FINAL REPORT

Validation of an In Vitro Bioaccessibility Test Method for Estimation of Bioavailability of Arsenic from Soil and Sediment

ESTCP Project ER-200916

December 2012

Susan Griffin
U.S. EPA Region 8

Yvette Lowney
Exponent, Inc.

ESTCP

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
01-05-2012	Final	2008-2012

4. TITLE AND SUBTITLE

Validation of an In Vitro Bioaccessibility Test Method for the Estimation of the Bioavailability of Arsenic from Soil and Sediment

5a. CONTRACT NUMBER
NA

5b. GRANT NUMBER
NA

5c. PROGRAM ELEMENT NUMBER
NA

6. AUTHOR(S)
Griffin, Susan
Lowney, Yvette

5d. PROJECT NUMBER
ER-0916

5e. TASK NUMBER
NA

5f. WORK UNIT NUMBER
NA

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
USEPA Region 8, 1595 Wynkoop St, Denver CO 80202
Exponent, Inc., 4141 Arapahoe Ave. Suite 101, Boulder CO 80303

8. PERFORMING ORGANIZATION REPORT NUMBER
NA

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Environmental Security Technology Certification Program (ESTCP)
901 North Stuart Street
Suite 303
Arlington VA 22203-1821

10. SPONSOR/MONITOR'S ACRONYM(S)
ESTCP

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
ER-0916

12. DISTRIBUTION/AVAILABILITY STATEMENT
Available for public release; distribution is unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

A method is described for measuring the in vitro bioaccessibility (IVBA) of arsenic in soil or soil-like media, and using the measured IVBA value to estimate the relative bioavailability (RBA) in swine or monkeys using empiric regression models developed from 20 (swine) or 17 (monkey) calibration soils. The correlation coefficients for the models are high (0.85 for swine, 0.87 for monkey), and the precision of the method is high, both within and between laboratories. This method is the most thoroughly tested, calibrated, and validated IVBA approach for estimation of arsenic RBA that has been described to date.

15. SUBJECT TERMS
Arsenic, RBA, IVBA

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Susan Griffin
U	U	U	UU	28	19b. TELEPHONE NUMBER *(Include area code)* 303-312-6651

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ACRONYMS

ABA	absolute bioavailability
ºC	degrees Celsius
CSF	cancer slope factor
DI	de-ionized
EPA	U.S. Environmental Protection Agency
g	grams
g/mL	grams per milliliter
HAH	hydroxylamine hydrochloride
HDPE	high-density polyethylene
IVBA	*in vitro* bioaccessibility
IVIVC	*in vivo*-*in vitro* correlation
L	liter
mg/kg	milligrams per kilogram
mL	milliliter
N	Normal
NIST	National Institute of Standards and Technology
OU1	Operable Unit 1
ppm	parts per million = mg/L or mg/kg
RAM	relative arsenic mass
RBA	relative bioavailability
RfD	reference dose
SOP	standard operating procedure
ug/kg	microgram per kilogram
ug/L	microgram per liter

ACKNOWLEDGEMENTS

The work described in the report was accomplished through the efforts of a team of scientists. The co-investigators for this project were Susan Griffin of the U.S. Environmental Protection Agency, and Yvette Lowney of Exponent, Inc. The co-investigators were supported throughout the study by John Drexler of the University of Colorado at Boulder, who performed all of the *in vitro* bioaccessibility and speciation analyses, and also provided many valuable discussions and insights. In addition, the project was supported by scientists from SRC, Inc. (William Brattin, Gary Diamond, and Penny Hunter) and from CDM Smith (Lynn Woodbury), who provided ongoing support in data reduction and modeling efforts, as well as authorship of project reports. Contact information for these individuals is provided in Appendix A.

EXECUTIVE SUMMARY

Accurate evaluation of the human health risk from ingestion of arsenic in soil or soil-like media requires knowledge of the relative bioavailability (RBA) of arsenic in the soil or soil-like material. Although RBA can be measured using studies in animals, such studies are generally slow and costly. An alternative strategy is to perform measurements of arsenic solubility in the laboratory. Typically, a sample of soil or sediment is extracted using a fluid that has properties that resemble a gastrointestinal fluid, and the amount of arsenic solubilized from the sample into the fluid under a standard set of extraction conditions is measured. The fraction of arsenic that is solubilized is referred to as the *in vitro* bioaccessibility (IVBA). The IVBA is then utilized to predict the *in vivo* RBA of arsenic in that sample, usually through an empiric correlation model.

The technology developed in this project is an IVBA-based method to accurately predict the RBA of arsenic in soil and soil-like materials. The method consists of two parts. In the first part, one gram of test material is extracted in 100 mL of extraction fluid for one hour at 37°C with constant end-over-end mixing. A sample of the extraction fluid is removed and analyzed for arsenic. The IVBA value is calculated as the mass of arsenic solubilized in the fluid divided by the mass of arsenic contained in the sample extracted. In the second part, the RBA of arsenic is estimated from the IVBA value using an empirical mathematical model:

$$RBA = a + b \cdot IVBA$$

The key performance objectives established for the project include the following:

1. The correlation coefficient (R) between the observed and predicted RBA should be no less than 0.8 (this corresponds to an R^2 value no less than 0.64)
2. The method should be precise, yielding reproducible measures of RBA in repeat analyses
3. The method should be implementable in multiple laboratories with good agreement (high precision) between laboratories

Test materials used to establish the correlation between IVBA and RBA included 20 materials where RBA had been measured in juvenile swine, and 17 samples where RBA had been measured in monkeys. Based on extensive and systematic investigation of a wide range of differing extraction conditions, it was found that no single method would yield high quality RBA predictions for the combined data set. However, each data set could be successfully modeled independently. For swine, the optimum extraction fluid is 0.4 M glycine at pH 1.5, and the best fit regression model is:

$$RBA(swine) = 19.7 + 0.622 \cdot IVBA_{pH1.5} \quad (R^2 = 0.723)$$

For monkey, the optimum extraction fluid is 0.4 M glycine plus 0.05 M phosphate at pH 7, and the best fit regression model is:

$$RBA(monkey) = 14.3 + 0.583 \cdot IVBA_{pH7} \quad (R^2 = 0.755)$$

The finding that the best-fit regression model occurs at pH 7 for monkey and pH 1.5 for swine suggests that there might be significant physiological differences between the animal species that result in this outcome. However, this study did not seek to investigate the reason why different extraction pH conditions yielded a better fit for swine and monkey, so no mechanistic explanation is available at this time.

The within- and between-laboratory precision of the IVBA method was evaluated by triplicate analysis of each of 12 soils for each of three extraction fluids by each of four laboratories. Within-laboratory precision was evaluated by examining the magnitude of the standard deviation for three replicate values for each of 12 test materials. Within-laboratory precision was typically less than 3%, with an average of 0.8% for all four laboratories. Between-laboratory precision was evaluated by examining the between-laboratory variability in the mean IVBA values for each test soil for each extraction condition. Between-laboratory variation in mean values was generally less than 7%, with an overall average of 3%. These results demonstrate the method is highly reproducible, both within and between laboratories.

The principal advantage of this IVBA-based method compared to measurement of RBA *in vivo* is that it is much less expensive and much more rapid. For example, a typical *in vivo* RBA study may cost up to $100,000 and require several months for assessment of two samples, while a typical IVBA study can perform 40-60 extractions in one day at a cost of about $100 per extraction. This has the additional advantage that multiple samples (20 or more) may be collected from a site to ensure a robust characterization of IVBA/RBA across the site.

The principle advantages of this IVBA method compared to other *in vitro* methods that have been described in the literature are that 1) the fluids and extraction conditions are simple, 2) the results have been calibrated against a larger data set than any other method, and 3) the method has been demonstrated to be reproducible both within and between laboratories.

1.0 INTRODUCTION

1.1 BACKGROUND

Arsenic is a naturally occurring element found in soil at background concentrations ranging from about 1 to 50 milligram per kilogram (mg/kg), depending on location (Shacklette and Boergnen 1984). Concentrations of arsenic higher than background occur in soil at many National Priority List sites, most often as a result of mining, smelting, leather tanning, wood preservation, or pesticide manufacture and/or application.

Exposures to elevated levels of arsenic in soil are of potential health concern for humans, both for cancer and non-cancer effects. Incidental ingestion of soil is typically the primary route of exposure to contaminants in soil, and quantitative risk assessment of this exposure route affects remedial decisions at sites with arsenic-contaminated soils.

Accurate assessment of the human health risks resulting from incidental ingestion of arsenic-containing soil requires knowledge of the bioavailability of arsenic from those soils. Oral bioavailability is defined in this report as the amount of arsenic that is absorbed into the body following ingestion of soil or soil-like materials that contain arsenic. This is also referred to as the oral absorption fraction.

Absorption of arsenic following oral ingestion of contaminated soil or sediment depends mainly on the physical and chemical attributes of the arsenic in the soil. Some forms of arsenic (e.g., sodium arsenate) are readily soluble in gastrointestinal fluid and are well absorbed into the blood in most species (Juhasz et al. 2006, ATSDR 2007). Other forms of arsenic (e.g., arsenic adsorbed to iron-containing particles in soil) that are not as readily dissolved are generally not as extensively absorbed. Because the form of arsenic in soil varies widely from site to site (depending mainly on source), the bioavailability of arsenic in soil also varies widely from site to site.

Gastrointestinal absorption of ingested arsenic may be described either in absolute or relative terms:

> Absolute bioavailability (ABA) is the ratio of the amount of arsenic absorbed to the amount ingested:
>
> ABA = (absorbed dose) / (ingested dose)
>
> This ratio is also referred to as the oral absorption fraction.
>
> Relative bioavailability (RBA) is the ratio of the absolute oral bioavailability of arsenic present in some test material (e.g., soil) to the absolute oral bioavailability of arsenic in an appropriate reference material:
>
> $RBA = ABA_{test} / ABA_{reference}$

Oral toxicity values for arsenic, including the oral reference dose and cancer slope factors, are based on studies of human populations exposed to arsenic in drinking water. Therefore, the most appropriate form of arsenic for use as a reference material is a readily soluble arsenic compound such as sodium arsenate.

When a reliable RBA value is available for a particular site medium (e.g., soil), the RBA can be used to adjust the default oral reference dose (RfD_{IRIS}) and oral cancer slope factor (CSF_{IRIS}) for arsenic to account for differences in absorption between arsenic ingested in water and arsenic ingested in the site medium, as follows:

$$RfD_{adj} = \frac{RfD_{IRIS}}{RBA}$$

$$CSF_{adj} = CSF_{IRIS} \cdot RBA$$

Alternatively, it is also acceptable to adjust the dose (rather than the toxicity factors) as follows:

$$Dose_{adj} = Dose_{default} \cdot RBA$$

In the risk assessment process, using the RBA to adjust the toxicity value or the dose results is mathematically equivalent and results in identical calculated risks.

In the absence of reliable site-specific data, the conservative default approach is to assume an RBA of 100% for arsenic in soil and sediment. However, studies performed to date indicate that this assumption is generally too high, with most measured RBA values ranging from 5% to 50% (Roberts et al. 2007, USEPA 2010). Hence, when site-specific arsenic RBA can be reliably measured, it often reduces the estimated health risk from arsenic in soil, and this in turn can result in substantial cost savings during site cleanup.

Arsenic RBA can be measured *in vivo* using animal models (e.g., swine, monkey, or mice), and this is the preferred strategy whenever feasible. However, the cost (up to $100,000) and time (up to 6 months) requirements of *in vivo* RBA tests often limit the application of these models to only the largest sites. Therefore, a faster, more economical yet dependable *in vitro* method for predicting *in vivo* RBA is highly desirable.

One such alternative strategy is to perform measurements of arsenic solubility in the laboratory. Typically, a sample of soil or sediment is extracted using a fluid that has properties that resemble a gastrointestinal fluid, and the amount of arsenic solubilized from the sample into the fluid under a standard set of extraction conditions is measured. The fraction of arsenic that is solubilized is referred to as the *in vitro* bioaccessibility (IVBA). The IVBA is then utilized to predict the *in vivo* RBA of arsenic in that sample, usually through an empiric correlation model.

1.2 OBJECTIVE OF THE DEMONSTRATION

The objective of this demonstration project was to develop, optimize, and validate an IVBA method to estimate RBA of arsenic from soil for use in human health risk assessments.

1.3 REGULATORY DRIVERS

EPA's Risk Assessment Guidance for Superfund Part A (EPA 1989) and EPA's Guidance for Evaluating the Bioavailability of Metals in Soils for Use in Human Health Risk Assessment (EPA 2007a) both indicate that it is acceptable and appropriate to make site-specific adjustments to exposure and risk estimates when reliable site-specific data are available to show that the absorption of a contaminant from site media (e.g., soil or sediment) is different than the absorption of that chemical in studies used to derive the toxicity values.

As noted above, when these data are derived from reliable studies in an appropriate animal model, the data are generally considered to be acceptable. However, use of RBA values derived using an *in vitro* methodology requires that the *in vitro* test method should be supported by the following attributes (EPA 2007b):

- The method should have undergone independent scientific peer review by disinterested persons who are experts in the field, knowledgeable in the method, and financially unencumbered by the outcome of the evaluation.
- There should be a detailed protocol with standard operating procedures (SOPs), a description of operating characteristics, and criteria for judging test performance and results.
- Data generated by the method should adequately measure or predict the toxic endpoint of interest and demonstrate a linkage between either the new test and an existing test or the new test and effects in the target species.
- There should be adequate test data for chemicals and products representative of those administered by the regulatory program or agency and for which the test is proposed.
- The method should generate data useful for risk assessment purposes, i.e., for hazard identification, dose-response assessment, and/or exposure assessment. Methods may be useful alone or as part of a battery or leveled approach.
- The specific strengths and limitations of the test must be clearly identified and described.
- The test method must be robust (relatively insensitive to minor changes in protocol) and transferable among properly equipped and staffed laboratories.
- The method should be time- and cost-effective.
- The method should be one that can be harmonized with similar testing requirements of other agencies and international groups.
- The method should be suitable for international acceptance.
- The method must provide adequate consideration for the reduction, refinement, and replacement of animal use.

The project reported here achieves these requirements and is expected to be acceptable to EPA for use in human health risk assessments of arsenic ingestion from soil or sediment.

2.0 TECHNOLOGY

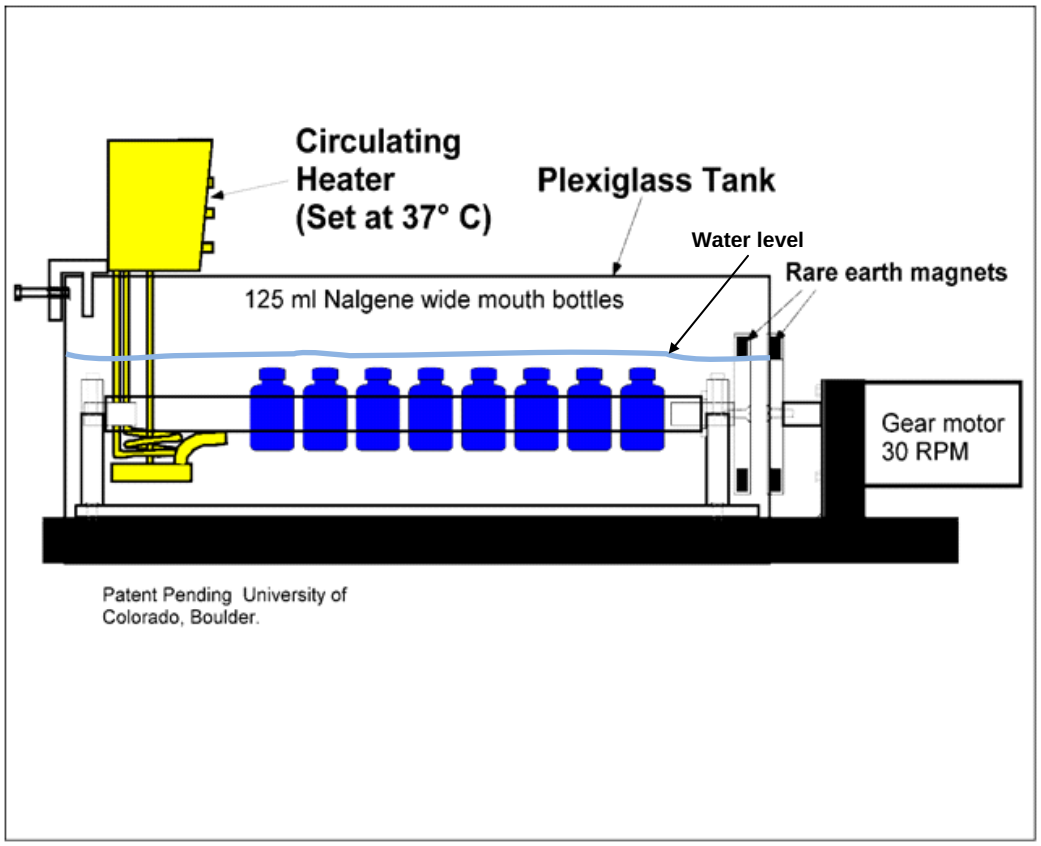
2.1 TECHNOLOGY DESCRIPTION

The technology developed during this project consists of an extraction system to measure the IVBA of arsenic in a test material under specified conditions, coupled with a set of mathematical models to predict the RBA of the test material from the measured IVBA value.

Extraction Device

Figure 2-1 illustrates the extraction device used in these studies. The device holds ten 125-milliliter (mL) wide-mouth high-density polyethylene (HDPE) bottles. These are rotated within a Plexiglas tank by an electric motor with a magnetic flywheel. The water bath must be filled such that the extraction bottles are fully immersed. Temperature in the water bath is maintained at 37 ± 2 degrees Celsius (ºC) using an immersion circulator heater. The 125-mL HDPE bottles must have an airtight screw-cap seal, and care must be taken to ensure that the bottles do not leak during the extraction procedure.

Figure 2-1. IVBA Extraction Device



Solutions and Reagents

All solutions are prepared utilizing America Society for Testing and Materials Type II de-ionized (DI) water. All reagents and water must be free of arsenic, and the final fluid must be tested to confirm that arsenic concentrations are less than one-fourth of the project-required detection limits of 20 micrograms per liter (µg/L) (e.g., 5 µg/L arsenic in the final fluid). Depending on the specific test requested, two extraction solutions may be required, as follows:

Extraction Fluid 1 consists of 0.4 M glycine pH 1.5 without other reagents, prepared as follows:

> To 1.937 liters (L) of DI water, add 60.06 grams (g) glycine (free base, reagent grade). Add 63 mL of trace-metal grade hydrochloric acid bringing the final solution volume to 2 L. Place the mixture in the water bath at 37ºC until the extraction fluid reaches 37°C. Standardize the pH meter using both pH 2.0 and pH 4.0 pH standard buffers using temperature compensation at 37ºC or buffers maintained at 37ºC. Add, dropwise, trace-metal grade, concentrated hydrochloric acid (12.1 N) until the solution pH reaches a value of 1.50 ± 0.05.

Extraction Fluid 2 consists of 0.4 M glycine pH 7.0 supplemented with 0.05 M phosphate, prepared as follows:

> To 2.0 L of DI water, add 60.06 g glycine and 14.196 g anhydrous dibasic sodium phosphate. Place the mixture in the water bath at 37ºC until the extraction fluid reaches 37ºC. Standardize the pH meter using both pH 4.0 and pH 7.0 pH standard buffers using temperature compensation at 37ºC or buffers maintained at 37ºC. Add, dropwise, concentrated sodium hydroxide solution or concentrated trace-metal grade hydrochloric acid until the solution pH reaches a value of 7.00 ± 0.05.

Cleanliness of all materials used to prepare and/or store the extraction fluid and buffer is essential. All non-disposable glassware and equipment used to prepare standards and reagents must be properly cleaned, acid-washed, and, triple-rinsed with de-ionized water before use. Disposable labware is recommended whenever possible.

Extraction Procedure

All test substances must be thoroughly mixed before use to ensure homogeneity. This mixing may be achieved using a roller mixer (several minutes) or by end-over-end mixing for about 30 seconds.

After mixing, measure 1.00 ± 0.05 g of test substrate and place in a clean 125 mL Nalgene bottle, ensuring that static electricity does not cause soil particles to adhere to the lip or outside threads of the bottle. If necessary, use an antistatic brush to eliminate static electricity before adding the media. Record the mass of substrate added to the bottle on the laboratory worksheet.

Measure 100 ± 0.5 mL of the designated extraction fluid using a graduated cylinder and transfer to the 125 mL wide-mouth HPDE bottle containing the test material. Hand-tighten each bottle top and shake/invert to ensure that no leakage occurs, and that no media is caked on the bottom of the bottle.

Place the bottle into the extraction device (Figure 1), making sure each bottle is secure and the lid(s) are tightly fastened. Fill the extractor with 125 mL bottles containing test materials or quality assurance samples. Ensure that the temperature of the water bath is 37 ± 2ºC.

Turn on the extractor and rotate end-over-end at 30 ± 2 revolutions per minute for 1 hour. After 1 hour, stop the extractor rotation and remove the bottles. Wipe them dry and place upright on the bench top. Measure and record the final pH of the fluid in the extraction bottle. If the final fluid pH is not within ± 1.0 pH units of the starting pH, the test must be discarded and the sample reanalyzed.

Draw extract directly from the top portion of the extraction bottle into a disposable 10 mL syringe with a Luer-Lok attachment. Attach a 0.45 µm cellulose acetate disk filter (25 millimeter in diameter) to the syringe, and filter the extract into a clean 15 mL polypropylene centrifuge tube or other appropriate sample vial for analysis.

Measure and record the final pH of the fluid in the extraction bottle. If the final fluid pH is not within ± 1.0 pH units of the starting pH, the test must be discarded and the sample reanalyzed.

Add 2 drops of trace-metal grade nitric acid to each 15 mL polypropylene centrifuge tube containing filtered extraction fluid and store in a refrigerator at 4ºC until analysis. Analysis for arsenic concentrations must occur within 1 week of extraction for each sample.

Sample Analysis

Extracts are analyzed for arsenic using EPA Method 6020. Dilute each sample 50:1 (200 microliters (µL) extract in 10 mL DI water) for analysis. This is needed to eliminate matrix effects, reduce interference from chlorine plus argon, and dilute arsenic concentrations into a more common analytical working range.

Quality Control/Quality Assurance

Quality control samples generated during extraction studies includes the following:

- A laboratory blank is a bottle containing 100 mL of extraction fluid put through the entire extraction process but with no added soil or test substrate. All laboratory blank samples should have arsenic concentrations of 10 ug/L or less.

- A blank-spike is a bottle containing 2.5 parts per million (ppm) (2.5 µg/mL) arsenic, prepared by adding 250 µL of 1,000 ppm National Institute of Standards and Technologoy (NIST) traceable inductively coupled plasma arsenic standard solution to 100 mL of

extraction fluid. Recovery of arsenic in the spiked samples should be between 85% and 115%.

Calculation of IVBA

The IVBA of arsenic in the test material is calculated as follows:

$$IVBA = \frac{C_{fluid} \cdot V_{fluid}}{C_{soil} \cdot M_{soil}}$$

where

C_{fluid} = Concentration of arsenic in the extraction fluid (µg/L)
V_{fluid} = Volume of extraction fluid (L)
C_{soil} = Concentration of arsenic in the test soil (µg/g), measured using EPA Method 3050
M_{soil} = Mass of soil placed in the extraction bottle (g)

Calculation of RBA

The RBA of arsenic in the test material is estimated from the IVBA value using an equation of the following form:

$$RBA = a + b \cdot IVBA$$

The values of the model parameters (a and b) are derived empirically using regression analysis to fit the model to a calibration data set of samples for which reliable values of IVBA and *in vivo* RBA have both been measured (see Section 2.2, below). For a prediction of RBA (as a percentage) measured in swine, the best extraction fluid is Fluid 1 (pH 1.5, no additions), and the best fit prediction model is:

$$RBA_{swine}\ (\%) = 19.7 + 0.62 \cdot IVBA_{pH\ 1.5}$$

For a prediction of RBA measured in monkeys, the best extraction fluid is Fluid 2 (pH 7.0 with phosphate), and the best fit prediction model is:

$$RBA_{monkey}(\%) = 14.3 + 0.58 \cdot IVBA_{pH\ 7}$$

The finding that the best-fit regression model occurs at pH 7 for monkey and pH 1.5 for swine suggests that there might be significant physiological differences between the animal species that result in this outcome. However, this study did not seek to investigate the reason why different extraction pH conditions yielded a better fit for swine and monkey, so no mechanistic explanation is available at this time.

2.2 TECHNOLOGY DEVELOPMENT

The IVBA extraction procedure described here was developed in a series of phases. Detailed reports on each phase of investigation are provided in **Appendix B**, and the main findings of each phase are summarized below.

Phase I of the project consisted of a literature review to indentify candidate test materials and to inventory soils that were available for testing. In order for a test material to be considered a candidate, a reliable RBA value for arsenic was required from studies in swine and/or monkey, and sufficient material had to be available to EPA for use in testing. Based on the review, a total of 48 test materials were identified. These soils were obtained and inventoried, and a detailed review of the RBA calculations was performed to ensure all values were correct and were based on the most reliable estimate of arsenic concentration in the test material.

Phase II of the project investigated the effect of a wide range of experimental variables in the IVBA extraction protocol on the measured IVBA values in a set of 31 test materials. These test materials were selected to provide a wide range of different mineralogical forms, including the most common forms encountered at Superfund sites, and a range of IVBA results, including some samples for which prior testing indicated that the IVBA result and the RBA result were not in good agreement. The extraction variables that were investigated in Phase II included the following:

- pH of the extraction fluid
- temperature of the water bath
- time of extraction
- buffer strength
- addition of oxyanions (e.g., phosphate [PO_4]) to the extraction fluid
- addition of hydroxylamine hydrochloride (HAH) to the extraction fluid
- filter pore size
- redox potential of the extraction fluid
- mass of test material used in the assay

Although all of these variables had effects on the IVBA of at least some test materials, the three variables that were judged to be most useful for further evaluation were the pH of the extraction fluid, the addition of PO_4 to the extraction fluid, and the addition of HAH to the extraction fluid. Thus, these three extraction variables were retained for multivariate evaluation in Phase III.

Phase III of the project consisted of a series of studies to measure arsenic IVBA for a selected set of test substrates using various combinations of the three key extraction variables identified in Phase II. In the Phase III study, these key variables were evaluated in a Latin square design (i.e., one parameter was varied at a time while all other parameters were kept constant). The following 21 different assay variable conditions were evaluated:

PO_4 (M)	0	0.05		0.2		0.8	
HAH (M)	0	0.1	0.25	0.1	0.25	0.1	0.25
pH 1.5	x	x	x	x	x	x	x
pH 5.0	x	x	x	x	x	x	x
pH 7.0	x	x	x	x	x	x	x

x Baseline IVBA extraction protocol

A set of 16 test materials were selected for evaluation. The objective of the Phase III work was to identify up to three different IVBA extraction conditions that provide the best predictive relationship between IVBA and RBA. When the test materials spiked with sodium arsenate were included in the data sets, good correlations between IVBA and RBA were apparent under several assay variable conditions. The following three IVBA extraction conditions were selected for further evaluation in Phase IV:

- pH 1.5, without PO_4 or HAH additions
- pH 7.0, without PO_4 or HAH additions
- pH 7.0, with 0.05M PO_4 and HAH additions (either 0.1 or 0.25M)

Phase IV and Phase V of the project consisted of finalizing the IVBA extraction conditions, testing the final extraction protocols on an expanded set of 39 test substrates, and assessing the degree of correlation between the IVBA values and the RBA values. The objective of this phase of the project was to develop a mathematical model that could reliably predict RBA from one or more IVBA measurements for a wide range of test materials. These mathematical models included the evaluation of IVBA measurements for the three IVBA extraction conditions both with and without accompanying arsenic mineralogy data.

Based on the model fittings developed in Phase V, the following step-wise approach was recommended for selecting RBA values for arsenic in soil:

Step 1: Perform risk calculations assuming the RBA for arsenic in soil is equal to the national or regional default value. If risks are below a level of concern, and it is not anticipated that a more refined estimate of RBA would change risk management decisions or influence soil cleanup strategies, then no further effort is necessary.

Step 2: If risks from arsenic (assuming a default RBA) are sufficient to influence soil cleanup decisions, then measure IVBA of arsenic in several soil samples from the site to obtain an improved estimate of RBA, collecting IVBA data using Extraction Fluid 1 (pH 1.5 without PO_4 or HAH additions). The resulting IVBA data can be utilized to predict RBA based on the swine model:

$$RBA = 19.7 + 0.62 \cdot IVBA_{pH\ 1.5}$$

Step 3: If risks from arsenic (assuming a site-specific RBA value based on IVBA measurements) are still sufficient to influence soil cleanup decisions, and if it is suspected

that a more accurate estimate of RBA might have significant impacts on the extent or cost of cleanup, then collect arsenic mineralogy data for several soil samples from the site to allow estimation of RBA using models that utilize both IVBA and mineralogy data on relative arsenic mass (RAM) in each of three mineralogic phase "bins" (see Phase IV/V report in Appendix B for description of mineralogic characterization of soils and phase binning methodology):

$$RBA = 0.573 \cdot IVBA_{pH\ 1.5} + 0.081 \cdot RAM_{Bin\ 1} + 0.236 \cdot RAM_{Bin\ 2} + 0.346 \cdot RAM_{Bin\ 3}$$

Phase VI of the project consisted of an inter-laboratory ("round-robin") testing of several IVBA extraction methods, and also the results of inter-laboratory testing of arsenic mineralogy by electron microprobe analysis. The objective of this phase was to evaluate the reliability and reproducibility of the laboratory protocols for obtaining arsenic IVBA and mineralogy data needed for RBA prediction. A total of 12 test materials were selected for evaluation in the IVBA inter-laboratory evaluation. Based on the arsenic IVBA inter-laboratory results, it was concluded that IVBA extractions for arsenic can be implemented by laboratories with high within-laboratory precision and good between-laboratory precision, well within limits that are generally considered to be acceptable. A total of 3 test materials were selected for evaluation in the arsenic mineralogy inter-laboratory evaluation. The mineralogy inter-laboratory evaluation showed that there was generally poor agreement between the laboratories. Thus it was concluded that, under present conditions, arsenic mineralogy is too costly and too variable to support the use of mathematical models that require mineralogy data to improve estimates of RBA. This effectively eliminates Step 3 (above) as a generally applicable approach to refining arsenic RBA estimates, at least until standard and reproducible methods for arsenic speciation can be developed.

