are affecting the measurement of any of the elements or distorting the accuracy of the reported values. If matrix effects are confirmed, the laboratory should consult with the data user when feasible for possible corrective actions which may include the use of alternative or modified test procedures so that the analysis is not impacted by the same interference.

9.9.1 Post digestion spike addition

If the MS/MSD recoveries are unacceptable, the same sample from which the MS/MSD aliquots were prepared should also be spiked with a post digestion spike. Otherwise, another sample from the same preparation should be used as an alternative. An analyte spike is added to a portion of a prepared sample, or its dilution, and should be recovered to within 80% to 120% of the known value. The spike addition should produce a minimum level of 10 times and a maximum of 100 times the lower limit of quantitation. If this spike fails, then the dilution test (Sec. 9.9.2) should be run on this sample. If both the MS/MSD and the post digestion spike fail, then matrix effects are confirmed.

9.9.2 Dilution test

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the lower limit of quantitation after dilution), an analysis of a 1:5 dilution should agree within ± 10% of the original determination. If not, then a chemical or physical interference effect should be suspected.

CAUTION: If spectral overlap is suspected, then the use of computerized compensation, an alternate wavelength, or comparison with an alternate method is recommended.

9.10 Ultra-trace analysis requires the use of clean chemistry preparation and analysis techniques. Several suggestions for minimizing analytical blank contamination are provided in Chapter Three.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Set up the instrument with proper operating parameters established as detailed below. The instrument should be allowed to become thermally stable before beginning (usually requiring at least 30 minutes of operation prior to calibration). For operating conditions, the analyst should follow the instructions provided by the instrument manufacturer.

10.1.1 Before using this procedure to analyze samples, data should be available documenting the initial demonstration of performance. The required data should document the location of the background points being used for correction; the determination of the linear dynamic ranges; a demonstration of the desired method sensitivity and instrument detection limits; and the determination and verification of interelement correction equations or other routines for correcting spectral interferences. These data should be generated using the same instrument, operating conditions, and calibration routine to be used for sample analysis. These data should be kept on file and be available for review by the data user or auditor.

10.1.2 Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference corrections need to be established for each individual target analyte on each particular instrument. All measurements (both target analytes and constituents which interfere with the target analytes) need to be within the instrument linear range where the correction equations are valid.

10.1.3 The lower limits of quantitation should be established for all wavelengths utilized for each type of matrix analyzed and for each preparation method used and for each instrument. These limits are considered the lowest reliable laboratory reporting concentrations and should be established from the lower limit of quantitation check sample and then confirmed using either the lowest calibration point or from a low-level calibration check standard.

10.1.3.1 Lower limit of quantitation check sample

The lower limit of quantitation check (LLQC) sample should be analyzed after establishing the lower laboratory reporting limits and on an as needed basis to demonstrate the desired detection capability. Ideally, this check sample and the low-level calibration verification standard will be prepared at the same concentrations with the only difference being the LLQC sample is carried through the entire preparation and analytical procedure. Lower limits of quantitation are verified when all analytes in the LLQC sample are detected within ± 30% of their true value. This check should be used to both establish and confirm the lowest quantitation limit.

10.1.3.2 The lower limits of quantitation determination using reagent water represents a best case situation and does not represent possible matrix effects of real-world samples. For the application of lower limits of quantitation on a project-specific basis with established data quality objectives, low-level matrix-specific spike studies may provide data users with a more reliable indication of the actual method sensitivity and minimum detection capabilities.

10.1.4 Specific recommended wavelengths are listed in Table 1. Other wavelengths may be substituted if they can provide the needed sensitivity and are corrected for spectral interference. Because of differences among various makes and models of spectrometers, specific instrument operating conditions are not provided. The instrument and operating conditions utilized for determination must be capable of providing data of acceptable quality for the specific project and data user. The analyst should follow the instructions provided by the instrument manufacturer unless other conditions provide similar or better performance for a given task.

For radial viewed plasma, operating conditions for aqueous solutions usually vary from:

- 1100 to 1200 watts forward power,
- 14 to 18 mm viewing height,
- 15 to 19 L/min argon coolant flow,
- 0.6 to 1.5 L/min argon nebulizer flow,
- 1 to 1.8 mL/min sample pumping rate with a 1 minute preflush time and measurement time near 1 sec per wavelength peak for sequential instruments and a rinse time of 10 sec per replicate with a 1 sec per replicate read time for simultaneous instruments.

For an axial viewed plasma, the conditions will usually vary from:

- 1100 to 1500 watts forward power,
- 15 to 19 L/min argon coolant flow,
- 0.6 to 1.5 L/min argon nebulizer flow,
- 1 to 1.8 mL/min sample pumping rate with a 1 minute preflush time and measurement time near 1 sec per wavelength peak for sequential instruments and a rinse time of 10 sec per replicate with a 1 sec per replicate read time for simultaneous instruments.

One recommended way to achieve repeatable interference correction factors is to adjust the argon aerosol flow to reproduce the Cu/Mn intensity ratio at 324.754 nm and 257.610 nm respectively. This can be performed before daily calibration and after the instrument warm-up period.

10.1.5 Plasma optimization

The plasma operating conditions need to be optimized prior to use of the instrument. The purpose of plasma optimization is to provide a maximum signal to background ratio for some of the least sensitive elements in the analytical array. The use of a mass flow controller to regulate the nebulizer gas flow or source optimization software greatly facilitates the procedure. This routine is not required on a daily basis, it is only required when first setting up a new instrument, or following a change in operating conditions. The following procedure is recommended, or follow the manufacturer's recommendations.

10.1.5.1 Ignite the radial plasma and select an appropriate incident radio frequency (RF) power. Allow the instrument to become thermally stable before beginning, about 30 to 60 minutes of operation. While aspirating a 1000 µg/L solution of yttrium, follow the instrument manufacturer's instructions and adjust the aerosol carrier gas flow rate through the nebulizer so a definitive blue emission region of the plasma extends approximately from 5 to 20 mm above the top of the load coil. Record the nebulizer gas flow rate or pressure setting for future reference. The yttrium solution can also be used for coarse optical alignment of the torch by observing the overlay of the blue light over the entrance slit to the optical system.

10.1.5.2 After establishing the nebulizer gas flow rate, determine the solution uptake rate of the nebulizer in mL/min by aspirating a known volume of a calibration blank for a period of at least three minutes. Divide the volume aspirated by the time in minutes and record the uptake rate. Set the peristaltic pump to deliver that rate in a steady even flow.

10.1.5.3 Profile the instrument to align it optically as it will be used during analysis. The following procedure is written for vertical optimization in the radial mode, but it also can be used for horizontal optimization.

Aspirate a solution containing 10 µg/L of several selected elements. As, Se, Tl, and Pb are the least sensitive of the elements and most in need of optimization. However, other elements may be used, based on the judgement of the analyst or project-specific protocols. (V, Cr, Cu, Li and Mn also have been used with success.) Collect intensity data at the wavelength peak for each analyte at 1 mm intervals from 14 to 18 mm above the load coil. (This region of the plasma is referred to as the analytical zone.) Repeat the process using the calibration blank. Determine the net signal to blank intensity ratio for each analyte for each viewing height setting. Choose the height for viewing the plasma that provides the

best net intensity ratios for the elements analyzed or the highest intensity ratio for the least sensitive element. For optimization in the axial mode, follow the instrument manufacturer's instructions.

10.1.5.4 The instrument operating conditions finally selected as being optimum should provide the most appropriate instrument responses that correlate to the desired target analyte sensitivity while meeting the minimum quality control criteria noted in this method or as specified in the project-specific planning documents.

10.1.5.5 If the instrument operating conditions, such as incident power or nebulizer gas flow rate, are changed, or if a new torch injector tube with a different orifice internal diameter is installed, then the plasma and viewing height should be re-optimized.

10.1.5.6 After completing the initial optimization of operating conditions, and before analyzing samples, the laboratory should establish and initially verify an interelement spectral interference correction routine to be used during sample analysis with interference check standards that closely match the anticipated properties of the expected sample matrices, i.e., for saltwater type matrices the interference check standard should contain components that match the salinities of the proposed sample matrix. A general description of spectral interferences and the analytical requirements for background correction, in particular, are discussed in Sec. 4.2.

10.1.5.7 Before daily calibration, and after the instrument warmup period, the nebulizer gas flow rate should be reset to the determined optimized flow. If a mass flow controller is being used, it should be set to the recorded optimized flow rate. In order to maintain valid spectral interelement correction routines, the nebulizer gas flow rate should be the same (< 2% change) from day to day.

10.2 For operation with organic solvents, the use of the auxiliary argon inlet is recommended, as is the use of solvent-resistant tubing, increased plasma (coolant) argon flow, decreased nebulizer flow, and increased RF power, to obtain stable operation and precise measurements.

10.3 All analyses require that a calibration curve be prepared to cover the appropriate concentration range based on the intended application and prior to establishing the linear dynamic range. Usually, this means the preparation of a calibration blank and mixed calibration standard solutions (Sec. 7.4), the highest of which would not exceed the anticipated linear dynamic range of the instrument. Check the instrument calibration by analyzing appropriate QC samples as follows.

10.3.1 Individual or mixed calibration standards should be prepared from known primary stock standards every six months to one year as needed based on the concentration stability as confirmed from the ICV analyses. The analysis of the ICV, which is prepared from a source independent of the calibration standards, is necessary to verify the instrument performance once the system has been calibrated for the desired target analytes. It is recommended that the ICV solution be obtained commercially as a certified traceable reference material such that an expiration date can be assigned. Alternately, the ICV solution can be prepared from an independent source on an as needed basis depending on the ability to meet the calibration verification criteria. If the ICV analysis is outside of the acceptance criteria, at a minimum the calibration standards must be

prepared fresh and the instrument recalibrated prior to beginning sample analyses. Consideration should also be given to preparing fresh ICV standards if the new calibration cannot be verified using the existing ICV standard.

NOTE: This method describes the use of both a low-level and mid-level ICV standard analysis. For purposes of verifying the initial calibration, only the mid-level ICV needs to be prepared from a source other than the calibration standards.

10.3.1.1 The calibration standards should be prepared using the same type of acid or combination of acids and at similar concentrations as will result in the samples following processing.

10.3.1.2 The response of the calibration blank should be less than the response of the typical laboratory lower limit of quantitation for each desired target analyte. Additionally, if the calibration blank response or continuing calibration blank verification is used to calculate a theoretical concentration, this value should be less than the level of acceptable blank contamination as specified in the approved quality assurance project planning documents. If this is not the case, the reason for the out-of-control condition must be found and corrected, and the sample analyses should not proceed or the previous ten samples should be reanalyzed.

10.3.2 For the initial and daily instrument operation, calibrate the system according to the instrument manufacturer's guidelines using the mixed calibration standards as noted in Sec. 7.4. The calibration curve should be prepared daily with a minimum of a calibration blank and a single standard at the appropriate concentration to effectively outline the desired quantitation range. The resulting curve should then be verified with mid-level and low-level initial calibration verification standards as outlined in Sec. 10.3.3.

Alternatively, the calibration curve can be prepared daily with a minimum of a calibration blank and three non-zero standards that effectively bracket the desired sample concentration range. If low-level as compared to mid- or high-level sample concentrations are expected, the calibration standards should be prepared at the appropriate concentrations in order to demonstrate the instrument linearity within the anticipated sample concentration range. For all multi-point calibration scenarios, the lowest non-zero standard concentration should be considered the lower limit of quantitation.

NOTE: Regardless of whether the instrument is calibrated using only a minimum number of standards or with a multi-point curve, the upper limit of the quantitation range may exceed the highest concentration calibration point and can be defined as the "linear dynamic" range, while the lower limit can be identified as the "lower limit of quantitation limit" (LLQL) and will be either the concentration of the lowest calibration standard (for multi-point curves) or the concentration of the low level ICV/CCV check standard. Results reported outside these limits would not be recommended unless they are qualified as estimated. See Sec. 10.4 for recommendations on how to determine the linear dynamic range. The guidance in this section and Sec. 10.3.3 provide options for defining the lower limit of quantitation.

10.3.2.1 To be considered acceptable, the calibration curve should have a correlation coefficient greater than or equal to 0.998. When using a multi-point calibration curve approach, every effort should be made to attain an acceptable correlation coefficient based on a linear response for each desired

target analyte. If the recommended linear response cannot be attained using a minimum of three non-zero calibration standards, consideration should be given to adding more standards, particularly at the lower concentrations, in order to better define the linear range and the lower limit of quantitation. Conversely, the extreme upper and lower calibration points may be removed from the multi-point curve as long as three non-zero points remain such that the linear range is narrowed and the non-linear upper and/or lower portions are removed. As with the single point calibration option, the multi-point calibration should be verified with both a mid- and low-level ICV standard analysis using the same 90 - 110% and 70 - 130% acceptance criteria, respectively.

10.3.2.2 Many instrument software packages allow multi-point calibration curves to be "forced" through zero. It is acceptable to use this feature, provided that the resulting calibration meets the acceptance criteria, and can be verified by acceptable QC results. Forcing a regression through zero should NOT be used as a rationale for reporting results below the calibration range defined by the lowest standard in the calibration curve.

10.3.3 After initial calibration, the calibration curve should be verified by use of an initial calibration verification (ICV) standard analysis. At a minimum, the ICV standard should be prepared from an independent (second source) material at or near the mid-range of the calibration curve. The acceptance criteria for this mid-range ICV standard should be ±10% of its true value. Additionally, a low-level initial calibration verification (LLICV) standard should be prepared, using the same source as the calibration standards, at a concentration expected to be the lower limit of quantitation. The suggested acceptance criteria for the LLICV is ± 30% of its true value. Quantitative sample analyses should not proceed for those analytes that fail the second source standard initial calibration verification. However, analyses may continue for those analytes that fail the criteria with an understanding these results should be qualified and would be considered estimated values. Once the calibration acceptance criteria is met, either the lowest calibration standard or the LLICV concentration can be used to demonstrate the lower limit of quantitation and sample results should not be quantitated below this lowest standard. In some cases depending on the stated project data quality objectives, it may be appropriate to report these results as estimated, however, they should be qualified by noting the results are below the lower limit of quantitation. Therefore, the laboratory's quantitation limit cannot be reported lower than either the LLICV standard used for the single point calibration option or the low calibration and/or verification standard used during initial multi-point calibration. If the calibration curve cannot be verified within these specified limits for the mid-range ICV and LLICV analyses, the cause needs to be determined and the instrument recalibrated before samples are analyzed. The analysis data for the initial calibration verification analyses should be kept on file with the sample analysis data.

10.3.4 Both the single and multi-point calibration curves should be verified at the end of each analysis batch and after every 10 samples by use of a continuing calibration verification (CCV) standard and a continuing calibration blank (CCB). The CCV should be made from the same material as the initial calibration standards at or near the mid-range concentration. For the curve to be considered valid, the acceptance criteria for the CCV standard should be ±10% of its true value and the CCB should contain target analytes less than the established lower limit of quantitation for any desired target analyte. If the calibration cannot be verified within the specified limits, the sample analysis must be discontinued, the cause determined and the instrument recalibrated. All samples following the last acceptable CCV/CCB must be reanalyzed. The analysis data for the CCV/CCB should be kept on file with the sample analysis data.

The low-level continuing calibration verification (LLCCV) standard should also be analyzed at the end of each analysis batch. A more frequent LLCCV analysis, i.e., every 10 samples, may be necessary if low-level sample concentrations are anticipated and the system stability at low end of the calibration is questionable. In addition, the analysis of a LLCCV on a more frequent basis will minimize the number of samples for re-analysis should the LLCCV fail if only run at the end of the analysis batch. The LLCCV standard should be made from the same source as the initial calibration standards at the established lower limit of quantitation as reported by the laboratory. The acceptance criteria for the LLCCV standard should be ± 30% of its true value. If the calibration cannot be verified within these specified limits, the analysis of samples containing the affected analytes at similar concentrations cannot continue until the cause is determined and the LLCCV standard successfully analyzed. The instrument may need to be recalibrated or the lower limit of quantitation adjusted to a concentration that will ensure a compliant LLCCV analysis. The analysis data for the LLCCV standard should be kept on file with the sample analysis data.

10.4 The linear dynamic range is established when the system is first setup, or whenever significant instrument components have been replaced or repaired, and on an as needed basis only after the system has been successfully calibrated using either the single or multi-point standard calibration approach.

The upper limit of the linear dynamic range needs to be established for each wavelength utilized by determining the signal responses from a minimum of three, preferably five, different concentration standards across the range. The ranges which may be used for the analysis of samples should be judged by the analyst from the resulting data. The data, calculations and rationale for the choice of range made should be documented and kept on file. A standard at the upper limit should be prepared, analyzed and quantitated against the normal calibration curve. The calculated value should be within 10% (±10%) of the true value. New upper range limits should be determined whenever there is a significant change in instrument response. At a minimum, the range should be checked every six months. The analyst should be aware that if an analyte that is present above its upper range limit is used to apply an interelement correction, the correction may not be valid and those analytes where the interelement correction has been applied may be inaccurately reported.

NOTE: Many of the alkali and alkaline earth metals have non-linear response curves due to ionization and self-absorption effects. These curves may be used if the instrument allows it; however the effective range must be checked and the second order curve fit should have a correlation coefficient of 0.998 or better. Third order fits are not acceptable. These non-linear response curves should be revalidated and/or recalculated on a daily basis using the same calibration verification QC checks as a linear calibration curve. Since these curves are much more sensitive to changes in operating conditions than the linear lines, they should be checked whenever there have been moderate equipment changes. Under these calibration conditions, quantitation is not acceptable above or below the calibration standards. Additionally, a non-linear curve should be further verified by calculating the actual recovery of each calibration standard used in the curve. The acceptance criteria for the calibration standard recovery should be ±10% of its true value for all standards except the lowest concentration. A recovery of ± 30% of its true value should be achieved for the lowest concentration standard.

10.5 The analyst should (1) verify that the instrument configuration and operating conditions satisfy the project-specific analytical requirements and (2) maintain quality control data that demonstrate and confirm the instrument performance for the reported analytical results.

11.0 PROCEDURE

11.1 Preliminary treatment of most matrices is necessary because of the complexity and variability of sample matrices. Groundwater and other aqueous samples designated for a dissolved metal determination which have been prefiltered and acidified will not need acid digestion. However, all associated QC samples (i.e., method blank, LCS and MS/MSD) must undergo the same filtration and acidification procedures. Samples which are not digested must either use an internal standard or be matrix-matched with the standards. Solubilization and digestion procedures are presented in Chapter Three, "Inorganic Analytes."

11.2 Profile and calibrate the instrument according to the instrument manufacturer's recommended procedures, using the typical mixed calibration standard solutions described in Sec. 7.4. Flush the system with the calibration blank (Sec. 7.5.1) between each standard or as the manufacturer recommends. (Use the average intensity of multiple exposures for both standardization and sample analysis to reduce random error.) The calibration curve should be prepared as detailed in Sec. 10.3.2.

11.3 Regardless of whether the initial calibration is performed using a single high standard and the calibration blank or the multi-point option, the laboratory should analyze an LLCCV (Sec. 10.3.4). For all analytes and determinations, the laboratory must analyze an ICV and LLICV (Sec. 10.3.3) immediately following daily calibration. It is recommended that a CCV LLCCV, and CCB (Sec. 10.3.4) be analyzed after every ten samples and at the end of the analysis batch.

11.4 Rinse the system with the calibration blank solution (Sec. 7.5.1) before the analysis of each sample. The rinse time will be one minute. Each laboratory may establish a reduction in this rinse time through a suitable demonstration. Analyze the samples and record the results.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 The quantitative values must be reported in appropriate units, such as micrograms per liter (µg/L) for aqueous samples and milligrams per kilogram (mg/kg) for solid samples. If dilutions were performed, the appropriate corrections must be applied to the sample values. All results should be reported with up to three significant figures.

12.2 If appropriate, or required, calculate results for solids on a dry-weight basis as follows:

(1) A separate determination of percent solids must be performed.

(2) The concentrations determined in the digest are to be reported on the basis of the dry weight of the sample.

$$\text{Concentration (dry weight)(mg/kg)} = \frac{C \times V}{W \times S}$$

Where,

C = Digest Concentration (mg/L)
V = Final volume in liters after sample preparation
W = Weight in kg of wet sample

$$S = \frac{\%\ \text{Solids}}{100}$$

Calculations must include appropriate interference corrections (see Sec. 4.2 for examples), internal-standard normalization, and the summation of signals at 206, 207, and 208 m/z for lead (to compensate for any differences in the abundances of these isotopes between samples and standards).

12.3 Results must be reported in units commensurate with their intended use and all dilutions must be taken into account when computing final results.

13.0 METHOD PERFORMANCE

13.1 Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance criteria for users of the methods. Instead, performance criteria should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method. These performance data are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

13.2 In an EPA round-robin study, seven laboratories applied the ICP technique to acid-digested water matrices that had been spiked with various metal concentrates. Table 4 lists the true values, the mean reported values, and the mean percent relative standard deviations. These data are provided for guidance purposes only.

13.3 Performance data for aqueous solutions and solid samples from a multilaboratory study are provided in Tables 5 and 6. These data are provided for guidance purposes only.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste*

Reduction available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th St. NW, Washington, D.C. 20036, http://www.acs.org.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* available from the American Chemical Society at the address listed in Sec. 14.2.

16.0 REFERENCES

1. C. L. Jones, *et al*., "An Interlaboratory Study of Inductively Coupled Plasma Atomic Emission Spectroscopy Method 6010 and Digestion Method 3050," EPA-600/4-87-032, U.S. Environmental Protection Agency, Las Vegas, NV, 1987.

17.0 TABLES, DIAGRAMS, FLOW CHARTS, AND VALIDATION DATA

The following pages contain the tables referenced by this method. A flow diagram of the procedure follows the tables.

TABLE 1

RECOMMENDED WAVELENGTHS AND ESTIMATED INSTRUMENTAL DETECTION LIMITS

Element	Wavelength[a] (nm)	Estimated IDL[b] (µg/L)
Aluminum	308.215	30
Antimony	206.833	21
Arsenic	193.696	35
Barium	455.403	0.87
Beryllium	313.042	0.18
Boron	249.678 x2	3.8
Cadmium	226.502	2.3
Calcium	317.933	6.7
Chromium	267.716	4.7
Cobalt	228.616	4.7
Copper	324.754	3.6
Iron	259.940	4.1
Lead	220.353	28
Lithium	670.784	2.8
Magnesium	279.079	20
Manganese	257.610	0.93
Mercury	194.227 x2	17
Molybdenum	202.030	5.3
Nickel	231.604 x2	10
Phosphorus	213.618	51
Potassium	766.491	See note c
Selenium	196.026	50
Silica (SiO_2)	251.611	17
Silver	328.068	4.7
Sodium	588.995	19
Strontium	407.771	0.28
Thallium	190.864	27
Tin	189.980 x2	17
Titanium	334.941	5.0
Vanadium	292.402	5.0
Zinc	213.856 x2	1.2

TABLE 1
(continued)

[a] The wavelengths listed (where x2 indicates second order) are recommended because of their sensitivity. Other wavelengths may be substituted (e.g., in the case of an interference) if they provide the needed sensitivity and are treated with the same corrective techniques for spectral interference.

[b] The estimated instrumental detection limits shown are provided for illustrative purposes only. Each laboratory must determine IDLs and MDLs, as necessary, for their specific application of the method. These IDLs represent radial plasma data and axial plasma IDLs may be lower.

[c] Highly dependent on operating conditions and plasma position.

TABLE 2

POTENTIAL INTERFERENCES AND ANALYTE CONCENTRATION EQUIVALENTS (mg/L) ARISING FROM INTERFERENCE AT THE 100-mg/L LEVEL

Analyte	Wavelenth (nm)	Interferant[a,b]									
		Al	Ca	Cr	Cu	Fe	Mg	Mn	Ni	Ti	V
Aluminum	308.215	--	--	--	--	--	--	0.21	--	--	1.4
Antimony	206.833	0.47	--	2.9	--	0.08	--	--	--	0.25	0.45
Arsenic	193.696	1.3	--	0.44	--	--	--	--	--	--	1.1
Barium	455.403	--	--	--	--	--	--	--	--	--	--
Beryllium	313.042	--	--	--	--	--	--	--	--	0.04	0.05
Cadmium	226.502	--	--	--	--	0.03	--	--	0.02	--	--
Calcium	317.933	--	--	0.08	--	0.01	0.01	0.04	--	0.03	0.03
Chromium	267.716	--	--	--	--	0.003	--	0.04	--	--	0.04
Cobalt	228.616	--	--	0.03	--	0.005	--	--	0.03	0.15	--
Copper	324.754	--	--	--	--	0.003	--	--	--	0.05	0.02
Iron	259.940	--	--	--	--	--	--	0.12	--	--	--
Lead	220.353	0.17	--	--	--	--	--	--	--	--	--
Magnesium	279.079	--	0.02	0.11	--	0.13	--	0.25	--	0.07	0.12
Manganese	257.610	0.005	--	0.01	--	0.002	0.002	--	--	--	--
Molybdenum	202.030	0.05	--	--	--	0.03	--	--	--	--	--
Nickel	231.604	--	--	--	--	--	--	--	--	--	--
Selenium	196.026	0.23	--	--	--	0.09	--	--	--	--	--
Sodium	588.995	--	--	--	--	--	--	--	--	0.08	--
Thallium	190.864	0.30	--	--	--	--	--	--	--	--	--
Vanadium	292.402	--	--	0.05	--	0.005	--	--	--	0.02	--
Zinc	213.856	--	--	--	0.14	--	--	--	0.29	--	--

[a] Dashes indicate that no interference was observed even when interferents were introduced at the following levels:

Al at 1000 mg/L	Cu at 200 mg/L	Mn at 200 mg/L
Ca at 1000 mg/L	Fe at 1000 mg/L	Ti at 200 mg/L
Cr at 200 mg/L	Mg at 1000 mg/L	V at 200 mg/L

[b] The data shown above as analyte concentration equivalents are not the actual observed concentrations. To obtain those data, add the listed concentration to the interferant figure.

[c] Interferences will be affected by background choice and other interferences may be present.

TABLE 3

MIXED STANDARD SOLUTIONS

Solution	Elements
I	Be, Cd, Mn, Pb, Se and Zn
II	Ba, Co, Cu, Fe, and V
III	As and Mo
IV	Al, Ca, Cr, K, Na, Ni, Li, and Sr
V	Ag[a], Mg, Sb, and Tl
VI	P

[a] See the note in Sec. 7.4.

TABLE 4

EXAMPLE ICP PRECISION AND ACCURACY DATA[a]

Element	Sample No. 1				Sample No. 2				Sample No. 3			
	True Conc. (µg/L)	Mean Conc. (µg/L)	RSD[b] (%)	Accuracy[d] (%)	True Conc. (µg/L)	Mean Conc. (µg/L)	RSD[b] (%)	Accuracy[d] (%)	True Conc. (µg/L)	Mean Conc. (µg/L)	RSD[b] (%)	Accuracy[d] (%)
Be	750	733	6.2	98	20	20	9.8	100	180	176	5.2	98
Mn	350	345	2.7	99	15	15	6.7	100	100	99	3.3	99
V	750	749	1.8	100	70	69	2.9	99	170	169	1.1	99
As	200	208	7.5	104	22	19	23	86	60	63	17	105
Cr	150	149	3.8	99	10	10	18	100	50	50	3.3	100
Cu	250	235	5.1	94	11	11	40	100	70	67	7.9	96
Fe	600	594	3.0	99	20	19	15	95	180	178	6.0	99
Al	700	696	5.6	99	60	62	33	103	160	161	13	101
Cd	50	48	12	96	2.5	2.9	16	116	14	13	16	93
Co	700	512	10	73	20	20	4.1	100	120	108	21	90
Ni	250	245	5.8	98	30	28	11	93	60	55	14	92
Pb	250	236	16	94	24	30	32	125	80	80	14	100
Zn	200	201	5.6	100	16	19	45	119	80	82	9.4	102
Se[c]	40	32	21.9	80	6	8.5	42	142	10	8.5	8.3	85

These data are provided for guidance purposes only.

[a] Not all elements were analyzed by all laboratories.

[b] RSD = relative standard deviation.

[c] Results for Se are from two laboratories.

[d] Accuracy is expressed as the mean concentration divided by the true concentration times 100.

TABLE 5

EXAMPLE ICP-AES PRECISION AND ACCURACY FOR AQUEOUS SOLUTIONS

Element	Mean Conc. (mg/L)	n	RSD (%)	Accuracy (%)
Al	14.8	8	6.3	100
Sb	15.1	8	7.7	102
As	14.7	7	6.4	99
Ba	3.66	7	3.1	99
Be	3.78	8	5.8	102
Cd	3.61	8	7.0	97
Ca	15.0	8	7.4	101
Cr	3.75	8	8.2	101
Co	3.52	8	5.9	95
Cu	3.58	8	5.6	97
Fe	14.8	8	5.9	100
Pb	14.4	7	5.9	97
Mg	14.1	8	6.5	96
Mn	3.70	8	4.3	100
Mo	3.70	8	6.9	100
Ni	3.70	7	5.7	100
K	14.1	8	6.6	95
Se	15.3	8	7.5	104
Ag	3.69	6	9.1	100
Na	14.0	8	4.2	95
Tl	15.1	7	8.5	102
V	3.51	8	6.6	95
Zn	3.57	8	8.3	96

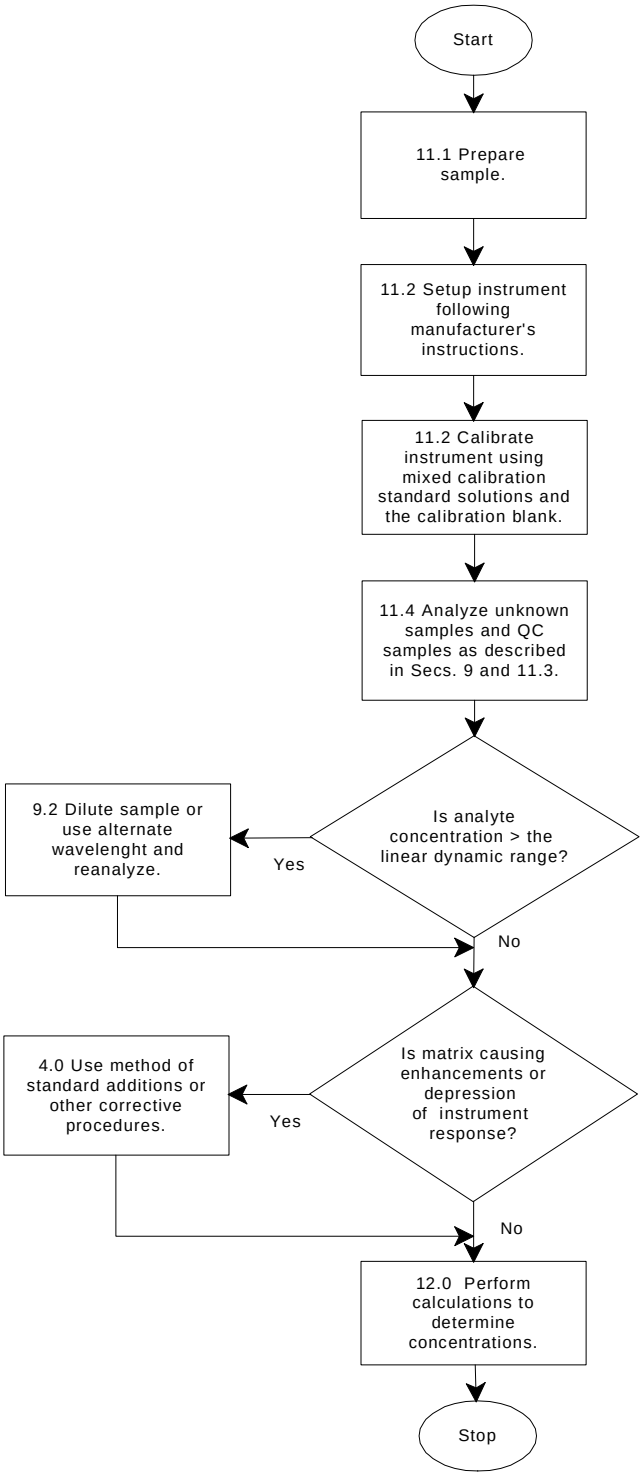
These performance values are independent of sample preparation because the labs analyzed portions of the same solutions and are provided for illustrative purposes only.
n= Number of measurements.
Accuracy is expressed as a percentage of the nominal value for each analyte in acidified, multi-element solutions.
These data are provided for guidance purposes only.

TABLE 6

EXAMPLE ICP-AES PRECISION AND BIAS FOR SOLID WASTE DIGESTS

	Spiked Coal Fly Ash (NIST-SRM 1633a)				Spiked Electroplating Sludge			
Element	Mean Conc. (mg/L)	n	RSD (%)	Bias (% AA)	Mean Conc. (mg/L)	n	RSD (%)	Bias (% AA)
Al	330	8	16	104	127	8	13	110
Sb	3.4	6	73	96	5.3	7	24	120
As	21	8	83	270	5.2	7	8.6	87
Ba	133	8	8.7	101	1.6	8	20	58
Be	4.0	8	57	460	0.9	7	9.9	110
Cd	0.97	6	5.7	101	2.9	7	9.9	90
Ca	87	6	5.6	208	954	7	7.0	97
Cr	2.1	7	36	106	154	7	7.8	93
Co	1.2	6	21	94	1.0	7	11	85
Cu	1.9	6	9.7	118	156	8	7.8	97
Fe	602	8	8.8	102	603	7	5.6	98
Pb	4.6	7	22	94	25	7	5.6	98
Mg	15	8	15	110	35	8	20	84
Mn	1.8	7	14	104	5.9	7	9.6	95
Mo	891	8	19	105	1.4	7	36	110
Ni	1.6	6	8.1	91	9.5	7	9.6	90
K	46	8	4.2	98	51	8	5.8	82
Se	6.4	5	16	73	8.7	7	13	101
Ag	1.4	3	17	140	0.75	7	19	270
Na	20	8	49	130	1380	8	9.8	95
Tl	6.7	4	22	260	5.0	7	20	180
V	1010	5	7.5	100	1.2	6	11	80
Zn	2.2	6	7.6	93	266	7	2.5	101

These performance values are independent of sample preparation because the labs analyzed portions of the same digests and are provided for illustrative purposes only.
n = Number of measurements.
Bias for the ICP-AES data is expressed as a percentage of atomic absorption spectroscopy (AA) data for the same digests.
These data are provided for guidance purposes only.


Start
11.1 Prepare sample.
11.2 Setup instrument following manufacturer's instructions.
11.2 Calibrate instrument using mixed calibration standard solutions and the calibration blank.
11.4 Analyze unknown samples and QC samples as described in Secs. 9 and 11.3.
Is analyte concentration > the linear dynamic range?
Yes
9.2 Dilute sample or use alternate wavelenght and reanalyze.
No
Is matrix causing enhancements or depression of instrument response?
Yes
4.0 Use method of standard additions or other corrective procedures.
No
12.0 Perform calculations to determine concentrations.
Stop

Title: ICP – Atomic Emission Spectroscopy
[6010B]

Approvals (Signature/Date):

Peter Moreton 03/29/2007
Lab Director

Natasha Zahedi 03/31/2007
Operations Manager

Rene Boongaling 03/28/2007
QA Manager

This SOP was previously identified as SOP No. SF-IN-0814-09.

Facility Distribution No. _____ **Distributed To:_____**

SF-IN-0814-09A

ICP – Atomic Emission Spectroscopy

Reference – SW-846 Method 6010B, Revision 2, December 1996

I hereby acknowledge that I have read and understood this revision of the SOP and will not deviate from it. Full Signature Approvals are kept on file with STL's QA Standard Practice Records.

Approved By:

Peter Moreton / Laboratory Director

Rene Boongaling / Quality Assurance

Natasha Zahedi / Operations Manager

Note: SOP Format

The basis of this SOP is EPA SW-846 Method: 6010B

The direct text from these methods forms the body of this SOP and is printed in italics and highlighted with a grey background.

STL-SF deviations from these methods are highlighted in **bold red text** in electronic versions and **bold grey text** in printed versions**.** In addition, STL-SF deviations are inset from the Reference Method text.

The SOP is structured as follows:

SECTION A - SAFETY, WASTE MANAGEMENT AND POLLUTION PREVENTION

SECTION B – EPA METHOD 6010B

SECTION C – REVISION HISTORY AND ACCOMPANYING DOCUMENTS

A1 –SAFETY

"Employees must abide by the policies and procedures in the Corporate Safety Manual, Radiation Safety Manual and this document."

A1.1 Specific Safety Concerns or Requirements

The ICP / ICP-MS instruments contain zones that have elevated temperatures. The analyst needs to be aware of the locations of those zones, and must cool them to room temperature prior to working on them.

There are areas of high voltage in both the gas chromatograph and the mass spectrometer. Depending on the type of work involved, either turn the power to the instrument off, or disconnect it from its source of power.

A1.2 Primary Materials Used

The following is a list of the materials used in this method, which have a serious or significant hazard rating. NOTE: This list does not include all materials used in the method. The table contains a summary of the primary hazards listed in the MSDS for each of the materials listed in the table. A complete list of materials used in the method can be found in the reagents and materials section. Employees must review the information in the MSDS for each material before using it for the first time or when there are major changes to the MSDS.

Material (1)	Hazards	Exposure Limit (2)	Signs and symptoms of exposure
Hydrochloric Acid	Corrosive Poison	5ppm-Ceiling	Inhalation of vapors can cause coughing, choking, inflammation of the nose, throat, and upper respiratory tract, and in severe cases, pulmonary edema, circulatory failure, and death. Can cause redness, pain, and severe skin burns. Vapors are irritating and may cause damage to the eyes. Contact may cause severe burns and permanent eye damage.
Nitric Acid	Corrosive Oxidizer Poison	2ppm-TWA 4ppm-STEL	Nitric acid is extremely hazardous; it is corrosive, reactive, an oxidizer, and a poison. Inhalation of vapors can cause breathing difficulties and lead to pneumonia and pulmonary edema, which may be fatal. Other symptoms may include coughing, choking, and irritation of the nose, throat, and respiratory tract. Can cause redness, pain, and severe skin burns. Concentrated solutions cause deep ulcers and stain skin a yellow or yellow-brown color. Vapors are irritating and may cause damage to the eyes. Contact may cause severe burns and permanent eye damage.

A2.0 WASTE MANAGEMENT AND POLLUTION PREVENTION

A2.1 All waste will be disposed of in accordance with Federal, State and Local regulations. Where reasonably feasible, technological changes have been made to minimize the potential for pollution to the environment. Employees will abide by this method and the policies in section 13 of the Corporate Safety Manual for "Waste Management and Pollution Prevention." Waste Streams Produced by the Method

The following waste streams are produced when this method is carried out.