2.3 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

Advantages and Limitations Compared to *In Vivo* RBA Measurements

As noted previously, *in vivo* measurements of arsenic RBA typically require up to 6 months to plan and complete and can cost up to $100,000. Because of this, *in vivo* methods are typically limited to assessing a small number of samples at a site (e.g., 1-4). In contrast, the primary advantage of the IVBA approach described here is that it is rapid (40 or more samples per day) and inexpensive (typically about $50 to $100 per IVBA extraction). As a result, the *in vitro* IVBA methods can be applied to a large number of samples (e.g., 10-50), allowing a more robust characterization of arsenic RBA at a site.

The principal limitation of the *in vitro* method is that the RBA value predicted from an IVBA measurement may not be identical to the RBA value that would have been derived had an *in vivo* study been performed. Rather, the predicted RBA value is what would be typical for a sample with the measured IVBA. However, it is important to recognize that *in vivo* RBA values have measurement error which introduces uncertainty in to the estimate of the RBA, and the prediction error from the IVBA approach is about the same magnitude as the measurement error in a typical *in vivo* RBA estimate. Also, in practice, the small number of soil samples usually assessed using *in*

vivo methods introduces additional uncertainty in site-wide characterization of RBA because this small number of samples cannot allow assessment of variability in RBA across the site.

Advantages and Limitations Compared to Other *In Vitro* IVBA-Based Prediction Models

A number of other researchers have described *in vitro* systems for measuring the extractability of arsenic from soil or other soil-like materials (see Table 2-1). The principal advantages of the method described here compared to other published methods include the following:

- The current method utilizes a single extraction step. This is in contrast to methods that utilize two or more sequential extraction steps, each intended to represent differing parts of the gastrointestinal system.
- The current method utilizes simple extraction fluids. This is in contrast to methods that seek to create extraction fluids that closely mimic various gastrointestinal fluids, including the presence of a number of biochemical constituents such as enzymes and metabolites.
- The current method is based on a more extensive and systematic testing of extraction conditions to identify the optimal conditions that most other approaches.
- The current method utilizes a larger set of calibration samples to establish the *in vitro-in vivo* correlation (IVIVC) between IVBA and RBA than any other approach. Indeed, some methods provide no information on IVIVC. The use of a large calibration data set is important because finding a successful model for a small set of samples appears to be substantially easier than finding a model for a wide variety of samples.
- The current method has undergone inter-laboratory validation, while, to our knowledge, no other approaches have been subjected to true inter-laboratory validation.

In summary, the current method is distinguished primarily by its simplicity, reliability, and degree of validation.

Table 2-1. Overview of Published IVBA Procedures for Arsenic

Reference	Phases	Gastric fluid pH	Gastric extraction time	Intestinal fluid pH	Intestinal Extraction time	Test Material: Extraction fluid ratio	Gastric Solution	Intestinal Solution	Method Complexity	IVIVC Calibration soils	Round Robin Validation ?
Basta et al. (2007)	2 (stomach/ intestinal)	1.8	1.0	5.5	1.0	1:150	HCl, NaCl, pepsin	$NaHCO_3$ (Na_2CO_3), bile extract, pancreatin	Moderate	15	No
Bruce et al. (2007)	2 (stomach/ intestinal)	1.3	1.0	7.0	3.0	0.4:40	HCl, pepsin, sodium citrate, malic acid, lactic acid and acetic acid	Na_2HCO_3, bile extract, pancreatin	High	9	No
Buckley (1997)	5 (*)	1.8	1.0	7.0	5.0	unknown	HCl, $CaCl_2$, KCl, NaCl, $MgCl_2$, $FeCl_3$, KI, $NaPO_4$	Na_2HCO_3, $KHCO_3$	High	None	No
CBR (1993)	2 (stomach/ intestinal)	2.0	1.0	6.9	1.5	1:0.03	HCl	Na_2HCO_3/NaOH,	High	None	No
Ellickson et al. (2001)	3 (saliva/stomach/ intestinal)	1.4	2.0	6.5	2.0	0.05:100	HCl, NaCl, pepsin	Na_2HCO_3	High	1	No
Juhasz et al. (2007)	1 (stomach)	1.5	1.0	--	--	1:100	HCl, Glycine	--	Low	12	No
Medlin (1997)	2 (stomach/ intestinal)	1.5	1.0	---	3.0	1:110	HCl, pepsin, citrate, malate, lactic and acetic acids	Na_2HCO_3, bile extract, pancreatin	High	6	No
Oomen et al. (2002)	1 (stomach)	1.5	1.0	---	---	1:100	HCl, glycine	---	Low	None	No
Oomen et al. (2002)	2 (stomach/ intestinal)	2.0	2.0	7.5	6.0	2:100	HCl, pepsin, mucin	Na_2HCO_3, trypsin, pancreatine, bile extract	High	None	No
Wragg et al. (2002)	3 (saliva/stomach/ intestinal)	1.2	1.0	6.3	4.0	0.6:13.5	HCl, pepsin, mucin, BSA	Na_2HCO_3, pancreatine, lipase, bovine serum albumin, bile extract	High	9-11	Partial
Oomen et al. (2002)	2 (stomach/ intestinal)	4.0	3.0	6.5	5.0	1:2.5	HCl, pepsin, mucin, cellobiose, proteose, peptone starch	Na_2HCO_3, pancreatine	High	None	No
Oomen et al. (2002)	5 (*)	2.5	1.5	6.8	6.0	1:25	HCl, pepsin, lipase	Na_2HCO_3, pancreatine	High	None	No
Rodriguez et al. (1999)	2 (stomach/ intestinal)	1.8	1.0	5.5	1.0	1:150	HCl, NaCl, pepsin	Na_2HCO_3, bile extract, pancreatin	Moderate	15	No
Ruby et al. (1996)	2 (stomach/ intestinal)	2.5	1.0	7.0	3.0	1:100	HCl, pepsin, citrate, malate, lactic and acetic acids	Na_2HCO_3, bile extract, pancreatin	High	3	No

*Extensive extraction procedure, including saliva, esophagus, stomach, small and large intestine steps.

3.0 PERFORMANCE OBJECTIVES

Performance objectives are the primary criteria for evaluating the success or failure of a new technology. They provide the basis for evaluating the performance and costs of the technology. Meeting these performance objectives is essential for successful demonstration and validation of the technology.

Table 3-1 provides a summary of the performance objectives that were established at the outset of the project. The extent to which these objectives were obtained is discussed below.

Table 3-1. Performance Objectives

Performance Objective	Data Requirements	Success Criteria
1) Identify principal variables affecting assay results.	Test the effect of pH, temperature of the bath, time of extraction, fluid composition (ionic strength, competitive binding agents), and filter pore size on metal extraction in the IVBA system.	Either the comparison between *in vivo* RBA and the IVBA RBA yield a correlation coefficient of 0.8 or better using linear regression, or specific mineralogical forms can be identified which react differently to one or more variables of interest in the IVBA system.
2) Determine up to 3 combinations of assay variables that are most likely to improve the predictive relationship between IVBA and RBA.	Key variables of interest identified in the previous step will be evaluated in a Latin square design. Assay variables of combinations that yield the highest R^2 and, secondarily, the lowest intercept, would be selected for further evaluation in a more demanding optimization evaluation.	Either regression analysis relating *in vivo* RBA to IVBA yields a correlation coefficient of 0.8 or better, or specific mineralogical forms can be identified which yield a correlation coefficient of 0.8 or better when run in two or more combinations of optimized variables.
3) Determine final protocol and test on all soils.	Test materials will be assayed using up to 3 test protocols identified from the previous step. Multiple assessments of each test material set will be conducted to assess reproducibility of results from each protocol. Analyze data by a series of regression models for each test protocol relating IVBA and RBA for each test material.	A single protocol is generated or several protocols are generated specific to the mineralogical form of arsenic.
4) Quantify the intra- and inter-laboratory reproducibility of the optimized protocol.	In a round-robin analysis, 3 independent laboratories will test each of several soils following the new optimized IVBA protocol. The results will be input into a regression model that best describes the relationship between IVBA and RBA for that protocol. All of the data on within and between-laboratory variability, the between laboratory correlation results for IVBA and RBA, and the results of the QC samples (blanks and duplicates) included in the analysis.	The initial acceptance criterion for precision will be defined as the high end of the precision achieved by the primary laboratory. Absolute percent error, root-mean percent error and percent predictive error will also be calculated to evaluate predictive performance following methods described in Malinowski et al. (1997). Acceptance criteria and control limits will be based on limits established by Brattin and Drexler (2007).

Performance Objective 1: Identify Principal Variables Affecting IVBA Results

Objective

The objective of this phase of the study was to determine which experimental conditions of the extraction procedure had the largest effects on measured IVBA values.

Data Collection

Data that were collected are detailed in the Phase II report (see Appendix B). In brief, variables that were studied included pH of the extraction fluid, temperature of the bath, time of extraction, fluid ionic strength, oxyanion (phosphate) addition, hydroxylamine addition, filter pore size, redox potential, and soil mass. The effect of varying these parameters was evaluated individually, holding all other extraction conditions constant.

Data Evaluation and Success Criteria

Although all of these variables had effects on the IVBA of at least some test materials (see Phase 2 report, Appendix B), the three that were considered to be the strongest determinants of IVBA were pH, phosphate concentration, and hydroxylamine hydrochloride concentration. Some variables impacted the IVBA of nearly all test materials in a similar fashion, while others impacted some test soils more than others. These data satisfy the success criteria identified in Table 2.

Performance Objective 2: Identify Optimized Combinations of Key Variables

Objective

The objective of this phase of the investigation was to evaluate various combinations of the three variables identified in Phase II in order to help focus in on the optimum IVBA extraction conditions (that is, to find the extraction conditions that yielded the highest correlation between the IVBA values and RBA values for the same samples).

Data Collection

Data were collected for 16 different test soils under 21 different combinations of extraction pH, phosphate concentration, and hydroxylamine concentration (a total of 336 extractions).

Data Evaluation and Success Criteria

The primary success criterion established for this phase was that one or more extraction conditions yielded a correlation coefficient of 0.8 or better. (Note: a correlation coefficient of 0.8 is equivalent to a linear regression R^2 value of 0.64). As discussed in the Phase III Report (see Appendix B), this criterion was achieved in 11 cases when the RBA data were measured in

swine, in 14 cases when the RBA data were measured in monkey, and in 11 cases when the data sets were combined. These results satisfy the performance criteria established in Table 2.

Performance Objective 3: Determine a Final Protocol and Test on All Soils

Objective

The objective of this phase was to test a selected subset of extraction conditions using the largest number of test soils available. The purpose was to determine if the good correlations established on the subset of samples evaluated in Phase III remained robust when new test soils were added to the data set.

Data Collected

The data collected during this phase of the project included measurement of IVBA for a set of 35 test materials under three different extraction conditions: pH 1.5 (no additions), pH 7 (no additions), and pH 7 plus 0.05 M phosphate and 0.1 M hydroxylamine.

Data Evaluation and Success Criteria

The data generated were evaluated by fitting regression models relating IVBA and RBA for each extraction condition. The success criterion was a correlation coefficient of 0.8 or higher (an R^2 value of 0.64 or higher). As discussed in the Phase IV/V Report (see Appendix B), this objective was achieved using pH 1.5 IVBA data for the swine data set ($R^2 = 0.72$) and using pH 7 IVBA data for the monkey data set, either with phosphate and hydroxylamine ($R^2 = 0.75$) or without any other additions ($R^2 = 0.71$). No extraction condition was found that satisfied this criterion when the swine and monkey data were combined.

Although not part of the original work plan, an additional data evaluation effort was performed to determine if addition of arsenic speciation data would result is a more reliable way to predict RBA values. The speciation data used were obtained by the University of Colorado at Boulder, and consisted of the amount of arsenic in each of 16 different arsenic-containing mineral phases. This effort identified a model that yielded an R^2 value of 0.90 for the swine data set, 0.82 for the monkey data set, and 0.72 for the combined data set. On this basis, it was concluded that a modeling approach that utilized both IVBA data and speciation data could yield predictions that were somewhat more reliable than if only IVBA data were used.

Performance Objective 4: Evaluate Inter-Laboratory Reproducibility

Objective

The objective of this phase of the study was to quantify the intra- and inter-laboratory reproducibility of the methods for obtaining arsenic IVBA and speciation data.

Data Collected for IVBA measurements

A set of 12 test materials was distributed to each of four laboratories (including the University of Colorado reference laboratory), along with an SOP detailing the proper technique for obtaining arsenic IVBA measurements (see Appendix C). Each laboratory measured the IVBA of each material in triplicate, for each of three different extraction fluids (a total of 108 extractions per laboratory).

Data Collected for Arsenic Speciation

Three samples of soil were selected for inter-laboratory arsenic speciation studies. Each soil was prepared in a polished puck, and the same puck was provided to each of two individuals for evaluation. The data collected was the observed amount of arsenic in each of 16 phases in each of the three samples.

Data Evaluation and Success Criteria for IVBA Data

The success criterion for within-laboratory precision on IVBA measurements was defined as the high end of the precision achieved by the reference laboratory (University of Colorado Boulder). This value is 6%. The results for the three round-robin laboratories (see Phase VI Report, Appendix B) were all within this value, and overall within-laboratory precision for each laboratory was similar to that of the reference laboratory.

No *a priori* criterion was established for between-laboratory precision, since this value is generally established empirically from the results of inter-laboratory testing. Although there is no standard rule, the acceptance criterion is often set at about twice the observed between-laboratory standard deviation. In this case, the between laboratory precision was very good (an average of about 5%), indicating that a suitable acceptance criterion for other laboratories would likely be no larger than about 10%.

Data Evaluation and Success Criteria for Speciation Data

Because the use of speciation data was not a part of the original work plan, no success criteria for this assessment were defined. However, the results of the effort (see Phase VI report, Appendix B) revealed that there were large differences between laboratories, and that the results were too variable to be considered useful. Therefore, it was concluded that obtaining the phase data needed to predict RBA using a model that employed both IVBA and phase data was not feasible, unless substantial additional effort was invested in refining the SOP and/or providing additional training. The reason for the low precision in the analysis of arsenic phase data is likely the result of random Poisson statistical variation combined with differing degrees of operator experience. These results do not rule out the possibility of achieving better reproducibility at some point in the future as training and methodology (including instrumentation) improve.

4.0 SITE DESCRIPTION

The site selected for the technology demonstration is Operable Unit 1 (OU1) of the Hill Air Force Base (AFB) in Utah. Detailed information about the site is provided in CH2M Hill (2011). Relevant information for the purposes of this report is summarized in the sections below.

4.1 SITE LOCATION AND HISTORY

OU1 is located along the east side of Hill AFB and comprises Landfills 3 and 4, Chemical Disposal Pits 1 and 2, Fire Training Areas 1 and 2, and associated on- and off-base groundwater and sediment contamination (see Figure 4-1). Historically, industrial operations at the base included the use of numerous chemicals, metals, degreasing solvents, and hydrocarbon fuel products that were disposed of in on-base pits and landfills, resulting in soil and groundwater contamination.

4.2 SITE GEOLOGY/HYDROGEOLOGY

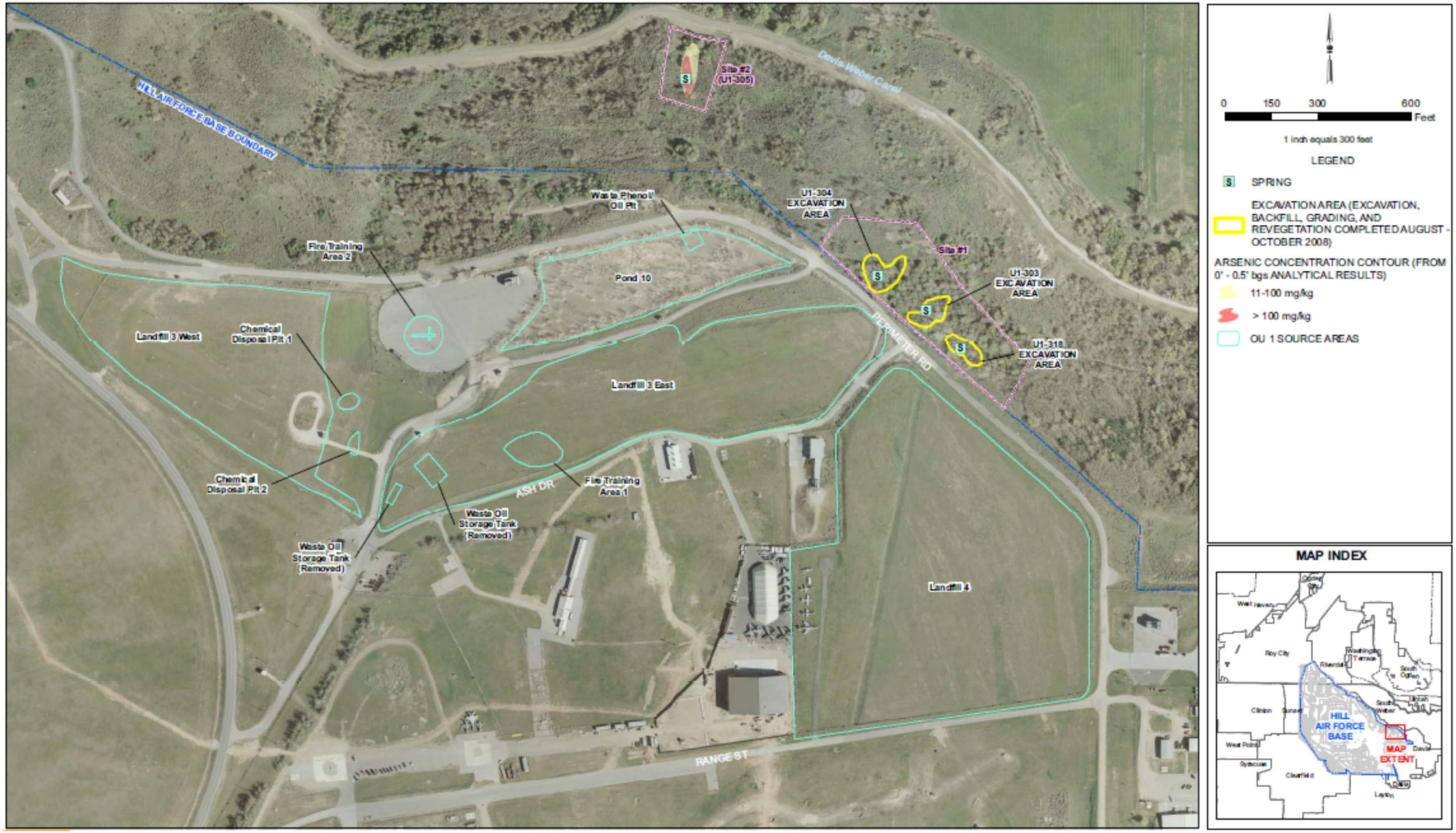
Geochemically reduced shallow groundwater exists within OU1 and is thought to be exacerbated by (1) oxygen depletion due to the degradation of hydrocarbons disposed of in the OU1 source areas and (2) the construction of a landfill cap that limits the movement of oxygen-bearing precipitation into the shallow groundwater. The reducing conditions resulted in the mobilization of naturally occurring metals (including iron, manganese, and arsenic) from soil into groundwater. Metals dissolved in the shallow on-base groundwater were transported through higher-permeability subsurface soil overlying low-permeability clay deposits. These dissolved metals precipitated along several hillside springs and seeps immediately north of the OU1 source area, as shown in Figure 4-1. Former Springs U1-303, U1-304, U1-305, and U1-318 ceased flowing in 2001 because of engineering controls applied to the upgradient shallow groundwater on Hill AFB. Figure 4-2 illustrates the previously described processes on a conceptual cross-section of the OU1 source area and hillside.

The locations of former Springs U1-303, U1-304, and U1-318 are referred to collectively as "Site 1" and former Spring U1-305 is referred to as "Site 2" (see Figure 4-1). Currently, the arsenic-contaminated sediment at Site 2 appears as stained surface soil along the steep hill slope. Red stains reflect the presence of iron-rich minerals that formed when the metals-bearing groundwater came into contact with the atmosphere as it emerged from the springs on the hillside. Because the springs no longer flow, the contaminated spring sediments are currently more akin to surface soil than subaqueous sediment from the perspective of environmental fate and transport and potential human exposures.

4.3 CONTAMINANT DISTRIBUTION

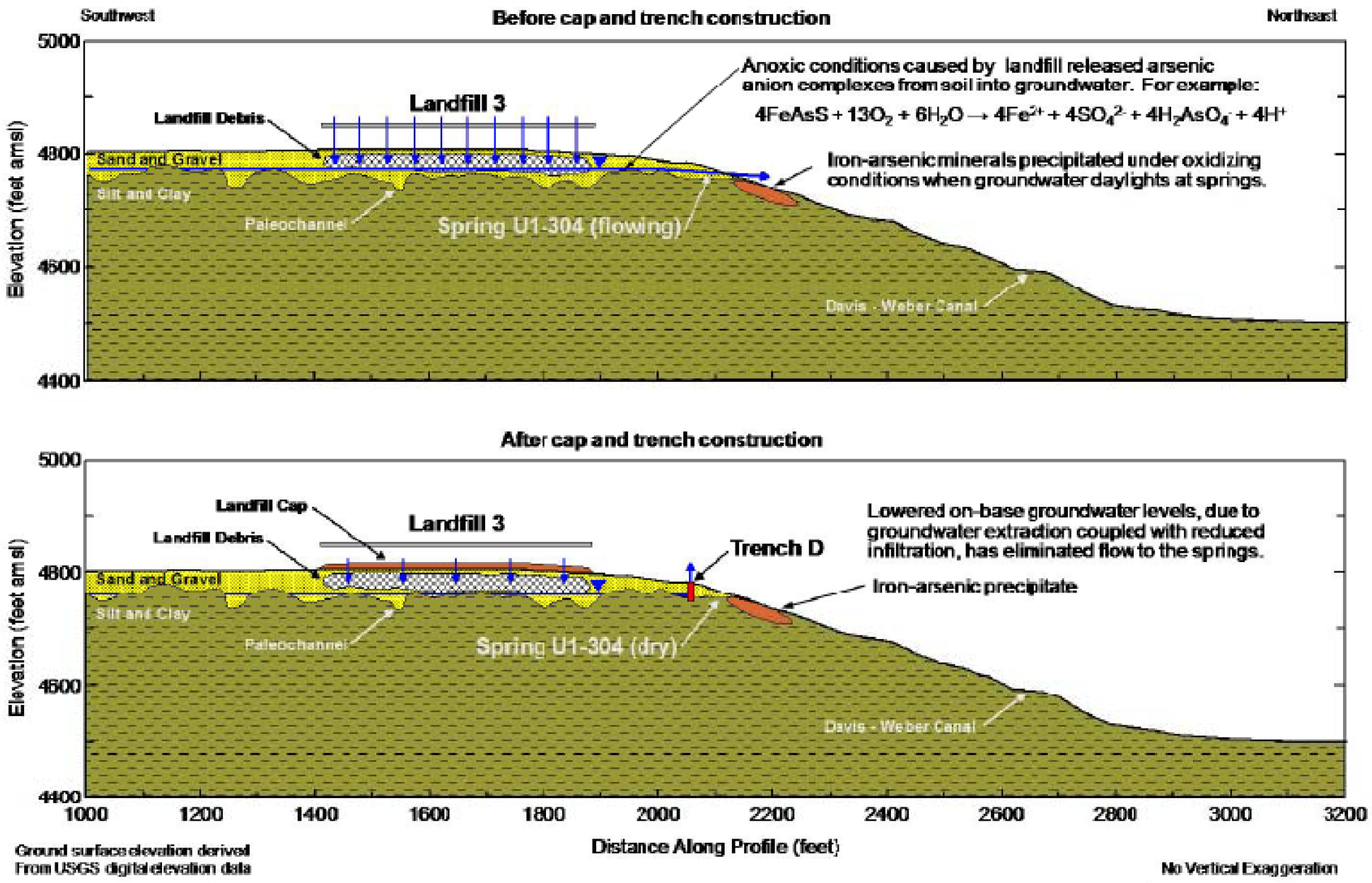
Sampling was conducted in August 2009 to collect samples of surface sediments at Site 2 for evaluation of arsenic speciation and bioavailability. In order to achieve spatial representation, samples were collected from three separate zones along the more contaminated dry channel that extends downhill from U1-305. Four sediment samples were collected from each zone. The Site 2 sample locations are presented in Figure 4-3.

Figure 4-1. Operable Unit 1 Source Areas



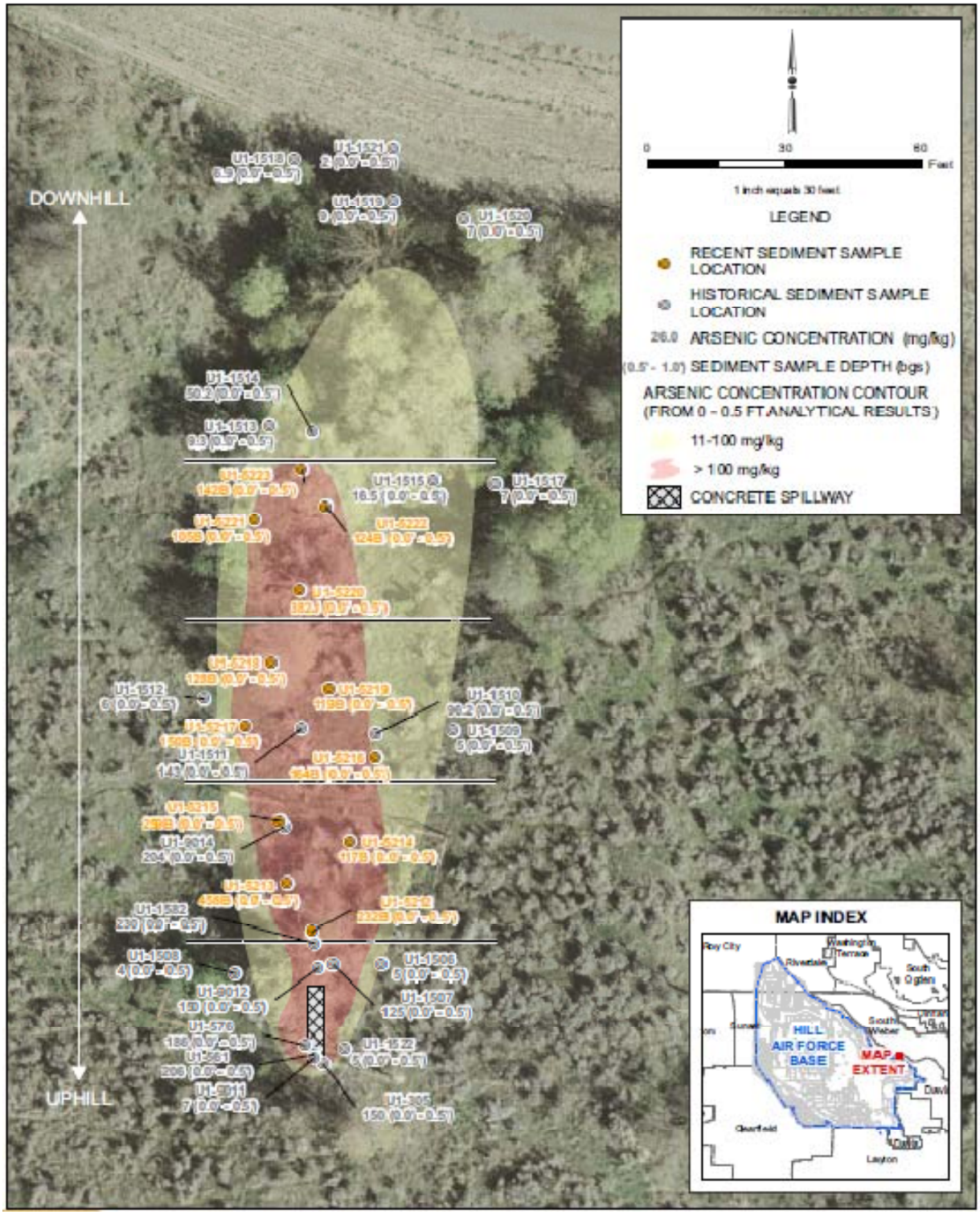
Source: Figure 1-2 from CH2M Hill (2011)

Figure 4-2. Conceptual Model for OU1 Springs



Source: Figure 1-3 from CH2M Hill (2011)

Figure 4-3. Site 2 Sediment Sample Locations



Source: Appendix A, Figure 3 from CH2M Hill (2011)

Before sediment collection, the sample locations were cleared of surface vegetation and debris. Grab samples (0-6 inches in depth) were collected using a stainless steel hand auger and homogenized in a stainless steel bowl. Following homogenization, a 4-ounce aliquot was placed into glass jar for total arsenic analysis by EPA Method SW6020B. Arsenic concentrations detected in the 12 soil samples at Site 2 ranged from 105 to 458 mg/kg (Figure 4-3).

Following receipt of the total arsenic results, six of the 12 collected sediment samples were selected for arsenic IVBA testing and arsenic speciation. These six samples were selected to provide total arsenic concentrations across the range of concentrations detected in the 12 samples and also to provide good spatial representation across Site 2. Before IVBA analysis, these samples were sieved to yield the fine fraction (< 250 μm). The following table summarizes the total arsenic concentration measured in each of the six sieved samples selected for IVBA analysis:

Sample ID	Arsenic Concentration (mg/kg)
U1-5212	205
U1-5213	138
U1-5216	192
U1-5218	137
U1-5221	118
U1-5223	172

5.0 TEST DESIGN

5.1 CONCEPTUAL EXPERIMENTAL DESIGN

The technology demonstration at the Hill AFB consists of measuring the IVBA of arsenic in several sediment samples collected from Site 2 of OU1, using the measured IVBA values to predict the site-specific RBA of arsenic in these samples, and then comparing the estimated human health risks using the default RBA and the site-specific RBA. Appendix A of the *Supplemental Human Health Risk Assessment (HHRA) for the Operable Unit 1 (OU1) Hillside of Hill Air Force Base, Utah* (CH2M HILL 2011) provides detailed information on the site-specific arsenic bioavailability evaluation.

5.2 BASELINE CHARACTERIZATION

In the absence of site-specific data, the national default (baseline) assumption used in the risk assessment is that the RBA of arsenic in soil and sediment is 100%.

5.3 TREATABILITY OR LABORATORY STUDY RESULTS

No treatability studies were performed as part of this project.

5.4 DESIGN AND LAYOUT OF TECHNOLOGY COMPONENTS

No technology components were deployed to the site as part of this project.

5.5 FIELD TESTING

No field testing was performed as part of this project.

5.6 SAMPLING RESULTS

IVBA and Speciation Results

Arsenic IVBA testing and arsenic speciation of the six selected samples was performed by the Laboratory for Environmental and Geological Studies at the University of Colorado, Boulder. Attachment C of CH2M HILL (2011) provides detailed information on the arsenic IVBA procedures and speciation methodology utilized to evaluate these samples. In brief, arsenic IVBA was determined based on pH 1.5 extraction fluid conditions in accordance with the standard extraction procedure. IVBA results are summarized below:

Sample ID	IVBA (pH 1.5)
U1-5212	13%
U1-5213	5%
U1-5216	18%
U1-5218	17%
U1-5221	28%
U1-5223	12%

Arsenic speciation was performed using an electron microprobe. The results were expressed as the length-weighted frequency and as the relative arsenic mass in a variety of identified arsenic-bearing phases. Nearly all of the identifiable arsenic in the samples was associated with iron as iron oxide/hydroxide (FeOOH).

RBA Prediction

Attachment D of CH2M HILL (2011) provides detailed information on the how the site-specific RBA for arsenic was predicted from the IVBA and arsenic speciation results. In brief, site-specific RBA values were predicted using a regression model that that was based on a set of eight soil samples where the predominant form of arsenic was FeOOH. The resulting best-fit model was:

$$RBA = 14.465 + 0.159 \cdot IVBA(pH\ 1.5)$$

Based on this prediction model, site-specific RBA values for arsenic ranged from 15% to 19%, with a median (and mean) value of 17%.

At the time of the HHRA, Phase IV of this project had not yet been completed. If RBA values were predicted utilizing the recommended model identified in the Phase IV report (see Appendix B), the predicted site-specific RBA values for arsenic would have ranged from 23% to 37%, with a median (and mean) value of 29%.

Impact on Risk

In the human health risk assessment, risks from arsenic in Site 2 were evaluated for two receptor populations (hypothetical residents and visitors/trespassers) based on particulate inhalation and ingestion exposure scenarios. The following table illustrates how the estimated cancer risks from ingestion exposures to arsenic in sediment at Site 2 differ depending upon the selected arsenic RBA value:

Receptor	Cancer Risk Estimates		
	National default RBA of 100%	Site-specific predicted RBA of 17% (a)	Site-specific predicted RBA of 29% (b)
Hypothetical resident	4E-04	7E-05	1E-04
Visitor/trespasser	6E-06	1E-06	2E-06

(a) Based on FeOOH model provided in Attachment D of the HHRA
(b) Based on model provided Phase IV Report

As seen, compared to the default, use of site-specific RBA values derived from pH 1.5 IVBA measurements resulted in a decrease of risk estimates from above EPA's typical level of concern (>1E-04) to within EPA's typical risk range (1E-04 to 1E-06), such that remedial actions would not be needed.

6.0 PERFORMANCE ASSESSMENT

The performance of the IVBA approach for estimation of RBA of a specific soil sample cannot be evaluated without performing an independent *in vivo* study of RBA on the same test soil. However, based on the regression model for swine data (see Phase IV/V report, Appendix B), it is expected that an RBA value estimated from IVBA is likely to be accurate within about 10% of the value that would have been obtained by measurement *in vivo*.

As noted above, the performance of the IVBA approach at a site-wide level is likely to be superior to an approach based on *in vivo* RBA, since the IVBA approach allows for the evaluation of a large number of samples, while the *in vivo* method is generally restricted to only a few samples (often only one or two).

7.0 COST ASSESSMENT

7.1 COST MODEL

Table 7-1 summarizes the cost elements in obtaining an IVBA value needed to calculate an RBA value for a sample of soil or sediment.

Table 7-1.
Cost Model for Conducting an IVBA Test for
Estimating the RBA of Arsenic from Soil

Cost Element	Unit Cost
Collect samples for analysis	$200-$300 (a)
Dry and sieve samples for analysis	$15-$25
Analyze fine-grained soil samples for total arsenic (b)	$20-$30
Conduct IVBA Assay (extract sample, measure arsenic in extraction fluid)	$75-$125 (c)

(a) Cost not tracked as part of this demonstration, typical range is provided
(b) Sample digestion by EPA Method 3050 followed by sample analysis by EPA Method 6020
(c) Cost per sample usually decreases as sample number increases

Cost Element: Collect and Prepare Soil Samples

The cost of sample collection and preparation was not tracked in this demonstration. Samples may be collected using traditional field sampling techniques and may be either grab samples or composites (the latter is generally preferred for risk assessment purposes). A sampling round specific for the IVBA study may sometimes be needed, but often the samples for IVBA analysis may be selected from samples already collected as part of the remedial investigation (assuming that holding times are not exceeded). Costs of sample collection vary widely from site to site, but are often in the $200-$300 per sample range.

Once collected, the samples are shipped to a laboratory for preparation and analysis. Preparation steps include drying, mixing and (usually) sieving to a particle size of ≤ 250 μm, since this is the particle size that is generally believed to be of greatest concern for ingestion by the hand-to-mouth exposure pathway. Costs of drying and sieving are typically about $15-25 per sample.

Cost Element: Analyze Soil Samples for Total Arsenic

Once the sample is dried and sieved, the sample is digested according to EPA Method 3050, followed by analysis according to EPA method 6020 (inductively coupled plasma/mass spectroscopy). The total cost of digestion and analysis varies from laboratory to laboratory, but typical costs are about $20-$30 per analysis. Duplicate analyses of IVBA test materials are generally recommended to help ensure reliable calculations.

Cost Element: Conduct IVBA Assay(s)

Each soil requires extraction in one or more extraction fluids. Assuming the goal is to predict RBA as measured in the swine bioassay (see Phase IV/V Report, Appendix B), extraction in pH

1.5 fluid is recommended. In general, each sample should be extracted in duplicate to help ensure the IVBA value is precise.

Several commercial laboratories currently offer the IVBA extraction assay. The cost of the analysis varies between laboratories, but the following breakdown is representative:

- Setup $300-$500
- IVBA Extraction $30-$50 per extraction
- Fluid analysis $30-$50 per analysis

Because of the setup cost, there is generally an economy of scale, with decreased cost per sample as the number of samples increases. For a site where 20 test soils were collected, the total cost would be about $1,500-$2,500 for singlicate analysis (about $75-$125 per sample), and $2,700-$4,500 for duplicate analysis (about $135-$225 per sample).

7.2 COST DRIVERS

The principal cost driver for this technology is the number of independent samples needed to adequately characterize the IVBA of arsenic at a site. If available site information suggests the site is likely to be relatively homogeneous with respect to arsenic mineralogy and soil chemistry, then a relatively small number of samples (e.g., 4-6) may be sufficient to derive a reliable and robust estimate of RBA. However, if available site information suggests the site is likely to be relatively heterogeneous (e.g., differing types of mine waste and/or differing soil types at different locations across the site), then it may be necessary to collect and analyze a larger number of samples (e.g., 20-40 or possibly more) to obtain reliable and representative information.

7.3 COST ANALYSIS

The total cost of implementing an IVBA-based approach for estimation of site-specific RBA values for arsenic is provided in Table 7-2, along with a comparison to the cost of obtaining RBA measures using an *in vivo* animal study. The site where the approach is implemented is assumed to be heterogeneous in arsenic types that are present, such that a total of 20 samples are required to achieve good spatial coverage.

Table 7-2.
Cost Analysis for Conducting an IVBA Study at a Heterogeneous Site (N = 20)

Cost Element	*IVBA*		*In Vivo*	
	Unit Cost	**Total Cost**	**Unit Cost**	**Total Cost**
Collect samples for analysis	$200-$300	$4,000-$6,000	$200-$300	$4,000-$6,000
Dry/sieve samples for analysis	$15-$25	$300-$500	$15-$25	$300-$500
Analyze soil samples for arsenic	$20-$30	$800-$1,200	$20-$30	$800-$1,200
Measure IVBA or RBA	$75-$125	$1,500-$2,500	$40,000-$60,000	$800,000-$1,200,000
Total cost		**$6,200-$9,600**		**$804,700-$1,207,100**

As shown, the total cost of estimating RBA for 20 samples using the IVBA method is less than $10,000, while the cost of obtaining the same data via *in vivo* studies may exceed $1,000,000. In

addition, the IVBA studies could be completed within weeks, while the *in vivo* studies would likely require a year or more to complete.

8.0 IMPLEMENTATION ISSUES

8.1 REGULATORY ACCEPTANCE

As noted above, the EPA and other regulatory agencies generally accept and support the concept of incorporating reliable RBA data into site-specific risk assessments (e.g., see http://www.epa.gov/superfund/health/contaminants/bioavailability/bio_guidance.pdf), but do not automatically accept an *in vitro*-based approach for estimation of RBA.

In order to maximize the probability of regulatory acceptance of the IVBA-based method for arsenic, this project has been performed using an approach similar to the approach that was previously followed to develop and gain regulatory acceptance for an IVBA-based method for estimating the RBA of lead in soil (EPA 2007a). This approach involves frequent presentations to and discussions with EPA's Bioavailability Subcommittee of the Technical Review Workgroup (TRW) to ensure they accept the approach that is being developed and to incorporate any recommendations they may offer, as well as consideration of the guidelines for acceptance of *in vitro* methods described in EPA (2007a). An arsenic *in vitro* method validation assessment report (Griffin 2012) has been prepared and submitted to EPA to document that the method meets all specified method validation criteria and regulatory acceptance criteria for *in vitro* methods specified in the bioavailability guidance (EPA 2007a).

In accordance with this approach, from 2009 through 2012 Dr. Griffin has attended several meetings of the TRW subcommittee to present the current progress and findings of the project. We have also been in discussions with the co-chairs of this subcommittee on bringing the method development, validation, and SOPs to the TRW for national acceptance. National acceptance of the arsenic *in vitro* methodology developed through the ESTCP grant should remove all federal and state regulatory barriers to the use of IVBA tests to adjust bioavailability factors in risk assessment equations and cleanup level development.

8.2 PROCUREMENT OF IVBA ANALYSES

Although IVBA extractions are not a routine service provided by all analytical laboratories, there are several laboratories that currently have the equipment and provide the services. This includes both commercial laboratories as well as several EPA regional laboratories (see Phase VI Report, Appendix B).

8.3 PROCUREMENT OF ARSENIC SPECIATION DATA

Characterization of the arsenic mineral phases present in soil or sediment at a site is often useful in identifying the probable source of the arsenic contamination. In addition, as discussed previously, arsenic speciation data may be used to improve the accuracy of RBA predictions (see Phase IV/V Report, Appendix B). However, at present, there are only a limited number of laboratories with the equipment and expertise to perform arsenic speciation studies in soils and sediments, and the round robin tests from this demonstration project indicate that the approach is not yet sufficiently standardized for this to be practical at present. In the future, as the database

of samples with paired RBA and IVBA measurements increases, it may be appropriate to revisit the utility of using arsenic speciation data to strengthen RBA predictions from *in vitro* measurements.

9.0 REFERENCES

ATSDR. 2007. Toxicological Profile for Arsenic. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. August 2007.

Basta, N.T., Foster, J.N., Dayton, E.A., Rodriguez, R.R., and Casteel, S.W. 2007. The Effect of Dosing Vehicle on Arsenic Bioaccessibility in Smelter-Contaminated Soils. J. Envir. Science and Health. Part A., 42, pp. 1275-1281.

Bruce, S., Noller, B., Matanitobua, V., and Ng, J. 2007. In Vitro Physiologically Based Extraction Test (PBET) and Bioaccessibility of Arsenic and Lead from Various Mine Waste Materials. J. Tox. And Envir. Health. Part A., pp. 1700-1711.

Buckley, B.T. 1997. Estimates of Bioavailability of Metals in Soil with Synthetic Biofluids: Is This a Replacement for Animal Studies? In: IBC Conference on Bioavailability. 1998, Scottsdale, Arizona, USA.

CBR (CB Research International). 1993. Report: Development of a Physiologically Relevant Extraction Procedure. Sidney, British Columbia, Canada.

Drexler, J.W. 1998. An In Vitro Method That Works! A Simple, Rapid, and Accurate Method for Determination of lead Bioavailability. EPA Workshop, Durham, North Carolina.

Drexler, J.W. 1997. Validation of an In Vitro method: A tandem approach to Estimating the Bioavailability of Lead and Arsenic in Humans. IBC Conference on Bioavailability. Scottsdale, Arizona.

Drexler, J.W. 2000. Bioavailability/bioaccessibility of metals. Paper read at The ITRC Fall 2000 Conf.: New Environ. Technol. and Market Opportunities, at San Antonio, Texas. 16–20 Oct. 2000.

Drexler,J. and Brattin,W. 2007. An In Vitro Procedure for Estimation of Lead Relative Bioavailability: With Validation. Human and Ecological Risk Assessment. 13(2), pp. 383-401.

Ellickson, K.M., Meeker, R.J., Gallo, M.A., Buckley, B.T., and Lioy, P.J. 2001. Oral Bioavailability of Lead and Arsenic from a NIST Standard Reference Soil Material., Arch. Environ. Contam. Toxicol., 40, pp. 128-135.

EPA (U.S. Environmental Protection Agency). 1989. Risk Assessment Guidance for Superfund Volume I Human Health Evaluation Manual. Office of Emergency and Remedial Response, U.S. Environmental Protection Agency, Washington D.C. EPA/540/1-89/002.

EPA. 2007a. Guidance for Evaluating the Oral Bioavailability of Metals in Soils for Use in Human Health Risk Assessment. U.S. Environmental Protection Agency, Office of Solid Waste

and Emergency Response. Washington, DC 20460. OSWER 9285.7-80. Available online at: http://www.epa.gov/superfund/health/contaminants/bioavailability/bio_guidance.pdf.

EPA. 2007b. Framework for Metals Risk Assessment. Office of the Science Advisor Risk Assessment Forum. EPA/120/R-07/001.

EPA. 2010. Relative Bioavailability of Arsenic in Soils at 11 Hazardous Waste Sites Using an *In Vivo* Juvenile Swine Method. Bioavailability Subcommittee of the Technical Review Workgroup, Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, DC. OSWER Directive #9200.0-76. June 2010. Available online at: http://epa.gov/superfund/bioavailability/pdfs/as_in_vivo_rba_main.pdf.

Griffin, S. 2012. Validation Assessment of *In Vitro* Arsenic Bioaccessibility Assay for Predicting Relative Bioavailability of Arsenic in Soils and Soil-like Materials at Superfund Sites. November 2012.

Juhasz, A.L., Smith, E., Weber, J., Rees, M., Rofe, A., Kuchel, T., Sansom, L., and Ravi Naidu, R.. 2006. In Vivo Assessment of Arsenic Bioavailability in Rice and Its Significance for Human Health Risk Assessment. Environ. Health Perspect. 114(12): 1826-1831.

Juhasz, A.L., Smith, E., Weber, J., Rees, M., Rofe, A., Kuchel, T., Sansom, L., and Naidu, R. 2007. In Vitro Assessment of Arsenic Bioaccessibility in Contaminated (Anthropogenic and Geogenic) Soils. Chemosphere 69: 69–78.

Malinowski H, Marroum P, Uppoor VR, et al. 1997. Draft guidance for industry extended release solid oral dosage forms. In: Young D, Devane J, and Butler J (eds), Vitro-in Vivo Correlations. Plenum Press, New York, NY, USA

Medlin, E.A. 1997. An In Vitro Method for Estimating the Relative Bioavailability of Lead in Humans. Masters thesis. Department of Geological Sciences, University of Colorado, Boulder.

Oomen, A.G., Hack, A., Minekus, M., Zeijdner, E., Cornelis, C., Schoeters, G., Verstraete, W., Van de Weile, T., Wragg, J., Rompelberg, C.J.M., Sips, A.J.A.M., and Van Wijnen, J. 2002. Comparison of Five In Vitro Digestion Models to study the Bioaccessibility of Soil Contaminants. Envirn. Sci Tech. 36: 3326-3334.

Roberts, S.M., Munson, J.W., Lowney, Y.W., and Ruby, M.V. 2007. Relative Oral Bioavailability of Arsenic from Contaminated Soils Measured in the Cynomolgus Monkey. Toxicological Sciences 95:281-288.

Rodriguez, R.R., Basta, N.T., Casteel, S.W., and Pace, L.W. 1999. An In Vitro Gastrointestinal Method to Estimate Bioavailable Arsenic in Contaminated Soils and Solid Media. Environ. Sci. Technol. 33, 642-649.

Ruby, M.W., Davis, A., Schoof, R., Eberle, S., and Sellstone, C.M. 1996. Estimation of Lead and Arsenic Bioavailability Using a Physiologically Based Extraction Test. Environ. Sci. Technol. 30(2):422–430.

APPENDICES

Appendix A: Points of Contact

POINT OF CONTACT	ORGANIZATION	Phone E-mail	Role in Project
Susan Griffin	EPA, Region 8 15195 Wynkoop Street Denver, CO 80202	303-312-6651 griffin.susan@epa.gov	Co-investigator
Yvette Lowney	Exponent, Inc. 4141 Arapahoe Ave., Suite 101 Boulder, CO 80303	303-245-7070 ylowney@exponent.com	Co-investigator
John Drexler	University of Colorado Dept. of Geological Sciences Benson Earth Science Bldg. 2200 Colorado Ave. Boulder, CO 80309	303-492-5251 drexlerj@colorado.edu	Laboratory director
William Brattin	SRC, Inc. 999 18th Street, Suite 1150 Denver CO 80202	303-357-3121 brattin@srcinc.com	Contributing scientist
Gary Diamond	SRC, Inc. 8191 Cedar St Akron, NY 14001	716-542-7140 diamond@srcinc.com	Contributing scientist
Lynn Woodbury	CDM Smith 555 17th Street, Suite 1100 Denver, CO 80202	303.383.2382 woodburyL@cdmsmith.com	Contributing scientist

Appendix B: Phase Reports

Phase I Report:
Soil Characterization

Phase II Report:
Identification Of Principal Variables Affecting IVBA Assay Results

Phase III Report:
Multi-Variate Evaluation of Key Variables Affecting IVBA Assay Results

Phase IV/V Report:
Selection of Optimum RBA Prediction Methods

Phase VI Report:
Results of Round-Robin Evaluation of IVBA Testing and Arsenic Speciation

Appendix C: Standard Operating Procedures

Standard Operating Procedure:

In Vitro Bioacessibility (IVBA) Procedure for Arsenic

Standard Operating Procedure:

Arsenic Speciation

APPENDICES

Appendix A: Points of Contact

POINT OF CONTACT	ORGANIZATION	Phone E-mail	Role in Project
Susan Griffin	EPA, Region 8 15195 Wynkoop Street Denver, CO 80202	303-312-6651 griffin.susan@epa.gov	Co-investigator
Yvette Lowney	Exponent, Inc. 4141 Arapahoe Ave., Suite 101 Boulder, CO 80303	303-245-7070 ylowney@exponent.com	Co-investigator
John Drexler	University of Colorado Dept. of Geological Sciences Benson Earth Science Bldg. 2200 Colorado Ave. Boulder, CO 80309	303-492-5251 drexlerj@colorado.edu	Laboratory director
William Brattin	SRC, Inc. 999 18th Street, Suite 1150 Denver CO 80202	303-357-3121 brattin@srcinc.com	Contributing scientist
Gary Diamond	SRC, Inc. 8191 Cedar St Akron, NY 14001	716-542-7140 diamond@srcinc.com	Contributing scientist
Lynn Woodbury	CDM Smith 555 17th Street, Suite 1100 Denver, CO 80202	303.383.2382 woodburyL@cdmsmith.com	Contributing scientist

Appendix B: Phase Reports

Phase I Report:
Soil Characterization

IVBA SOIL CHARACTERIZATION REPORT

A SUPPORTING DOCUMENT FOR THE IN VITRO OPTIMIZATION METHOD TO PREDICT THE RELATIVE ORAL BIOAVAILABILITY OF ARSENIC

October 5, 2009

Prepared by:

USEPA Region 8
15195 Wynkoop Street
Denver, CO 80202

Dr. John Drexler
Department of Geological Sciences
Benson Earth Science
2200 Colorado Ave.
Boulder, CO 80309

SRC, Inc.
999 18th Street, Suite 1975
Denver, CO 80202

Exponent
4141 Arapahoe Avenue, Suite 101
Boulder, CO 80303

THE UNIVERSITY OF COLORADO AT BOULDER — THE LABORATORY FOR ENVIRONMENTAL AND GEOLOGICAL STUDIES

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

1.0 INTRODUCTION

The U.S Environmental Protection Agency (EPA) is presently working to develop an optimized and validated *in vitro* bioaccessability (IVBA) method that may be used to reliably estimate the *in vivo* relative bioavailability (RBA) or arsenic in soils from Superfund sites (USEPA 2009). Because many of the initial phases of IVBA method development and optimization will involve selecting representative soil samples for testing, there is a need to understand the range and variability of the soils available. Thus, the objective of this report is to characterize relevant features of the candidate soils available for the work planned.

2.0 INVENTORY OF TEST MATERIALS

The inventory of available test materials includes forty-eight test materials (Table 1) from Superfund or National Priority List (NPL) sites for which *in vivo* RBA values had been determined in monkeys (Roberts et al. 2007) or in swine (Casteel and SRC 2009a,b, USEPA 2005, USEPA 1996). These soils constitute the set of available soils that are candidates for use in the development and validation of the IVBA method for arsenic. These soils were collected from various residential and industrial sites across the U.S. that have been impacted by arsenic-containing pesticides, mining-related wastes, and/or other industrial releases. Table 1 lists each test material's name, site of origin, and available mass. Descriptions of the sites and collection procedures for the soils (where available) are provided in Appendix A.

Throughout the remainder of this report, test materials assayed in swine (referred to henceforth as EPA soils) are discussed separately from materials assayed in monkeys (referred to henceforth as Roberts soils), This distinction is made because, in general, RBA estimates based on the EPA soil assays have yielded higher estimates than those based on the Roberts soil assays. The reasons for the differences are not currently understood and may be contributed by differences in test materials (soil composition, arsenic mineralogy), or assay protocols (e.g., single dose versus steady-state). Although studies are underway to directly compare assay results for the same test materials, until a better understanding of the differences in RBA results is achieved, separate optimization evaluations will be conducted for EPA and Roberts soils.

3.0 SOIL CHARACTERISTICS

Soil characteristics reviewed in this report include metals content, arsenic mineralogy, particle size and relative bioavailability.

3.1 Soil Metal Concentrations

Soil metal concentrations (Table 2a) were measured in EPA test materials between 1996 and 2009 (Casteel and SRC 2009a,b, USEPA 2005, USEPA 1996a), and in 2007 for Roberts test materials (Roberts et al. 2007). All soils except the Barber Orchard soils were sieved <250-µm prior to metals analysis. The Barber Orchard soils were sieved prior to arsenic analysis but not for other metals analyses. Soil digestion and analysis

procedures for arsenic were not consistent between studies, and were often not reported precisely for EPA studies. Details regarding soil digestion and analysis methods for arsenic are shown in Table 2b.

The variability in arsenic concentrations was higher in EPA soils than in Roberts soils, however average arsenic concentrations in Roberts and EPA soils were roughly similar, averaging ~575 mg/kg (range: 123 - 1490 mg/kg) in Roberts soils and ~675 mg/kg (range: 16 - 10,000 mg/kg) in EPA soils. Most materials also exhibited high iron and lead concentrations.

3.2 Mineralogy

Arsenic mineralogy of soils was evaluated using electron microprobe analysis (EMPA). Mineralogy was determined in EPA soils initially in 2005 (USEPA 2005), but some soils were reanalyzed in 2009. Roberts soils were analyzed for mineralogy in 2007 (Roberts et al. 2007). Mineralogy of all soils was evaluated by Dr John Drexler at the Laboratory for Environmental and Geological Studies at the University of Colorado, Boulder.

The As-bearing phases in all of the test materials were characterized by EMPA following the method of Davis et al. (1993). Briefly, the EMPA procedure uses an electron microprobe with combined energy dispersive spectrometer (EDS) and multiple wavelength dispersive spectrometers (WDS) to evaluate the elemental composition of arsenic-bearing particles. A 1-2 gram split of dried sample is placed in a 2.5-cm plastic mold and impregnated with epoxy. Once the sample is hardened, it is polished and carbon coated. Instrument response is calibrated using a certified mineral or pure metal standard and counting times are chosen to provide 3-sigma detection limits of between 100-200 ppm. Elemental concentrations are corrected using matrix correction factors (ZAF) and concentration errors are determined to be acceptable if less than 5%.

Relative arsenic mass (percentage of total arsenic in a sample that was present in that phase) was then determined for each sample. Results are shown in Table 3.

The majority of EPA and Roberts soils contained predominately oxide forms of arsenic, including Fe, Mn and Pb oxides. Iron sulfates were also present in both sets of soils, though only about a quarter of samples contained substantial (>25%) arsenic mass associated with iron sulfates. EPA and Roberts soils each contained a few samples with unique arsenic forms, such as arsenic trioxide (in 5 EPA samples collected from a single site), and in the Roberts data set, an arsenopyrite sample (CAMT), a sample from a cattle dip vat site (FLCDV) and a Hawaiian volcano soil (HIVS) containing 72% iron aluminum silicates.

Uncertainty regarding the representativeness of the EMPA sample to the test material as a whole is moderate, because on a mass basis, only a small fraction of the total sample is evaluated by electron microprobe. However, every measure is taken to create a representative sample for EMPA.

3.3 Matrix Association

The physical location of an arsenic-bearing grain relative to the particle it is associated with is expected to have a significant impact on its bioavailability, since the arsenic must be exposed to gastrointestinal fluids in order for the arsenic to be dissolved and absorbed. Arsenic grain association may be characterized into four categories:

Liberated- A grain of arsenic-containing material is not attached to or contained within any other particle.

Rimmed- A condition in which arsenic is present on the outer surface of a particle, usually as a consequence of adsorption or precipitation.

Cemented- The arsenic-containing particle is associated with other particles or phases that do not contain arsenic.

Included- The arsenic-containing particle is entirely contained within another particle

For liberated, rimmed and cemented matrix associations, the arsenic is exposed at the surface of some or all of the particles and hence the arsenic is available to be dissolved by gastrointestinal fluids. Particles that are fully included in other particles are not exposed to external fluids and are expected to have zero or very low bioavailability, although this depends in part on the size of the particle and the surrounding matrix as well.

Matrix association data for arsenic-bearing particles measured in EPA and Roberts soils is summarized in Table 4. All soils have been sieved <250 µm prior to analysis. In both EPA and Roberts soils, most of the arsenic was either liberated or cemented, and very little arsenic was included.

3.4 Particle Size Distribution

The arsenic-bearing particles in test materials can occur in a wide range of sizes. Because the process of dissolving arsenic from a particle into gastrointestinal fluid occurs only at the surface of the particle, and because the ratio of surface area to volume decreases as a function of increasing particle size, it is thought that large particles are likely to have a lower bioavailability than smaller particles of the same mineral. Size distributions of arsenic-containing particles for each test material sample are summarized in Table 5. The majority of particles in most EPA and Roberts soils was <100 µm diameter.

3.5 Relative Bioavailability

Relative bioavailability was measured *in vivo* in a swine model (EPA soils) and/or a monkey model (Roberts soils). Subsequently, *in vitro* bioavailability (IVBA) assays were performed on all samples, following methods of Drexler and Brattin (2007), to compare to *in vivo* RBA values. Swine *in vivo* tests showed a wide range of RBA values for the test materials (Table 6), from 8% to over 100%. Monkey *in vivo* tests showed a narrower RBA range, from 5% to 33%.

As shown in Figures 1 and 2, the overall RBA to IVBA correlation was weak for test materials assayed in swine ($R^2 = 0.14$) as well as monkeys ($R^2 = 0.07$). IVBA estimates tended to be higher than RBA estimates for arsenic trioxide and lead oxide-dominated EPA soils, and IVBA estimates were lower than RBA estimates for iron sulfate, iron oxide and manganese oxide-dominated EPA soils. IVBA estimates tended to be higher than RBA measures for most of the Roberts soils.

4.0 IMPLICATIONS FOR IVBA STUDIES

About 45% of the EPA soils have arsenic concentrations <150 mg/kg. USEPA (2005) reported that soils with these low concentrations had greater uncertainty associated with RBA estimates and in resulted in poorer IVBA to RBA correlations. Thus, soils with low arsenic concentrations may not be optimal for use in development of the IVBA method for arsenic.

Mineralogical forms in the soils are diverse, with about half the EPA and Roberts soils containing predominately oxides of iron, manganese or lead, which tend to have low bioavailability (<40%), and another third of the EPA soils containing a large proportion of arsenic trioxide, which is typically characterized as having moderate to high bioavailability (40% - 60%) based on USEPA (2009). Mineralogy of remaining Roberts soils was more diverse, and have not previously been categorized in terms of expected RBA. Development of the IVBA method should ensure that the diversity of mineralogical forms from both EPA and Roberts soils is tested to enable wide-spread application of the IVBA method to future sites.

Bioavailability will also vary depending on the matrix association and particle size distribution. In most cases, the arsenic associated with the majority of oxides and sulfates was liberated, implying that the arsenic is readily accessible and will be dissolved and available for extraction/absorption following GI digestion. Particle sizes in the samples evaluated tended to be ≤100 µm, again suggesting that the arsenic will be readily bioaccessible.

The interactions of arsenic with major soil ions (e.g., iron) during IVBA extractions or absorption *in vivo* are not well understood. All of the soils contain other metals in addition to arsenic, most notably calcium, iron, aluminum and magnesium. Iron can bond strongly with arsenic, which can lower arsenic bioavailability (Davis et al. 1996). Roberts et al. (2007) found a strong inverse relationship between RBA amd the fraction of arsenic present as iron sulfate mineral phases in soils used in tests with cynomolgus monkeys. However, a similar relationship has not been observed in test materials assayed in juvenile swine (USEPA 2005), and other researchers that have evaluated the effect of iron additions on arsenic IVBA have found little to no significant difference in IVBA to RBA correlation (CBR 1993, Rodriguez et al. 1999, Datta et al. 2007). Other metals/metalloids have not been evaluated specifically with respect to impacts on arsenic IVBA.

5.0 REFERENCES

Casteel and SRC. 2009a. Relative Bioavailability of Arsenic in Barber Orchard Soils. Prepared for USEPA Office of Superfund Remediation Technology Innovation. September 30, 2009.

Casteel and SRC. 2009b. Relative Bioavailability of Arsenic in NIST 2710. Prepared for USEPA Office of Superfund Remediation Technology Innovation. March 20, 2009.

CBR (CB Research International). 1993. Report: Development of a physiologically relevant extraction procedure, Sidney, BC, Canada.

CDM Federal. 2001. Data summary report for arsenic bioavailability study soil sampling conducted May 11 and May 17, 2001 in Butte and Walkerville, Montana. Report addressed to Sara Sparks, USEPA Region VIII, by CDM Federal Programs Corporation, October 19, 2001.

Datta, R., Makris, K.C., and Sarkar, D. 2007. Arsenic fractionation and bioaccessibility in two alkaline Texas soils incubated with sodium arsenate. Arch. Environ. Contam. Toxicol. 52(4): 475-482.

Davis, A., M.V. Ruby M. Bloom, R. Schoof, G. Freeman, P.D. Bergstrom. 1996. Mineralogic constraints on the bioavailability of arsenic in smelter-impacted soils. Environmental Science and Technology 30:392-399.