- **Aqueous waste generated from analysis. This material may have a pH of less than 2.0.**
- **Solvent waste generated from analysis**
- **Solid waste generated from analysis.**
- **Expired Standards**

A2.2 Disposal and removal of waste from STL San Francisco will be handled by a professional waste management company in accordance with the requirements specified in the STL Corporate Health & Safety Manual.

A2.3 Pollution Prevention – STL San Francisco will adhere to the pollution prevention requirements listed in the STL Corporate Safety Manual. No specific method modification in this procedure reflects additional pollution prevention.

SECTION B – EPA METHOD 6010B

METHOD 6010B

1.0 SCOPE AND APPLICATION

1.1 Inductively coupled plasma-atomic emission spectrometry (ICP-AES) determines trace elements, including metals, in solution. The method is applicable to all of the elements listed in Table 1. All matrices, excluding filtered groundwater samples but including ground water, aqueous samples, TCLP and EP extracts, industrial and organic wastes, soil's, sludge's, sediments, and other solid wastes, require digestion prior to analysis. Groundwater samples that have been pre-filtered and acidified will not need acid digestion. Samples which are not digested must either use an internal standard or be matrix matched with the standards. Refer to Chapter Three for the appropriate digestion procedures.

1.2 Table 1 lists the elements for which this method is applicable. Detection limits, sensitivity, and the optimum and linear concentration ranges of the elements can vary with the wavelength, spectrometer, matrix and operating conditions. Table 1 gives the recommended analytical wavelengths and the estimated instrumental detection limits for the elements in clean aqueous matrices. The instrument detection limit data may be used to estimate instrument and method performance for other sample matrices. Elements and matrices other than those listed in the Table may be analyzed by this method if performance at the concentration levels of interest (see Section 8.0) is demonstrated.

Table 1: Recommended Wavelengths and Estimated Instrument Detection Limits

Element	Wavelength	STL SF Wavelength	Estimated IDL
Aluminum	308.215	237.313	30
Antimony	206.836	188.98	21
Arsenic	193.696		35
Barium	455.403	493.408	.87
Beryllium	313.042	234.861	.18
Boron	249.678x2	NR	3.8
Cadmium	226.502		2.3
Calcium	317.933	373.69	6.7
Chromium	267.716	205.560	4.7
Cobalt	228.616	230.786	4.7
Copper	324.752	327.395	3.6
Iron	259.940	273.954	4.1
Lead	220.353		28
Magnesium	279.079		2.8
Manganese	257.610		20
Mercury	194.227x2	NR	.93
Molybdenum	202.031		17
Nickel	231.604 x2		5.3
Phosphorus	213.618	NR	51

Table 1: Recommended Wavelengths and Estimated Instrument Detection Limits			
Element	**Wavelength**	**STL SF Wavelength**	**Estimated IDL**
Potassium	**766.490**	**404.72/769.89**	
Selenium	**196.026**		50
Silica	**251.611**	**NR**	17
Silver	**328.068**		4.7
Sodium	**588.995**	**589.597**	19
Strontium	**407.771**	**NR**	.28
Thallium	**190.864**	**190.794**	27
Tin	**189.980x2**		17
Titanium	**334.941**	**NR**	5
Vanadium	**292.402**		5
Zinc	**213.856x2**	**206.200**	1.2

1.3 Users of the method should state the data quality objectives prior to analysis and must document and have on file the required initial demonstration performance data described in the following sections prior to using the method for analysis.

DOC's are updated annually

1.4 Use of this method is restricted to spectroscopist's who are knowledgeable in the correction of spectral, chemical, and physical interferences described in this method.

2.0 SUMMARY OF METHOD

2.1 Prior to analysis, samples must be solubilized or digested using appropriate Sample Preparation Methods (e.g. Chapter Three). When analyzing groundwater samples for dissolved constituents, acid digestion is not necessary if the samples are filtered and acid preserved prior to analysis.

Refer to SOP's 8.05 (Dissolved Waters), 8.08 (Total Waters) and 8.10 (Soils) for appropriate sample digestion methods.

2.2 This method describes multi-elemental determinations by ICP-AES using sequential or simultaneous optical systems and axial or radial viewing of the plasma.

The Varian Vista Pro uses a simultaneous optical system in axial mode.

The instrument measures characteristic emission spectra by optical spectrometry. Samples are nebulized and the resulting aerosol is transported to the plasma torch. Element-specific emission spectra are produced by radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer, and the intensities of the emission lines are monitored by photosensitive devices. Background correction is required for trace element determination. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. In one mode of analysis the position used should be as free as possible from spectral interference and should reflect the same change in background intensity as occurs

at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result. The possibility of additional interferences named in Section 3.0 should also be recognized and appropriate corrections made; tests for their presence are described in Section 8.5.

Alternatively, users may choose multivariate calibration methods. In this case, point selections for background correction are superfluous since whole spectral regions are processed.

3.0 INTERFERENCES

3.1 Spectral interferences are caused by background emission from continuous or recombination phenomena, stray light from the line emission of high concentration elements, overlap of a spectral line from another element, or unresolved overlap of molecular band spectra.

3.1.1 Background emission and stray light can usually be compensated for by subtracting the background emission determined by measurements adjacent to the analyte wavelength peak. Spectral scans of samples or single element solutions in the analyte regions may indicate when alternate wavelengths are desirable because of severe spectral interference. These scans will also show whether the most appropriate estimate of the background emission is provided by an interpolation from measurements on both sides of the wavelength peak or by measured emission on only one side. The locations selected for the measurement of background intensity will be determined by the complexity of the spectrum adjacent to the wavelength peak. The locations used for routine measurement must be free of off-line spectral interference (inter-element or molecular) or adequately corrected to reflect the same change in background intensity as occurs at the wavelength peak. For multivariate methods using whole spectral regions, background scans should be included in the correction algorithm. Off-line spectral interferences are handled by including spectra on interfering species in the algorithm.

3.1.2 To determine the appropriate location for off-line background correction, the user must scan the area on either side adjacent to the wavelength and record the apparent emission intensity from all other method analyte's. This spectral information must be documented and kept on file.

The manufacturer's technical representative set up the background positions, wavelength selection and IEC's as part of the initial method development. All spectral scans and information are saved on the instrument.

The location selected for background correction must be either free of off-line inter-element spectral interference or a computer routine must be used for automatic correction on all determinations. If a wavelength other than the recommended wavelength is used, the analyst must determine and document both the overlapping and nearby spectral interference effects from all method analytes and common elements and provide for their automatic correction on all analyses.

The IEC table used corrects for interfering peaks and background effects.

Tests to determine spectral interference must be done using analyte concentrations that will adequately describe the interference. Normally, 100 mg/L single element solutions are sufficient; however, for analytes such as iron that may be found at high concentration, a more appropriate test would be to use a concentration near the upper analytical range limit.

3.1.3 Spectral overlaps may be avoided by using an alternate wavelength or can be compensated by equations that correct for interelement contributions. Instruments that use equations for interelement correction **require** the interfering elements be analyzed at the same time as the element of interest. When operative and uncorrected, interferences will produce false positive determinations and be reported as analyte concentrations. More extensive information on interferant effects at various wavelengths and resolutions is available in reference wavelength tables and books. Users may apply interelement correction equations determined on their instruments with tested concentration ranges to compensate (off line or on line) for the effects of interfering elements. Some potential spectral interference observed for the recommended wavelengths are given in Table 2. For multivariate methods using whole spectral regions, spectral interferences are handled by including spectra of the interfering elements in the algorithm. The interferences listed are only those that occur between method analytes. Only interferences of a direct overlap nature are listed. These overlaps were observed with a single instrument having a working resolution of 0.035 nm.

3.1.4 When using interelement correction equations, the interference may be expressed as analyte concentration equivalents (i.e. false analyte concentrations) arising from 100 mg/L of the interference element. For example, assume that As is to be determined (at 193.696 nm) in a sample containing approximately 10 mg/L of Al. According to Table 2, 100 mg/L of Al would yield a false signal for As equivalent to approximately 1.3mg/L. Therefore, the presence of 10 mg/L of Al would result in a false signal for As equivalent to approximately 0.13 mg/L. The user is cautioned that other instruments may exhibit somewhat different levels of interference than those shown in Table 2. The interference effects must be evaluated for each individual instrument since the intensities will vary.

3.1.5 Interelement corrections will vary for the same emission line among instruments because of differences in resolution, as determined by the grating, the entrance and exit slit widths, and by the order of dispersion. Interelement corrections will also vary depending upon the choice of background correction points. Selecting a background correction point where an interfering emission line may appear should be avoided when practical. Interelement corrections that constitute a major portion of an emission signal may not yield accurate data. Users should not forget that some samples may contain uncommon elements that could contribute spectral interferences.

3.1.6 The interference effects must be evaluated for each individual instrument whether configured as a sequential or simultaneous instrument. For each instrument, intensities will vary not only with optical resolution but also with operating conditions (such as power, viewing height and argon flow rate). When using the recommended wavelengths, the analyst is required to determine and document for each wavelength the effect from referenced interferences (Table 2) as well as any other suspected interference that may be specific to the instrument or matrix. The analyst is encouraged to utilize a computer routine for automatic correction on all analyses.

3.1.7 Users of sequential instruments must verify the absence of spectral interference by scanning over a range of 0.5 nm centered on the wavelength of interest for several samples. The range for lead, for example, would be from 220.6 to 220.1 nm. This procedure must be repeated whenever a new matrix is to be analyzed and when a new calibration curve using different instrumental conditions is to be prepared. Samples that show an elevated background emission across the range may be background corrected by applying a correction factor equal to the emission adjacent to the line or at two points on either side of the line and interpolating between them. An alternate wavelength that does not exhibit a background shift or spectral overlap may also be used.

The Varian VistaPro is a simultaneous instrument and this step is not conducted.

3.1.8 If the correction routine is operating properly, the determined apparent analyte(s) concentration from analysis of each interference solution should fall within a specific concentration range around the calibration blank. The concentration range is calculated by multiplying the concentration of the interfering element by the value of the correction factor being tested and divided by 10. If after the subtraction of the calibration blank the apparent analyte concentration falls outside of this range in either a positive or negative direction, a change in the correction factor of more than 10% should be suspected.

This step is not conducted. A daily ISCAB solution is run at the start of the 1st run.

The cause of the change should be determined and corrected and the correction factor updated. The interference check solutions should be analyzed more than once to confirm a change has occurred. Adequate rinse time between solutions and before analysis of the calibration blank will assist in the confirmation.

Run single element interferences to verify

3.1.9 When interelement corrections are applied, their accuracy should be verified, daily, by analyzing spectral interference check solutions.

The spectral interference check solution (ICSAB) is run daily at the beginning of each sequence and is composed by mixing the interference test solution "A" with the analyte spiking standard "B". The ICSAB is made by adding 10ml of the VHG calibration solution (100/1000/4000 ppm of reported analytes - Fe, Ca, Mg, Mn/ Na, K) and 100 ml of the 10,000 ppm interference solution (Fe, Ca , Al, Mg) to 2000 ml of 2% HNO_3 and 5% HCL.

If the correction factors or multivariate correction matrices tested on a daily basis are found to be within the 20% criteria for 5 consecutive days, the required verification frequency of those factors in compliance may be extended to a weekly basis.

The ISCAB is run once daily.

Also, if the nature of the samples analyzed is such they do not contain concentrations of the interfering elements at ± one reporting limit from zero, daily verification is not required. All interelement spectral correction factors or multivariate correction matrices must be verified and updated every six months or when an instrumentation change, such as in the torch, nebulizer, injector, or plasma conditions occurs. Standard solution should be inspected to ensure that there is no contamination that may be perceived as a spectral interference.

The ICSAB is run at the beginning of the first sequence of the day.

3.1.10 When interelement corrections are not used, verification of absence of interferences is required.

3.1.10.1 One method is to use a computer software routine for comparing the determinative data to limits files for notifying the analyst when an interfering element is detected in the sample at a concentration that will produce either an apparent false positive concentration, (i.e., greater than) the analyte instrument

detection limit, or false negative analyte concentration, (i.e., less than the lower control limit of the calibration blank defined for a 99% confidence interval).

3.1.10.2 Another method is to analyze an Interference Check Solution(s) which contains similar concentrations of the major components of the samples (>10mg/L) on a continuing basis to verify the absence of effects at the wavelengths selected. These data must be kept on file with the sample analysis data. If the check solution confirms an operative interference that is > 20% of the analyte concentration, the analyte must be determined using (1) analytical and background correction wavelengths (or spectral regions) free of the interference, (2) by an alternative wavelength, or (3) by another documented test procedure.

3.2 Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they must be reduced by diluting the sample or by using a peristaltic pump, by using an internal standard or by using a high solids nebulizer. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, affecting aerosol flow rate and causing instrumental drift. The problem can be controlled by wetting the argon prior to nebulization, using a tip washer, using a high solids nebulizer or diluting the sample. Also, it has been reported that better control of the argon flow rate, especially to the nebulizer, improves instrument performance: this may be accomplished with the use of mass flow controllers. The test described in Section 8.5.1 will help determine if a physical interference is present.

The nebulizer is visually checked by taking out of the spray chamber and placing it a small tube and starting the pump. The spray is checked for symmetry and the nebulizer cleaned with by backflushing with acid and de-onized water. Nebulizer checks are done in concert with torch cleaning.

3.3 Chemical interferences include molecular compound formation, ionization effects, and solute vaporization effects. Normally, these effects are not significant with the ICP technique, but if observed, can be minimized by careful selection of operating conditions (incident power, observation position, and so forth), by buffering of the sample, by matrix matching, and by standard addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element.
3.4 Memory interferences result when analytes in a previous sample contribute to the signals measured in a new sample. Memory effects can result from sample deposition on the uptake tubing to the nebulizer and from the build up of sample material in the plasma torch and spray chamber. The site where these effects occur is dependent on the element and can be minimized by flushing the system with a rinse blank between samples. The possibility of memory interferences should be recognized within an analytical run and suitable rinse times should be used to reduce them. The rinse times necessary for a particular element must be estimated prior to analysis. This may be achieved by aspirating a standard containing elements at a concentration ten times the usual amount or at the top of the linear dynamic range. The aspiration time for this sample should be the same as a normal sample analysis period, followed by analysis of the rinse blank at designated intervals. The length of time required to reduce analyte signals to within a factor of two of the method detection limit should be noted. Until the required rinse time is established, this method suggests a rinse period of at least 60 seconds between samples and standards.

If memory interference is suspected, the sample must be reanalyzed after a rinse period of sufficient length. Alternate rinse times may be established by the analyst based upon their DQOs.

Rinse times are automatically controlled by the ICP software using a routine called "SmartRinse" which determines rinse times by continuously scanning the rinse solution and confirming the absence of memory effects prior to sample introduction.

3.5 Users are advised that high salt concentrations can cause analyte signal suppressions and confuse interference tests. If the instrument does not display negative values, fortify the interference check solution with the elements of interest at 0.5 to 1 mg/L and measure the added standard concentration accordingly. Concentrations should be within 20% of the true spiked concentration or dilution of the samples will be necessary. In the absence of measurable analyte, overcorrection could go undetected if a negative value is reported as zero.

This step is not performed

3.6 The dashes in Table 2 indicate that no measurable interferences were observed even at higher interferant concentrations. Generally, interferences were discernible if they produced peaks, or background shifts, corresponding to 2 to 5% of the peaks generated by the analyte concentrations.

4.0 APPARATUS AND MATERIALS

4.1 Inductively coupled argon plasma emission spectrometer:

4.1.1 Computer-controlled emission spectrometer with background correction.

4.1.2 Radio-frequency generator compliant with FCC regulations.

4.1.3 Optional mass flow controller for argon nebulizer gas supply.

4.1.4 Optional peristaltic pump.

4.1.5 Optional Autosampler.

4.1.6 Argon gas supply - high purity.

4.2 Volumetric flasks and pipettes of suitable precision and accuracy.

5.0 REAGENTS

5.1 Reagent or trace metals grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. If the purity of a reagent is in question analyze for contamination. If the concentration of the contamination is less than the MDL then the reagent is acceptable.

5.1.1 Hydrochloric acid (conc.), HCl.

5.1.2 Hydrochloric acid (1:1), HCl. Add 500mL concentrated HCl to 400mL water and dilute to 1 liter in an appropriately sized beaker.

5.1.3 Nitric acid (conc.), HNO_3

5.1.4 Nitric acid (1:1), HNO_3. Add 500mL concentrated HNO_3 to 400mL water and dilute to 1 liter in an appropriately sized beaker.

5.2 Reagent Water. All references to water in the method refer to reagent water unless otherwise specified. Reagent water will be interference free. Refer to Chapter One for a definition of reagent water.

5.3 Standard stock solutions may be purchased or prepared from ultra- high purity grade chemicals or metals (99.99% pure or greater). All salts must be dried for 1 hour at 105˚C, unless otherwise specified.

Note: This section does not apply when analyzing samples that have been prepared by Method 3040.

CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.

Typical stock solution preparation procedures follow: Concentrations are calculated based upon the weight of pure metal added, or with the use of the element fraction and the weight of the metal salt added.

For metals: Concentration (ppm) = weight (mg)/ volume (L)
For metal salts: Concentration (ppm) = (weight (mg) x mole fraction) / volume (L)

All calibration and check solutions are purchased as custom mixes and no solutions are made from metal salts.

5.3.1 Aluminum solution, stock, 1mL = 1000 µg Al: Dissolve 1.000 g of aluminum metal, weighed accurately to at least four significant figures, in an acid mixture of 4.0mL of (1:1) HCl and 1.0mL of concentrated $HN0_3$ in a beaker. Warm the beaker slowly. When dissolution is complete, transfer solution quantitatively to a 1-liter flask, add an additional 10.0mL of (1:1) HCl and dilute to volume with reagent water.

NOTE: Weight of analyte is expressed to four significant figures for consistency with the weights below because rounding to two decimal places can contribute up to 4 % error for some of the compounds.

5.3.2 Antimony solution, stock, 1mL = 1000 µg Sb: Dissolve 2.6673 g $K(SbO)C_4 H_4 O_6$ (element fraction Sb = 0.3749), weighed accurately to at least four significant figures, in water, add 10mL (1:1) HCl, and dilute to volume in a 1,000mL volumetric flask with water.

5.3.3 Arsenic solution, stock, 1mL = 1000 µg As: Dissolve 1.3203 g of $As_2 O_3$ (element fraction

As = 0.7574), weighed accurately to at least four significant figures, in 100mL of water containing 0.4 g NaOH. Acidify the solution with 2mL concentrated HNO_3 and dilute to volume in a 1,000mL volumetric flask with water.

5.3.4 Barium solution, stock, 1mL = 1000 µg Ba: Dissolve 1.5163 g $BaCl_2$ (element fraction Ba = 0.6595), dried at 250˚C for 2 hours, weighed accurately to at least four significant figures, in 10mL water with 1mL (1:1) HCl. Add 10.0mL (1:1) HCl and dilute to volume in a 1,000mL volumetric flask with water.

5.3.5 Beryllium solution, stock, 1mL = 1000 µg Be: Do not dry. Dissolve 19.6463g $BeSO_4H_4O_2$ (element fraction Be = 0.0509), weighed accurately to at least four significant figures, in water, add 10.0mL concentrated HNO_3 , and dilute to volume in a 1,000mL volumetric flask with water.