Drexler, J.W., and Brattin,W. 2007. An *in vitro* procedure for estimation of lead relative bioavailability: with validation. Human and Ecological Risk Assessment, 13(2), pp.383-401.

NIST. 2003. Certificate of Analysis, Standard Reference Material® 2710 – Montana Soil, Highly Elevated Trace Element Concentrations. National Institute of Standards and Technology, Gaithersburg, MD. Certificate Issue Date: July 18, 2003.

Roberts, S.M., J.W. Munson, Y.W. Lowney and M.V. Ruby. 2007. Relative Oral Bioavailability of Arsenic from Contaminated Soils Measured in the Cynomolgus Monkey. Toxicological Sciences 95:281-288.

Rodriguez, R.R., Basta, N.T., Casteel, S.W., and Pace, L.W. 1999. An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and solid media. Environ. Sci. Technol. 33, 642-649.

USEPA. 2009. Workplan to Optimize an In Vitro Method to Predict the Relative Oral Bioavailability of Arsenic. March 13, 2009.

USEPA 2005. Estimation of relative bioavailability of arsenic in soils and soil-like materials using in vivo and in vitro methods (Draft 2005). U.S. Environmental Protection Agency, Region 8, Denver, CO.

USEPA. 1996. Bioavailability of Arsenic and Lead in Environmental Substrates. EPA/910/R-96/002. February 1996.

TABLES

Table 1. Inventory of Test Materials Available for IVBA Optimization

Sample Name	Site Name	Location	Phase[1]	Experiment[1]	Available Mass (grams)	Note
EPA Soils						
Aberjona River TM1	Wells G & H Superfund Site	Woburn, MA	III	4	100	[b]
Aberjona River TM2	Wells G & H Superfund Site	Woburn, MA	III	4	100	[b]
ACC Dislodgeable Arsenic	n/a (study sponsored by ACC)	Unknown	III	6	10	[d]
ACC Utility Pole Soil	n/a (study sponsored by ACC)	Unknown	III	7	10	[d]
Aspen Berm	Smuggler Mountain NPL Site	Aspen, CO	II	5	3	[c]
Aspen Residential	Smuggler Mountain NPL Site	Aspen, CO	II	5	5.4	[h]
Bingham Creek Channel Soil	Kennecott NPL Site	Salt Lake City, UT	II	2	1488.8	[h]
Butte TM1 (Phase II study)	Silver Bow Creek/Butte Area NPL Site	Butte, MT	II	6	0	--
Butte TM1 (Phase III study)	Silver Bow Creek/Butte Area NPL Site	Butte, MT	III	3	890.7	[h]
Butte TM2	Silver Bow Creek/Butte Area NPL Site	Butte, MT	III	3	887.5	[h]
CA Gulch AV Slag (Phase II study)	California Gulch NPL Site	Leadville, CO	II	8	6	[c]
CA Gulch AV Slag (Pilot Study)	California Gulch NPL Site	Leadville, CO	Pilot	10	6	[c]
CA Gulch Fe/Mn PbO	California Gulch NPL Site	Leadville, CO	II	7	99.4	[h]
CA Gulch Phase I Residential Soil	California Gulch NPL Site	Leadville, CO	II	7	259.3	[h]
Clark Fork Tailings	Milltown Reservoir Sediments NPL Site	Milltown, MT	Pilot	15	10	[d]
El Paso TM1	El Paso/Dona Ana County Metals Survey Site	El Paso, TX / Dona Ana County, NM	III	5	10	[d]
El Paso TM2	El Paso/Dona Ana County Metals Survey Site	El Paso, TX / Dona Ana County, NM	III	5	10	[d]
Jasper County High Lead Mill	Jasper County Superfund Site	Jasper County, MO	II	4	587.9	[h]
Midvale Slag	Midvale Slag NPL Site	Midvale, UT	II	6	448	[h]
Murray Smelter Slag	Midvale Slag NPL Site	Murray City, UT	II	4	33.3	[h]
Murray Smelter Soil	Midvale Slag NPL Site	Murray City, UT	II	11	142.4	[h]
Palmerton Location 2	New Jersey Zinc NPL Site	Palmerton, PA	II	9	595.9	[h]
Palmerton Location 4	New Jersey Zinc NPL Site	Palmerton, PA	II	9	370.8	[h]

Table 1. Inventory of Test Materials Available for IVBA Optimization

Sample Name	Site Name	Location	Phase[1]	Experiment[1]	Available Mass (grams)	Note
EPA Soils						
VBI70 TM1	Vasquez Boulevard and I-70 NPL Site	Denver, CO	III	1	1129.3	[h]
VBI70 TM2	Vasquez Boulevard and I-70 NPL Site	Denver, CO	III	1	917.4	[b]
VBI70 TM3	Vasquez Boulevard and I-70 NPL Site	Denver, CO	III	1	1490.9	[h]
VBI70 TM4	Vasquez Boulevard and I-70 NPL Site	Denver, CO	III	2	650	[h]
VBI70 TM5	Vasquez Boulevard and I-70 NPL Site	Denver, CO	III	2	691.9	[h]
VBI70 TM6	Vasquez Boulevard and I-70 NPL Site	Denver, CO	III	2	0	[e]
Tacoma Smelter Slag	Ruston/North Tacoma Superfund Site	Tacoma, WA	---	---	<5 ?	[f]
Tacoma Smelter Soil	Ruston/North Tacoma Superfund Site	Tacoma, WA	---	---	<5 ?	[f]
MS-1 (EPA)	Barber Orchard Superfund Site	Waynesville, NC	---	---	Unknown	
MS-4 (EPA)	Barber Orchard Superfund Site	Waynesville, NC	---	---	Unknown	
MS-5 (EPA)	Barber Orchard Superfund Site	Waynesville, NC	---	---	Unknown	
MS-8 (EPA)	Barber Orchard Superfund Site	Waynesville, NC	---	---	Unknown	
NIST 2710	NIST 2710 standard	Montana	---	---	Unknown	

Table 1. Inventory of Test Materials Available for IVBA Optimization

Sample Name	Site Name	Location	Phase[1]	Experiment[1]	Available Mass (grams)	Note
Roberts Soils						
CAMT	California mine tailings	California	---	---	1000	[g]
CORS	VB/I-70 (Colorado residential soil)	Denver, CO	---	---	40	[g]
COSCS	Colorado smelter composite soil (Globeville)	Denver, CO	---	---	226	[g]
COSS	Colorado smelter soil (Smeltertown)	Denver, CO	---	---	1515	[g]
FLCDV	Florida cattle dip vat soil	Florida	---	---	115	[g]
FLCPS	Florida Inglis	Florida	---	---	555	[g]
HIVS	Hawaii Volcanic soil	Hawaii	---	---	210	[g]
MTSS	Montana smelter soil	Montana	---	---	1008	[g]
NYOS	New York orchard soil	New York	---	---	4020	[g]
NYPS1	New York pesticide plant soil, A1B20 (5B)	New York	---	---	137	[g]
NYPS2	New York pesticide plant soil, T5E3 (8B)	New York	---	---	1700	[g]
NYPS3	New York pesticide plant soil, T15-E4 (13B)	New York	---	---	1000	[g]
WAOS	Washington orchard soil	Washington	---	---	5010	[g]
WISS	Rodriguez #8 soil (western iron slag soil)	western U.S.	---	---	10	[g]
MS-1 (Roberts)	Barber Orchard Superfund Site	Waynesville, NC	---	---	Unknown	
MS-4 (Roberts)	Barber Orchard Superfund Site	Waynesville, NC	---	---	Unknown	
MS-5 (Roberts)	Barber Orchard Superfund Site	Waynesville, NC	---	---	Unknown	
MS-8 (Roberts)	Barber Orchard Superfund Site	Waynesville, NC	---	---	Unknown	

Table 1. Inventory of Test Materials Available for IVBA Optimization

Sample Name	Site Name	Location	Phase[1]	Experiment[1]	Available Mass (grams)	Note

Notes:
ACC = American Chemistry Council.
1 From USEPA (2005)
[b] Stan Casteel sent all soil directly to John Drexler. Estimated mass.
[c] bottle is empty.
[d] John Drexler reported that he has ~10 g.
[e] Sample not found.
[f] Roseanne will send/sent all soil directly to John Drexler.
[g] Yvette Lowry has possession of these soils; will ship directly to John Drexler.
[h] SRC has/had possession of these soils; shipped to John Drexler on 3/30/09

Table 2a. Soil Metal Concentrations of Test Materials

Sample Name	As	Al	Sb	Ba	Be	Cd	Ca	Cr	Co	Cu	Fe	Pb	Mg	Mn	Hg	Ni	K
EPA Soils																	
Aberjona River TM1	**676**	15,000	4.30	75	0.96	15.0	9,100	680.0	32.0	840	73,000	410	2,000	510	2.90	28.0	690
Aberjona River TM2	**313**	11,000	3.70	98	0.62	16.0	10,000	620.0	46.0	540	38,000	350	2,600	610	1.10	35.0	770
ACC Dislodgeable Arsenic	**74**	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
ACC Utility Pole Soil	**320**	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Aspen Berm	**66.9**	5,070	5.2	1,640	1.30	41.9	37,200	7.7	17.1	145	33,700	14,200	14,300	2,220	0.77	29.8	1,090
Aspen Residential	**16.7**	8,440	11.40	1,030	0.82	47.4	17,300	10.4	11.1	52	23,000	3,870	6,890	934	0.23	21.9	2,140
Bingham Creek Channel Soil	**149**	10,100	18.70	152	0.73	8.7	8,500	17.9	7.9	1,720	22,500	6,330	5,970	376	-	15.1	4,150
Butte TM1 (Phase II study)	**234**	7,540	10.60	134	0.56	42.2	15,700	6.9	9.2	838	48,500	8,530	2,950	12,800	2.20	8.0	3,560
Butte TM1 (Phase III study)	**234**	14,100	1.20	165	0.88	42.3	15,700	16.7	5.5	837	55,800	7,980	3,510	13,200	2.30	6.3	4,170
Butte TM2	**367**	14,067	3.37	211	0.78	7.7	3,363	25.6	8.7	3,130	39,800	492	3,950	732	0.42	12.0	3,680
CA Gulch AV Slag (Phase II study)	**1050**	20,800	57.2	2,430	1.20	12.8	117,000	43.1	53.8	2,080	207,000	10,600	6,360	6,910	0.11	7.1	7,390
CA Gulch AV Slag (Pilot Study)	**1050**	20,800	57.2	2,430	1.20	12.8	117,000	43.1	53.8	2,080	207,000	10,600	6,360	6,910	0.11	7.1	7,390
CA Gulch Fe/Mn PbO	**110**	11,900	6.00	266	1.00	38.5	3,930	7.5	6.9	165	27,500	4,320	2,520	1,190	4.90	7.5	1,770
CA Gulch Phase I Residential Soil	**203**	8,670	1.8	605	0.60	59.9	20,100	9.1	2.0	657	68,120	7,510	9,521	7,090	1.26	5.6	1,500
Clark Fork Tailings	**181**	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
El Paso TM1	**74**	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
El Paso TM2	**73**	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Jasper County High Lead Mill	**16.4**	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Midvale Slag	**591**	10,500	71.9	637	0.58	24.5	93,200	142.0	33.0	1,330	202,000	8,170	6,180	1,640	0.74	.31U	4,250
Murray Smelter Slag	**695**	9,370	55.7	2,140	0.86	30.9	89,600	34.0	45.4	2,100	170,000	11,700	11,200	2,640	1.00	16.7	2,430
Murray Smelter Soil	**310**	6,520	20.0	584	0.48b	23.8	69,000	16.4	11.5	856	38,700	3,200	15,000	863	0.52	10.4	2,040
Palmerton Location 2	**110**	7,750	6.00	6,850	1.40	195.0	1,160	30.3	18.8	462	25,900	3,230	725	6,320	1.70	15.0	515
Palmerton Location 4	**134**	7,850	7.40	1,090	2.00	319.0	2,480	26.6	17.4	350	26,700	2,150	684	9,230	1.10	26.8	512
VBI70 TM1	**312**	8,440	2.0	163	0.60	6.8	5,095	40.4	5.6	30	12,800	733	2,770	328	0.73	9.4	2,600
VBI70 TM2	**983**	9,255	6.00	275	0.80	8.8	7,285	20.5	6.1	47	14,800	824	2,885	429	1.34	10.3	2,750
VBI70 TM3	**390**	8,915	2.50	208	0.65	2.6	4,475	19.6	5.5	32	12,750	236	2,510	424	0.71	8.3	2,350
VBI70 TM4	**813**	7,495	3.00	324	0.60	5.0	5,895	19.7	5.0	53	15,250	541	2,315	525	0.68	9.6	1,750
VBI70 TM5	**368**	7,305	2.00	106	0.45	1.1	2,920	16.7	4.7	19	12,100	157	2,085	207	0.36	8.2	2,000
VBI70 TM6	**516**	5,740	4.5	136	0.50	5.0	3,010	12.9	4.5	32	11,800	264	1,755	275	0.34	7.3	2,200

Table 2a. Soil Metal Concentrations of Test Materials

Sample Name	As	Al	Sb	Ba	Be	Cd	Ca	Cr	Co	Cu	Fe	Pb	Mg	Mn	Hg	Ni	K
EPA Soils																	
Tacoma Smelter Slag	**10100**	18200	3350	274	6.9	16	41700	401	269	5220	224000	3780	6910	850	---	93	---
Tacoma Smelter Soil	**1600**	65000	145	526	ND	7	16500	91	17	2670	34800	1350	8630	768	---	59	---
MS-1 (EPA)	**290**	23000	9.9 U	45	0.3 U	5.0 U	1900	27	1.9	69	23000	1200	1200	230	---	4.7	770
MS-4 (EPA)	**388**	51000	9.9 U	370	0.98	5.0 U	6500	58	13	110	39000	1200	9700	840	---	24	5200
MS-5 (EPA)	**382**	35000	9.9 U	210	0.98	4.9 U	1900	41	12	190	33000	1200	5500	1200	---	21	2100
MS-8 (EPA)	**364**	34000	9.9 U	280	1.1	4.9 U	6400	39	12	76	31000	1400	6900	1400	---	21	2900
NIST 2710	**626**	6%	38.4	707	---	21.8	1%	---	---	2950	3%	5532	1%	1%	32.6	14.3	2%
Roberts Soils																	
CAMT	**300**	---	10 U	---	1 U	1.8	---	41.6	---	61.6	---	35.8	---	---	0.51	29.9	---
CORS	**1230**	---	---	---	---	---	---	---	---	---	13700	---	---	---	---	---	---
COSCS	**394**	---	12.9	---	1 U	436.5	---	23.1	---	85	19667	655	---	---	7.67	16.3	---
COSS	**1492**	---	30.1	---	1 U	52.75	---	11.9	---	1950	---	11550	---	---	60.15	80.8	---
FLCDV	**150**	---	10 U	---	1 U	1 U	---	8	---	6.5	---	20 U	---	---	0.02 U	4 U	---
FLCPS	**268**	---	110	---	1 U	1 U	---	9.4	---	154	---	3770	---	---	1.39	4.7	---
HIVS	**724**	---	16.9	---	1.3 U	9.1	---	177	---	175	73200	797	---	1180	1.07	247	---
MTSS	**647**	---	18.7	---	1 U	10	---	17.9	---	2190	---	435	---	---	0.53	9.4	---
NYOS	**123**	---	10 U	---	1 U	1 U	---	25.8	---	41.6	---	695	---	---	0.14	11.8	---
NYPS1	**1000**	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
NYPS2	**339**	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
NYPS3	**549**	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
WAOS	**301**	---	10 U	---	1 U	1 U	---	33.6	---	36.5	21600	2890	---	---	0.04	17	---
WISS	**1412**	---	95.4	---	1 U	126	---	30.1	---	2720	---	6810	---	---	6.32	23.4	---
MS-1 (Roberts)	**290**	23000	9.9 U	45	0.3 U	5.0 U	1900	27	1.9	69	23000	1200	1200	230	---	4.7	770
MS-4 (Roberts)	**388**	51000	9.9 U	370	0.98	5.0 U	6500	58	13	110	39000	1200	9700	840	---	24	5200
MS-5 (Roberts)	**382**	35000	9.9 U	210	0.98	4.9 U	1900	41	12	190	33000	1200	5500	1200	---	21	2100
MS-8 (Roberts)	**364**	34000	9.9 U	280	1.1	4.9 U	6400	39	12	76	31000	1400	6900	1400	---	21	2900

Notes:

All units in mg/kg except where shown (some NIST 2710 results displayed as %).

U or < = undetected. Detection limit shown.

ND = not detected.

--- = no data available.

All soils were sieved <250um for arsenic analysis. Other analyses were performed on <250um sieved samples for all soils except Barber Orchard

Table 2a. Soil Metal Concentrations of Test Materials

Sample Name	Se	Ag	Na	Tl	V	Zn
EPA Soils						
Aberjona River TM1	5.80	0.9	ND	1.70	49.0	3,300
Aberjona River TM2	3.80	1.1	<500	4.40	43.0	4,500
ACC Dislodgeable Arsenic	---	---	---	---	---	---
ACC Utility Pole Soil	---	---	---	---	---	---
Aspen Berm	2.00	92.3	249	1.80	11.5	6,580
Aspen Residential	0.38	18.9	114	0.27	16.0	4,110
Bingham Creek Channel Soil	<17	17.2	314	<17	22.0	-
Butte TM1 (Phase II study)	0.27	40.5	530	1.80	27.0	12,100
Butte TM1 (Phase III study)	1.00	37.0	3,950	10.10	55.8	12,500
Butte TM2	0.91	8.1	777	0.85	48.7	2,457
CA Gulch AV Slag (Phase II study)	61.30	21.2	4,080	1.80	37.2	67,300
CA Gulch AV Slag (Pilot Study)	61.30	21.2	4,080	1.80	37.2	67,300
CA Gulch Fe/Mn PbO	0.80	16.7	279	3.70	17.9	2,650
CA Gulch Phase I Residential Soil	1.90	43.0	6,560	<0.5	33.7	13,738
Clark Fork Tailings	---	---	---	---	---	---
El Paso TM1	---	---	---	---	---	---
El Paso TM2	---	---	---	---	---	---
Jasper County High Lead Mill	---	---	---	---	---	---
Midvale Slag	39.70	0.11U	7,910	8.10	10.1U	33,300
Murray Smelter Slag	43.90	18.3	836	12.60	73.6	49,500
Murray Smelter Soil	6.80	11.1	532.0b	4.80	28.3	10,400
Palmerton Location 2	11.80	9.5	667	1.90	53.1	6,500
Palmerton Location 4	6.90	5.1	2,100	0.85	49.8	19,100
VBI70 TM1	1.00	0.7	200	<1	22.3	374
VBI70 TM2	2.00	<0.5	200	<1	24.8	449
VBI70 TM3	<1	<0.5	200	<1	22.7	342
VBI70 TM4	2.00	<0.5	200	<1	23.7	661
VBI70 TM5	<1	<0.5	<100	<1	21.5	120
VBI70 TM6	<1	<0.5	<100	<1	18.9	261

Table 2a. Soil Metal Concentrations of Test Materials

Sample Name	Se	Ag	Na	Tl	V	Zn
EPA Soils						
Tacoma Smelter Slag	ND	18.2	5000	ND	58	11400
Tacoma Smelter Soil	ND	15	16500	ND	99	332
MS-1 (EPA)	20 U	0.5 U	99 U	9.9 U	45	50
MS-4 (EPA)	20 U	0.99 U	200 U	9.9 U	85	170
MS-5 (EPA)	20 U	0.49 U	99 U	9.9 U	67	140
MS-8 (EPA)	20 U	0.49 U	99 U	9.9 U	63	210
NIST 2710	---	35.3	1%	---	76.6	6952
Roberts Soils						
CAMT	1 U	2 U	---	1 U	---	107
CORS	---	---	---	---	---	---
COSCS	13.95	2.55	---	15.15	---	1200
COSS	4.85	88.1	---	1.95	---	15500
FLCDV	1 U	2 U	---	1 U	---	3.6
FLCPS	2.5	13.9	---	11.6	---	127
HIVS	2.7 U	2.7 U	---	5.5 U	---	1820
MTSS	2 U	16.5	---	2 U	---	1160
NYOS	2 U	2 U	---	2 U	---	71.8
NYPS1	---	---	---	---	---	---
NYPS2	---	---	---	---	---	---
NYPS3	---	---	---	---	---	---
WAOS	2 U	2 U	---	2 U	---	312
WISS	9.2	36.1	---	10 U	---	2710
MS-1 (Roberts)	20 U	0.5 U	99 U	9.9 U	45	50
MS-4 (Roberts)	20 U	0.99 U	200 U	9.9 U	85	170
MS-5 (Roberts)	20 U	0.49 U	99 U	9.9 U	67	140
MS-8 (Roberts)	20 U	0.49 U	99 U	9.9 U	63	210

Notes:

All units in mg/kg except where shown

U or < = undetected. Detection limit sh

All soils were sieved <250um for arsen

Table 2b. Soil Digestion and Analysis Method Details

Sample Name	Digestion Method No.	Analysis Method No. or name	No. replicates analyzed	Notes
EPA Soils				
Aberjona River TM1	unknown	6010B and 6020	4	2 samples measured by ICP-MS, 2 by ICP-AES; average taken of all samps.
Aberjona River TM2	unknown	6010B and 6020	4	2 samples measured by ICP-MS, 2 by ICP-AES; average taken of all samps.
ACC Dislodgeable Arsenic	unknown	6010B	2	
ACC Utility Pole Soil	unknown	6010B	2	
Aspen Berm	EPA 200.7	6010B	unknown	no records exist, however the USEPA (2005) Appx C notes that soils were analyzed by ICP-AES method 200.7.
Aspen Residential	EPA 200.7	6010B	unknown	no records exist, however the USEPA (2005) Appx C notes that soils were analyzed by ICP-AES method 200.7.
Bingham Creek Channel Soil	EPA 200.7	6010B	unknown	Original report says "standard EPA CLP methods were used for analysis". USEPA (2005) Appx C notes that soils were analyzed by ICP-AES method 200.7.
Butte TM1 (Phase II study)	EPA 200.7	6010B	2	Original report cited 239 ppm As based on 2 replicates (unknown method). Butte Soil TM1 was reanalyzed twice more throughout the course of USEPA (2005) project.
Butte TM1 (Phase III study)	EPA 200.7	6010B	4	Original report cited 239 ppm As based on 2 replicates (unknown method). Butte Soil TM1 was reanalyzed twice more throughout the course of USEPA (2005) project.
Butte TM2	unknown	6010B	3	
CA Gulch AV Slag (Phase II study)	EPA 200.7	6010B	unknown	Original report says "standard EPA CLP methods were used for analysis". USEPA (2005) Appx C notes that soils were analyzed by ICP-AES method 200.7.
CA Gulch AV Slag (Pilot Study)	EPA 200.7	6010B	unknown	Original report says "standard EPA CLP methods were used for analysis". USEPA (2005) Appx C notes that soils were analyzed by ICP-AES method 200.7.

Table 2b. Soil Digestion and Analysis Method Details

Sample Name	Digestion Method No.	Analysis Method No. or name	No. replicates analyzed	Notes
EPA Soils				
CA Gulch Fe/Mn PbO	EPA 200.7	6010B	unknown	Original report says "standard EPA CLP methods were used for analysis". USEPA (2005) Appx C notes that soils were analyzed by ICP-AES method 200.7.
CA Gulch Phase I Residential Soil	EPA 200.7	6010B	unknown	Original report says "standard EPA CLP methods were used for analysis". USEPA (2005) Appx C notes that soils were analyzed by ICP-AES method 200.7.
Clark Fork Tailings	unknown	unknown	unknown	
El Paso TM1	unknown	6010B	unknown	
El Paso TM2	unknown	6010B	unknown	
Jasper County High Lead Mill	EPA 200.7	6010B	unknown	Original report says "standard EPA CLP methods were used for analysis". USEPA (2005) Appx C notes that soils were analyzed by ICP-AES method 200.7.
Midvale Slag	EPA 200.7	6010B	unknown	Original report says "standard EPA CLP methods were used for analysis". USEPA (2005) Appx C notes that soils were analyzed by ICP-AES method 200.7.
Murray Smelter Slag	unknown	6010B	2	No specific notes on As analysis, only on Pb analysis.
Murray Smelter Soil	unknown	6010B	2	No specific notes on As analysis, only on Pb analysis.
Palmerton Location 2	EPA 200.7	6010B	unknown	Original report says "standard EPA CLP methods were used for analysis". USEPA (2005) Appx C notes that soils were analyzed by ICP-AES method 200.7.
Palmerton Location 4	EPA 200.7	6010B	unknown	Original report says "standard EPA CLP methods were used for analysis". USEPA (2005) Appx C notes that soils were analyzed by ICP-AES method 200.7.
VBI70 TM1	3010 (for ICP-AES)	6010B, NAA, XRF	4 (2 reps analyzed for ICP, assume 1 rep each for NAA and XRF)	based on the mean of the XRF-2, ICP and NAA values

Table 2b. Soil Digestion and Analysis Method Details

Sample Name	Digestion Method No.	Analysis Method No. or name	No. replicates analyzed	Notes
EPA Soils				
VBI70 TM2	3010 (for ICP-AES)	6010B, NAA, XRF	4 (2 reps analyzed for ICP, assume 1 rep each for NAA and XRF)	based on the mean of the XRF-2, ICP and NAA values
VBI70 TM3	3010 (for ICP-AES)	6010B, NAA, XRF	4 (2 reps analyzed for ICP, assume 1 rep each for NAA and XRF)	based on the mean of the XRF-2, ICP and NAA values
VBI70 TM4	3010 (for ICP-AES)	6010B, NAA, XRF	4 (2 reps analyzed for ICP, assume 1 rep each for NAA and XRF)	based on the mean of the XRF-2, ICP and NAA values
VBI70 TM5	3010 (for ICP-AES)	6010B, NAA, XRF	4 (2 reps analyzed for ICP, assume 1 rep each for NAA and XRF)	based on the mean of the XRF-2, ICP and NAA values
VBI70 TM6	3010 (for ICP-AES)	6010B, NAA, XRF	4 (2 reps analyzed for ICP, assume 1 rep each for NAA and XRF)	based on the mean of the XRF-2, ICP and NAA values
Tacoma Smelter Slag	Potassium Hydroxide fusion (no number associated with method)	HG-ICP-AES	3 (?)	No. samples specifically stated for Pb isotope analyses, but not metals. St dev and LCL/UCL are reported, however.
Tacoma Smelter Soil	Potassium Hydroxide fusion (no number associated with method)	HG-ICP-AES	3 (?)	No. samples specifically stated for Pb isotope analyses, but not metals. St dev and LCL/UCL are reported, however.
MS-1 (EPA)	EPA 200.8	6020	1	
MS-4 (EPA)	EPA 200.8	6020	1	
MS-5 (EPA)	EPA 200.8	6020	1	
MS-8 (EPA)	EPA 200.8	6020	1	
NIST 2710	----	NAA, XRF	20	

Table 2b. Soil Digestion and Analysis Method Details

Sample Name	Digestion Method No.	Analysis Method No. or name	No. replicates analyzed	Notes
Roberts Soils				
CAMT	3050B	6010	5	Digestion and rep info from Y. Lowney, pers. comm.
CORS	3050B	6010	5	Digestion and rep info from Y. Lowney, pers. comm.
COSCS	3050B	6010	5	Digestion and rep info from Y. Lowney, pers. comm.
COSS	3050B	6010	5	Digestion and rep info from Y. Lowney, pers. comm.
FLCDV	3050B	6010	5	Digestion and rep info from Y. Lowney, pers. comm.
FLCPS	3050B	6010	5	Digestion and rep info from Y. Lowney, pers. comm.
HIVS	3050B	6010	5	Digestion and rep info from Y. Lowney, pers. comm.
MTSS	3050B	6010	5	Digestion and rep info from Y. Lowney, pers. comm.
NYOS	3050B	6010	5	Digestion and rep info from Y. Lowney, pers. comm.
NYPS1	3050B	6010	5	Digestion and rep info from Y. Lowney, pers. comm.
NYPS2	3050B	6010	5	Digestion and rep info from Y. Lowney, pers. comm.
NYPS3	3050B	6010	5	Digestion and rep info from Y. Lowney, pers. comm.
WAOS	3050B	6010	5	Digestion and rep info from Y. Lowney, pers. comm.
WISS	3050B	6010	5	Digestion and rep info from Y. Lowney, pers. comm.
MS-1 (Roberts)	EPA 200.8	6020	1	
MS-4 (Roberts)	EPA 200.8	6020	1	
MS-5 (Roberts)	EPA 200.8	6020	1	
MS-8 (Roberts)	EPA 200.8	6020	1	

Table 2b. Soil Digestion and Analysis Method Details

Sample Name	Digestion Method No.	Analysis Method No. or name	No. replicates analyzed	Notes

Notes:
6010 = ICP-AES
6020 = ICP-MS
AES = atomic emission spectrometry
HG = hydride generation
ICP = inductively coupled plasma
NAA = neutron activation analysis
XRF = x-ray fluorescence

Table 3. Relative Arsenic Mass Associated with Mineralogical Phase

Sample	Fe Sulfate	Fe Oxide (FeO, FeOOH)	Mn Oxide (MnO, MnOOH)	Pb Oxide	Phos-phates	Sulfosalts	Heavily As-enriched iron oxide (FeAsO, AsFeOOH)	Arsenic Trioxide (As2O3)	Slag	Barite	As bromide
EPA Soils											
Aberjona River TM1	31.05	68.79	—	—	—	—	—	—	—	—	Not Meas.
Aberjona River TM2	81.82	16.40	—	—	—	—	—	—	—	—	Not Meas.
ACC Dislodgeable Arsenic	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
ACC Utility Pole Soil	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
Aspen Berm	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
Aspen Residential	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
Bingham Creek Channel Soil	45.82	10.70	0.39	34.34	8.50	—	—	—	—	—	Not Meas.
Butte TM1 (Phase II study)	53.36	20.42	16.27	—	7.61	2.27	—	—	—	—	Not Meas.
Butte TM1 (Phase III study)	—	—	—	—	—	—	—	—	—	—	Not Meas.
Butte TM2	18.28	39.28	—	—	—	41.87	—	—	—	—	Not Meas.
CA Gulch AV Slag (Phase II study)	0.25	—	—	84.15	—	—	—	—	5.08	—	Not Meas.
CA Gulch AV Slag (Pilot Study)	0.25	—	—	84.15	—	—	—	—	5.08	—	Not Meas.
CA Gulch Fe/Mn PbO	4.89	23.15	65.65	—	5.36	—	—	—	—	—	Not Meas.
CA Gulch Phase I Residential Soil	10.65	28.57	36.20	—	14.98	—	—	—	3.77	0.85	Not Meas.
Clark Fork Tailings	24.32	40.25	0.97	—	16.25	16.09	2.05	—	—	—	Not Meas.
El Paso TM1	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
El Paso TM2	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
Jasper County High Lead Mill	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
Midvale Slag	0.04	0.15	—	87.20	—	1.36	—	—	11.25	—	Not Meas.
Murray Smelter Slag	9.90	26.64	0.04	48.77	—	—	—	—	13.91	—	Not Meas.
Murray Smelter Soil	5.80	2.90	—	86.77	—	—	—	—	2.41	—	Not Meas.
Palmerton Location 2	0.68	21.16	39.63	—	27.11	—	—	—	—	11.42	Not Meas.
Palmerton Location 4	—	4.87	10.15	42.39	0.20	—	37.71	—	—	0.24	Not Meas.
VBI70 TM1	0.06	3.24	1.77	31.84	8.48	—	—	54.28	—	—	Not Meas.