5.3.6 Boron solution, stock, 1 mL = 1000 µg B: Do not dry. Dissolve 5.716 g anhydrous H_3BO_3 (B fraction = 0.1749), weighed accurately to at least four significant figures, in reagent water and dilute in a 1-L volumetric flask with reagent water. Transfer immediately after mixing in a clean polytetrafluoroethylene (PTFE) bottle to minimize any leaching of boron from the glass volumetric container. Use of a non-glass volumetric flask is recommended to avoid boron contamination from glassware.

5.3.7 Cadmium solution, stock, 1mL = 1000 µg Cd: Dissolve 1.1423 g CdO (element fraction Cd = 0.8754), weighed accurately to at least four significant figures, in a minimum amount of (1:1) HNO_3. You should heat to increase the rate of dissolution. Add 10.0mL concentrated HNO_3 and dilute to volume in a 1,000mL volumetric flask with water.

5.3.8 Calcium solution, stock, 1mL = 1000 µg Ca: Suspend 2.4969g $CaCO_3$ (element Ca fraction = 0.4005), dried at 180˚C for 1 hour before weighing, weighed accurately to at least four significant figures, in water and dissolve cautiously with a minimum amount of (1:1) HNO_3 . Add 10.0mL concentrated HNO_3 and dilute to volume in a 1,000mL volumetric flask with water.

5.3.9 Chromium solution, stock, 1mL = 1000 µg Cr: Dissolve 1.9231 g CrO_3 (element fraction Cr = 0.5200), weighed accurately to at least four significant figures, in water. When solution is complete, acidify with 10mL concentrated HNO_3 and dilute to volume in a 1,000mL volumetric flask with water.

5.3.10 Cobalt solution, stock, 1mL = 1000 µg Co: Dissolve 1.00g of cobalt metal, weighed accurately to at least four significant figures, in a minimum amount of (1:1) HNO_3 Add 10.0mL (1:1) HCl and dilute to volume in a 1,000mL volumetric flask with water.

5.3.11 Copper solution, stock, 1mL = 1000 µg Cu: Dissolve 1.2564g CuO (element fraction Cu = 0.7989), weighed accurately to at least four significant figures), in a minimum amount of (1:1) HNO_3. Add 10.0mL concentrated HNO_3 and dilute to volume in a 1,000mL volumetric flask with water.

5.3.12 Iron solution, stock, 1mL = 1000 µg Fe: Dissolve 1.4298 g Fe_2O_3 (element fraction Fe = 0.6994), weighed accurately to at least four significant figures, in a warm mixture of 20mL (1:1) HCl and 2mL of concentrated HNO_3 . Cool, add an additional 5.0mL of concentrated HNO_3, and dilute to volume in a 1,000mL volumetric flask with water.

5.3.13 Lead solution, stock, 1mL = 1000 µg Pb: Dissolve 1.5985 g $Pb(NO_3)_2$ (element fraction

Pb = 0.6256), weighed accurately to at least four significant figures, in a minimum amount of (1:1) HNO_3. Add 10mL (1:1) HNO_3 and dilute to volume in a 1,000mL volumetric flask with water.

5.3.14 Lithium solution, stock, 1mL = 1000 µg Li: Dissolve 5.3248g lithium carbonate (element fraction Li = 0.1878), weighed accurately to at least four significant figures, in a minimum amount of (1:1) HCl and dilute to volume in a 1,000mL volumetric flask with water.

5.3.15 Magnesium solution, stock, 1mL = 1000 µg Mg: Dissolve 1.6584g MgO (element fraction Mg = 0.6030), weighed accurately to at least four significant figures, in a minimum amount of (1:1) HNO_3. Add 10.0mL (1:1) concentrated HNO_3 and dilute to volume in a 1,000mL volumetric flask with water.

5.3.16 Manganese solution, stock, 1mL = 1000 µg Mn: Dissolve 1.00g of manganese metal, weighed accurately to at least four significant figures, in acid mixture (10mL concentrated HCl and 1mL concentrated HNO_3) and dilute to volume in a 1,000mL volumetric flask with water.

5.3.17 Mercury solution, stock, 1mL = 1000 µg Hg: Do not dry, highly toxic element. Dissolve 1.354g $HgCl_2$ (Hg fraction = 0.7388) in reagent water. Add 50.0mL concentrated HNO_3 and dilute to volume in 1-L volumetric flask with reagent water.

5.3.18 Molybdenum solution, stock, 1mL = 1000 µg Mo: Dissolve 1.7325 g $(NH_4)_6\ Mo_7O_{24}$.4H2O (element fraction Mo = 0.5772), weighed accurately to at least four significant figures, in water and dilute to volume in a 1,000mL volumetric flask with water.

5.3.19 Nickel solution, stock, 1mL = 1000 µg Ni: Dissolve 1.00g of nickel metal weighed accurately to at least four significant figures, in 10.0mL hot concentrated HNO_3 ,cool and dilute to volume in a 1,000mL volumetric flask with water.

5.3.20 Phosphate solution, stock, 1mL = 1000 µg P: Dissolve 4.3937g anhydrous KH_2PO_4 (element fraction P = 0.2276), weighed accurately to at least four significant figures in water. Dilute to volume in a 1,000mL volumetric flask with water.

5.3.21 Potassium solution, stock, 1mL = 1000 µg K: Dissolve 1.9069g KCl (element fraction K = 0.5244) dried at 110˚C, weighed accurately to at least four significant figures, in water, and dilute to volume in a 1,000mL volumetric flask with water.

5.3.22 Selenium solution, stock, 1mL = 1000 µg Se: Do not dry. Dissolve 1.6332g H_2SeO_3 (element fraction Se = 0.6123), weighed accurately to at least four significant figures, in water and dilute to volume in a 1,000mL volumetric flask with water.

5.3.23 Silica solution, stock, 1mL = 1000 µg SiO_2: Do not dry. Dissolve 2.964g NH_4SiF_6, weighed accurately to at least four significant figures, in 200mL (1:20) HCl with heating at 85˚C to effect dissolution. Let solution cool and dilute to volume in a 1-L volumetric flask with reagent water.

5.3.24 Silver solution, stock, 1mL = 1000 µg Ag: Dissolve 1.5748g $AgNO_3$ (element fraction Ag = 0.6350), weighed accurately to at least four significant figures, in water and 10mL concentrated HNO_3. Dilute to volume in a 1,000mL volumetric flask with water.

5.3.25 Sodium solution, stock, 1mL = 1000 µg Na: Dissolve 2.5419 g NaCl (element fraction

Na = 0.3934), weighed accurately to at least four significant figures, in water. Add 10.0mL concentrated HNO_3 and dilute to volume in a 1,000mL volumetric flask with water.

5.3.26 Strontium solution, stock, 1mL = 1000 µg Sr: Dissolve 2.4154 g of strontium nitrate ($Sr(NO_3)_2$) (element fraction Sr = 0.4140), weighed accurately to at least four significant figures, in a 1L flask containing 10mL of conc. HCl and 700mL of DI water. Dilute to volume in a 1,000mL volumetric flask with water.

5.3.27 Thallium solution, stock, 1mL = 1000 µg Tl: Dissolve 1.3034g $TlNO_3$ (element fraction Tl = 0.7672), weighed accurately to at least four significant figures, in water. Add 10.0mL concentrated HNO_3 and dilute to volume in a 1,000mL volumetric flask with water.

5.3.28 Tin solution, stock, 1mL = 1000 µg Sn: Dissolve 1.000g Sn shot, weighed accurately to at least 4 significant figures, in 200mL (1:1) HCl with heating to affect dissolution. Let solution cool and dilute with (1:1) HCl in a 1-L volumetric flask.

5.3.29 Vanadium solution, stock, 1mL = 1000 µg V: Dissolve 2.2957g NH_4VO_3 (element fraction V = 0.4356), weighed accurately to at least four significant figures, in a minimum amount of concentrated HNO_3 and Heat to increase the rate of dissolution. Add 10.0mL concentrated HNO_3 and dilute to volume in a 1,000mL volumetric flask with water.

5.3.30 Zinc solution, stock, 1mL = 1000 µg Zn: Dissolve 1.2447g ZnO (element fraction Zn = 0.8034), weighed accurately to at least four significant figures, in a minimum amount of dilute HNO_3. Add 10.0mL concentrated HNO_3 and dilute to volume in a 1,000mL volumetric flask with water.

All elemental standards are in the form of pre-made custom metals mixes are purchased from CPI or equivalent. Source purity is > 99.99% and are analyzed for trace impurities. An example solution containing analytes and interference checks has the following concentrations:

Example Mixed Calibration Solution	
Compound	**Concentration ug/ml**
Ag	0.100
Ca	500
Cr	0.100
Mg	500.0
Ni	0.200
Ti	10.0
Al	500.0
Cd	0.050
Cu	0.100
Mo	10.0
P	500.0
V	0.200
As	0.100
Cl	3600
Fe	500.0

Example Mixed Calibration Solution	
Compound	**Concentration ug/ml**
Mn	0.100
Se	0.100
Zn	0.100
C	1000.0
Co	0.200
K	500.0
Na	500.0
S	500.0

5.4 Mixed calibration standard solutions - Prepare mixed calibration standard solutions by combining appropriate volumes of the stock solutions in volumetric flasks (Table 3). Add the appropriate types and volumes of acids so that the standards are matrix matched with the sample digestate's. Prior to preparing the mixed standards, each stock solution should be analyzed separately to determine possible spectral interference or the presence of impurities. Care should be taken when preparing the mixed standards to ensure that the elements are compatible and stable together. Transfer the mixed standard solutions to FEP fluorocarbon or previously unused polyethylene or polypropylene bottles for storage. Fresh mixed standards should be prepared, as needed, with the realization that concentration can change on aging.

Metals working standards are good for 6 month. All standards are prepared using solutions within the defined expiration dates. Solution preparation is documented in the Metals Standard Preparation Logbook (Attachment I).

NOTE: If the addition of silver to the recommended acid combination results in an initial precipitation, add 15mL of water and warm the flask until the solution clears. Cool and dilute to 100mL with water. For this acid combination, the silver concentration should be limited to 2 mg/L. Silver under these conditions is stable in a tap-water matrix for 30 days. Higher concentrations of silver require additional HCl.

5.5 Two types of blanks are required for the analysis for samples prepared by any method other than 3040. The calibration blank is used in establishing the analytical curve, and the method blank is used to identify possible contamination resulting from varying amounts of the acids used in the sample processing.

The first sample in every sequence is labeled "Blank" and is a standard reagent blank. Method blanks (QC Blanks) that have gone through the requisite digestion step are labeled as such.

5.5.1 The calibration blank is prepared by acidifying reagent water to the same concentrations of the acids found in the standards and samples. Prepare a sufficient quantity to flush the system between standards and samples. The calibration blank will also be used for all initial and continuing calibration blank determinations (see Sections 7.3 and 7.4).

5.5.2 The method blank must contain all of the reagents in the same volumes as used in the processing of the samples. The method blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

5.6 The Initial Calibration Verification (ICV) is prepared by the analyst by combining compatible elements from a standard source different than that of the calibration standard and at concentrations within the linear working range of the instrument (see Section 8.6.1 for use).

The ICV is prepared by taking 10 ml of the CPI 100/1000/4000 ppm solution (Reported analytes / Fe, Ca, Mg, Mn / Na, K) and making up to a final volume of 2000 ml in 2% HNO_3 and 5% HCL

5.7 The Continuing Calibration Verification (CCV) should be prepared in the same acid matrix using the same standards used for calibration at a concentration near the mid-point of the calibration curve (see Section 8.6.1 for use).

The CCV is the same solution as the ICV.

5.8 The interference check solution is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest, particularly those with known interferences at 0.5 to 1 mg/L. In the absence of measurable analyte, overcorrection could go undetected because a negative value could be reported as zero. If the particular instrument will display overcorrection as a negative number, this spiking procedure will not be necessary.

A low level check of known concentration is prepared near the RL to verify low level hits from a custom stock CRI (Contract Required) standard.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material in Chapter Three, Inorganic Analytes, Sections 3.1 through 3.3.

7.0 PROCEDURE

7.1 Preliminary treatment of most matrices is necessary because of the complexity and variability of sample matrices. Groundwater samples which have been pre-filtered and acidified will not need acid digestion. Samples which are not digested must either use an internal standard or be matrix matched with the standards**.** Solubilization and digestion procedures are presented in Sample Preparation Methods (Chapter Three, Inorganic Analytes).

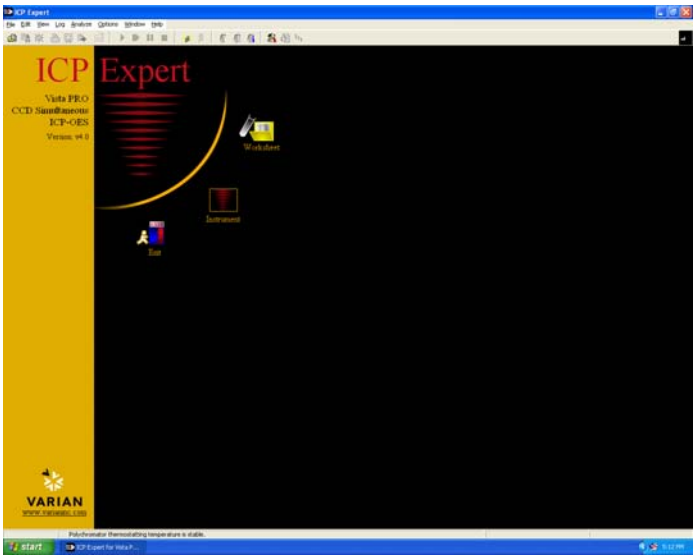
7.2 Set up the instrument with proper operating parameters established as detailed below. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 minutes of operation prior to calibration). Operating conditions - The analyst should follow the instructions provided by the instrument manufacturer.

ANALYTICAL PROCEDURE

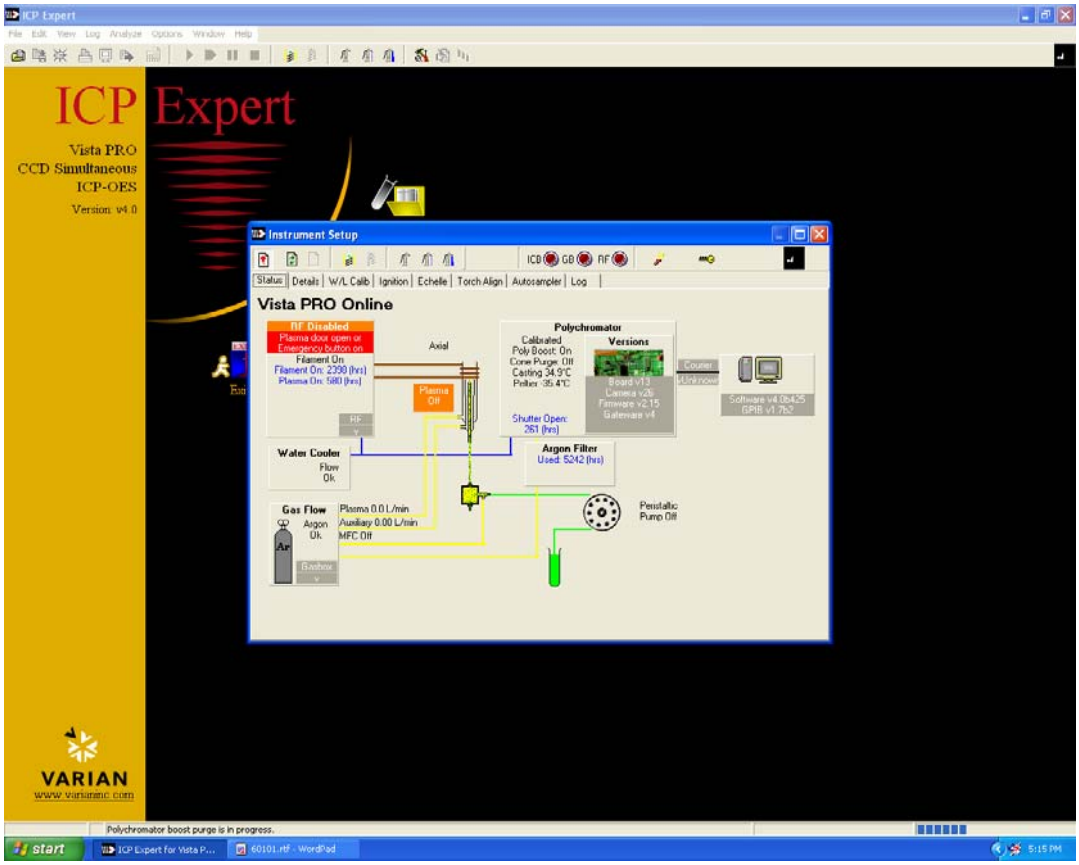
Start Up and Sequence Set-Up

Before starting ICP, check the following:

- **Torch** – make sure it looks clean. The torch generally needs daily cleaning.
- **Peristaltic tubing and pump**, check tubing for wrinkles, flattening, smashed tubing. Replace as necessary to ensure excellent pump performance.
- **Drainage bottle**--if full, empty it in the waste drum.

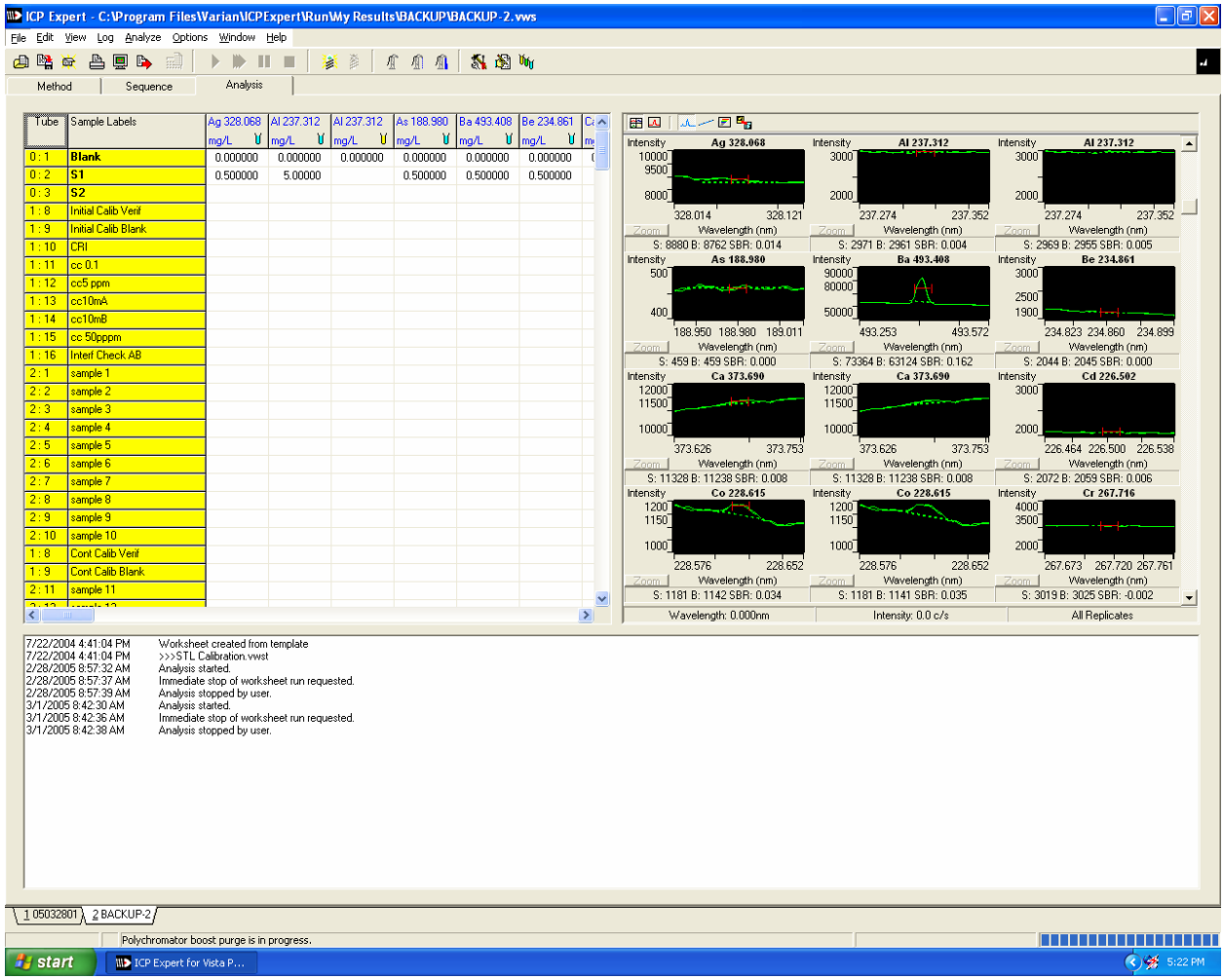


- **Argon and Nitrogen tanks** – Check pressure/volume
- **Run Ionization Solution –**
- **Run ICPExpert Software** and click on Instruments to check instrument status (e.g. chiller etc). A flow diagram of the instrumentation is shown with the present status of the equipment.