Table 3. Relative Arsenic Mass Associated with Mineralogical Phase

Sample	Fe Sulfate	Fe Oxide (FeO, FeOOH)	Mn Oxide (MnO, MnOOH)	Pb Oxide	Phos-phates	Sulfosalts	Heavily As-enriched iron oxide (FeAsO, AsFeOOH)	Arsenic Trioxide (As2O3)	Slag	Barite	As bromide
EPA Soils											
VBI70 TM2	0.04	3.04	0.21	70.03	4.45	—	—	22.07	—	—	Not Meas.
VBI70 TM3	—	7.68	5.29	5.51	1.50	—	—	79.53	—	—	Not Meas.
VBI70 TM4	0.15	1.83	0.43	9.84	0.97	—	—	86.06	—	—	Not Meas.
VBI70 TM5	0.02	2.80	0.30	—	—	—	—	96.80	—	—	Not Meas.
VBI70 TM6	—	0.52	—	18.21	0.13	—	—	79.92	—	—	Not Meas.
Tacoma Smelter Slag	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
Tacoma Smelter Soil	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
MS-1 (EPA)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
MS-4 (EPA)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
MS-5 (EPA)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
MS-8 (EPA)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
NIST 2710	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.

Table 3. Relative Arsenic Mass Associated with Mineralogical Phase

Sample	Fe Sulfate	Fe Oxide (FeO, FeOOH)	Mn Oxide (MnO, MnOOH)	Pb Oxide	Phos-phates	Sulfosalts	Heavily As-enriched iron oxide (FeAsO, AsFeOOH)	Arsenic Trioxide (As2O3)	Slag	Barite	As bromide
Roberts Soils											
CAMT	2.3	27.2	—	—	—	Not Meas.	—	—	—	Not Meas.	—
CORS	0.1	1.7	0.3	10.3	0.2	Not Meas.	—	87.3	—	Not Meas.	—
COSCS	1.4	1.5	—	24.7	—	Not Meas.	3	—	—	Not Meas.	35.8
COSS	76.7	22.2	—	—	—	Not Meas.	—	—	—	Not Meas.	—
FLCDV	—	14.4	—	—	—	Not Meas.	—	—	—	Not Meas.	—
FLCPS	64.8	35.2	—	—	—	Not Meas.	—	—	—	Not Meas.	—
HIVS	—	22.9	3	2.3	—	Not Meas.		—	—	Not Meas.	—
MTSS	23.1	55.9	0.4	—	—	Not Meas.	12.3	—	1.9	Not Meas.	—
NYOS	—	6.9	54.5	37.2	—	Not Meas.	—	—	—	Not Meas.	—
NYPS1	0.5	32.1	3	8.1	0.1	Not Meas.	54.5	—	—	Not Meas.	—
NYPS2	—	100	—	—	—	Not Meas.	—	—	—	Not Meas.	—
NYPS3	—	99.9 (37.5)	0.1 (8.8)	—	—	Not Meas.	—	—	—	Not Meas.	—
WAOS	—	1.3	0.04	98.6	—	Not Meas.	—	—	—	Not Meas.	—
WISS	9.3	3.5	—	66.4	0.02	Not Meas.	10.6	—	—	Not Meas.	—
MS-1 (Roberts)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
MS-4 (Roberts)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
MS-5 (Roberts)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
MS-8 (Roberts)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.

Notes:

--- = mineralogical form was not found.

"Not Meas." = not measured.

All units expressed as %

Table 3. Relative Arsenic Mass Associated with Mineralogical Phase

Sample	Arseno-pyrite	Lead (metal) oxide	As (metals) oxide	As (metals) sulfate	Calcium arsenate (CaAsO4)	Clay	Iron aluminum silicate	Pyrite	Zinc (metal) oxide	Other
EPA Soils										
Aberjona River TM1	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	0.16
Aberjona River TM2	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	1.78
ACC Dislodgeable Arsenic	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
ACC Utility Pole Soil	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
Aspen Berm	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
Aspen Residential	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
Bingham Creek Channel Soil	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	0.25
Butte TM1 (Phase II study)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	0.07
Butte TM1 (Phase III study)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	—
Butte TM2	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	0.57
CA Gulch AV Slag (Phase II study)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	10.52
CA Gulch AV Slag (Pilot Study)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	10.52
CA Gulch Fe/Mn PbO	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	0.94
CA Gulch Phase I Residential Soil	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	4.97
Clark Fork Tailings	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	0.07
El Paso TM1	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
El Paso TM2	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
Jasper County High Lead Mill	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
Midvale Slag	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	—
Murray Smelter Slag	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	0.73
Murray Smelter Soil	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	2.12
Palmerton Location 2	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	—
Palmerton Location 4	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	4.43
VBI70 TM1	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	0.33

Table 3. Relative Arsenic Mass Associated with Mineralogical Phase

Sample	Arseno-pyrite	Lead (metal) oxide	As (metals) oxide	As (metals) sulfate	Calcium arsenate (CaAsO4)	Clay	Iron aluminum silicate	Pyrite	Zinc (metal) oxide	Other
EPA Soils										
VBI70 TM2	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	0.17
VBI70 TM3	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	0.49
VBI70 TM4	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	0.71
VBI70 TM5	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	0.08
VBI70 TM6	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	1.22
Tacoma Smelter Slag	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
Tacoma Smelter Soil	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
MS-1 (EPA)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
MS-4 (EPA)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
MS-5 (EPA)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
MS-8 (EPA)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
NIST 2710	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.

Table 3. Relative Arsenic Mass Associated with Mineralogical Phase

Sample	Arseno-pyrite	Lead (metal) oxide	As (metals) oxide	As (metals) sulfate	Calcium arsenate (CaAsO4)	Clay	Iron aluminum silicate	Pyrite	Zinc (metal) oxide	Other
Roberts Soils										
CAMT	70.4	—	—	—	—	—	—	—	—	Not Meas.
CORS	—	—	0.2	—	—	—	—	—	—	Not Meas.
COSCS	—	3.3	30	—	—	—	—	—	—	Not Meas.
COSS	—	—	—	—	—	—	—	—	—	Not Meas.
FLCDV	—	—	—	—	—	85.5	—	—	—	Not Meas.
FLCPS	—	—	—	—	—	—	—	—	—	Not Meas.
HIVS	—	—	—	—	—	—	71.8	—	—	Not Meas.
MTSS	—	—	6.4	—	—	—	—	—	—	Not Meas.
NYOS	—	1.4	—	—	—	—	—	—	—	Not Meas.
NYPS1	—	—	—	—	1.7	—	—	—	—	Not Meas.
NYPS2	—	—	—	—	—	—	—	—	—	Not Meas.
NYPS3	—	—	—	—	—	—	—	—	—	Not Meas.
WAOS	—	—	—	—	—	—	—	—	—	Not Meas.
WISS	—	2.5	—	7.5	—	—	—	0.3	0.1	Not Meas.
MS-1 (Roberts)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
MS-4 (Roberts)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
MS-5 (Roberts)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.
MS-8 (Roberts)	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.	Not Meas.

Table 4. Matrix Association Frequency of Arsenic

Sample	Species	Frequency of Occurance (%)				
		Total	Liberated	Cemented	Rimmed	Included
EPA Soils						
ABERJONA RIVER TM 1	FeOOH	69%	28%	69%	2%	0%
	Pyrite	13%	0%	100%	0%	0%
	Sulfate	16%	47%	27%	0%	27%
	ZnMO	2%	0%	100%	0%	0%
Total			27%	67%	2%	4%
ABERJONA RIVER TM 2	FeOOH	10%	25%	75%	0%	0%
	FeSO4	18%	36%	64%	0%	0%
	Pyrite	24%	21%	79%	0%	0%
	ZnMO	49%	17%	83%	0%	0%
Total			22%	78%	0%	0%
BINGHAM CRK CHANNEL SOIL	Barite	0%	100%	0%	0%	0%
	FeOOH	7%	87%	13%	0%	0%
	FeSO4	81%	50%	46%	4%	0%
	MnOOH	1%	40%	60%	0%	0%
	PbAsO	1%	67%	33%	0%	0%
	Phosphate	10%	76%	14%	10%	0%
Total			55%	40%	4%	0%
BUTTE SOIL TM 1	Barite	0%	100%	0%	0%	0%
	FeOOH	5%	54%	26%	6%	14%
	FeSO4	25%	55%	37%	4%	4%
	MnOOH	19%	31%	54%	12%	3%
	Phosphate	1%	13%	42%	0%	46%
Total			46%	40%	6%	8%
BUTTE SOIL TM 2	AsMSO4	1%	0%	0%	100%	0%
	Clay	1%	100%	0%	0%	0%
	FeOOH	55%	55%	43%	3%	0%
	FeSO4	23%	53%	34%	13%	0%
	Pyrite	5%	71%	29%	0%	0%
	Slag	1%	100%	0%	0%	0%
	SulfoSalt	15%	95%	0%	5%	0%
Total			61%	33%	6%	0%

Table 4. Matrix Association Frequency of Arsenic

Sample	Species	Frequency of Occurance (%)				
		Total	Liberated	Cemented	Rimmed	Included
CA GULCH SITE AV SLAG	AsMO	0%	100%	0%	0%	0%
	FeSO4	0%	0%	0%	20%	80%
	PbAsMO	3%	5%	0%	0%	95%
	PbAsO	15%	14%	0%	0%	86%
	PbMO	0%	80%	0%	20%	0%
	PbMS / PbMSO4	0%	100%	0%	0%	0%
	Slag	82%	100%	0%	0%	0%
Total			85%	0%	0%	15%
CA GULCH SITE FeMnPb OXIDE	Barite	2%	0%	14%	0%	86%
	FeOOH	49%	59%	40%	1%	0%
	FeSO4	13%	81%	19%	0%	0%
	MnOOH	19%	18%	76%	6%	0%
	PbMO	1%	0%	100%	0%	0%
	Phosphate	17%	56%	38%	3%	3%
Total			52%	44%	2%	2%
CA GULCH SITE PHASE I RESIDENTIAL SOIL	Barite	1%	17%	0%	0%	83%
	FeOOH	35%	76%	18%	0%	6%
	FeSO4	14%	90%	3%	0%	7%
	MnOOH	16%	81%	19%	0%	0%
	PbMO	1%	0%	0%	0%	100%
	Phosphate	29%	74%	2%	0%	24%
	Slag	4%	100%	0%	0%	0%
Total			78%	10%	0%	12%
CLARK FORK RIVER SITE	FeAsO	1%	0%	100%	0%	0%
	FeOOH	17%	47%	43%	10%	0%
	FeSO4	11%	56%	29%	15%	0%
	MnOOH	2%	94%	0%	7%	0%
	Phosphate	16%	42%	50%	7%	2%
	Slag	3%	100%	0%	0%	0%
	SulfoSalt	3%	82%	13%	0%	7%
Total			59%	28%	12%	1%

Table 4. Matrix Association Frequency of Arsenic

Sample	Species	Frequency of Occurance (%)				
		Total	Liberated	Cemented	Rimmed	Included
MIDVALE SLAG	FeOOH	0%	100%	0%	0%	0%
	FeSO4	0%	100%	0%	0%	0%
	PbAsO	6%	34%	0%	0%	66%
	Slag	93%	100%	0%	0%	0%
	SulfoSalt	0%	100%	0%	0%	0%
Total			96%	0%	0%	4%
MURRAY SLAG	FeOOH	0%	100%	0%	0%	0%
	FeSO4	0%	50%	50%	0%	0%
	MnOOH	1%	29%	14%	57%	0%
	PbAsO	4%	46%	5%	28%	21%
	PbMO	1%	38%	0%	0%	63%
	Slag	94%	100%	0%	0%	0%
Total			97%	0%	1%	1%
MURRAY SOIL	AsMO	0%	100%	0%	0%	0%
	FeOOH	1%	0%	100%	0%	0%
	FeSO4	0%	100%	0%	0%	0%
	PbAsO	12%	30%	5%	2%	64%
	PbMO	2%	33%	33%	0%	33%
	Slag	84%	100%	0%	0%	0%
Total			89%	2%	0%	8%
PALMERTON LOCATION 2	Barite	10%	82%	18%	0%	0%
	FeOOH	14%	67%	33%	0%	0%
	FeSO4	1%	100%	0%	0%	0%
	MnOOH	61%	43%	47%	10%	0%
	Phosphate	14%	50%	50%	0%	0%
Total			51%	42%	6%	0%

Table 4. Matrix Association Frequency of Arsenic

Sample	Species	Frequency of Occurance (%)				
		Total	Liberated	Cemented	Rimmed	Included
PALMERTON LOCATION 4	Barite	1%	100%	0%	0%	0%
	FeOOH	7%	84%	13%	4%	0%
	MnOOH	62%	38%	58%	3%	0%
	PbAsO	16%	0%	0%	0%	100%
	PbMO	5%	100%	0%	0%	0%
	Phosphate	1%	100%	0%	0%	0%
	ZnSiO4	2%	50%	50%	0%	0%
Total			41%	40%	3%	16%
VBI70 TM 1	As2O3	3%	100%	0%	0%	0%
	AsMO	0%	0%	100%	0%	0%
	Clay	2%	50%	50%	0%	0%
	FeOOH	10%	68%	28%	4%	0%
	FeSO4	0%	100%	0%	0%	0%
	MnOOH	15%	26%	69%	5%	0%
	Pb solder	1%	100%	0%	0%	0%
	PbAsO	20%	37%	54%	10%	0%
	PbMO	0%	0%	100%	0%	0%
	Phosphate	49%	9%	91%	0%	0%
Total			28%	69%	3%	0%
VBI70 TM 2	As2O3	5%	29%	57%	0%	14%
	Clay	4%	0%	100%	0%	0%
	FeOOH	19%	54%	46%	0%	0%
	FeSO4	1%	100%	0%	0%	0%
	MnOOH	4%	40%	60%	0%	0%
	PbAsO	42%	24%	74%	2%	0%
	PbMO	2%	0%	0%	100%	0%
	Phos	22%	14%	82%	4%	0%
	Slag	2%	100%	0%	0%	0%
Total			29%	67%	3%	1%

Table 4. Matrix Association Frequency of Arsenic

Sample	Species	Frequency of Occurance (%)				
		Total	Liberated	Cemented	Rimmed	Included
VBI70 TM 3	As2O3	8%	38%	50%	13%	0%
	Clay	7%	86%	14%	0%	0%
	FeOOH	29%	46%	54%	0%	0%
	MnOOH	23%	23%	68%	9%	0%
	Pb solder	1%	0%	100%	0%	0%
	PbAsO	6%	0%	67%	33%	0%
	PbMO	3%	100%	0%	0%	0%
	PbMO	1%	0%	100%	0%	0%
	Phosphate	21%	10%	85%	5%	0%
	Slag	1%	100%	0%	0%	0%
Total			34%	60%	6%	0%
VBI70 TM 4	As2O3	24%	85%	12%	3%	0%
	AsSbO	2%	67%	0%	33%	0%
	Clay	6%	100%	0%	0%	0%
	FeOOH	29%	60%	38%	3%	0%
	FeSO4	4%	50%	33%	17%	0%
	MnOOH	10%	7%	86%	7%	0%
	Pb solder	1%	100%	0%	0%	0%
	PbAsO	3%	25%	75%	0%	0%
	PbMO	1%	0%	100%	0%	0%
	Phosphate	14%	50%	35%	15%	0%
	Slag	6%	100%	0%	0%	0%
Total			63%	32%	6%	0%

Table 4. Matrix Association Frequency of Arsenic

Sample	Species	Frequency of Occurance (%)				
		Total	Liberated	Cemented	Rimmed	Included
VBI70 TM 5	As2O3	34%	46%	40%	14%	0%
	Barite	2%	0%	100%	0%	0%
	FeOOH	37%	47%	32%	21%	0%
	FeSO4	1%	0%	100%	0%	0%
	MnOOH	3%	0%	100%	0%	0%
	Paint	1%	100%	0%	0%	0%
	Pb solder	16%	13%	44%	13%	31%
	PbMO	1%	100%	0%	0%	0%
	Slag	6%	33%	17%	50%	0%
Total			39%	39%	17%	5%
VBI70 TM 6	As2O3	24%	93%	3%	3%	0%
	AsMO	1%	0%	100%	0%	0%
	AsSbO	6%	100%	0%	0%	0%
	Clay	1%	100%	0%	0%	0%
	FeOOH	6%	43%	57%	0%	0%
	Pb solder	1%	100%	0%	0%	0%
	PbAsO	38%	77%	15%	9%	0%
	PbMO	16%	0%	100%	0%	0%
	Phosphate	2%	50%	50%	0%	0%
	Pyrite	1%	100%	0%	0%	0%
	Slag	6%	100%	0%	0%	0%
Total			69%	27%	4%	0%
Roberts Soils						
CAMT	Arsenopyrite	8%	67%	0%	0%	33%
	FeOOH	65%	86%	4%	6%	0%
	Pyrite	21%	43%	52%	0%	4%
	FeSO4	6%	100%	0%	0%	0%
Total			76%	14%	4%	4%

Table 4. Matrix Association Frequency of Arsenic

Sample	Species	Frequency of Occurance (%)				
		Total	Liberated	Cemented	Rimmed	Included
COSS	Anglesite	10%	94%	6%	0%	0%
	Cerussite	1%	100%	0%	0%	0%
	FeOOH	29%	94%	2%	4%	0%
	PbMO	10%	0%	100%	0%	0%
	PbSiO4	10%	0%	100%	0%	0%
	FeSO4	40%	99%	1%	0%	0%
Total			77%	22%	1%	0%
FLCDV	Clay	95%	27%	0%	73%	0%
	Anglesite	1%	100%	0%	0%	0%
	Brass	1%	100%	0%	0%	0%
	FeOOH	3%	100%	0%	0%	0%
	Galena	1%	100%	0%	0%	0%
Total			31%	0%	69%	0%
HIVS	Clay	55%	100%	0%	0%	0%
	FeOOH	42%	23%	75%	2%	0%
	MnOOH	1%	100%	0%	0%	0%
	PbAsO	2%	50%	50%	0%	0%
Total			67%	33%	1%	0%
MTSS	AsFeOOH	8%	91%	0%	0%	9%
	AsMO	2%	100%	0%	0%	0%
	FeOOH	32%	67%	12%	21%	0%
	MnOOH	1%	0%	100%	0%	0%
	Slag	29%	89%	0%	0%	0%
	Sulfosalt	8%	27%	0%	0%	73%
	FeSO4	19%	52%	0%	48%	0%
Total			69%	5%	16%	7%

Table 4. Matrix Association Frequency of Arsenic

Sample	Species	Frequency of Occurance (%)				
		Total	Liberated	Cemented	Rimmed	Included
NYOS	Anglesite	1%	100%	0%	0%	0%
	Brass	4%	100%	0%	0%	0%
	Cerussite	1%	0%	100%	0%	0%
	FeOOH	7%	75%	0%	25%	0%
	Galena	2%	100%	0%	0%	0%
	MnOOH	78%	17%	72%	9%	0%
	PbAsO	5%	67%	33%	0%	0%
	PbMO	1%	100%	0%	0%	0%
	Phosphate	2%	0%	100%	0%	0%
Total			29%	61%	9%	0%
NYPF1	Clay	1%	0%	100%	0%	0%
	AsFeOOH	25%	45%	55%	0%	0%
	CaAsO	1%	100%	0%	0%	0%
	FeOOH	50%	58%	37%	5%	0%
	MnOOH	7%	0%	100%	0%	0%
	PbAsO	6%	100%	0%	0%	0%
	Phosphate	5%	50%	33%	17%	0%
	FeSO4	6%	29%	71%	0%	0%
Total			51%	46%	3%	0%

Table 4. Matrix Association Frequency of Arsenic

Sample	Species	Frequency of Occurance (%)				
		Total	Liberated	Cemented	Rimmed	Included
NYPF2	FeOOH	100%	33%	61%	6%	0%
Total			33%	61%	6%	0%
NYPF3	FeOOH	99%	40%	48%	13%	0%
	MnOOH	1%	0%	100%	0%	0%
Total			39%	48%	13%	0%
WAOS	FeOOH	6%	17%	75%	8%	0%
	PbAsO	88%	17%	71%	13%	0%
	Phosphate	2%	50%	50%	0%	0%
	MnOOH	5%	11%	78%	11%	0%
Total			17%	71%	12%	0%

Notes:
Data displayed represents all data currently available. Some soils not analyzed.

Table 5. Particle Size Distribution

Sample Name	≤ 5 [4]	5 - 101 [5]	102 - 250 [6]
EPA Soils			
Aberjona River TM1	33%	61%	5%
Aberjona River TM2	59%	39%	2%
ACC Dislodgeable Arsenic	Not Meas.	Not Meas.	Not Meas.
ACC Utility Pole Soil	Not Meas.	Not Meas.	Not Meas.
Aspen Berm	Not Meas.	Not Meas.	Not Meas.
Aspen Residential	Not Meas.	Not Meas.	Not Meas.
Bingham Creek Channel Soil	71%	29%	<1%
Butte TM1 (Phase II study)	21%	69%	10%
Butte TM1 (Phase III study)	21%	69%	10%
Butte TM2	18%	79%	4%
CA Gulch AV Slag (Phase II study)	21%	34%	39%
CA Gulch AV Slag (Pilot Study)	21%	34%	39%
CA Gulch Fe/Mn PbO	35%	63%	2%
CA Gulch Phase I Residential Soil	22%	69%	9%
Clark Fork Tailings	34%	64%	1%
El Paso TM1	Not Meas.	Not Meas.	Not Meas.
El Paso TM2	Not Meas.	Not Meas.	Not Meas.
Jasper County High Lead Mill	Not Meas.	Not Meas.	Not Meas.
Midvale Slag	3%	36%	60%
Murray Smelter Slag	14%	58%	28%
Murray Smelter Soil	18%	70%	11%
Palmerton Location 2	40%	60%	---
Palmerton Location 4	21%	78%	<1%
VBI70 TM1	81%	19%	<1%
VBI70 TM2	59%	41%	---
VBI70 TM3	49%	51%	---
VBI70 TM4	45%	55%	<1%
VBI70 TM5	48%	52%	---
VBI70 TM6	63%	37%	---
Tacoma Smelter Slag	Not Meas.	Not Meas.	Not Meas.
Tacoma Smelter Soil	Not Meas.	Not Meas.	Not Meas.
MS-1 (EPA)	Not Meas.	Not Meas.	Not Meas.
MS-4 (EPA)	Not Meas.	Not Meas.	Not Meas.
MS-5 (EPA)	Not Meas.	Not Meas.	Not Meas.
MS-8 (EPA)	Not Meas.	Not Meas.	Not Meas.
NIST 2710	Not Meas.	Not Meas.	Not Meas.

Table 5. Particle Size Distribution

Sample Name	≤ 5 [4]	5 - 101 [5]	102 - 250 [6]
Roberts Soils			
CAMT	4%	57%	37%
CORS	Not Meas.	Not Meas.	Not Meas.
COSCS	Not Meas.	Not Meas.	Not Meas.
COSS	Not Meas.	Not Meas.	Not Meas.
FLCDV	1%	41%	57%
FLCPS	Not Meas.	Not Meas.	Not Meas.
HIVS	Not Meas.	Not Meas.	Not Meas.
MTSS	6%	55%	38%
NYOS	6%	76%	16%
NYPS1	Not Meas.	Not Meas.	Not Meas.
NYPS2	Not Meas.	Not Meas.	Not Meas.
NYPS3	Not Meas.	Not Meas.	Not Meas.
WAOS	3%	58%	33%
WISS	Not Meas.	Not Meas.	Not Meas.
MS-1 (Roberts)	Not Meas.	Not Meas.	Not Meas.
MS-4 (Roberts)	Not Meas.	Not Meas.	Not Meas.
MS-5 (Roberts)	Not Meas.	Not Meas.	Not Meas.
MS-8 (Roberts)	Not Meas.	Not Meas.	Not Meas.

Notes:

Data displayed represents all data currently available. Some soils were not analyzed, "Not Meas." = not measured.

--- = particlesin this size class were not found.

4 Roberts et al. (2007) reports ≤ 4 um.

5 EPA soils reports 4 - 106 um while Roberts reported 5 - 101 um um

6 EPA soils reports 101-250 um while Roberts reported 106 - 250 um

Table 6. IVBA and RBA Measures and Dominant Mineralogical Form(s)

Sample Name	Soil As (mg/kg)	Dominant Mineralogy	As RBA [1]	As RBA St Error	As IVBA[2]	IVBA St Error
EPA Soils						
Aberjona River TM1	676	FeAsO	0.38	0.02	0.14	0.02
Aberjona River TM2	313	FeAsSO	0.52	0.02	0.34	0.01
ACC Dislodgeable Arsenic	74	Unknown	0.26	0.01	Not Meas.	Not Meas.
ACC Utility Pole Soil	320	Unknown	0.47	0.03	Not Meas.	Not Meas.
Aspen Berm	66.9	Unknown	1.00	0.47	Not Meas.	Not Meas.
Aspen Residential	16.7	Unknown	1.28	0.52	Not Meas.	Not Meas.
Bingham Creek Channel Soil	149	FeAsSO / PbAsO	0.39	0.08	0.16	0.03
Butte TM1 (Phase II study)	234	FeAsSO	0.09	0.03	0.09	0.08
Butte TM1 (Phase III study)	234	FeAsSO	0.18	0.03	0.09	0.08
Butte TM2	367	FeAsO / Sulfosalts	0.24	0.03	0.13	0.06
CA Gulch AV Slag (Phase II study)	1050	PbAsO	0.13	0.04	0.23	0.06
CA Gulch AV Slag (Pilot Study)	1050	PbAsO	0.18	0.02	0.23	0.06
CA Gulch Fe/Mn PbO	110	MnAsO	0.57	0.12	0.19	0.06
CA Gulch Phase I Residential Soil	203	MnAsO / FeAsO	0.08	0.03	0.12	0.15
Clark Fork Tailings	181	FeAsO	0.51	0.06	0.54	0.01
El Paso TM1	74	Unknown	0.44	0.03	0.75	0.02
El Paso TM2	73	Unknown	0.37	0.03	0.42	0.09
Jasper County High Lead Mill	16.4	Unknown	3.27	1.05	Not Meas.	Not Meas.
Midvale Slag	591	PbAsO	0.23	0.04	0.29	0.1
Murray Smelter Slag	695	PbAsO	0.55	0.10	0.55	0.15
Murray Smelter Soil	310	PbAsO	0.33	0.05	0.67	0.06
Palmerton Location 2	110	MnAsO / AsPO / FeAsO	0.49	0.10	0.09	0.04
Palmerton Location 4	134	FeAsO / PbAsO	0.61	0.11	0.28	0.03
VBI70 TM1	312	As2O3 / PbAsO	0.40	0.04	0.54	0.03
VBI70 TM2	983	PbAsO	0.42	0.04	0.47	<0.01
VBI70 TM3	390	As2O3	0.37	0.03	0.51	<0.01
VBI70 TM4	813	As2O3	0.24	0.02	0.32	0.05
VBI70 TM5	368	As2O3	0.21	0.02	0.20	0.01
VBI70 TM6	516	As2O3	0.24	0.03	0.32	0.02
Tacoma Smelter Slag	10100	Unknown	0.42	Not Determined	Not Meas.	Not Meas.
Tacoma Smelter Soil	1600	Unknown	0.78	Not Determined	Not Meas.	Not Meas.
MS-1 (EPA)	290	PbAsO	0.31	0.04	0.21	Not Determined
MS-4 (EPA)	388	PbAso/MnOOH/FeOOH	0.41	0.018	0.19	Not Determined
MS-5 (EPA)	382	PbAsO/Cobaltite/FeOOH	0.49	0.047	0.2	Not Determined
MS-8 (EPA)	364	PbAsO	0.53	0.023	0.33	Not Determined
NIST 2710	626	Unknown	0.44	0.023	0.55	Not Determined

Table 6. IVBA and RBA Measures and Dominant Mineralogical Form(s)

Sample Name	Soil As (mg/kg)	Dominant Mineralogy	As RBA [1]	As RBA St Error	As IVBA[2]	IVBA St Error
Roberts Soils						
CAMT	300	Arsenopyrite	0.19	0.02	0.13	Not Determined
CORS	1230	As2O3	0.17	0.08	0.41	Not Determined
COSCS	394	As(metal)oxide/PbAsO	0.18	0.06	0.67	Not Determined
COSS	1492	FeAsO	0.05	0.04	0.07	Not Determined
FLCDV	150	Clay	0.31	0.04	0.17	Not Determined
FLCPS	268	FeAsO/FeOOH	0.07	0.03	0.09	Not Determined
HIVS	724	FeAlSi	0.05	0.01	0.065	Not Determined
MTSS	647	FeOOH/FeAsSO	0.13	0.05	0.45	Not Determined
NYOS	123	MnOOH/PbAsO	0.15	0.08	0.36	Not Determined
NYPS1	1000	AsFeOOH/FeOOH	0.19	0.05	0.56	Not Determined
NYPS2	339	FeOOH	0.28	0.1	0.72	Not Determined
NYPS3	549	FeOOH	0.2	0.1	0.41	Not Determined
WAOS	301	PbAsO	0.24	0.09	1.03	Not Determined
WISS	1412	PbAsO/AsFeOOH	0.13	0.07	0.46	Not Determined
MS-1 (Roberts)	290	PbAsO	0.26	0.05	0.21	Not Determined
MS-4 (Roberts)	388	PbAsO/MnOOH/FeOOH	0.24	0.03	0.19	Not Determined
MS-5 (Roberts)	382	PbAsO/Cobaltite/FeOOH	0.33	0.07	0.2	Not Determined
MS-8 (Roberts)	364	PbAsO	0.2	0.05	0.33	Not Determined

Notes:

Not Meas. = not measured.

1 As reported by Roberts et al. (2007), USEPA 1996, 2005 and Casteel and SRC 2009a,b, respectively.

2 PhII&III values as reported in USEPA 2005. El Paso soils were not reported in the document.

FIGURES

Figure 1. RBA-IVBA Correlation for EPA Soils

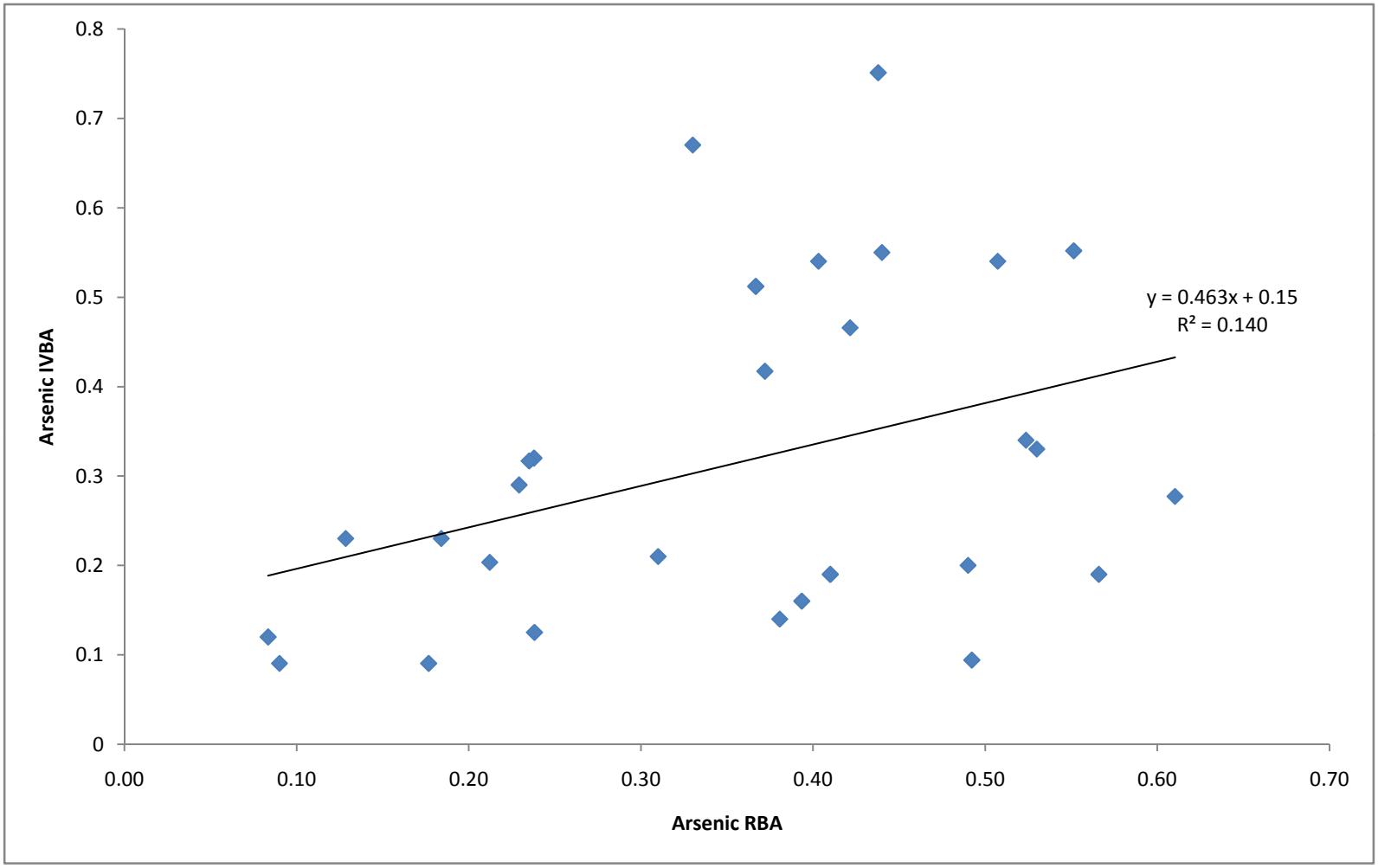
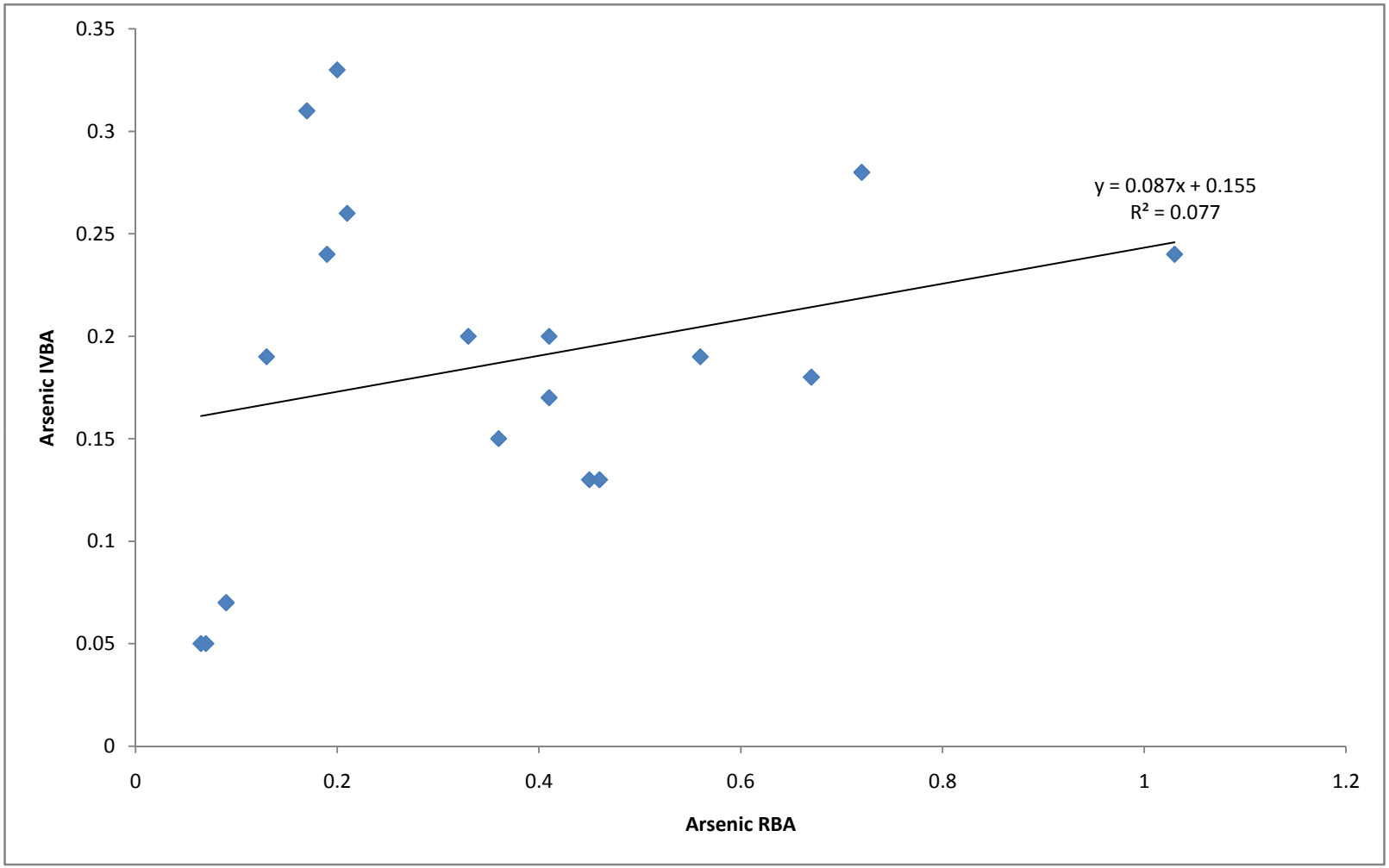


Figure 2. RBA-IVBA Correlation for Roberts Soils



APPENDICES

APPENDIX A. TEST MATERIAL DESCRIPTIONS

Below are descriptions of the test material collection procedures, where available. Descriptions are listed by site.

1.0 MATERIALS ASSAYED IN SWINE

Aberjona River

Twelve sediment samples were collected from multiple locations along the Aberjona River. The sampling locations of the 12 sediment samples spanned four basic regions of the Aberjona River. Sediment samples 1-3 were collected from the Halls Brook Holding Area, samples 4-6 were collected from the Wells G&H 38-acre Wetland, samples 7-9 were collected from the Cranberry Bog, and samples 10-12 were from Davidson Park. Samples were selected to cover a range of arsenic concentrations in sediments, and were also selected to provide reasonable spatial representativeness across the site.

In preparation for *in vivo* RBA, one portion of each of the 12 samples was coarse-sieved through a 1 cm screen to remove large debris (sticks, leaf matter, stones, etc.). This screening was performed on the un-dried samples.

Samples 1, 6 and 7 were composited for the Aberjona TM1 RBA assay. Samples 2, 5 and 8 were composited for the Aberjona TM2 RBA assay.

Aspen

The Aspen Berm sample is a composite of nine sampling locations collected from the Racquet Club property including a berm, parking lot, and vacant lot between the tennis court and the Park Circle. The Aspen Residential sample is a composite of 9 sampling locations at residential properties within the study area. The samples were collected by the EPA remedial project manager and EPA toxicologist for the site.

Bingham

Soil samples were collected from a residential area (Jordan View Estates) located along Bingham Creek in the community of West Jordan, Utah. The Residential sample is a composite of 22 individual yard-soil samples. The Channel sample is a composite of 28 soil samples from the Bingham Creek channel adjacent to residential yards.

Butte

The test materials (Butte TM1 and Butte TM2) are two soil samples from Butte, Montana. Test Material 1 (USEPA sample number 8-37926) is a composite collected from the Butte Priority Soils Operable Unit (BPSOU) of the Silver Bow Creek/ Butte Area NPL Site in Butte, Montana. The sampling investigation focused on four source areas: the Little Mina-1, Little Mina-2, West Ruby, and North Emma waste rock dumps. At each source area, five sub-samples were collected and composited, and these were then further composited across source areas to yield the sample used in the study.

Test Material 2 (USEPA sample number BPSOU-0501-ASBIO) was collected by CDM in May 2001 (CDM Federal 2001). This soil sample is a composite collected from a residential property located adjacent to a railroad grade in Butte, Montana. A total of 5 soil samples from this property were composited.

CA Gulch

The Phase I Residential soil sample is a composite of surface soils (0-6 inches) from 28 residential properties within Leadville. Each sub-sample was selected to have a lead concentration of at least 6,000 mg/kg with actual values ranging from 6,380 mg/kg to 21,380 mg/kg.

Fe-Mn-Pb-oxide soil sample is a composite of three surface soil samples (0-6 inches) collected near the Lake Fork Trailer Park, southwest of Leadville near the Arkansas River.

The AV Smelter Slag sample was collected from the toe of a steep slope on the northern edge of a large pile of water-quenched slag that remained on the property of the former Arkansas Valley Smelter, located west of Leadville.

Jasper

Soil samples were collected from 3 locations at the Jasper County, Missouri Superfund site. Each sample was a composite of 4 subsamples collected from four, 1-foot square areas covering a 2-foot by 2-foot area at each sampling location. The depth of the soil collected was 1 to 2 inches. All samples consisted of dry, dusty leaf debris and organic soil.

Midvale

The slag sample was collected from 4 locations of Pile D (Water Quenched Slag) located in the northern portion of Midvale Slag Operable Unit 2.

Murray

Two samples from the Murray Smelter Superfund site were collected. The samples included a sample of slag and a sample of soil.

NIST 2710

NIST SRM 2710 is a standard reference material. From NIST (2003): The soil was collected from the top 4 inches of pasture land along Silver Bow Creek near Butte, Montana. The soil is a native Montana soil that has been contaminated with mine tailings deposits. The collection site is ~6.5 miles south of settlings ponds that feed the creek. The creek periodically floods, depositing mine tailings with high concentrations of copper, manganese and zinc at the collection site.

The sample was prepared by air drying in an oven for 3 days at room temperature. The material was then passed over a vibrating 2mm screen to remove plant material, rocks and large chunks of aggregated soil. Material remaining on the screen was deaggregated and rescreened. The combined material passing the screen was ground in a ball mill to

pass a 74-um screen, radiation sterilized and blended for 24 hours to achieve a high degree of homogeneity. This prepared soil as provided by NIST was used as-is for the bioavailability study, without further preparation.

Palmerton

Soil samples were collected from 4 locations at the Palmerton site. Each sample was a composite of 4 subsamples collected from four, 1-foot square areas covering a 2-foot by 2-foot area at each sampling location. The depth of soil collected was 1 to 2 inches. All samples consisted of dry, dusty leaf debris and organic soil. After screening, USEPA Region III selected two of the four samples for analysis in the swine bioavailability assay. These were referred to as "Location 2" and "Location 4."

VB I-70

Test Materials 1 through 5 were samples of residential yard soils that were collected during risk-based sampling activities at the site. Samples were selected to cover a range of arsenic concentrations in soil, and were also selected to provide reasonable spatial representativeness across the site, including samples from the Swansea/Elyria, Cole and Clayton neighborhoods. Soils with arsenic concentrations less than 200-250 mg/kg were not included because the mass of soil required for swine dosing was not available. Test Material 6 was prepared by mixing clean site soil (As < 10 mg/kg) with sufficient PAX (an arsenical herbicide that is considered to be a potential source material) to yield a concentration of approximately 516 mg/kg arsenic.

Each test material was prepared by combining all soil samples that had been collected from the selected property. For example, for properties that had been sampled during the risk-based sampling program (Test Materials 1 and 2), over 100 subsamples of soil were combined. For properties selected from the Phase 3 program (Test Materials 3-5), three samples (each a composite of 10 subsamples) were combined.

All sub-samples from a property were composited using a stainless steel bowl and mixing spoon. The composites were then air dried, homogenized, and sieved to < 250 um. Test Material 6 was also prepared by drying and sieving prior to the addition of the PAX.

Tacoma

The Soil sample is a composite of 15 surface (0 - 3 inch depth) soils that were collected in the residential area surrounding the former Asarco smelter in Tacoma, Washington. The Slag sample is a composite of slag was collected in the residential area surrounding the former Asarco smelter in Tacoma, Washington. Decontaminated stainless steel hand auger, mixing bowl, spoon and sample containers were used to collect the samples. Soil samples were collected from two residential properties (vacant lots) within one to two blocks of the smelter stack where previous investigation indicated elevated arsenic concentrations and access was granted. Five random samples were collected from one site and ten from the other.

2.0 BARBER ORCHARD SOILS

Barber Orchard soils were assayed *in vivo* in both monkeys and swine. Single samples of Barber Orchard soils were collected and prepared, then subsamples were distributed to the laboratories for each *in vivo* assay (no further preparation was done on the soil after subsampling). Grab samples were collected from the Barber Orchard site located near Waynesville, Haywood County, NC. The property was used as a commercial apple orchard from 1903 until the mid-1980s. In the late 1980s, some of the land was parceled off and sold for residential properties, church properties, and commercial or light industrial property. The majority of the remaining acreage is slated for residential development. In 1999, elevated concentrations of arsenic, lead and organic pesticides were found in the soil.

USEPA Region 4 collected the soil from the Barber Orchard site. Samples were placed in large stainless steel mixing bowls and then homogenized. Homogenized soil was shipped to USEPA's Office of Research and Development, National Exposure Research Laboratory (NERL) for processing. Samples were spread out in drying trays, placed in an air-drying oven and dried for ~4 days at <40 ºC. Soils were then added to a vibrating 2 mm stainless steel sieve screen to remove plant material, rocks and large chunks of aggregated soil. Material remaining on the screen was deaggregated and rescreened. Soil was then screened to <250 µm. The soil was passed through a riffler five times and a 200-g aliquot was collected in a pre-cleaned 250 mL high-density polyethylene bottle.

3.0 MATERIALS ASSAYED IN MONKEYS

From Roberts et al. (2007): Soil samples were obtained from selected arsenic-contaminated sites. Samples were sought from sites that varied in arsenic contamination source (e.g., wood treatment, herbicide use, mining) and in geographic region. Each soil sample was dried and sieved to 250 um. The 250-um sieved soil was stored in sealed containers at room temperature until utilized.

Phase II Report:
Identification Of Principal Variables Affecting IVBA Assay Results

ARSENIC IVBA OPTIMIZATION PROJECT

PHASE II REPORT:

IDENTIFICATION OF PRINCIPAL VARIABLES

AFFECTING IVBA ASSAY RESULTS

September 7, 2010

Prepared by:

U.S. Environmental Protection Agency
Region 8
15195 Wynkoop Street
Denver, CO 80202

Dr. John Drexler
Department of Geological Sciences
Benson Earth Science
2200 Colorado Ave.
Boulder, CO 80309

SRC, Inc.
999 18th Street, Suite 1975
Denver, CO 80202

Exponent
4141 Arapahoe Avenue, Suite 101
Boulder, CO 80303

This page intentionally left blank to facilitate double-sided printing.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

The goal of the arsenic *in vitro* bioaccessibility assay (IVBA) optimization project is to conduct a systematic evaluation of the parameters that influence the dissolution of arsenic from soils and develop an *in vitro* method that is optimized to predict the *in vivo* relative oral bioavailability (RBA) of arsenic in soil measured in animal studies. This project is being conducted in multiple phases (EPA 2009). Phase II of the IVBA optimization project consisted of a series of studies in which a number of experimental variables associated with the *in vitro* method were tested to characterize their effect on arsenic IVBA. The objective of the Phase II work was to identify assay variables that strongly affected IVBA outcomes.

2.0 METHODS

2.1 Test Materials Selected

A variety of test materials were selected for evaluation as part of Phase II. Table 2-1 summarizes the test materials that were evaluated in one or more Phase II studies. These test materials included several "synthetic" soils (constructed by spiking standard reference soils or soils from uncontaminated sites with specific arsenic mineral phases) and several contaminated site soils (obtained from various arsenic-contaminated sites across the United States). These test materials were selected to provide:

- a wide range of different mineralogical forms, including the most common forms encountered at Superfund sites, and
- a range of IVBA results, including some samples for which prior testing indicated that the IVBA result and the RBA result were not in good agreement.

2.2 Baseline IVBA Procedure

In order to evaluate the effects of alternate assay variables on arsenic IVBA, a "baseline" IVBA condition for each test material was established using the Relative Bioaccessability Leaching Procedure (RBALP) method, which has been validated for use in predicting RBA of lead from soil (Drexler and Brattin 2007).

The baseline RBALP involves placing 1 gram of the test substrate into a 125-mL wide-mouth high density polyethylene (HDPE) bottle to which is added 100 ± 0.5 mL of the extraction fluid (0.4 M glycine, pH 1.5). The samples are rotated end-over-end at 30 ± 2 revolutions per minute (rpm) for 1 hour while submerged in a water bath maintained at 37ºC. After 1 hour, the bottles are removed, dried, and placed upright on the bench top to allow the soil to settle to the bottom. A 15-mL sample of supernatant fluid is removed directly from the extraction bottle into a disposable 20-cc syringe, and then filtered through a 25 mm cellulose acetate filter with 0.45 um

pores to remove any particulate matter. Filtered samples of extraction fluid are then analyzed for arsenic by inductively coupled plasma mass spectroscopy (ICP-MS) using EPA Method 6020. IVBA is then calculated as the amount of arsenic present in the dissolved phase divided by the total amount of arsenic that was present in the test material.

Table 2-1 provides the baseline IVBA values for each test material evaluated in Phase II.

2.3 Variables Investigated

Experimental variables of potential importance were identified through previous experience with applications of the RBALP assay, and through a review of the literature. The list of experimental variables of potential interest included:

1. pH of the extraction fluid
2. temperature of the water bath
3. time of extraction
4. buffer strength
5. presence of oxyanions
6. presence of hydroxlyamine
7. filter pore size
8. redox potential of the extraction fluid
9. mass of test material used in the assay

In Phase II, these variables were investigated to determine if they either had a large impact on IVBA in general (i.e., changing the variable would be expected to change IVBA for most or all test materials), or a large impact on specific types of test materials (e.g., some soils are affected differently than other soils).

2.4 Data Quality Constraints

In order to ensure that conclusions drawn from Phase II studies are reliable, results for test materials where the standard deviation of IVBA values measured across multiple RBALP tests was greater than 10% (on an absolute basis) were not used. This is because it is not possible to reliably recognize effects of changing an assay variable if the baseline condition is not stable. The cause of high variability in IVBA measurements for soils is usually unknown, but might be attributed to analytical variability, heterogeneity in the test material, and/or potential laboratory errors.

As shown in Table 2-1, four test materials evaluated in Phase II had a baseline IVBA standard deviation greater than or equal to 10% – Anaconda Tailings, Bingham Creek Residential, Trail, and VB I70 TM6. The results summary presented below focuses only on those test materials

that meet the data quality constraints identified above. Appendix A provides the results for all test materials evaluated in Phase II.

3.0 STUDY CONDITIONS AND RESULTS

3.1 Extraction Fluid pH

As described above, the baseline RBALP method employs a pH of 1.5. In a series of tests, the pH of the extraction fluid was varied from 1.5 to 7.5. Figure 3-1 presents the arsenic IVBA results as a function of the extraction fluid pH. In this figure, Panel A presents the IVBA results for each test material at varying extraction fluid pH values and Panel B presents a comparison of IVBA results to the baseline condition (i.e., pH of 1.5). As shown, nearly all test materials showed decreasing IVBA with increasing pH, with the magnitude of the decrease differing by test material (i.e., IVBA decreased less than 3% for some test materials and decreased more than 50% for other test materials).

3.2 Temperature of the Bath

The baseline RBALP method employs a water bath temperature of 37ºC (the temperature of the human body). Metal solubility is expected to increase with temperature, and any exothermic or endothermic reactions between soil and extraction fluid are also likely to be affected by a temperature change. In this test, the IVBA at a bath temperature of 20ºC was compared to the IVBA under baseline conditions (37ºC). Figure 3-2 presents the arsenic IVBA results as a function of the temperature of the water bath. In this figure, Panel A presents the IVBA results for each test material at varying bath temperatures and Panel B presents a comparison of IVBA results to the baseline condition (i.e., 37ºC). As shown, with the exception of NIST 2711, all test materials showed increasing IVBA with increasing bath temperature, with the magnitude of the increase differing by test material.

3.3 Time of Extraction

The baseline RBALP method employs an extraction time of 1 hour (similar to the estimated residence time of food in the human stomach after fasting) (Washington et al. 2001). In three separate studies, the extraction time was varied from 10 minutes to 48 hours. In the first study, the extraction time was evaluated at 10, 20, 30, 40, and 60 minutes. In the second study, the extraction time was evaluated at 1, 2, and 4 hours. In the third study, the extraction time was evaluated at 2, 4, 6, 8, 16, 24, and 48 hours. Figure 3-3 presents the arsenic IVBA results as a function of the extraction time. In this figure, Panel A presents results from the first study, Panel B presents results from the second study, and Panel C presents results from the third study. In general, IVBA values for most test materials tended to increase somewhat as a function of increased extraction time. One exception is the Midvale Slag test material, which showed

higher IVBA values at the 4 hour extraction time compared to the 1 hour extraction time (see Panel B).

3.4 Buffer Concentration

The baseline RBALP method employs 0.4 M glycine buffer. The function of glycine in the extraction fluid is to act as a pH buffer. In this test, the effect of decreasing glycine concentration to 0.2 M was investigated under two baseline pH conditions (pH 1.5 and 2.5). Glycine buffer concentrations of 0.13 M and 0.3 M were also evaluated for some test materials at a pH of 1.5. Results are shown in Figure 3-4. As shown, for most test materials, decreasing the glycine concentration generally tended to increase IVBA slightly (usually about 5-10%).

3.5 Oxyanion Addition

Because arsenic in solution usually exists as an oxyanion, addition of other oxyanions to the extraction fluid may enhance the solubilization of arsenic from soil-like materials by competition between arsenic and other oxyanions for cationic adsorption sites in the soil. Rodriguez et al. (2003) found that adding 0.1 M sodium phosphate doubled arsenic IVBA on average compared to an extraction without sodium phosphate. In addition, for several of the test materials evaluated in this Phase II study, research funded by the Strategic Environmental Research Development Program (SERDP) demonstrated differential effects with the addition of phosphate to the extraction fluid, with the addition causing only limited effects for some test materials and profound effects for others.

Figure 3-5 shows the effect of adding 0.2 M or 0.8 M phosphate on the arsenic IVBA result for various test materials. In this figure, Panel A presents the IVBA results for each test material at varying phosphate concentrations and Panel B presents a comparison of IVBA results to the baseline condition (i.e., no phosphate added). As shown, phosphate additions increased IVBA for all test materials examined, although the magnitude of the effect was variable, with the increases ranging from 0.5% to over 100%. There were no significant differences in the magnitude or direction of change in IVBA among the various forms of phosphate forms tested (see Appendix A).

Limited tests were also performed using other oxyanions, including fulvic and humic acids and silicate. Results of these tests are provided in Appendix A. In general, the addition of these other oxyanions also tended to affect IVBA, but they were less effective than phosphate.

3.6 Hydroxylamine Addition

Hydroxylamine hydrochloride (HAH) has been used to extract trace metals that are adsorbed to the surface of iron or manganese oxide particles in soil (Tessier 1979, Chao and Zhou 1983, Shuman 1982). The basic reaction is:

$$2MnO_2(s) + 6H^+ + 2NH_2OH \rightarrow 2Mn^{2+} + 6H_2O + N_2$$

Dissolution of the surface layer of iron and manganese minerals by HAH thereby tends to liberate (solubilize) associated trace metals. The effectiveness of the dissolution appears to be primarily dependent on pH. In general, with the exception of manganese, time and HAH molarity have been shown to have little effect on metal removal (Bermond and Eustache 1993; Krasnodebska-Ostrega et al. 2001).

Metals released by this reaction may tend to re-adsorb to soil particles. Amacher and Kotuby-Amacher (1994) and Jackson and Miller (2000) noted that the addition of phosphate with HAH tended to decrease re-adsorption. Rodriguez et al. (2003) showed that additions of 0.025 M phosphate to 0.25 M HAH increased the amount of arsenic extracted in the IVBA system and improved RBA-IVBA correlations compared to other extraction techniques (e.g., deionized water, sodium acetate, phosphate, ammonium oxalate).

In the Phase II studies reported here, 0.25 M HAH was added (without the addition of phosphate) to the extraction fluid under two baseline pH conditions (pH 1.5 and 7.5). Figure 3-6 shows the results. In this figure, Panel A presents the IVBA results for each test material with and without HAH at a pH of 1.5, Panel B presents the IVBA results for each test material with and without HAH at a pH of 7.5, and Panel C presents a comparison of IVBA results to the baseline condition (i.e., no HAH added). As shown, at a pH of 1.5 (Panel A), the addition of HAH tended to have a limited effect on IVBA results (only a slight increase was noted for most test materials). At a pH of 7.5 (Panel B), effects were more profound, with variable results across test materials (i.e., IVBA increased for some test materials when HAH was added and decreased for others).

3.7 Filter Pore Size

The baseline RBALP method employs a filter with 0.45 pore pores when filtering the extraction fluid. Whittemore and Langmuir (1974) suggest that the pore size of the filter used to filter the extraction fluid could affect the amount of arsenic measured in the filtrate. That is, if the extraction fluid contains nano-sized particles of soil, then a smaller filter pore size may remove particles that are passed by a filter with larger pore size, resulting in lower measured IVBA values. In order to evaluate this possibility, a test was performed comparing IVBA measurements obtained using three different filter pore sizes (0.2, 0.45, and 3.0 um). For comparison, supernatant was also centrifuged at 5,500 rpm (approximately 300xg) for 15

minutes, but not filtered. Three replicates were evaluated for each test material for each method. Figure 3-7 shows mean arsenic concentrations in the extraction solution after filtration or centrifugation. The results show that the amount of arsenic measured in solution was not significantly influenced by either preparation method (filtration vs. centrifugation) or filter pore size.

3.8 Redox Potential

Redox potential (the tendency for chemical species to undergo oxidation or reduction) may be a factor that influences how much arsenic goes into solution during extraction. The Eh of the baseline RBALP system (pH 1.5) is approximately +550 mV ± 20 mV. In order to investigate the effects of Eh on IVBA, the extraction fluid was modified by addition of 0.25 M sodium hypochlorite (bleach), 0.25 M bile, or 0.25 M HAH, both at pH 1.5 and at pH 7.5. Table 3-1 shows the Eh values of the extraction fluid at the start and at the end of the IVBA extraction procedure at pH of 1.5 (Panel A) and pH 7.5 (Panel B). As seen, addition of bleach resulted in an increase of several hundred mV in the starting Eh, with a tendency for the Eh to return toward the base case as oxidation occurred during the extraction. Addition of bile had relatively little effect on Eh, and addition of HAH resulted in a decrease of about several hundred mV.

Figure 3-8 shows the effect of these agents on arsenic IVBA at a pH of 1.5 (upper row) and 7.5 (lower row). In this figure, Panel A (left column) presents the IVBA results for each test material with and without bleach, Panel B (center column) presents the IVBA results for each test material with and without bile, and Panel C (right column) presents a comparison of IVBA results with treatment to the baseline condition (i.e., no redox agents added). (Note: results for HAH were presented previously in Figure 3-6.) As shown, at a pH of 1.5, changes in redox potential had relatively little effect on the IVBA of most test materials. At a pH of 7.5, the addition of bleach had a variable effect (i.e., IVBA increased for some test materials when bleach was added, but showed no change or decreased for others). The addition of bile at a pH of 7.5 had relatively little effect on the IVBA of most test materials, but significantly decreased IVBA for the CAMT and WAOS test materials.

3.9 Soil Mass

The baseline RBALP method employs a soil mass of 1.0 g of soil material in 100 mL of extraction fluid. This soil:fluid ratio is expected to minimize any saturation effects that might occur during the extraction procedure. However, in order to investigate whether dissolution of some forms of arsenic might be limited by saturation kinetics, IVBA tests were performed using soil masses of 0.2 to 1.4 g in 100 mL of extraction fluid. Figure 3-9 shows how changing soil mass affects IVBA for a number of test materials. As seen, most materials did not show any change in IVBA outcomes, although a few test materials (e.g., PbAsO, NIST 2710A, NYPS2)

showed declining IVBA outcomes with increased mass. This suggests that the IVBA of some phases of arsenic may tend to be saturation limited.

4.0 IDENTIFICATION OF KEY EXPERIMENTAL VARIABLES

As noted above, the objective of the Phase II study was to identify a subset of key experimental variables for further multi-variate evaluation and optimization in Phase III. In accord with the project workplan (EPA 2009), in order to maximize available resources, a total of three key experimental variables will be retained for further evaluation in Phase III.

Defining which variables are "key" is based on judgment. In general, a key variable is one that changes arsenic IVBA for some test materials differently (different magnitude and/or different direction) than for others. A variable for which IVBA changes for all test materials in a similar direction to a similar magnitude is of less interest because this effectively only shifts the relationship between RBA and IVBA by a constant, and does not alter the strength (correlation coefficient) of the IVBA-RBA relationship.