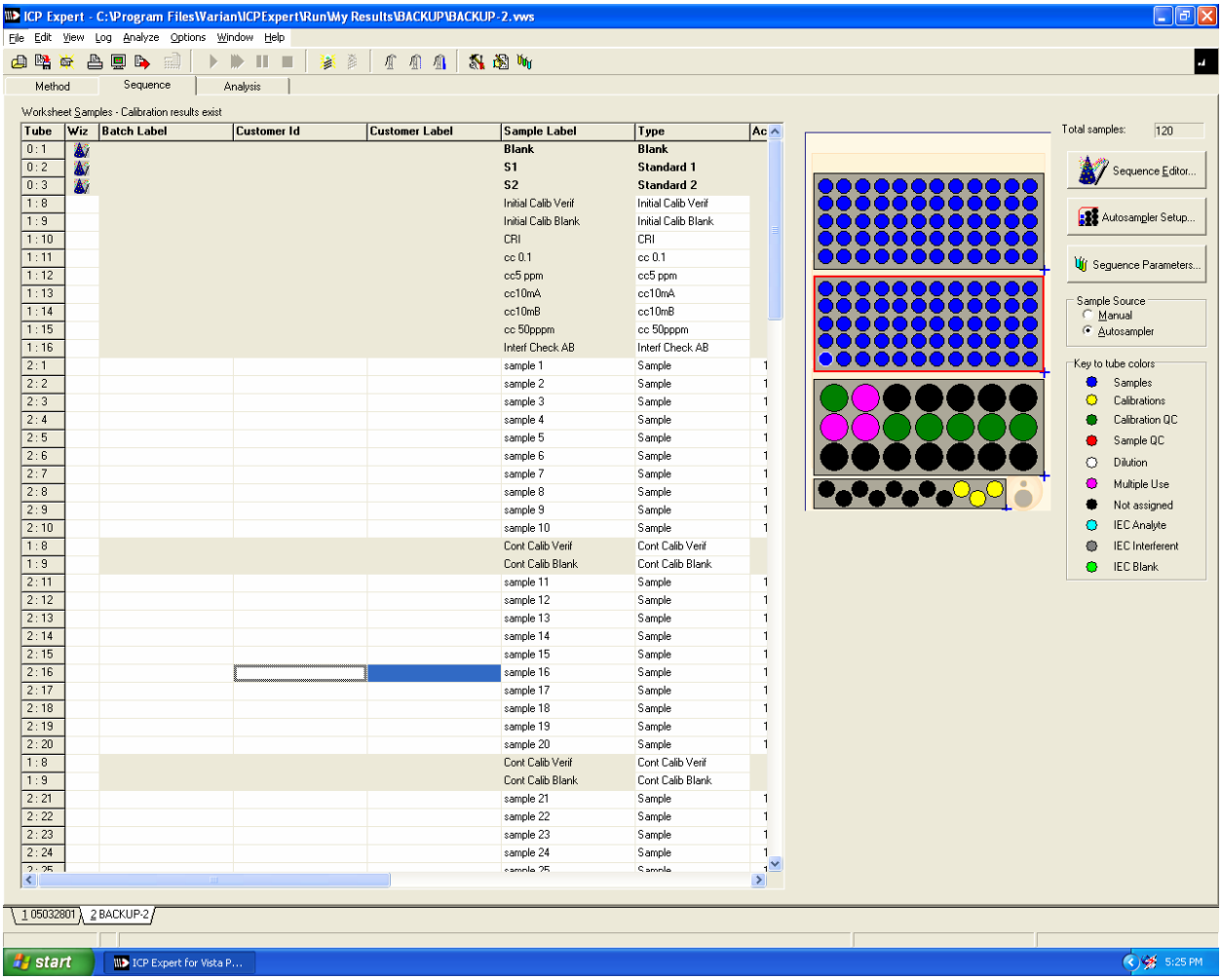
- Open the FoxPro window by clicking on the FoxPro Icon. When FoxPro window has opened enter the planned sequence information and save in the format 05032801 (i.e. First sequence of March 28, 2005).

- Open the blank VistaPro template in ICPExpert and open the saved FoxPro sequence. The planned sequence will appear in the left hand column colored in yellow.



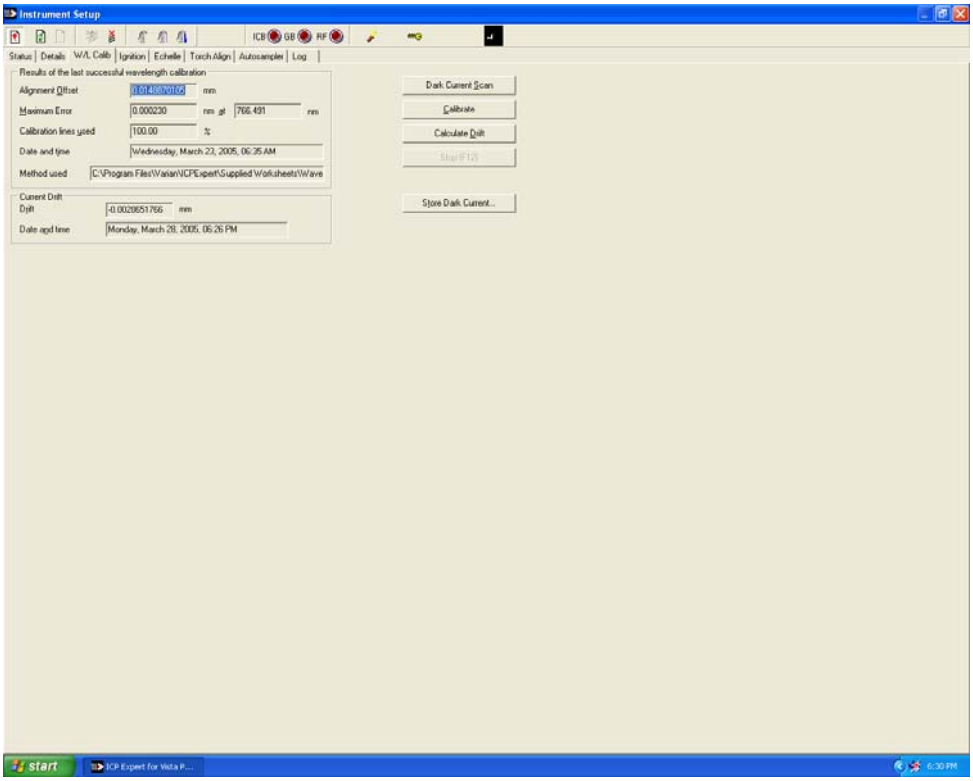
The sample ID's outlined in the yellow column should coincide with the planned sequence. Check samples against this *sequence* when loading the autosampler by checking the sequence section of the ICPExpert software (see below).

NOTE: Each sample vial is labeled with a pre-printed sample ID label, similar to the labels used in the digestion. Verify that the label on the sample vial is the same as on the digestion tube and prep sheet when transferring samples from the digestion tube to the autosampler tube.



Daily Test

After cleaning the torch (if required) the instrument should be tuned by running the manufacturer-supplied tune solution. Open the ICPExpert software to the Instrument Setup window and click on the W/L Calib tab. With the pump pumping tune solution click on the calibrate button and let the software conduct the tune sequence.



Daily Operation

The first run of the day should have the following sample sequence pattern.

Method | Sequence

Tube	Sample Labels	
0 : 1	**Blank**	**Acid Reagent Blank**
0 : 2	**S1**	**0.5ppm Calibration Standard**
0 : 3	**S2**	**100 ppm Calibration Standard**
1 : 8	Initial Calib Verif	**ICV**
1 : 9	Initial Calib Blank	
1 : 10	CRI	**Low level Check Standard**
1 : 11	cc 0.1	**Calibration, linear range, and interference check standards**
1 : 12	cc5 ppm	
1 : 13	cc10mA	
1 : 14	cc10mB	
1 : 15	cc 50pppm	
1 : 16	Interf Check AB	
2 : 1	sample 1	**Method Blank**
2 : 2	sample 2	**LCS**
2 : 3	sample 3	**LCSD**
2 : 4	sample 4	**Client Sample**
2 : 5	sample 5	**MS**
2 : 6	sample 6	**MSD**
2 : 7	sample 7	**Client Samples**
2 : 8	sample 8	
2 : 9	sample 9	
2 : 10	sample 10	
1 : 8	Cont Calib Verif	
1 : 9	Cont Calib Blank	
2 : 11	sample 11	

For all subsequent sequences add a CCB and CCV sample every 10 samples and at the end of the run add a CCB, CCV, and ICSAB.

NOTE: Each sample vial is labeled with a pre-printed sample ID label, similar to the labels used in the digestion. Verify that the label on the sample vial is the same as on the digestion tube and prep sheet when transferring samples from the digestion tube to the autosampler tube.

6.4 Shutdown

If the automatic shutdown procedure is used as in the case of overnight operation the VistaPro will shut the torch off, power down the plasma and stop the pump.

Manual shut down of the VistaPro can be accomplished by following the same sequence using the buttons in the Instrument window.

Do not shut off the Argon or Nitrogen. All gasses are to remain on. If the machine runs out of Argon, there is a 72 minute wait to restart the machine.

7.2.1 Before using this procedure to analyze samples, there must be data available documenting initial demonstration of performance. The required data document the selection criteria of background correction points; analytical dynamic ranges, the applicable equations, and the upper limits of those ranges; the method and instrument detection limits; and the determination and verification of interelement correction equations or other routines for correcting spectral interferences. This data must be generated using the same instrument, operating conditions and calibration routine to be used for sample analysis. These documented data must be kept on file and be available for review by the data user or auditor.

Analytical dynamic range files are electronically filed on the instrument. Daily 10 and 50 ppm checks are conducted and the data stored with the sequence files. Method detection limits are kept on file with the QA Group

7.2.2 Specific wavelengths are listed in Table 1. Other wavelengths may be substituted if they can provide the needed sensitivity and are corrected for spectral interference. Because of differences among various makes and models of spectrometers, specific instrument operating conditions cannot be provided. The instrument and operating conditions utilized for determination must be capable of providing data of acceptable quality to the program and data user. The analyst should follow the instructions provided by the instrument manufacturer unless other conditions provide similar or better performance for a task. Operating conditions for aqueous solutions usually vary from:

1100 to 1200 watts forward power,
14 to 18 mm viewing height
15 to 19 liters/min argon coolant flow,
0.6 to 1.5L/min argon nebulizer flow,
1 to 1.8mL/min sample pumping rate with
1 minute pre-flush time

measurement time near 1 second per wavelength peak for sequential instruments and 10 seconds per sample for simultaneous instruments.

For an axial plasma, the conditions will usually vary from 1100-1500 watts forward power,
15-19 liters/min argon coolant flow
0.6-1.5 L/min argon nebulizer flow
1-1.8mL/min sample pumping rate
1 minute pre-flush time

measurement time near 1 second per wavelength peak for sequential instruments and 10 seconds per sample for simultaneous instruments.

Reproduction of the Cu/Mn intensity ratio at 324.754 nm and 257.610 nm respectively, by adjusting the argon aerosol flow has been recommended as a way to achieve repeatable interference correction factors.

The VistaPro operates at the following conditions:

1.25 kW
Plasma Flow – 15 L/min
Auxillary Flow – 1.5 L/min
Nebulizer Flow – 0.7 L/min

7.2.3 The plasma operating conditions need to be optimized prior to use of the instrument. This routine is not required on a daily basis, but only when first setting up a new instrument or following a change in operating conditions. The following procedure is recommended. The purpose of plasma optimization is to provide a maximum signal to background ratio for some of the least sensitive elements in the analytical array. The use of a mass flow controller to regulate the nebulizer gas flow or source optimization software greatly facilitates the procedure.

7.2.3.1 Ignite the radial plasma and select an appropriate incident RF power. Allow the instrument to become thermally stable before beginning, about 30 to 60 minutes of operation. While aspirating a 1000ug/L solution of yttrium, follow the instrument manufacturer's instructions and adjust the aerosol carrier gas flow rate through the nebulizer so a definitive blue emission region of the plasma extends approximately from 5 to 20 mm above the top of the load coil. Record the nebulizer gas flow rate or pressure setting for future reference. The yttrium solution can also be used for coarse optical alignment of the torch by observing the overlay of the blue light over the entrance slit to the optical system.

Nebulizer gas flow check is not performed. Yttrium is not used.

7.2.3.2 After establishing the nebulizer gas flow rate, determine the solution uptake rate of the nebulizer in mL/min by aspirating a known volume of calibration blank for a period of at least three minutes. Divide the volume aspirated by the time in minutes and record the uptake rate; set the peristaltic pump to deliver the rate in a steady even flow.

Pump speed is set at 15 rpm. Flow rate is not monitored.

7.2.3.3 Profile the instrument to align it optically as it will be used during analysis. The following procedure can be used for both horizontal and vertical optimization in the radial mode, but is written for vertical.

Aspirate a solution containing 10ug/L of several selected elements. These elements can be As, Se, Tl or Pb as the least sensitive of the elements and most needing to be optimize or others representing analytical judgment (V, Cr, Cu, Li and Mn are also used with success).

Collect intensity data at the wavelength peak for each analyte at 1 mm intervals from 14 to 18 mm above the load coil. (This region of the plasma is referred to as the analytical zone.) ***Repeat the process using the calibration blank. Determine the net signal to blank intensity ratio for each analyte for each viewing height setting. Choose the height for viewing the plasma that provides the best net intensity ratios for the elements analyzed or the highest intensity ratio for the least sensitive element. For optimization in the axial mode, follow the instrument manufacturer's instructions*****.**

Mn (257.610) intensities are used during x-y alignment but the bold italic portion is not performed

7.2.3.4 The instrument operating condition finally selected as being optimum should provide the lowest reliable instrument detection limits and method detection limits.

7.2.3.5 If either the instrument operating conditions, such as incident power or nebulizer gas flow rate are changed, or a new torch injector tube with a different orifice internal diameter is installed, the plasma and viewing height should be re-optimized.

7.2.3.6 After completing the initial optimization of operating conditions, but before analyzing samples, the laboratory must establish and initially verify an interelement spectral interference correction routine to be used during sample analysis. A general description concerning spectral interference and the analytical requirements for background correction in particular are discussed in the section on interferences. Criteria for determining an interelement spectral interference is an apparent positive or negative concentration for the analyte that falls within ± one reporting limit from zero. The upper control limit is the analyte instrument detection limit. Once established the entire routine must be periodically verified every six months.
Only a portion of the correction routine must be verified more frequently or on a daily basis. Initial and periodic verification of the routine should be kept on file. Special cases where continual verification is required are described elsewhere.

7.2.3.7 Before daily calibration and after the instrument warm-up period, the nebulizer gas flow rate **must** be reset to the determined optimized flow. If a mass flow controller is being used, it should be set to the recorded optimized flow rate, In order to maintain valid spectral interelement correction routines the nebulizer gas flow rate should be the same (< 2% change) from day to day.

Nebulizer flow rate is software controlled through a mass flow controller.

7.2.4 For operation with organic solvents, use of the auxiliary argon inlet is recommended, as is solvent-resistant tubing, increased plasma (coolant) argon flow, decreased nebulizer flow, and increased RF power to obtain stable operation and precise measurements.

7.2.5 Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be established for each individual analyte line on each particular instrument. All measurements must be within the instrument linear range where the correction equations are valid.

7.2.5.1 Method detection limits must be established for all wavelengths utilized for each type of matrix commonly analyzed. The matrix used for the MDL calculation must contain analytes of known concentrations within 3-5 times the anticipated detection limit. Refer to Chapter One for additional guidance on the performance of MDL studies.

STL Corporate Policy is 1-10 Times

7.2.5.2 Determination of limits using reagent water represents a best case situation and does not represent possible matrix effects of real world samples.

7.2.5.3 If additional confirmation is desired, reanalyze the seven replicate aliquots on two more non consecutive days and again calculate the method detection limit values for each day. An average of the three values for each analyte may provide for a more appropriate estimate. Successful analysis of samples with added analytes or using method of standard additions can give confidence in the method detection limit values determined in reagent water.

7.2.5.4 The upper limit of the linear dynamic range must be established for each wavelength utilized by determining the signal responses from a minimum for three, preferably five, different concentration standards across the range.

Daily calibration checks are conducted with solutions containing 0.1, 5, 10, and 50 ppm of the target analytes.

One of these should be near the upper limit of the range. The ranges which may be used for the analysis of samples should be judged by the analyst from the resulting data. The data, calculations and rationale for the choice of range made should be documented and kept on file.

The 10 and 50 ppm upper range standards cover the normal reporting concentrations of the target analytes in all matrices. The 10 ppm solution is equivalent to 500 mg/Kg concentration on soil and a 10 mg/l concentration in water samples. The results are checked to see if they are within ±10%. Failing elements are restricted to being reported to the next lower passing calibration check.

The upper range limit should be an observed signal no more than 10% below the level extrapolated from lower standards. Determined analyte concentrations that are above the upper range limit must be diluted and reanalyzed. The analyst should also be aware that if an interelement correction from an analyte above the linear range exists, a second analyte where the interelement correction has been applied may be inaccurately reported. New dynamic ranges should be determined whenever there is a significant change in instrument response. For those analytes that periodically approach the upper limit, the range should be checked every six months. For those analytes that are known interferences, and are present at above the linear range, the analyst should ensure that the interelement correction has not been inaccurately applied.

NOTE: Many of the alkali and alkaline earth metals have non-linear response curves due to ionization and self absorption effects. These curves may be used if the instrument allows; however the effective range must be checked and the second order curve fit should have a correlation coefficient of 0.995 or better. Third order fits are not acceptable. These non-linear response curves should be revalidated and recalculated every six months. These curves are much more sensitive to changes in operating conditions than the linear lines and should be checked whenever there have been moderate equipment changes.

The VistaPro software uses a Correlation Co-efficient (R) value of 0.995 and will flag any failures of the curve to meet this standard.

7.2.6 The analyst must (1) verify that the instrument configuration and operating conditions satisfy the analytical requirements and (2) maintain quality control data confirming instrument performance and analytical results.

7.3 Profile and calibrate the instrument according to the instrument manufacturer's recommended procedures, using the typical mixed calibration standard solutions described in Section 5.4. Flush the system with the calibration blank between each standard or as the manufacturer recommends. (Use the average intensity of multiple exposures for both standardization and sample analysis to reduce random error.) The calibration curve must consist of a minimum of a blank and a standard.

The daily calibration curve consists of a reagent blank, S1 - 0.5ppm, 5ppm and 20ppm and S3 – 20ppm K.

7.4 For all analytes and determinations, the laboratory must analyze an ICV (Section 5.6), a calibration blank (Section 5.5.1), and a continuing calibration verification (CCV) (Section 5.7) immediately following daily calibration.

A calibration blank and either calibration verification (CCV) or an ICV must be analyzed after every tenth sample and at the end of the sample run.

Analysis of the check standard and calibration verification must verify that the instrument is within ± 10% of calibration with relative standard deviation <5% from replicate (minimum of two) integrations.

If the calibration cannot be verified within the specified limits, the sample analysis must be discontinued, the cause determined and the instrument recalibrated.

All samples following the last acceptable ICV, CCV or check standard must be reanalyzed. The analysis data of the calibration blank check standard, and ICV or CCV must be kept on file with the sample analysis data.

7.5 Rinse the system with the calibration blank solution (Section 5.5.1) before the analysis of each sample. The rinse time will be one minute. Each laboratory may establish a reduction in this rinse time through a suitable demonstration.

The "SmartRinse" mode is used to determine the appropriate rinse times between all samples.

7.6 Calculations: If dilutions were performed, the appropriate factors must be applied to sample values. All results should be reported with up to three significant figures.

7.7 The MSA should be used if interference is suspected or a new matrix is encountered. When the method of standard additions is used, standards are added at one or more levels to portions of a prepared sample. This technique compensates for enhancement or depression of an analyte signal by a matrix. It will not correct for additive interferences, such as contamination, interelement interferences, or baseline shifts. This technique is valid in the linear range when the interference effect is constant over the range, the added analyte responds the same as the endogenous analyte, and the signal is corrected for additive interferences. The simplest version of this technique is the single addition method. This procedure calls for two identical aliquots of the sample solution to be taken. To the first aliquot, a small volume of standard is added; while to the second aliquot, a volume of acid blank is added equal to the standard addition. The sample concentration is calculated by: multiplying the intensity value for the unfortified aliquot by the volume (Liters) and concentration (mg/L or mg/kg) of the standard addition to make the numerator; the difference in intensities for the fortified sample and unfortified sample is multiplied by the volume (Liters) of the sample aliquot for the denominator. The quotient is the sample concentration. For more than one fortified portion of the prepared sample, linear regression analysis can be applied using a computer or calculator program to obtain the concentration of the sample solution.

7.8 An alternative to using the method of standard additions is the internal standard technique. Add one or more elements not in the samples and verified not to cause an interelement spectral interference to the samples, standards and blanks; yttrium or scandium are often used. The concentration should be sufficient for optimum precision but not so high as to alter the salt concentration of the matrix. The element intensity is used by the instrument as an internal standard to ratio the analyte intensity signals for both calibration and quantitation. This technique is very useful in overcoming matrix interferences especially in high solids matrices.

Scandium (Wavelength 361.38) is used as the internal standard with a concentration of 2.5 ppm in all client and QC samples and the ionization buffer solution. The internal standard solution also contains cesium at a concentration of 0.4 ppm.

8.0 QUALITY CONTROL

8.1 All quality control data should be maintained and available for easy reference or inspection. All quality control measures described in Chapter One should be followed.

8.2 Dilute and reanalyze samples that exceed the linear calibration range or use an alternate, less sensitive line for which quality control data is already established.

8.3 Employ a minimum of one method blank per sample batch to determine if contamination or any memory effects are occurring. A method blank is a volume of reagent water carried through the same preparation process as a sample (refer to Chapter One).

In addition a low level check near the RL is also run to verify instrument sensitivity on a daily basis. The low level check has acceptance limits of 50 to 150%.

8.4 Analyze matrix spiked duplicate samples at a frequency of one per matrix batch. A matrix duplicate sample is a sample brought through the entire sample preparation and analytical process in duplicate.

8.4.1.1 The relative percent difference between spiked matrix duplicate determinations is to be calculated as follows:

$$RPD = \frac{D_1 * D_2}{D_1 * D_{2/2}} \times 100\%$$

Where:
RPD = relative percent difference.
D_1 = first sample value.
D_2 = second sample value (replicate).
(A control limit of ± 20% RPD or within the documented historical acceptance limits for each matrix shall be used for sample values greater than ten times the instrument detection limit.)

8.4.1.2 The spiked sample or spiked duplicate sample recovery is to be within ± 25% of the actual value or within the documented historical acceptance limits for each matrix.

8.5 It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration data for analyte elements. These tests, as outlined in Sections 8.5.1 and 8.5.2, will ensure that neither positive nor negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values.

8.5.1 Dilution Test: If the analyte concentration is sufficiently high (minimally, a factor of 10 above the instrumental detection limit after dilution), an analysis of a 1:5 dilution should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

This test is not performed.

8.5.2 Post Digestion Spike Addition: An analyte spike added to a portion of a prepared sample, or its dilution, should be recovered to within 75% to 125% of the known value. The spike addition should

produce a minimum level of 10 times and a maximum of 100 times the instrumental detection limit. If the spike is not recovered within the specified limits, a matrix effect should be suspected.

CAUTION: If spectral overlap is suspected, use of computerized compensation, an alternate wavelength, or comparison with an alternate method is recommended.

Does not apply unless client specified.

8.6 Check the instrument standardization by analyzing appropriate QC samples as follows.

8.6.1 Verify calibration with the Continuing Calibration Verification (CCV) Standard immediately following daily calibration, after every ten samples, and at the end of an analytical run. Check calibration with an ICV following the initial calibration (Section 5.6). At the laboratory's discretion, an ICV may be used in lieu of the continuing calibration verifications. If used in this manner, the ICV should be at a concentration near the mid-point of the calibration curve. Use a calibration blank (Section 5.5.1) immediately following daily calibration, after every 10 samples and at the end of the analytical run.

8.6.1.1 The results of the ICV and CCV's are to agree within 10% of the expected value; if not, terminate the analysis, correct the problem, and recalibrate the instrument.

8.6.1.2 The results of the check standard are to agree within 10% of the expected value; if not, terminate the analysis, correct the problem, and recalibrate the instrument.

8.6.1.3 The results of the calibration blank are to agree within three times the IDL.

If not, repeat the analysis two more times and average the results. If the average is not within three standard deviations of the background mean, terminate the analysis, correct the problem, recalibrate, and reanalyze the previous 10 samples.

If the blank is less than 1/10 the concentration of the action level of interest, and no sample is within ten percent of the action limit, analyses need not be rerun and recalibration need not be performed before continuation of the run.

8.6.2 Verify the interelement and background correction factors at the beginning of each analytical run. Do this by analyzing the interference check sample (Section 5.8). Results should be within ± 20% of the true value.

All QC performance is checked and documented using the Metals Checklist (see Attachment II). All QC must pass the performance standards as outlined in this SOP. Any failing QC must be investigated and the appropriate actions taken as outlined in Attachment III.

In addition to the prescribed QC outlined in the 6010 method. Blank spikes (Laboratory Control Sample or LCS) and Blank Spike Duplicates (LCSD) are prepared for each batch. All LCS and LCSD samples must have recoveries of ±20% of the known value. RPD's between the LCS/LCSD pairs are set at 20%.

9.0 METHOD PERFORMANCE

9.1 In an EPA round-robin Phase 1 study, seven laboratories applied the ICP technique to acid-distilled water matrices that had been spiked with various metal concentrates. Table 4 lists the true values, the mean reported values, and the mean percent relative standard deviations.

9.2 Performance data for aqueous solutions and solid samples from a multi-laboratory study (9) are provided in Tables 5 and 6.

SECTION C – REVISION HISTORY AND ACCOMPANYING DOCUMENTS

March 25, 2005 – Revision 7 – Ryan Korver

- Revision of SOP into new Reference Method Format.
- Addition of run log, tables and forms used in the analysis.

October 5, 2006 – Revision 8 – Rene Boongaling

- Added Yttrium as one of the internal standards

December 13, 2006 – Revision 9 – Rene Boongaling

- Changed SOP name to Standard Naming Format
- Analytical Procedures, Sequence Setup – Added the following note.
    - NOTE: Each sample vial is labeled with a pre-printed sample ID label, similar to the labels used in the digestion. Verify that the label on the sample vial is the same as on the digestion tube and prep sheet when transferring samples from the digestion tube to the autosampler tube.

March 28, 2007 – Revision 9A – Rene Boongaling

- Removed Yttrium

TABLE 2
POTENTIAL INTERFERENCES
ANALYTE CONCENTRATION EQUIVALENTS ARISING FROM
INTERFERENCE AT THE 100-mg/L LEVEL[c]

Analyte	Wavelength (nm)	Interferant[a,b] Al	Ca	Cr	Cu	Fe	Mg	Mn	Ni	Ti	V
Aluminum	308.215	--	--	--	--	--	--	0.21	--	--	1.4
Antimony	206.833	0.47	--	2.9	--	0.08	--	--	--	0.25	0.45
Arsenic	193.696	1.3	--	0.44	--	--	--	--	--	--	1.1
Barium	455.403	--	--	--	--	--	--	--	--	--	--
Beryllium	313.042	--	--	--	--	--	--	--	--	0.04	0.05
Cadmium	226.502	--	--	--	--	0.03	--	--	0.02	--	--
Calcium	317.933	--	--	0.08	--	0.01	0.01	0.04	--	0.03	0.03
Chromium	267.716	--	--	--	--	0.003	--	0.04	--	--	0.04
Cobalt	228.616	--	--	0.03	--	0.005	--	--	0.03	0.15	--
Copper	324.754	--	--	--	--	0.003	--	--	--	0.05	0.02
Iron	259.940	--	--	--	--	--	--	0.12	--	--	--
Lead	220.353	0.17	--	--	--	--	--	--	--	--	--
Magnesium	279.079	--	0.02	0.11	--	0.13	--	0.25	--	0.07	0.12
Manganese	257.610	0.005	--	0.01	--	0.002	0.002	--	--	--	--
Molybdenum	202.030	0.05	--	--	--	0.03	--	--	--	--	--
Nickel	231.604	--	--	--	--	--	--	--	--	--	--
Selenium	196.026	0.23	--	--	--	0.09	--	--	--	--	--
Sodium	588.995	--	--	--	--	--	--	--	--	0.08	--
Thallium	190.864	0.30	--	--	--	--	--	--	--	--	--
Vanadium	292.402	--	--	0.05	--	0.005	--	--	--	0.02	--
Zinc	213.856	--	--	--	0.14	--	--	--	0.29	--	--

[a] Dashes indicate that no interference was observed even when interferents were introduced at the following levels:

Al -	1000 mg/L	Mg -	1000 mg/L
Ca -	1000 mg/L	Mn -	200 mg/L
Cr -	200 mg/L	Tl -	200 mg/L
Cu -	200 mg/L	V -	200 mg/L
Fe -	1000 mg/L		

[b] The figures recorded as analyte concentrations are not the actual observed concentrations; to obtain those figures, add the listed concentration to the interferant figure.

[c] Interferences will be affected by background choice and other interferences may be present.

TABLE 3
MIXED STANDARD SOLUTIONS

Solution	Elements
I	Be, Cd, Mn, Pb, Se and Zn
II	Ba, Co, Cu, Fe, and V
III	As, Mo
IV	Al, Ca, Cr, K, Na, Ni,Li, and Sr
V	Ag (see "NOTE" to Section 5.4), Mg, Sb, and Tl
VI	P

TABLE 4. ICP PRECISION AND ACCURACY DATA[a]

Element	Sample No. 1				Sample No. 2				Sample No. 3			
	True Conc. (ug/L)	Mean Conc. (ug/L)	RSD[b] (%)	Accuracy[d] (%)	True Conc. (ug/L)	Mean Conc. (ug/L)	RSD[b]	Accuracy[d] (%)	True Conc. (ug/L)	Mean Conc. (ug/L)	RSD[b] (%)	Accuracy[d] (%)
Be	750	733	6.2	98	20	20	9.8	100	180	176	5.2	98
Mn	350	345	2.7	99	15	15	6.7	100	100	99	3.3	99
V	750	749	1.8	100	70	69	2.9	99	170	169	1.1	99
As	200	208	7.5	104	22	19	23	86	60	63	17	105
Cr	150	149	3.8	99	10	10	18	100	50	50	3.3	100
Cu	250	235	5.1	94	11	11	40	100	70	67	7.9	96
Fe	600	594	3.0	99	20	19	15	95	180	178	6.0	99
Al	700	696	5.6	99	60	62	33	103	160	161	13	101
Cd	50	48	12	96	2.5	2.9	16	116	14	13	16	93
Co	700	512	10	73	20	20	4.1	100	120	108	21	90
Ni	250	245	5.8	98	30	28	11	93	60	55	14	92
Pb	250	236	16	94	24	30	32	125	80	80	14	100
Zn	200	201	5.6	100	16	19	45	119	80	82	9.4	102
Se[c]	40	32	21.9	80	6	8.5	42	142	10	8.5	8.3	85

[a] Not all elements were analyzed by all laboratories.
[b] RSD = relative standard deviation.
[c] Results for Se are from two laboratories.
[d] Accuracy is expressed as the mean concentration divided by the true concentration times 100.

TABLE 5

ICP-AES PRECISION AND ACCURACY FOR AQUEOUS SOLUTIONS[a]

Element	Mean Conc. (mg/L)	N[b]	RSD[b] (%)	Accuracy[c] (%)
Al	14.8	8	6.3	100
Sb	15.1	8	7.7	102
As	14.7	7	6.4	99
Ba	3.66	7	3.1	99
Be	3.78	8	5.8	102
Cd	3.61	8	7.0	97
Ca	15.0	8	7.4	101
Cr	3.75	8	8.2	101
Co	3.52	8	5.9	95
Cu	3.58	8	5.6	97
Fe	14.8	8	5.9	100
Pb	14.4	7	5.9	97
Mg	14.1	8	6.5	96
Mn	3.70	8	4.3	100
Mo	3.70	8	6.9	100
Ni	3.70	7	5.7	100
K	14.1	8	6.6	95
Se	15.3	8	7.5	104
Ag	3.69	6	9.1	100
Na	14.0	8	4.2	95
Tl	15.1	7	8.5	102
V	3.51	8	6.6	95
Zn	3.57	8	8.3	96

[a]these performance values are independent of sample preparation because the labs analyzed portions of the same solutions

[b]N = Number of measurements for mean and relative standard deviation (RSD).

[c]Accuracy is expressed as a percentage of the nominal value for each analyte in acidified, multi-element solutions.

TABLE 6

ICP-AES PRECISION AND BIAS FOR SOLID WASTE DIGESTS[a]

Element	Spiked Coal Fly Ash (NIST-SRM 1633a) Mean Conc. (mg/L)	N[b]	RSD[b] (%)	Bias[c] (%AAS)	Spiked Electroplating Sludge Mean Conc. (mg/L)	N[b]	RSD[b] (%)	Bias[c] (%AAS)
Al	330	8	16	104	127	8	13	110
Sb	3.4	6	73	96	5.3	7	24	120
As	21	8	83	270	5.2	7	8.6	87
Ba	133	8	8.7	101	1.6	8	20	58
Be	4.0	8	57	460	0.9	7	9.9	110
Cd	0.97	6	5.7	101	2.9	7	9.9	90
Ca	87	6	5.6	208	954	7	7.0	97
Cr	2.1	7	36	106	154	7	7.8	93
Co	1.2	6	21	94	1.0	7	11	85
Cu	1.9	6	9.7	118	156	8	7.8	97
Fe	602	8	8.8	102	603	7	5.6	98
Pb	4.6	7	22	94	25	7	5.6	98
Mg	15	8	15	110	35	8	20	84
Mn	1.8	7	14	104	5.9	7	9.6	95
Mo	891	8	19	105	1.4	7	36	110
Ni	1.6	6	8.1	91	9.5	7	9.6	90
K	46	8	4.2	98	51	8	5.8	82
Se	6.4	5	16	73	8.7	7	13	101
Ag	1.4	3	17	140	0.75	7	19	270
Na	20	8	49	130	1380	8	9.8	95
Tl	6.7	4	22	260	5.0	7	20	180
V	1010	5	7.5	100	1.2	6	11	80
Zn	2.2	6	7.6	93	266	7	2.5	101

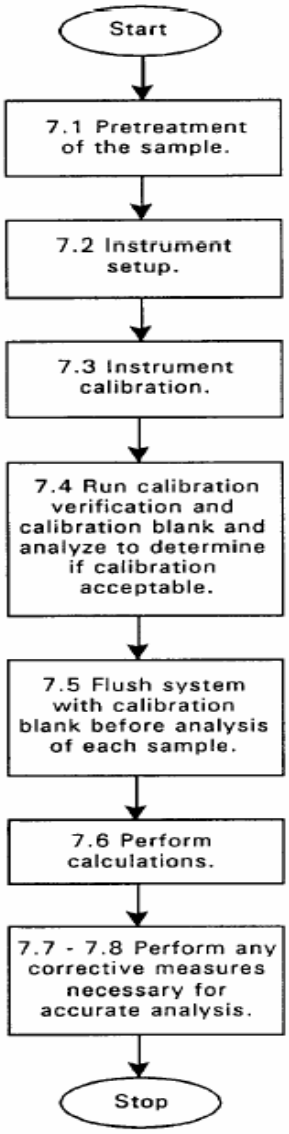
[a]These performance values are independent of sample preparation because the labs analyzed portions of the same digests.

[b]N = Number of measurements for mean and relative standard deviation (RSD).