Inspection of the Phase II results presented above shows that the pH of the extraction fluid (see Figure 3-1) is clearly one of the key experimental variables of interest for evaluation in Phase III. As seen, increasing the pH tended to decrease arsenic IVBA in almost all test materials, but the magnitude of the decrease tended to vary by test material.

The addition of competitive oxyanions such as phosphate (see Figure 3-5) generally tended to increase arsenic IVBA, and the magnitude of the effect differed by test material. The addition of other oxyanions, such as organic acids and silicate, were less effective than phosphate. Therefore, the concentration of phosphate in the extraction fluid is identified as a key experimental variable for evaluation in Phase III.

The addition of HAH (see Figure 3-6) tended to increase IVBA somewhat in most test materials. However, the test condition evaluated did not include the addition of HAH and phosphate. Rodriguez et al. (2003) showed that addition of HAH with phosphate tended to improve RBA-IVBA correlations relative to other extraction techniques. Therefore, HAH is identified as a key experimental variable for evaluation in Phase III.

Accordingly, the three experimental variables that are retained for multi-variate evaluation in Phase III are:

1. pH of the extraction fluid
2. concentration of phosphate in the extraction fluid
3. concentration of HAH in the extraction fluid

Other parameters of the baseline procedure will remain unchanged (i.e., extraction time should be maintained at 1 hour, water bath should be maintained at a temperature of 37°C, buffer concentration should be maintained at 0.4 M, filter size should be maintained at 0.45 um, soil mass should be maintained at 1 gram). However, if the optimal pH of the IVBA method for arsenic is determined to be in the neutral range (near pH 7), it may be appropriate to alter the buffer composition, since glycine is not a highly effect buffer at neutral pH values.

5.0 REFERENCES

Amacher, M.C. and Kotuby-Amacher, J. 1994. Selective extraction of arsenic from mine spoils, soils, and sediments. p. 256. In: 1994 Agronomy Abstracts. ASA, Madison, WI.

Anderson, D.W. and Schoenau, J.J. 1993. Chapter 38: Soil Humus Fractions. In: M.R. Carter (Ed.), *Soil Sampling and Methods of Analysis*. Lewis Publishers.

Bermond, A.P. and Eustache, S. 1993. Hydroxylamine extraction of trace metals in soils: kinetic aspects. *Envir. Technology* 14:359-365.

Chao, T.T. and Zhou, L. 1983. Extraction techniques for selective dissolution of amorphous iron oxides from soils and sediments. *Soil Sci. Soc. Am. J.* 47:225–232.

Drexler, J.W. and Brattin, W. 2007. An in vitro procedure for estimation of lead relative bioavailability: with validation. *Human and Ecological Risk Assessment,* 13(2):383-401.

EPA. 2009. Workplan to Optimize an *in vitro* Method to Predict the Relative Oral Bioavailability of Arsenic. Prepared by U.S. Environmental Protection Agency Region 8, Dr. John Drexler, Syracuse Research Corporation, and Exponent. March 13, 2009.

Jackson, B.P. and Miller, W.P. 2000. Effectiveness of phosphate and hydroxide for desorption of arsenic and selenium from iron oxides. *Soil Sci. Soc. Am. J.* 64:1616–1622.

Krasnodebska-Ostrega, B., Emons, H., and Golimowski, J. 2001. Selective leaching of elements associated with Mn-Fe oxides in forest soil, and comparison of two sequential extraction methods. *Fresenius J. Anal. Chem.* 371:385-390.

Rodriguez, R.R., N.T. Basta, S.W. Casteel, F.P. Armstrong, and Ward, D.C.. 2003. Chemical Extraction Methods to Assess Bioavailable Arsenic in Soil and Solid Media. *J. Environ. Qual.* 32:876-884.

Shuman, L.M. 1982. Separating soil iron- and manganese-oxide fractions for microelement analysis. *Soil Sci. Soc. Am. J.* 46:1099–1102.

Tessier, A., Campbell, P.G.C., and Bisson, N. 1979. Sequential extraction procedure for the separation of particulate trace metals. *Anal. Chem.* 51, 844-851.

Washington N, Washington C, and Wilson, C. 2001. Physiological Pharmaceutics: Barriers to drug absorption, 2nd ed. Taylor and Francis, New York, NY, USA.

Whittemore, D.O., and Langmuir, D. 1974. Ferric oxyhydroxide microparticles in water. *Envir. Health Perspectives* 9: 173-176.

This page intentionally left blank for double-sided printing

TABLE 2-1
TEST MATERIALS EVALUATED IN PHASE II

Test Material Identifier	Description	Predominant Minerology	Soil As Conc. (mg/kg)	Baseline As IVBA		Test Condition Evaluated in Phase II									
				n	Mean (%) ± StDev (%)	pH	Temp	Time	Glycine	PO4	OM & Si	HAH	Filter	Redox	Mass
Anaconda Flue Dust	Anaconda Flue Dust	FeAsO	1,663	1	15.0	X	X	X							
Anaconda Tailings	Anaconda Tailings	FeOOH	15,952	2	44.4 ± 31.6	X	X	X							
Barber Orchard MS-5	Barber Orchard MS-5	PbAsO/Cobaltite/FeOOH	382	3	19.3 ± 0.8	X									
BC Channel Soil	Bingham Creek Channel Soil, Salt Lake City, UT	Fe sulfate, PbAsO	193	26	16.3 ± 3.0	X		X	X			X		X	
BC Residential	Bingham Creek Residential, Salt Lake City, UT	--	51	4	44.8 ± 20.7				X						
BLM	Buearu of Land Management Soil	FeOOH/CaAsO	242	1	29.0					X					
Butte TM1	Silver Bow Creek/Butte Area NPL Site, Butte, MT	FeAsSO / FeAsO	276	31	8.2 ± 1.8	X						X		X	X
CA Gulch AV Slag	California Gulch, slag	PbAsO	1,050	18	25.2 ± 3.7				X						
CAMT	California mine tailings	Arsenopyrite	300	25	15.2 ± 4.7	X	X	X	X	X	X	X		X	X
CORS	VB/I-70 (CO residential soil)	As2O3	1,230	2	33.4 ± 6.4			X							
COW-Fe	Natural soil - Cave of the Winds, CO	FeAsO	689	4	0.93 ± 1.26	X		X	X	X		X	X	X	X
COW-Mn	Natural soil - Cave of the Winds, CO	MnAsO	490	2	1.5 ± 2.1	X	X	X		X			X		
CRM 205-225	TCLP Metals on Fly Ash	--	90	1	85.0					X					
Drexler-5	Spiked - CO sandy loam, low TOC, low CEC	NaAsO	1,239	5	95.0 ± 5.1	X		X			X	X		X	X
Drexler-6	Natural soil - Globeville site	AsMO-CaMO-PbAsO	1,414	4	71.7 ± 8.4	X	X	X				X		X	X
Drexler-7	Spiked - CO sandy loam, low TOC, low CEC	As2O3	1,767	2	88.2 ± 3.1	X		X				X		X	X
Drexler-8	Spiked - CO sandy loam, low TOC, low CEC	PbAsO	2,961	1	22.0 ±	X									X
Enargite	Enargite	Cu-Fe-As-S mineral	191	1	0 ±		X	X							
FLCDV	FL cattle dip vat soil	Clay	150	6	39.4 ± 3.5							X		X	
HIVS	Hawaii Volcanic soil	FeAl silicate	724	5	10.6 ± 4.2					X					
LS-paint	Carbonate soil with paint	Paint	71	3	31.5 ± 3.3				X						
Midvale Slag	Midvale Slag	PbAsO	591	24	34.0 ± 8.1		X	X							
MTSS	Montana smelter soil	FeOOH/FeSO4	647	7	48.1 ± 4.8	X		X							
Murray Smelter Flue Dust	Murray Smelter Flue Dust	--	41,051	2	98.0 ± 1.4			X							
Murray Smelter Soil	Murray Smelter Soil	PbAsO	310	21	71.5 ± 9.5				X						
NIST 2710	Certified std derived from Silver Bow Creek, MT	--	626	69	55.1 6.2	X	X	X	X				X		
NIST 2710A	Spiked PbAsO - NIST 2710	--	1,540	9	43.2 ± 2.3										X
NIST 2711	Certified std derived from Silver Bow Creek, MT	--	105	79	58.3 ± 9.4	X	X	X	X				X		
NYOS	New York orchard soil	MnOOH/PbAsO4	123	8	34.0 ± 2.3	X		X							
NYPS2	New York pesticide plant soil, T5E3 (8B)	FeOOH	339	13	58.9 ± 3.6	X		X							X
Refinery	Western Oil Refinery Soil	FeAsO	40	1	25.8				X						
Trail	Residential Soil Trail BC, Canada	FeAsO/Fe sulfate/Slag	82	3	36.3 ± 10.1				X						
VBI70 TM3	VB/I-70 TM3 (CO residential soil)	AsO	390	13	67.4 ± 9.5	X	X	X		X		X		X	X
VBI70 TM6	VB/I-70 TM6 (CO residential soil + PAX pesticide)	AsO	516	4	40.0 ± 14.4	X	X	X							
WAOS	Washington orchard soil	PbAsO	301	15	81.8 ± 6.5	X	X	X		X		X		X	X
					total # test materials evaluated:	20	12	21	12	8	2	10	4	10	14

Table 3-1 Eh of Extraction Fluid Following Additions

Panel A: pH 1.5 (starting Eh = 550 mV)

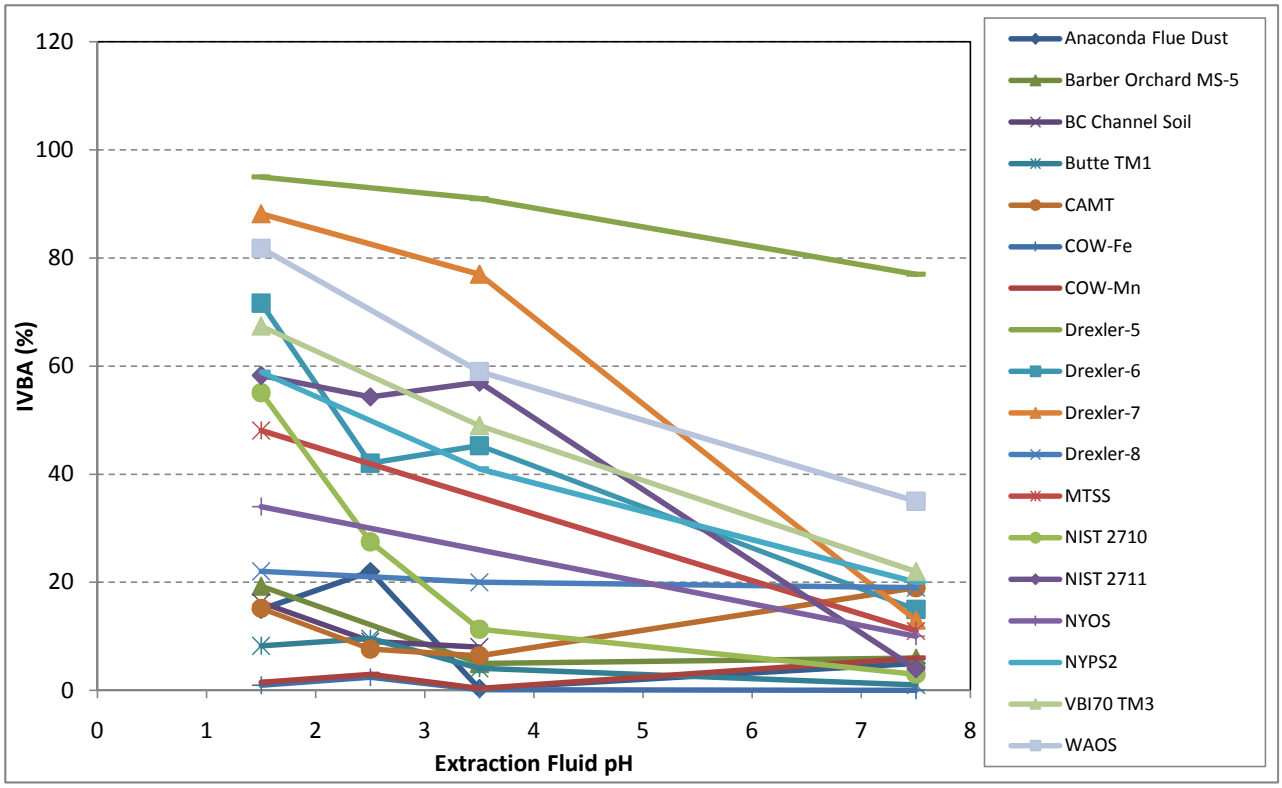
Test Material	Bleach		Bile		HAH	
	Initial Eh	Final Eh	Initial Eh	Final Eh	Initial Eh	Final Eh
BC CHANNEL SOIL	872	612	525	533	337	343
BUTTE TM1	872	708	525	569	337	345
CAMT	872	511	525	499	337	389
COW-FE	872	795	525	552	337	333
DREXLER-5	872	563	525	540	337	381
DREXLER-6	872	532	525	503	337	339
DREXLER-7	872	566	525	524	337	334
FLCDV	872	777	525	498	337	325
VBI70 TM3	872	567	525	557	337	341
WAOS	872	530	525	511	337	337

Panel B: pH 7.5 (starting Eh = 495 mV)

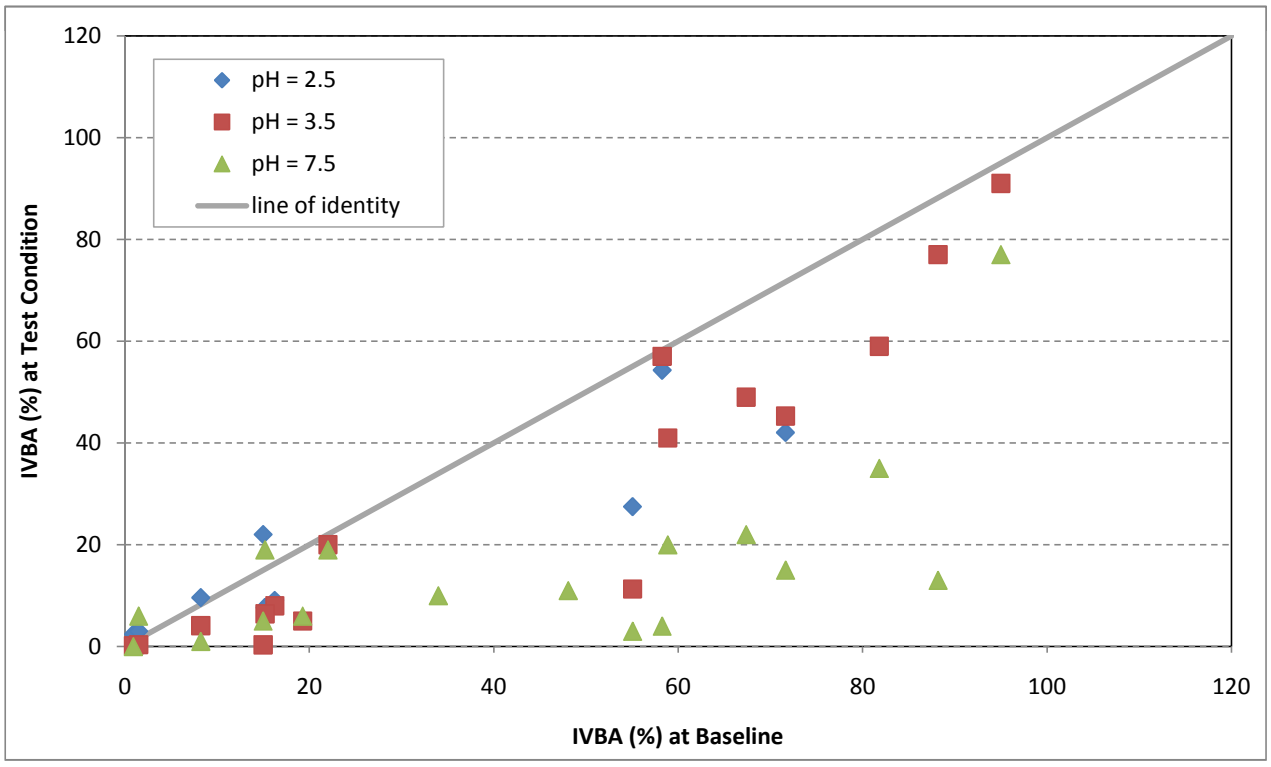
Test Material	Bleach		Bile		HAH	
	Initial Eh	Final Eh	Initial Eh	Final Eh	Initial Eh	Final Eh
BC CHANNEL SOIL	513	408	260	202	-131	-156
BUTTE TM1	513	426	260	215	-131	-155
CAMT	513	264	260	226	-131	-153
COW-FE	513	454	260	202	-131	-162
DREXLER-5	513	285	260	202	-131	-162
DREXLER-6	513	335	260	210	-131	-155
DREXLER-7	513	442	260	221	-131	-155
FLCDV	513	386	260	203	-131	-152
VBI70 TM3	513	263	260	226	-131	-154
WAOS	513	273	260	230	-131	-144

FIGURE 3-1. EFFECT OF EXTRACTION FLUID pH ON ARSENIC IVBA

Panel A: Arsenic IVBA at Varying Extraction Fluid pH Values



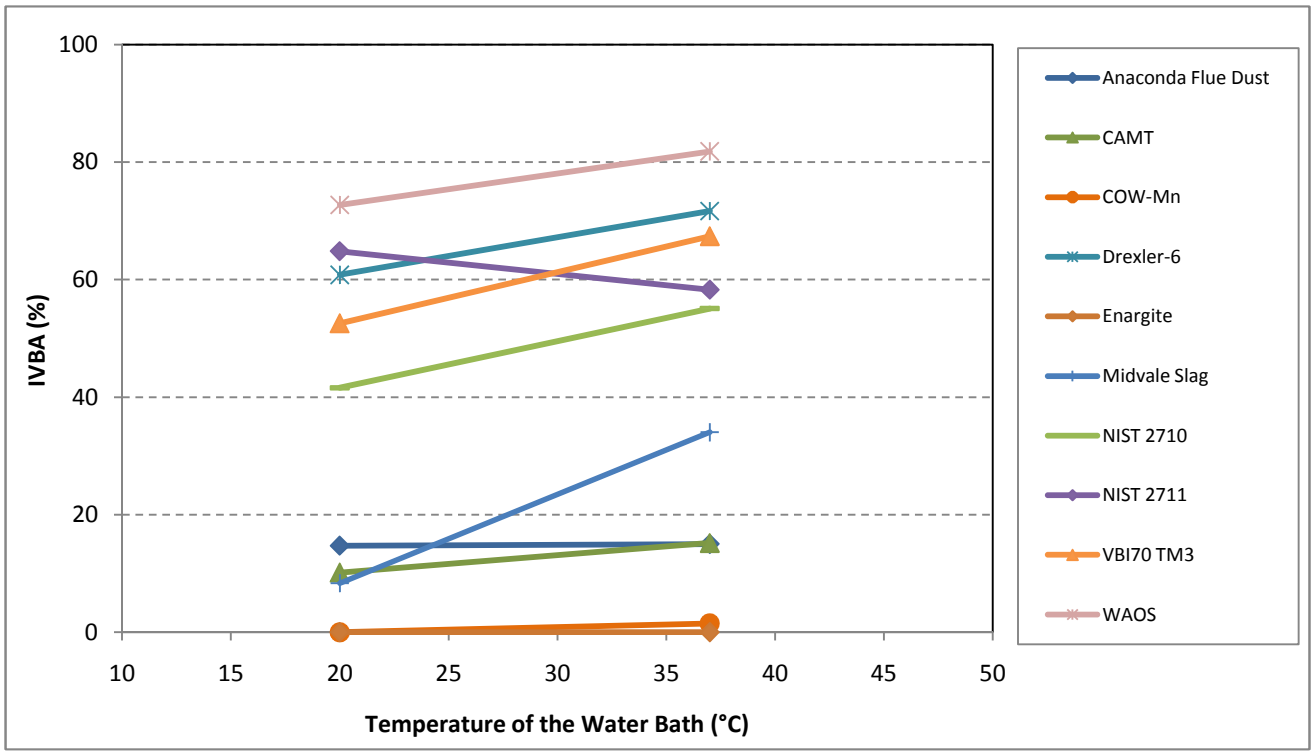
Panel B: Comparison to IVBA at Baseline Condition



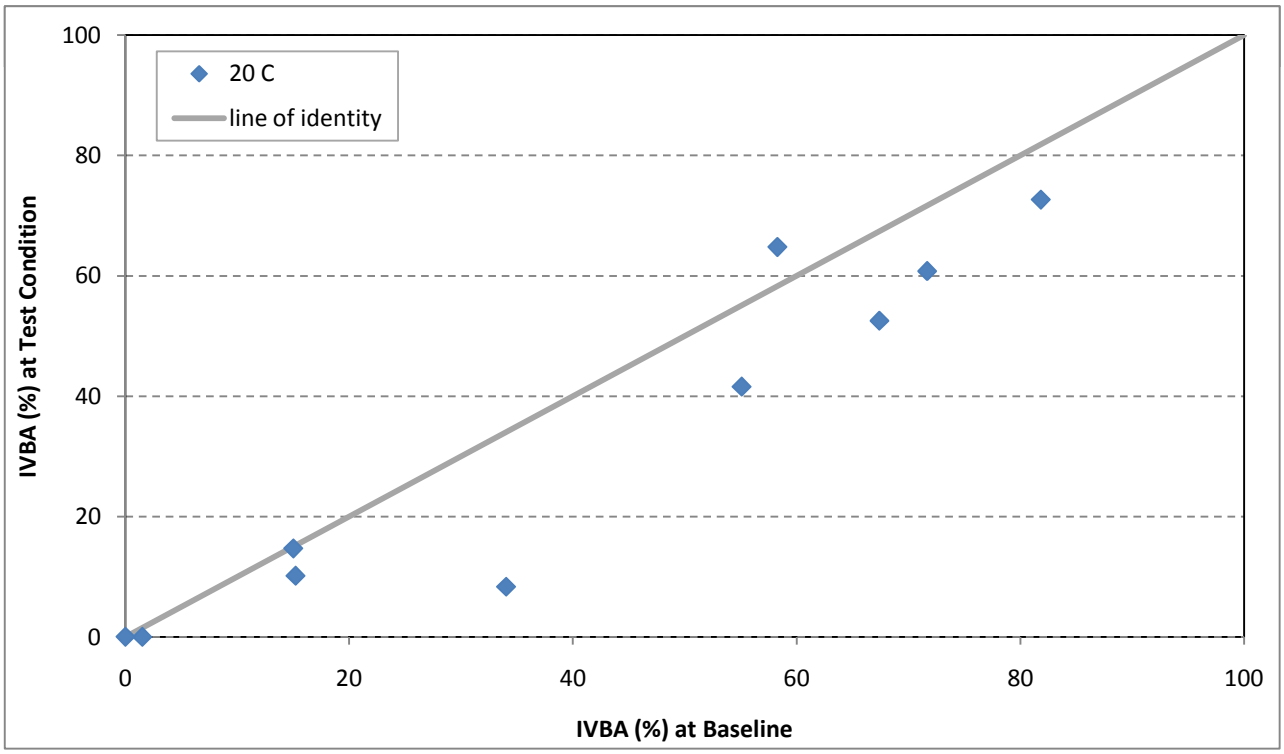
Baseline condition = pH 1.5

FIGURE 3-2. EFFECT OF TEMPERATURE ON ARSENIC IVBA

Panel A: Arsenic IVBA at Varying Water Bath Temperatures



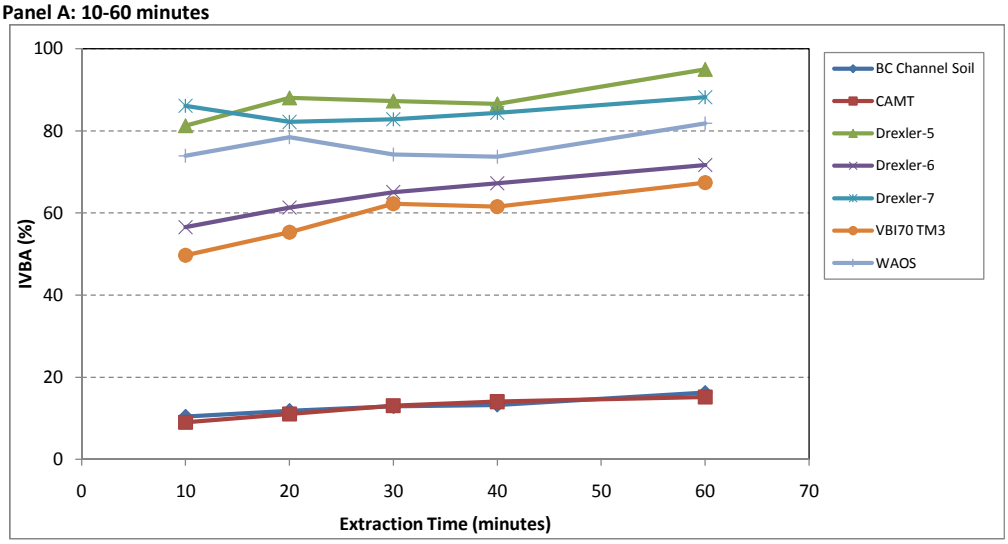
Panel B: Comparison to IVBA at Baseline Condition



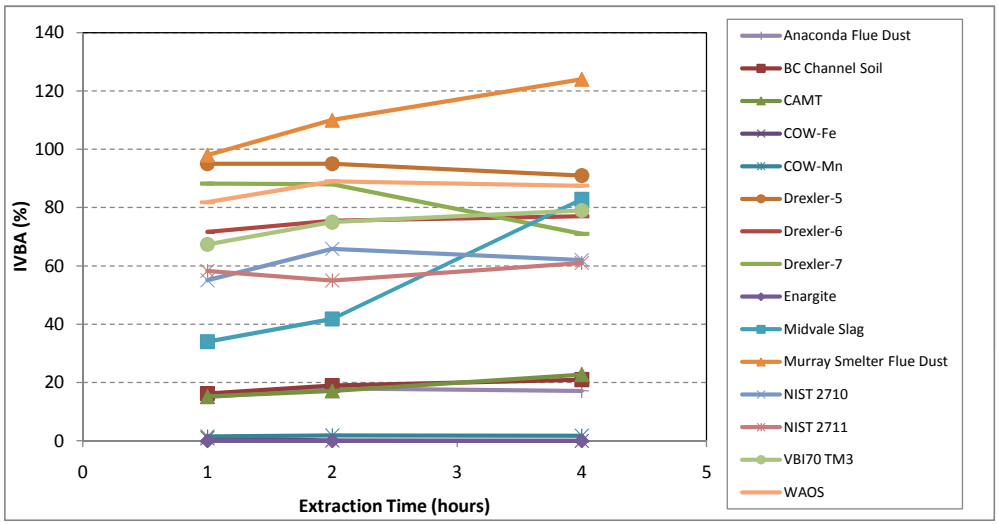
Baseline Condition = 37°C

FIGURE 3-3. EFFECT OF EXTRACTION TIME ON ARSENIC IVBA

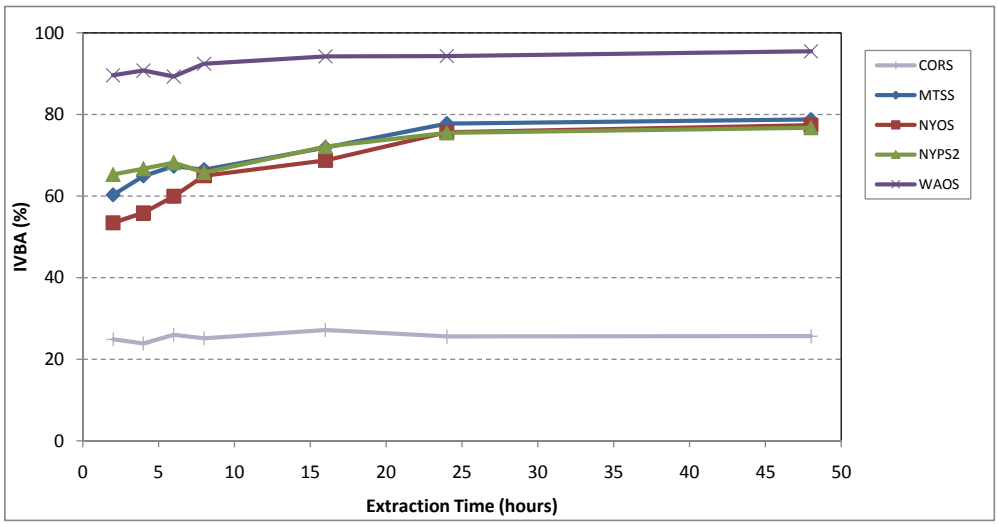
Panel A: 10-60 minutes



Panel B: 1-4 hours



Panel C: 1-48 hours

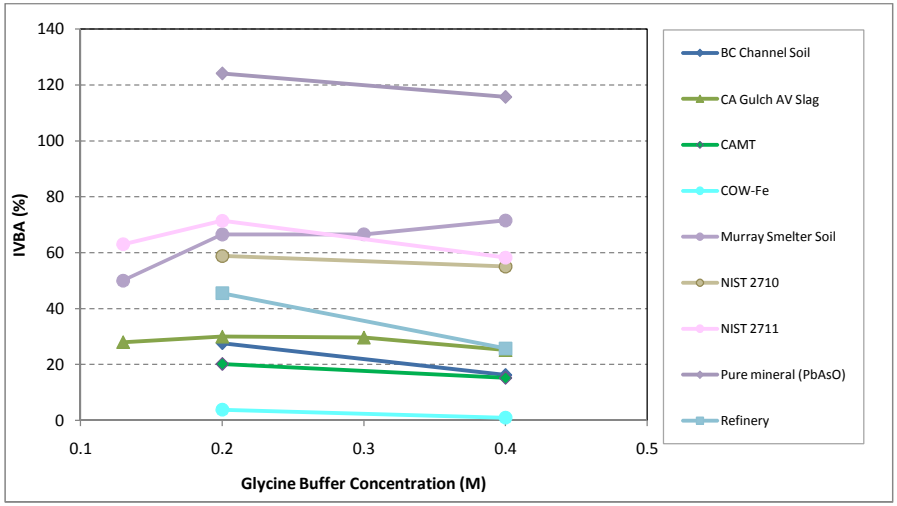


Baseline condition = 1 hour

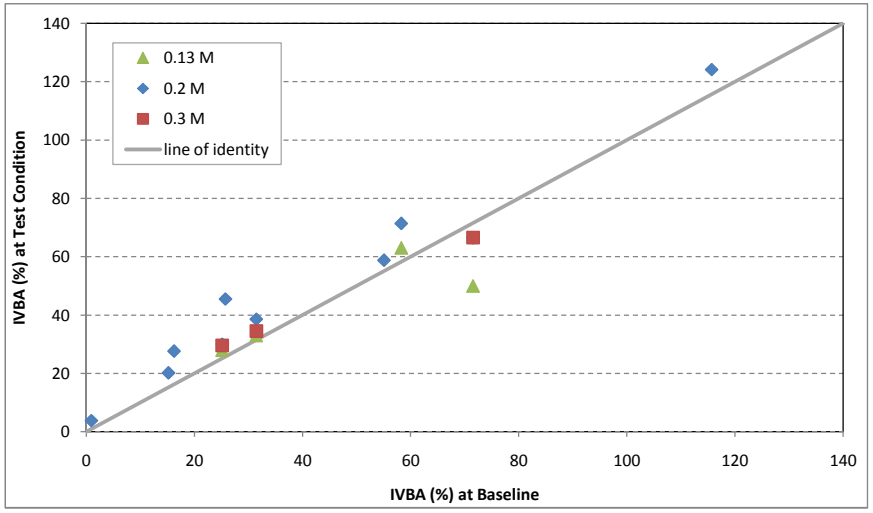
FIGURE 3-4. EFFECT OF GLYCINE BUFFER CONCENTRATION ON ARSENIC IVBA

pH = 1.5

Panel A: Arsenic IVBA at Varying Glycine Buffer Concentrations



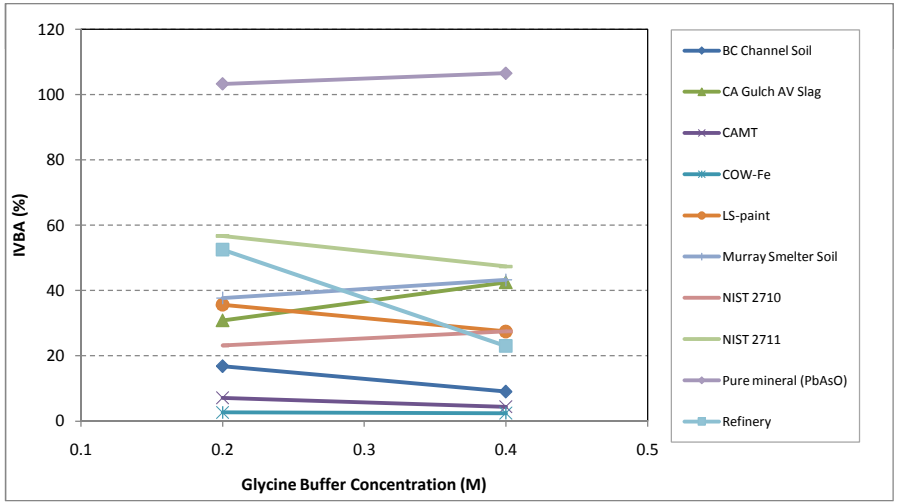
Panel B: Comparison to IVBA at Baseline Condition