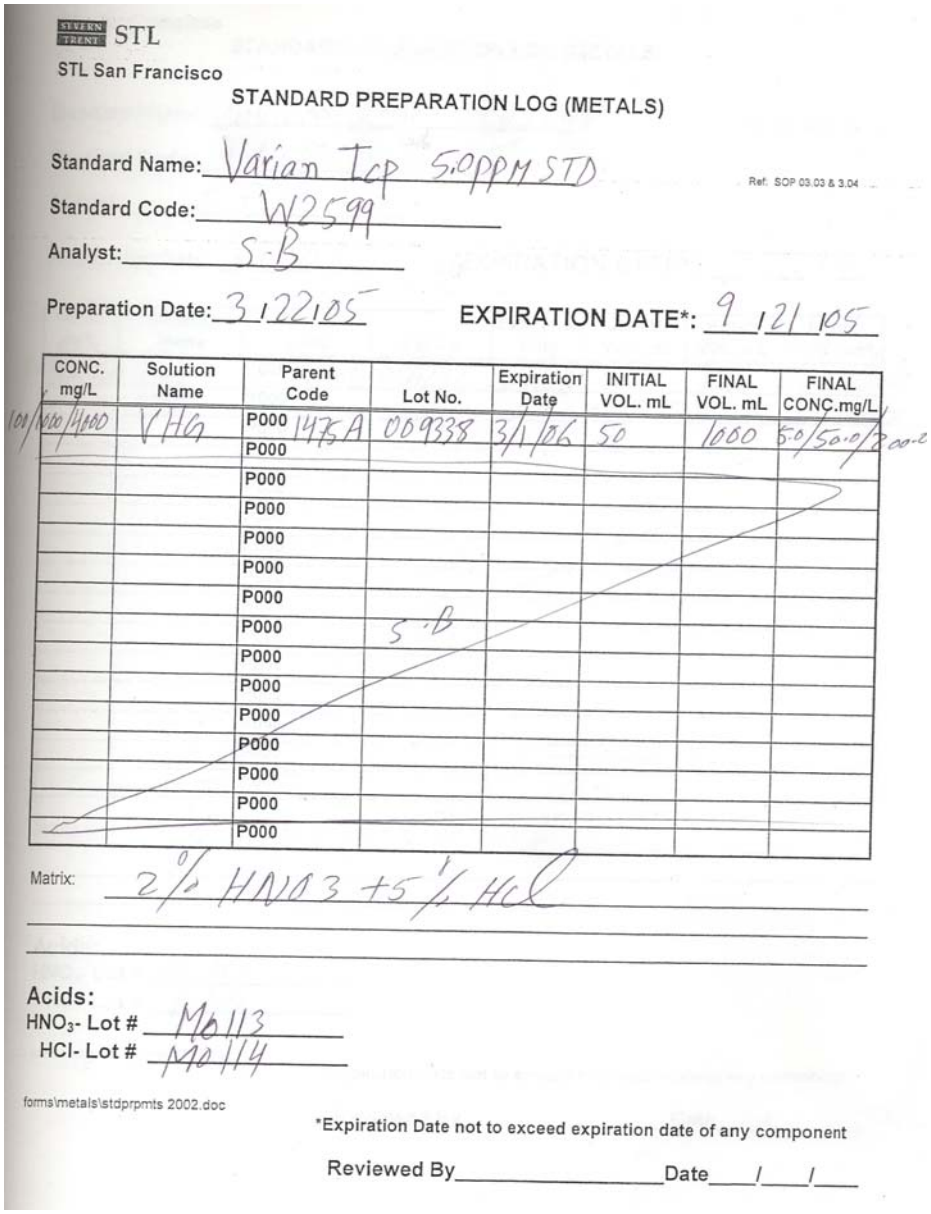
[c]Bias for the ICP-AES data is expressed as a percentage of atomic absorption spectroscopy (AA) data for the same digests.

METHOD 6010B

INDUCTIVELY COUPLED PLASMA-ATOMIC EMISSION SPECTROMETRY



Attachment I



SEVERN TRENT STL
STL San Francisco

STANDARD PREPARATION LOG (METALS)

Ref: SOP 03.03 & 3.04

Standard Name: Varian Icp 5.0PPM STD
Standard Code: W2599
Analyst: S-B
Preparation Date: 3/22/05 EXPIRATION DATE*: 9/21/05

CONC. mg/L	Solution Name	Parent Code	Lot No.	Expiration Date	INITIAL VOL. mL	FINAL VOL. mL	FINAL CONC.mg/L
10/100/1000	VHG	P000	1475A 009338	3/1/06	50	1000	5.0/50.0/200.0
		P000					
		P000					
		P000					
		P000					
		P000					
		P000					
		P000	S-B				
		P000					
		P000					
		P000					
		P000					
		P000					
		P000					
		P000					

Matrix: 2% HNO3 +5% HCl

Acids:
HNO_3- Lot # M0113
HCl- Lot # M0114

forms\metals\stdprpmts 2002.doc

*Expiration Date not to exceed expiration date of any component

Reviewed By_____Date___/___/___

Attachment II

STL San Francisco

Metals Data Review Checklist

Analytical Date (seq. start):_____/_____/_____

- O 6010B ICP- VARIAN EL04054059
- O 6020 ICP/MS- ELAN DRC-e

Review Items

A. Calibration/Instrument Run QC	Yes	No	NA	2nd Level
1. Instrument calibrated per manufacturer's instructions and at SOP specified levels?				
2. ICV/CCV analyzed at appropriate frequency and within control limits? (6010B = 90 -110%) Use comments to narrate N/A exceptions.				
3. ICB/CCB analyzed at appropriate frequency and within +/- RL? Use comments to narrate N/A exceptions				
4. CRI analyzed and met compliance?				
5. ICSAB run at required frequency and within SOP limits?				
B. Sample Results				
1. 1.Were samples reviewed for detect profile and IEC potential: As, Sb, Be Se and Tl at spectral level and analytical integrity (sequence position and complete run). NDs for Na, Fe, and Ca checked.				
2. Were samples with concentrations> the linear range for any parameter diluted and reanalyzed?				
3. All reported results bracketed by in control QC?				
4. Sample analyses performed to achieve TAT?				
C. Preparation/Matrix QC				
1. LCD/LCSD done per prep batch and within QC limits?				
2. Method blank done per prep batch and within +\- RL?				
3. MS/MSD run at required frequency and within limits?				
4. MSD or DU run at required frequency and RPD within SOP limits?				
D. Other				
1. Copies of corrective action report documented correctly and included?				
2. Current IDL/MDL/IEC data on file?				
3. Calculations checked for error?				
4. Transcriptions checked for error?				
5. All client/project specific requirements met?				
6. Date/time of analysis verified as correct?				

Primary Reviewer: Comments: **Date:**

2nd Level Reviewer: Comments: **Date:**

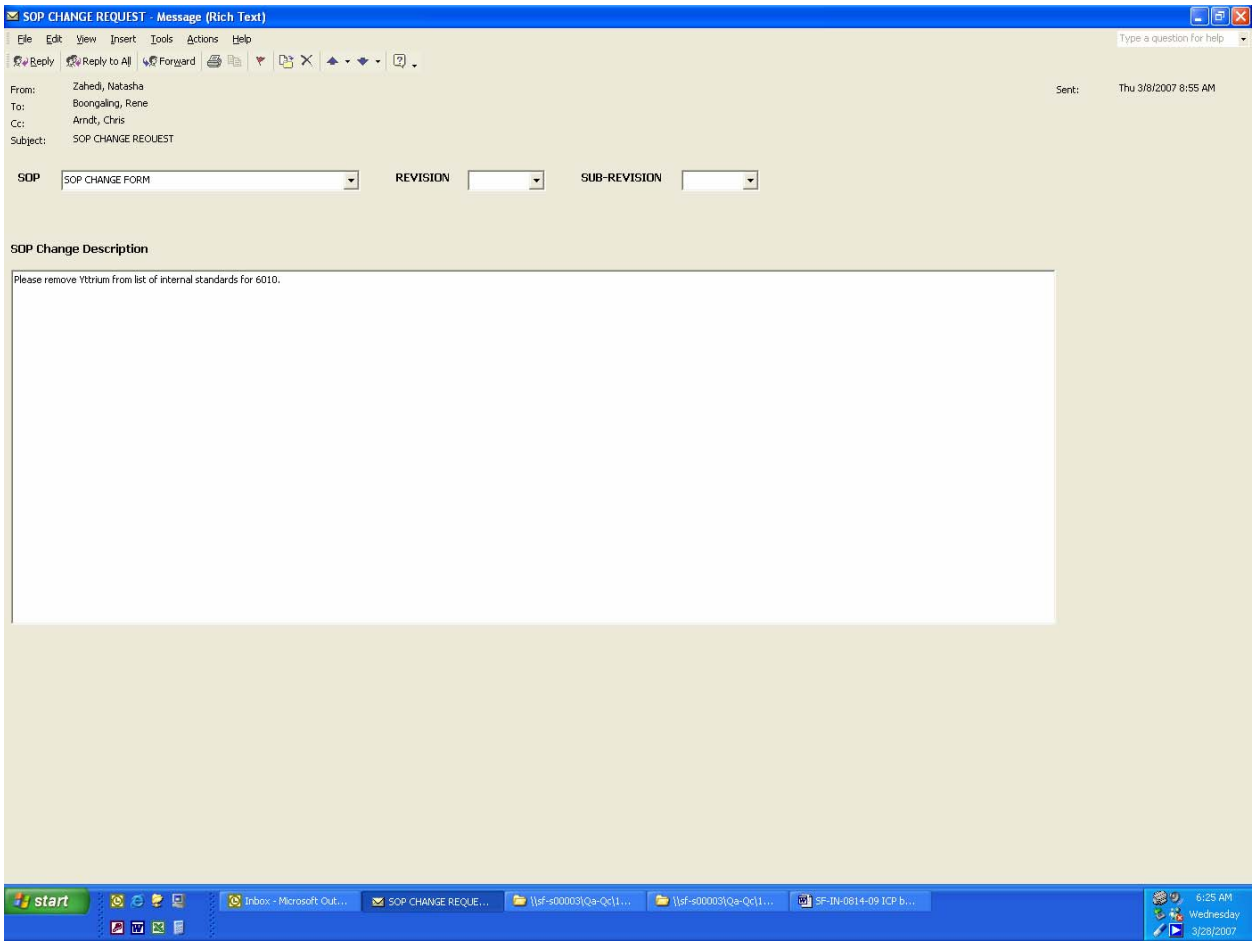
Controlled Document Form # MET-0001 Rev.1
E:\10 - QA Controlled Documents\10 - Forms\2005-Tracked Forms

Attachment III

EPA SW-846 6010 CORRECTIVE ACTION TABLE

Calibration	Frequency	Acceptance Criteria	Corrective Action
ICAL-5-pt. Low std @ RL	Daily	r ≥ 0.995	1. Recalibrate.
ICB & CCB	After ICAL & each CCV	<RL	1. RA. If passes, proceed. 2. If fails, rinse system. 3. RA CCB & samples back to last passing CCB.
ICSAB (interference check)	Run at beginning & end of daily run (within 8 hours)	80-120% of element values	1. Check calibration. 2. If out, check IECs. 3. If out, recalibrate.
ICV	Daily after calibration.	± 10% from expected Concentration, RSD <5%.	1. RA 2. If fails, recalibrate.
CRI-concentration @ RL	After calibration, prior to Sample analysis.	± 50% from expected conc. ± 20% for CDQMP. or refer to QAPP	1. RA CRI. 2. If fails, recalibrate. (Client requirements may vary)
Linearity Standard Check	After ICV, prior to sample	± 5% from expected. concentration	1. RA. 2. If fails, recalibrate.
CCV	Every 10 analyses &. end of sample sequence	± 10% from expected Concentration, RSD <5%.	1. RA CCV. 2. If fails, recalibrate. 3. RA affected samples-samples must be bracketed by passing CCVs.
QC Sample	**Frequency**	**Acceptance Criteria**	**Corrective Action**
Method Blank	1/batch, 20 samples or less	<RL <2x MDL :CDQMP <MDL: Navy or refer to QAPP	1. Check calculations. 2. RA. If passes, report. 3. No action if samples are N.D. or ≥10xMB. 4. Samples ≤10x, RA.
LCS	1/batch, 20 samples or less	± 20% , RPD 25%	1. Check calculations. 2. RA. If passes, report. 3. If fails, RX/RA batch.
LCSD	1/batch, 20 samples or less	± 20% , RPD 25%	1. Check calculations. 2. RA. If passes, report. 3. If fails, RX/RA batch.
MS	1/batch, 20 samples or less	Refer to Acceptance. Criteria Table or refer to QAPP	1. Check calculations. 2. Evaluate - trends, interferences, 4x rule. 3. MS's outside 75-125% indicate matrix problems with the spiked a. Flag recoveries <75% on Level II reports-mso. b. On level II reports flag recoveries outside 75-125% & with sample values above 4x spike level-msl. c. Flag recoveries >125% & RPD >20% on Level II reports-
MSD	1/batch, 20 samples or less	Refer to Acceptance. Criteria Table or refer to QAPP	1. Check calculations. 2. Evaluate - trends, interferences, 4x rule. 3. MSD's outside 75-125% indicate matrix problems with the spiked a. Flag recoveries <75% on Level II reports-mso. b. On level II reports flag recoveries outside 75-125% & with sample c. Flag recoveries >125% & RPD >20% on Level II

Attachment IV (Request for SOP Change)



Appendix A
Attachement 1
VB170 QAPP

QUALITY ASSURANCE PROJECT PLAN

FOR

Vasquez Blvd-I70
Bioavailability of Arsenic in Site Soils
Using Juvenile Swine as an Animal Model

September 1999

Prepared with technical assistance by:
ISSI Consulting Group, Inc.
Denver, CO

Program Approval Bonita Lavelle EPA RPM	date
Technical Approval Christopher Weis, PhD, DABT EPA Regional Toxicologist	date

TABLE OF CONTENTS

A3 Distribution List

This Vasquez Boulevard and I-70 Bioavailability of Arsenic in Juvenile Swine Project Plan and any revisions will be distributed as follows:

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Stan Casteel, DVM, PhD
Principal Investigator
Veterinary Medical Diagnostic Laboratory
College of Veterinary Medicine
University of Missouri - Columbia
Phone: (573) 882-6811

Vasquez Boulevard/Interstate-70 Site Working Group Members

A4. PROJECT TASK ORGANIZATION

A4.1 Project Task

EPA Region 8 is seeking to characterize the bioavailability of arsenic in fine particulate surface soils at the Vasquez Boulevard and I-70 (VB-I70) Study Area using juvenile swine as an animal model. This document serves as the Biological Media Sampling and Analysis Plan and Quality Assurance Project Plan (QAPP) for the project and presents the organization, objectives, functional activities and specific quality assurance and quality control activities associated with the bioavailability investigation. This QAPP includes study background information, project objectives and scope, analytical design and rationale, and data quality objectives (DQOs). It describes the specific protocols that will be followed for obtaining study materials, implementing the study, processing and storing of biological samples, preparing chain of custody forms, and conducting laboratory analyses. Surface soil (test substance) sampling, handling and partial analysis can be found in the *Pilot Soil Characterization Study* (EPA, 1999).

A4.2 Project Organization

The following lists key personnel who will serve as contacts and provide technical expertise during implementation of this Project Plan along with their designated roles and responsibilities.

Bonita Lavelle, EPA Remedial Project Manager, will be responsible for overall project management and coordination among EPA and its contractors and other interested parties

Christopher P. Weis, Ph.D., DABT. EPA Regional Toxicologist, will serve as the study design advisor and science manager for this project.

Stan Casteel, DVM, PhD, Principal Investigator, will be responsible for implementing and documenting all activities associated with dosing animals and collecting samples.

William Brattin, Ph.D., ISSI, Inc., will be responsible for technical management of ISSI's activities which include: preparing planning documents, providing technical oversight, and compiling and summarizing data generated during the investigation.

Tracy Hammon, M.S., ISSI, Inc., will be responsible for preparation of study investigation materials including; chain of custody forms, time details and dosing spreadsheets. In addition, Ms. Hammon will perform the data reduction for results from this study and calculate a bioavailability value for arsenic in juvenile swine.

Mary Goldade, M.S., ISSI, Inc., will serve as the QA officer for ISSI's role in this project.

John Drexler, Ph.D., University of Colorado, will be responsible for preparing samples for

analysis and for performing analytical measurements of surface soil samples for metals, phase speciation and in vitro bioaccessibility.

A5 PROBLEM DEFINITION and BACKGROUND

A5.1 Background

The VB-I70 study area is located north of downtown Denver in the state of Colorado. Due to the discovery of sporadic elevations in surficial arsenic levels, recent investigations have been initiated to determine the nature, extent, and public health implications of these findings in the residential areas of the site.

The Colorado Department of Public Health and Environment (CDPHE) collected approximately twenty-five soil samples from residential yards in the Vasquez Blvd-I70 study area during the summer of 1997. Samples were collected from yards north of Interstate 70 in the Swansea and Elyria neighborhoods. The samples indicated levels of arsenic from 12 to 1,300 mg/kg, and lead from 61 to 660 mg/kg. This discovery prompted further investigation to determine the extent of arsenic and lead present in this area.

During the spring of 1998, the USEPA Superfund Technical Assessment and Response Team (START) under contract 68-W5-0031 conducted further sampling and analysis in the area. Samples were again collected from residences in the Elyria and Swansea neighborhoods bounded by Colorado Boulevard on the east, the South Platte River on the west, 38th Avenue on the south, and 56th Avenue on the north. An additional 1200 residences were sampled, identifying 207 properties with arsenic greater than 70 mg/kg and 77 properties with lead greater than 500 mg/kg (UOS, 1998). Sampling efforts to date are continuing southward until the areal extent of the contamination is clearly defined. Residential arsenic concentrations in these follow-up investigations ranged higher than 10,000 mg/kg in selected yards.

A number of soils from this study area have undergone further characterization for metal speciation using electron microprobe analysis and *in vitro* bioaccessability (solubility testing). Arsenic found in soils at the VB/I-70 site was determined to be primarily in the form of arsenic trioxide (>95%). While *in vitro* solubility tests are presently experimental and therefore unsuitable for site specific adjustments in bioavailability, the tests indicated that solubility of the arsenic forms found in the VB/I-70 soils might be lower than expected. Based on the results of this further characterization, EPA will evaluate the *in vivo* bioavailability of arsenic in study area soils using juvenile swine as an animal model. This information will be used to help evaluate the potential risk to residents from exposure to arsenic in site soils.

A5.2 Problem Definition – Conceptual Model

An as yet unidentified source(s) has led to elevated residential soil concentrations of lead and arsenic, resulting in Comprehensive Environmental Response, Compensation, and Liability Act

(CERCLA or Superfund) actions by the Environmental Protection Agency (EPA) to assess and abate these hazards to human health and the environment. Accurate assessment of the human health risks resulting from oral exposure to metals requires knowledge of the amount of metal absorbed from the gastrointestinal tract into the body. This information is especially important for environmental media such as soil or metal extraction industry wastes, because metals in these media may exist, at least in part, in a variety of poorly water soluble minerals, and may also exist inside particles of inert matrix such as rock or slag. These chemical and physical properties may tend to influence (usually decrease) the absorption (bioavailability) of the metals when ingested. Therefore, reliable site-specific data on metal bioavailability in environmental media of concern may be expected to increase the accuracy and decrease the uncertainty in human health risk estimates. Preliminary *in vitro* bioaccessability testing on arsenic in site soils show low bioaccessability (solubility) values ranging from 3 - 26% as compared to EPA Region 8's default value of 80% for inorganic arsenic contamination in soils. In order to obtain more reliable information on the actual *in vivo* absorption of arsenic in these soils, EPA will run an *in vivo* study for arsenic bioavailability in juvenile swine as a plausible surrogate for arsenic absorption by humans.

This project plan will describe the efforts planned by EPA to evaluate the bioavailability of arsenic in soils from the study area using juvenile swine as an animal model. The overall approach will follow the methods developed by the EPA Region 8 and employed in the Phase II Bioavailability Studies (EPA, 1995).

A6 PROJECT TASK DESCRIPTION

A6.1 Study Goals

The study goal is to collect data that will allow a plausible estimate of *in vivo* relative arsenic absorption from site-specific soils when compared by statistical and biological means to arsenic absorption from a freely soluble arsenic form (sodium arsenate). This estimate will be used for risk assessment and possibly risk-based decision-making for the human health risk assessment at the VB/I-70 site.

In order to accomplish this goal the following general and specific quality objectives have been defined.

A6.2 Study Objectives

General Objective #1:

to determine quantitatively whether VB/I-70 soil arsenic is absorbed to a lesser or greater extent than freely soluble arsenic in water; and

General Objective #2:

to estimate a site specific absorption fraction for soil arsenic which is protective and plausibly applicable for human health risk assessment at the VB/I-70 site.

A7 QUALITY OBJECTIVES and CRITERIA for MEASUREMENT DATA

The Data Quality Objectives (DQO) process is an iterative process which is designed to focus on the decisions that must be made and to help ensure that the site activities acquire data that are logical, scientifically defensible, and cost effective. The DQO process is intended to:

- Ensure that task objectives are clearly defined;
- Determine anticipated uses of the data;
- Determine what environmental data are necessary to meet these objectives; and
- Ensure that the data collected are of adequate quantity and quality for the intended use.

A7.1 Study Objective DQOs

Two types of objectives are identified in this QAPP: general objectives and data quality objectives (DQOs). General objectives are statements of practical goals that, if realized, will substantially contribute to achieving the purpose of the study. Development of DQOs is a process that is intended to ensure that task objectives are clearly defined and that data collected are appropriate and of sufficient quality to satisfy the objectives. DQOs for each of the study objectives are provided below.

<u>General Objective #1:</u>

to determine quantitatively whether VB/I-70 soil arsenic is absorbed to a lesser or greater extent

than freely soluble arsenic in water:

General Objective #2:

to estimate a site specific absorption fraction for soil arsenic which is protective and plausibly applicable for human health risk assessment at the VB/I-70 site.

Specific Data Quality Objective Process

The three stages of the DQO process are identified below and a discussion of how they have been applied in the study described herein. The three stages are undertaken in an interactive and iterative manner, whereby all the DQO elements are continually reviewed and re-evaluated until there is reasonable assurance that suitable data for decision making will be attained.

- Stage I - Identify Decision Types: Stage I defines the types of decisions that will be made by identifying data uses, evaluating available data, developing a conceptual model, and specifying objectives for the project. The conceptual model facilitates identification of decisions that may be made, the end use of the data collected, and the potential deficiencies in the existing information.

- Stage II - Identify Data Uses/Needs: Stage II stipulates criteria for determining data adequacy. This stage involves specifying the quantity and quality of data necessary to meet the Stage I objectives. EPA's Data Useability for Risk Assessment Guidance (DURA) outlines general and specific recommendations for data adequacy. This includes identification of data uses and data types, and identification of data quality and quantity needs.

- Stage III - Design Data Collection Program: Stage III specifies the methods by which data of acceptable quality and quantity will be obtained for use in decision making. These methods are provided in the attached SOPs.

Through utilization of the DQO process, as defined in EPA guidance (EPA540-R-93-071 and -078, Sep 1993), this QAPP will use several terms that are specifically defined to avoid

confusion that might result from any misunderstanding of their use. For each of the tasks identified within this QAPP, a "Task Objective" is specifically defined. The Task Objective is a concise statement of the problem to be addressed by activities under this task. For each Task Objective, a decision (or series of decisions) is identified which addresses the problem contained in the Task Objective.

For each decision, the data necessary to make the decision are identified and described. For all analytical data, quality assurance objectives are specified that describe the minimum quality of data necessary to support the specified decision or test the hypotheses. These quality assurance objectives are specified as objectives for precision, accuracy, representativeness, comparability, and completeness. In addition, data review and validation procedures are specified in the QAPP that evaluate how well the analytical data meet these quality assurance objectives and whether or not the data are of sufficient quality for the intended usage.

The following sections apply the DQO process to the North Denver, Colorado Response, Stage I and Stage II, where Stage I and Stage II identify decision types and data uses/needs for the SAP. Stage III is discussed later and provides the specific task objectives, decisions, and rationale for resolving the decisions necessary to complete this Study.

DQO Stage I - Identifying Decision Types

Stage I of the DQO process identifies a primary question and secondary questions that need to be resolved at the completion of the sampling and analyses program.

- PRIMARY QUESTION 1: is VB/I-70 soil arsenic absorbed to a lesser or greater extent than freely soluble arsenic (sodium arsenate, NaAs)?

- PRIMARY QUESTION 2: is the data of sufficient quantity and quality to estimate a plausible value for relative arsenic bioavailability?

DQO Stage II - Identifying Data Uses/Needs

Stage II of the DQO process identifies data uses and needs. The primary uses of data are:

- Compare data from site test materials to data from a control material to develop a quantitative relative estimate of the bioavailability of soil arsenic when compared (using standard statistical analyses) to freely soluble arsenic.

- Using relative absorption data derived from the *in vivo* study, estimate a site specific relative bioavailability (RBA) for soil arsenic which is protective of human health and plausibly applicable for human health risk assessment at the VB/I-70 site.

In order to accomplish these uses, sample collection will be designed to ensure: 1) sufficient soil samples are tested during the course of the investigation; 2) that these soil samples are fully characterized to estimate their representativeness of arsenic at the site; 3) that sufficient biological samples are collected to support standard statistical comparison between dose groups and test substances; 4) that collection of biological samples is random within the study design; and 5) that sample handling and labeling ensures that analysis will be blind and otherwise according to Good Laboratory Practices of EPA..

Stage II of the DQO process also determines what type and quality of data are needed to answer the questions developed in Stage I. Within this QAPP, quantitative and qualitative limits are defined for precision, accuracy, representativeness, comparability and analytical completeness. Reporting limits for chemical analytes are set by the analytical laboratory based on matrix, historical data, and comparison to EPA limits for CLP and other methods. Quantitative limits are also defined for instrument and method detection limits, and for method reporting limits or method quantitation limits. The QA procedures outlined in this section are intended to ensure data quality and to administer corrective actions with the goal of producing data that satisfy the following requirements. General guidelines, policies, and procedures to achieve these objectives are presented below. Where additional, detailed, procedures are required to attain QA objectives and to describe specific methods, these are provided in the attached SOPs. The following PARCC requirements apply to more standard chemical analytical analyses:

Precision: Precision is defined as the agreement between a set of replicate measurements without assumption or knowledge of the true value. It is a measure of agreement among individual measurements of the same property under prescribed similar conditions. Agreement is expressed as either the relative percent difference (RPD) for duplicate measurements or the range and standard deviation for larger numbers of replicates. The RPD will be reported on required 5% laboratory duplicates, and a defined MDL will be reported as per EPA guidance in CFR, part 136, app. B (7 method-replicates on 3 non-consecutive days of a low-level [near MQL] standard, with MDL = 3 x SD).

Study personnel will prepare blind duplicate samples. A minimum of one blind duplicate

will be prepared for 5-10% of the samples collected. These blind duplicate samples will be specified in the study design.

Accuracy: Accuracy is a measure of the closeness of individual measurements to the "true" value. Accuracy usually is expressed as a percentage of that value. For a variety of analytical procedures, standard reference materials traceable to or available from National Institute of Standards and Technology (NIST, formerly National Bureau of Standards) or other sources can be used to determine accuracy of measurements. Accuracy will be measured as the percent recovery (%R) of an analyte in a reference standard or spiked samples (>3) that span the limit of linearity for the method.

Ideally, precision and accuracy estimates should represent the entire measurement process, including sampling, analysis, calibration, and other components. From a practical perspective, these estimates usually represent only a portion of the measurement process that occurs in the analytical lab.

Representativeness: Representativeness is the degree to which data accurately and precisely represent characteristics of a population, parameter variations at a sampling point, or an environmental condition. For this QAPP, samples representative of soils in the study area are to be selected from those previously tested for speciation and bioaccessibility.

Comparability: Data are comparable if study considerations, collection techniques, and measurement procedures, methods, and reporting are equivalent for the samples within a sample set. A qualitative assessment of data comparability will be made of applicable data sets. These criteria allow comparison of data from different sources. Comparable data will be obtained by specifying standard units for physical measurements and standard procedures for sample collection, processing, and analysis.

Completeness: Data are considered complete when a prescribed percentage of the total intended measurements and samples are obtained. Analytical completeness is defined as the percentage of valid analytical results requested, and >90% of analyzed samples should have results reported. For this sampling program, a minimum of 80 percent of the planned collection of individual samples must be obtained to achieve a satisfactory level of data completeness.

Method Detection Limits (applicable to chemical analyses only): Method detection limits (MDLs) are minimum values that can be reliably measured to identify the analyte as being

present in the matrix, vs method quantitation limits are the minimum values that can be quantitated with reasonable scientific confidence. The method will also have a maximum linear value in most situations, and analyses should occur within this limit of linearity range.

B. MEASUREMENT AND DATA ACQUISITION

B1 SAMPLING PROCESS DESIGN

This section provides an overview of the methods to be used in determining bioavailability of arsenic in site soils. Detailed protocols are provided in the attached SOPs.

The USEPA has been engaged in a multi-year investigation of the bioavailability of metals in soil and mine waste. This study has focused mainly on lead (Weis and LaVelle, 1991; Weis et al, 1994;, Casteel et al., 1997) but a number of studies were performed to investigate the relative bioavailability (RBA) of arsenic in a variety of test materials. This study will follow the sampling methods developed by EPA in previous studies.

Three representative site soil samples will be selected for inclusion in this study. These samples will be fully characterized to determine their physico-chemical characteristics including arsenic concentrations and to assess their representativeness for the site as a whole. The samples will then be administered to juvenile swine using a daily dosing protocol. Urine samples will be collected and analyzed for arsenic in order to determine the relative amount of arsenic absorbed from the animal groups dosed with soil vs animal groups dosed with freely soluble arsenic in the form of sodium arsenate (NaAs).

This study will be performed using young swine as the test species because the gastrointestinal system of swine is more nearly similar to humans than most other animal models. The animals will be housed individually in metabolic cages (cages designed to collect and separate urine and feces). Groups of randomly selected animals (N= 4) will be given oral doses of test material or sodium arsenate (NaAs) for a total of 12 days, with the dose for each day being administered in two equal portions given at 9:00 AM (after an overnight fast) and 3:00 PM (two hours before feeding). Doses will be based on measured group mean body weights, and will be adjusted every three days to account for animal growth.

The test materials have been intentionally left unidentified in this project plan so that the plan

may be used for multiple studies of test materials from this site. A memo documenting specific test materials will be prepared prior to the commencement of each study. All test materials which are used in the swine bioavailability study will undergo characterization and *in vitro* solubility testing. Characterization will include CLP metals analysis according to EPA method SW-846, evaluation of soil pH according to EPA method 9045C, measurement of total organic carbon according to EPA method 9060 and metals speciation according to SOP ISSI VBI70-09 (note: perlite will not be quantified). *In vitro* testing will be performed according to the SOP in Appendix A.6 in the Pilot-Soil Characterization Plan for this site (EPA, 1999).

For animals exposed by the oral route, dose material will be placed in the center of a small portion (about 5 grams) of moistened feed (referred to as a "doughball"), and administered to the animals by hand. All missed doses will be recorded and the time-weighted average dose calculation for each animal will be adjusted downward accordingly.

The following table shows the study design for evaluating the bioavailability of arsenic in site soils.

Group	Number of Animals	Material Administered	Dose Route	Dose (ug As/kg-day)
1	3	Control	Oral	0
2	4	NaAs	Oral	50
3	4	NaAs	Oral	125
4	4	Test Material #1	Oral	50
5	4	Test Material #1	Oral	125
6	4	Test Material #2	Oral	50
7	4	Test Material #2	Oral	125
8	4	Test Material #3	Oral	50
9	4	Test Material #3	Oral	125

Samples of urine and feces (48 hour composites) will be collected from each animal on days 6-7, 8-9, 10-11 during the study. Each collection of urine will be conducted by placing a stainless steel pan beneath each cage, which drains into a plastic storage bottle. Each collection pan will be fitted with a nylon screen to minimize contamination with feces, spilled food, or other debris.

Plastic diverters will be used to minimize urine dilution with drinking water spilled by the animals from the watering nozzle into the collection pan.

Aliquots of the urine and feces samples will be analyzed for total arsenic content. Measurement of urinary arsenic concentrations provides a measure of the amount of arsenic which was absorbed by the animal, whereas measurement of arsenic in feces provides a measure of the amount of arsenic which was not absorbed by the animal.

The amount of arsenic absorbed will be evaluated by measuring the amount of arsenic which was excreted in urine. The amount excreted in the urine can be expressed as the URINARY EXCRETION FRACTION (UEF). This is estimated by plotting mass recovered in urine per 48 hours divided by the amount given per 48 hours. The ratio of the urinary excretion fraction for some test material (e.g., arsenic in site soil) compared to the urinary excretion fraction for some readily absorbable form of arsenic (e.g., sodium arsenate) is a measure of the RELATIVE BIOAVAILABILITY (RBA):

RBA = UEF(test) / UEF(NaAs)

An RBA value of 1.0 means that arsenic in the test soil is just as well absorbed as sodium arsenate. An RBA value of 0.5 means that arsenic in the test soil is absorbed 50% as well as sodium arsenate.

The site-specific RBA is used to adjust the toxicity factors for arsenic as follows:

RfD (adjusted) = RfD (default) / RBA
Oral slope factor (adjusted) = Oral slope factor (default) * RBA

B2 SAMPLING METHODS REQUIREMENTS

The proposed sampling consists of the collection of approximately 105 samples of urine from exposed or control animals.

QA/QC samples will consist of blind spikes, media blanks and duplicate samples at a 5-10% rate, and measures of arsenic in other media to which the swine are exposed (e.g., water, feed). Every reasonable effort will be made to adhere strictly to specified TSOPs and Good Laboratory Practices as outlined by EPA in 40 CFR 792. Where deviation from TSOPs and/or GLP guidelines is unavoidable, documentation of the deviation and its potential impact on the outcome of the data collection effort will be documented. Detailed logbook notes will record information pertinent to each sample collection. These notes will be indexed and made available for review following sample collection.

B3 Sampling, Handling and Custody Requirement

Documentation of sample collection, handling, and shipment will include completion of chain-of-custody forms, use of time details and prepared forms, and entry of data and/or observations into a logbook. A chain-of-custody form shall accompany every shipment of samples to the analytical laboratory. The purpose of the chain-of-custody form is to establish the documentation necessary to trace possession from the time of collection to final disposal.

The chain-of-custody form will have the following information:

- Project number
- Sampler's signature
- Date of sample collection
- Collection Media (e.g., Urine)
- Sample identification number
- Analytical parameters

The shipping forms or transmittal memo will describe:

- Number of containers
- Sample preservative (dry ice for transit)
- Date and time of sample shipments

The labs will enter the following information upon receipt:

- Name of person receiving the sample
- Date of sample receipt

- Sample condition

All corrections to the chain-of-custody record will be **initialed and dated by the person making the corrections**. Each chain-of-custody form will include signatures of the appropriate individuals indicated on the form. The originals will accompany the samples to the laboratory, and copies documenting each custody change will be recorded and kept on file.

Chain-of-custody will be maintained until final disposition of the samples by the laboratory and acceptance of analytical results by EPA. One copy of the chain-of-custody will be kept by field personnel.

All required paper work, including sample container labels, chain-of-custody forms, custody seals and shipping forms will be fully completed in ink prior to overnight shipping of the samples to the laboratory.

Upon receipt, coolers containing the biological samples will be received by the laboratory sample custodian. The coolers will be opened and the contents inspected. Chain-of custody forms will be reviewed for completeness, and samples will be logged and assigned a unique laboratory sample number. Any discrepancies or abnormalities in samples will be noted.

The EPA Project Manager will maintain original log books and receive all data packages and reports.

B4 ANALYTICAL METHODS REQUIREMENTS

See the attached laboratory SOP for analytical methods and requirements.

B5 Quality Control Requirements

The project team organization ensures attainment of QA objectives by:

- Assigning responsibility for performing work according to specifications
- Providing oversight of quality-related activities for verification of conformance with

specifications

- Defining the relationships between management and personnel performing quality-related work Corrective Action

The Project Manager and Regional Toxicologist will prepare a summary of quality-related activities and problems. This summary will be forwarded to EPA for inclusion in the project file. If deficiencies in the program are identified, the Regional Toxicologist, in consultation with the Project Manager will identify recommendations for corrective action.

Communications. Lines of communication between project personnel and project management staff will be appropriate to enable timely response to events that have the potential to affect data quality. Project personnel will be provided with a project contact list that includes telephone numbers for both routine communications and emergency notifications.

Communications will also entail ensuring that information on sample collection, transportation, analysis, and storage; data acquisition, analysis, and reporting; personnel assignments and activities; and other information pertinent to the project are distributed to potentially affected personnel in a timely manner. Changes in procedures, equipment, personnel, or other program elements as a result of an accident or emergency that have the potential to affect data quality or achievement of overall program objectives will be communicated to the Project Manager in writing in a timely manner.

Copies of all written communications and written summaries of all substantive telephone conversations will be placed in a permanent project file maintained by the EPA Project Manager.

Laboratory Responsibilities. The laboratory and its staff will have the responsibility for processing all samples submitted according to the specific protocols for sample custody, holding times, analysis, reporting, and associated laboratory QA/QC. Laboratory spikes, duplicates, etc. will be performed.

B7 INSTRUMENT CALIBRATION and FREQUENCY

SOPs will identify requirements needed to be met by the field staff and laboratories to meet adequate instrument calibration frequency, and QA/QC for raw data and reports.

C. ASSESSMENT OVERSIGHT

C1 ASSESSMENTS and RESPONSE ACTIONS

The Principal Investigator will be on-site to oversee, implement and inspect study activities associated with the in life stages of the project. Enough sample will be taken and archived to allow for problems (such as loss or spoilage) from transportation or analytical labs.

D. DATA VALIDATION and USABILITY

D1 DATA REVIEW, VALIDATION and VERIFICATION REQUIREMENTS

Data validation will consist of a) establishing an absolute range, acceptance limits (screening criteria), and appropriate statistics for each data parameter, b) describing methods for determining the disposition of suspect data, and c) documenting final disposition of invalid or qualified data, including outliers.

Test Statistic: Qualitative professional judgement will be used to interpret the results of the chemical and biological data collected which is intended to be screening-level preliminary data.

Out-of-range chemical data will be excluded from the validated data set unless the appropriate data value can be positively established and documented. Other suspect data or samples will be examined in detail, including any irregularities in its collection and handling. In the absence of any clear indication that they are invalid (such as equipment failure or operator error), data outliers will remain in the validated data set but will be flagged as outliers per specified criteria (e.g., >3 x SD from the mean). Data points determined to be invalid will be permanently flagged in a clear and consistent manner in the original raw data set and removed from subsequent data summaries and files.

QA for data validation will ensure that the screening criteria are comprehensive, unambiguous, reasonable, and internally consistent; and that data validation activities are properly documented. Data discrepancy reports should be prepared describing any data problems observed and any data correction activities undertaken.

All data records should be cataloged and stored in their original form. Calibration adjustments

and adjustments to reduce data to standard conditions for comparability will be clearly documented, and raw data clearly distinguished from "corrected" data (i.e., data to which calibration and standardization adjustments have been applied).

Raw data and adjustments should be entered into a computer database and/or spreadsheet for correction, statistical analysis, manipulation, formatting, and summarizing to reduce the potential for human error.

D2 VALIDATION and VERIFICATION METHODS

Data reporting consists of communicating summarized data in a final form. QA for reporting consists of measures intended to avoid or detect human error and to correct identified errors. Such methods include specification of standard reporting formats and contents of measures to reduce data transcription errors. Study design and resulting data will undergo peer review by qualified reviewers capable of evaluating reasonableness of the data for the scientific design.

Reports: A report of all the summary study design characteristics, sample collections and analyses, data quality and results shall be presented by the analytical laboratories. Simple statistical tests of group treatment differences should be performed and presented as discussed above and will be conducted by EPA. All raw data and summary results of both data and summary statistics (means, standard deviations, ranges, etc.) should be tabulated by the laboratories. Study reports should be available within 60 days of receipt of acceptable laboratory results and reports.

QA records and project files will be maintained in accordance with standard project procedures. All QA records, logbooks, sample data forms, raw data summaries, and the like will be maintained until written directions for their disposal are provided.

D3 RECONCILIATION with DQOs

The project team will review any results which fall outside the DQOs and decide (per DURA 1992 and RAGS 1992) the extent of usability of results for the purposes of this investigation.

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