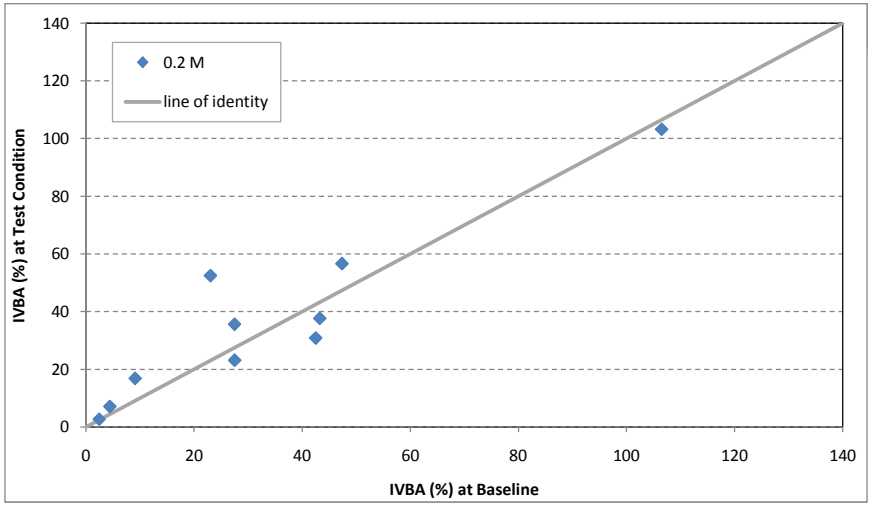
Baseline condition = pH 1.5, 0.4 M glycine

pH = 2.5

Panel A: Arsenic IVBA at Varying Glycine Buffer Concentrations



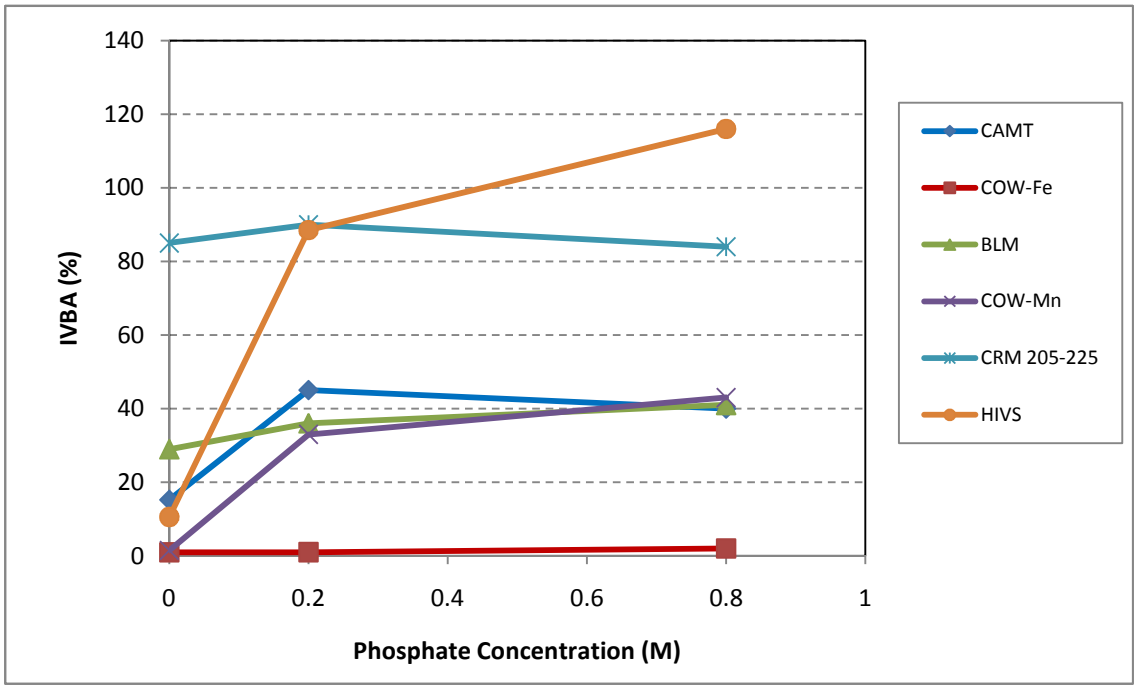
Panel B: Comparison to IVBA at Baseline Condition



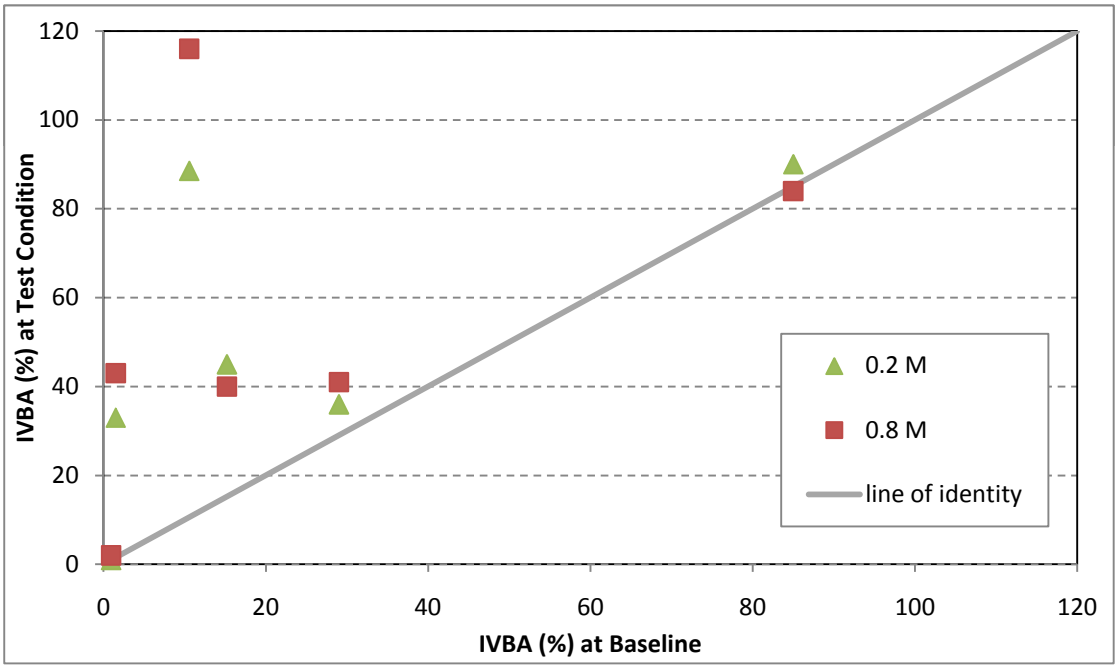
Baseline condition = pH 2.5, 0.4 M glycine

FIGURE 3-5. EFFECT OF PHOSPHATE ADDITION ON ARSENIC IVBA

Panel A: Arsenic IVBA at Varying Phosporic Acid Concentrations



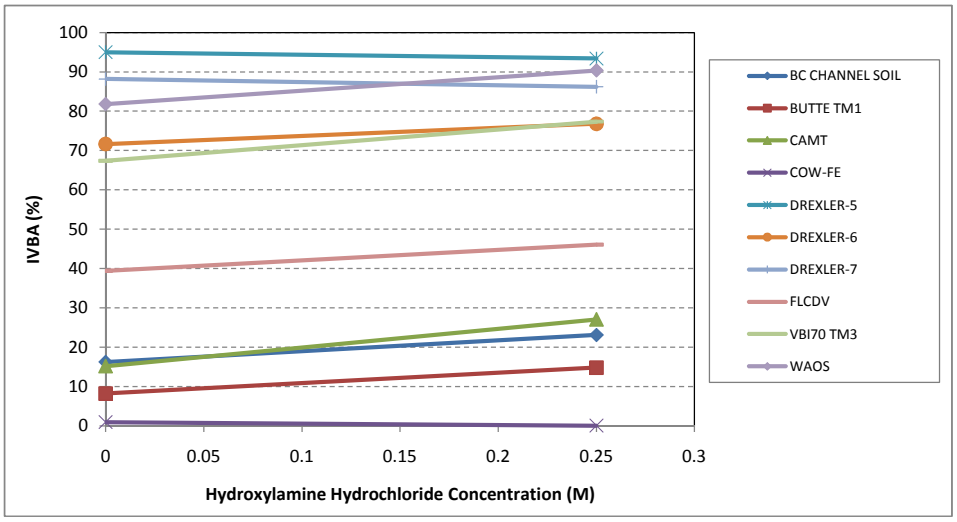
Panel B: Comparison to IVBA at Baseline Condition



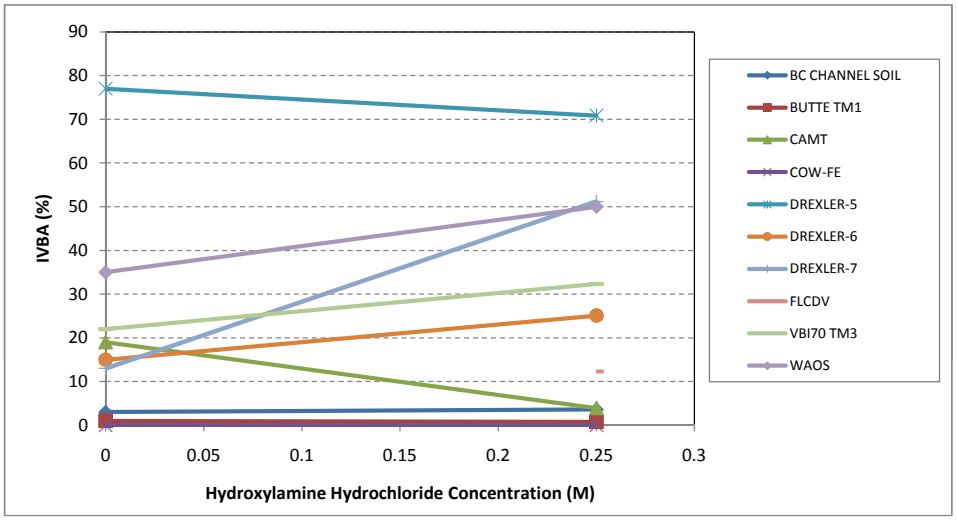
Baseline condition = no phosphate added

FIGURE 3-6. EFFECT OF HYDROXYLAMINE HYDROCHLORIDE ON ARSENIC IVBA

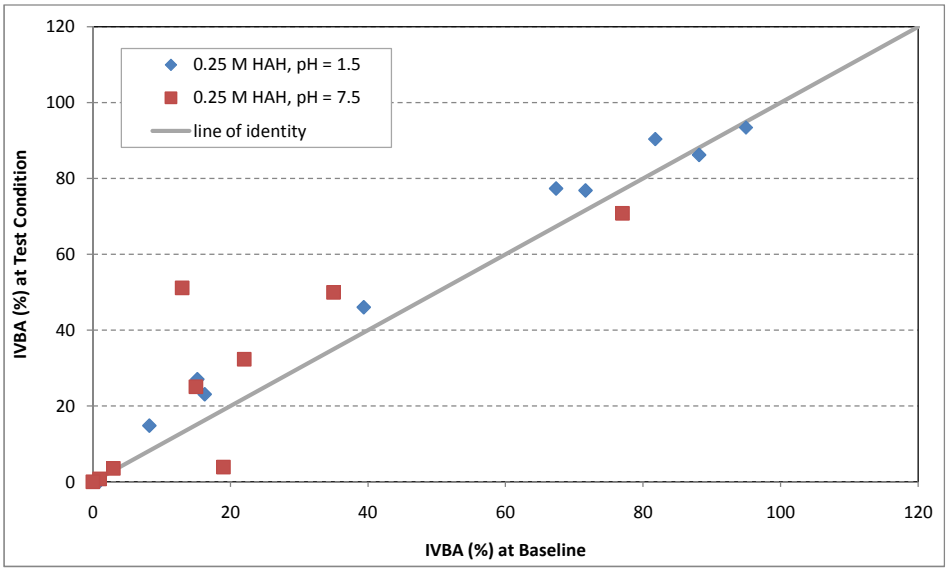
Panel A: Arsenic IVBA at Varying Hydroxylamine Hydrochloride Concentrations, pH = 1.5



Panel B: Arsenic IVBA at Varying Hydroxylamine Hydrochloride Concentrations, pH = 7.5

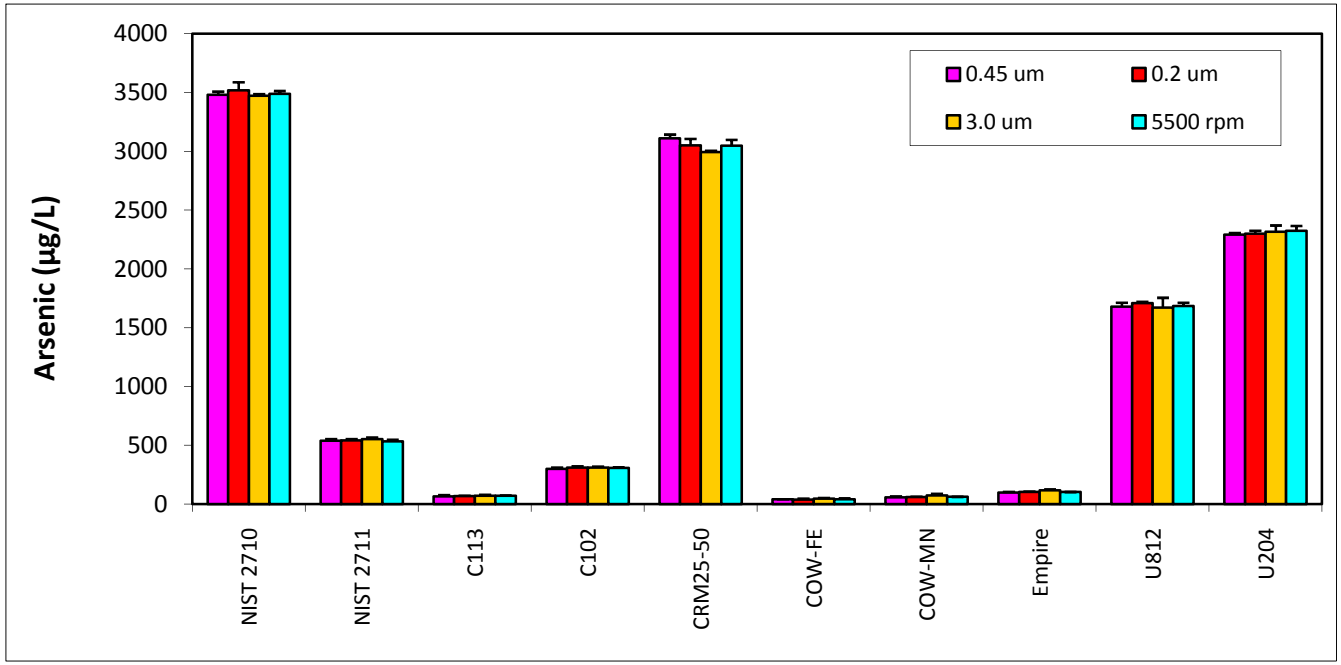


Panel C: Comparison to IVBA at Baseline Condition



Baseline condition = no hydroxylamine hydrochloride added

FIGURE 3-7. EFFECT OF FILTER PORE SIZE ON ARSENIC IVBA

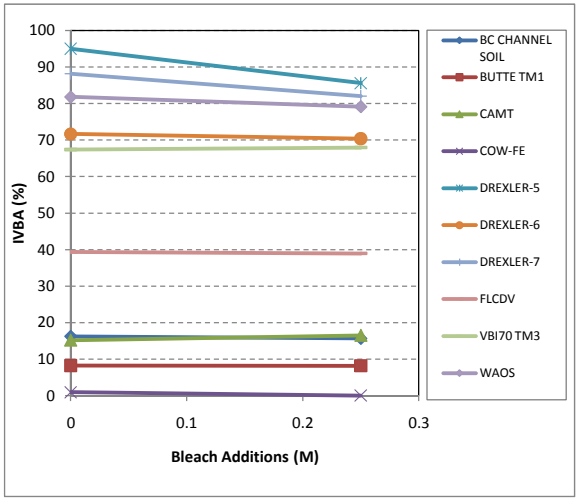


Baseline condition = 0.45 um

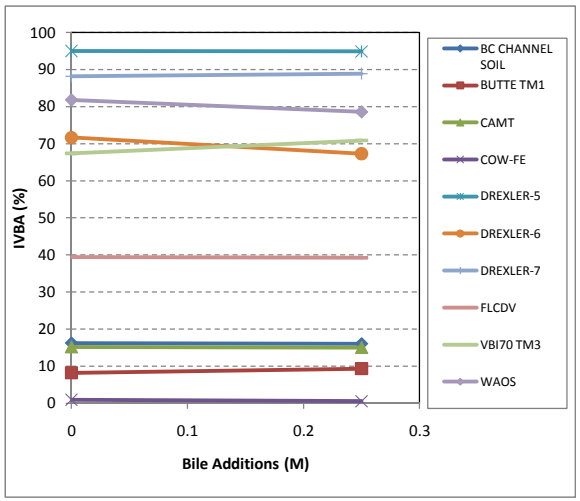
FIGURE 3-8. EFFECT OF REDOX AGENT ADDITION ON ARSENIC IVBA

pH = 1.5

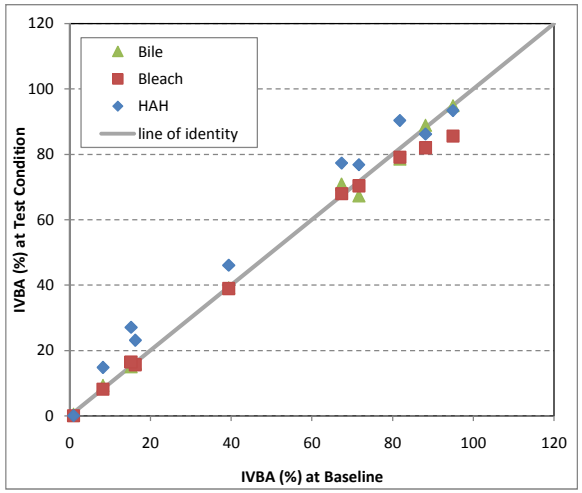
Panel A: Arsenic IVBA with and without the addition of bleach



Panel B: Arsenic IVBA with and without the addition of bile



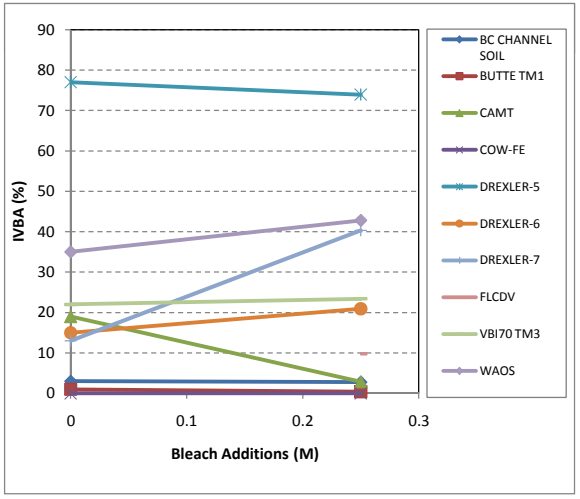
Panel C: Comparison to IVBA at Baseline Condition



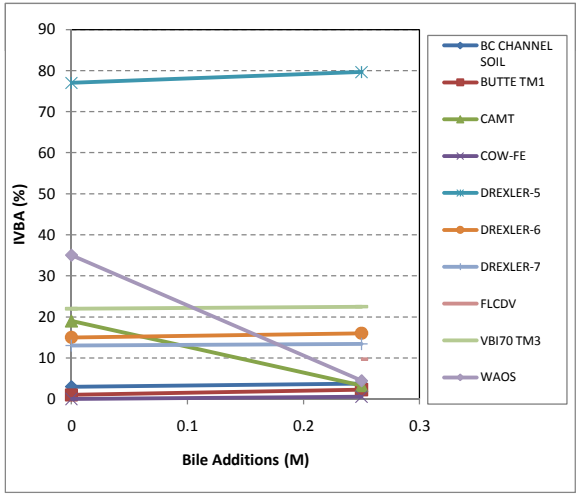
Baseline condition = no redox agents added, pH 1.5

pH = 7.5

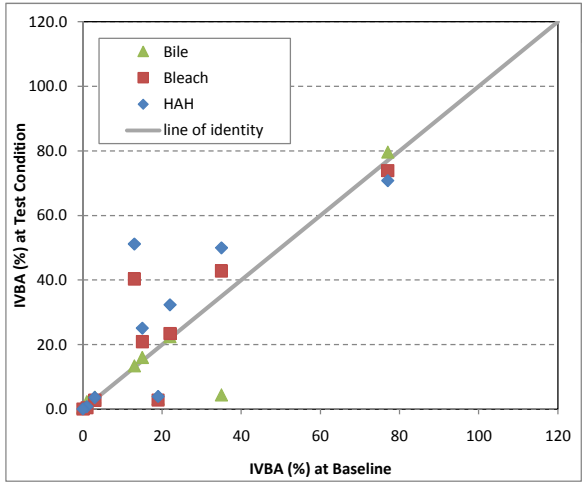
Panel A: Arsenic IVBA with and without the addition of bleach



Panel B: Arsenic IVBA with and without the addition of bile



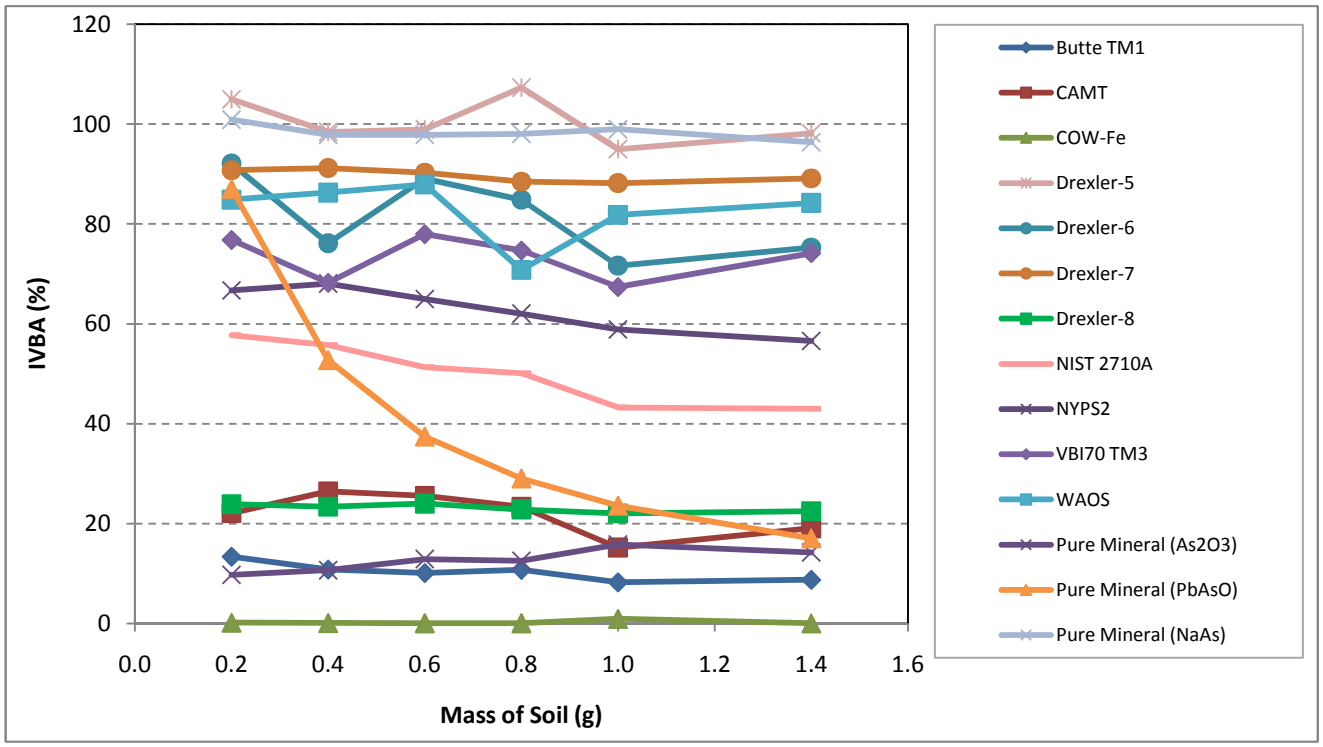
Panel C: Comparison to IVBA at Baseline Condition



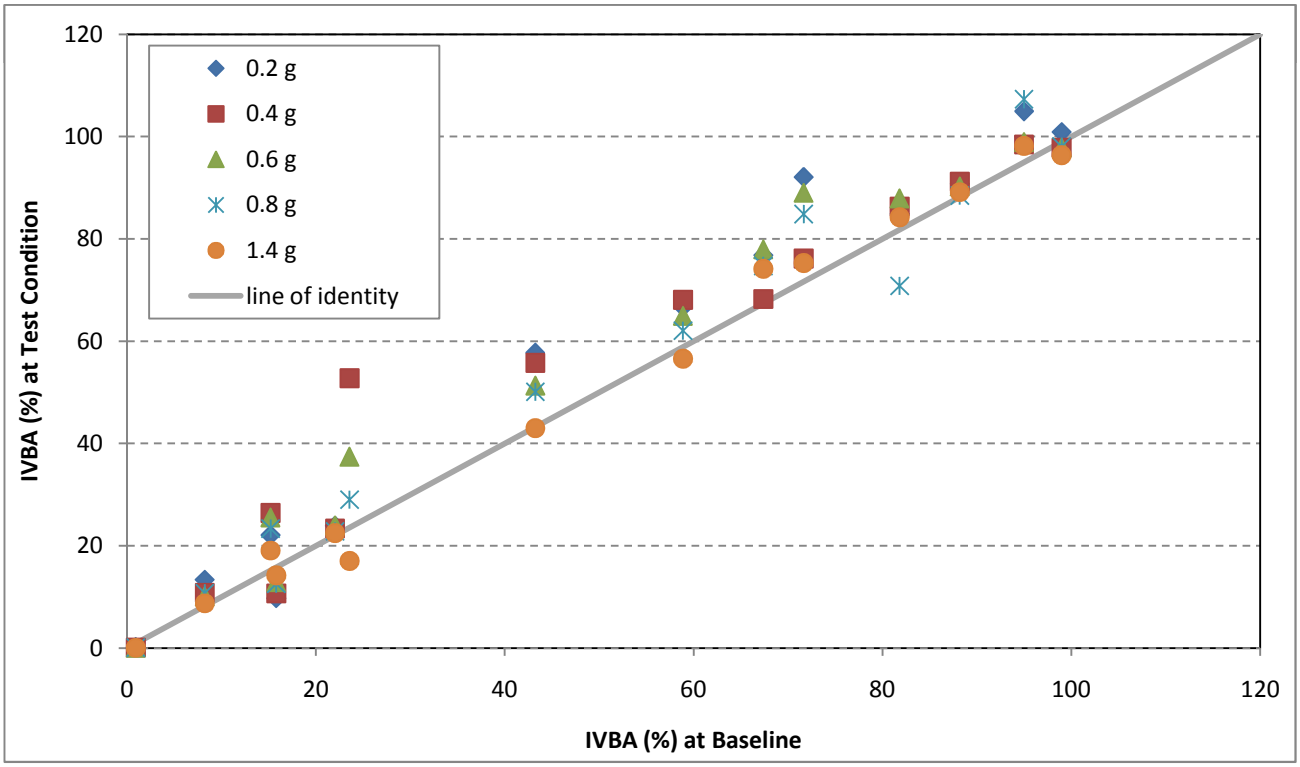
Baseline condition = no redox agents added, pH 7.5

FIGURE 3-9. EFFECT OF SOIL MASS ON ARSENIC IVBA

Panel B: Arsenic IVBA at Varying Soil Mass



Panel B: Comparison to IVBA at Baseline Condition



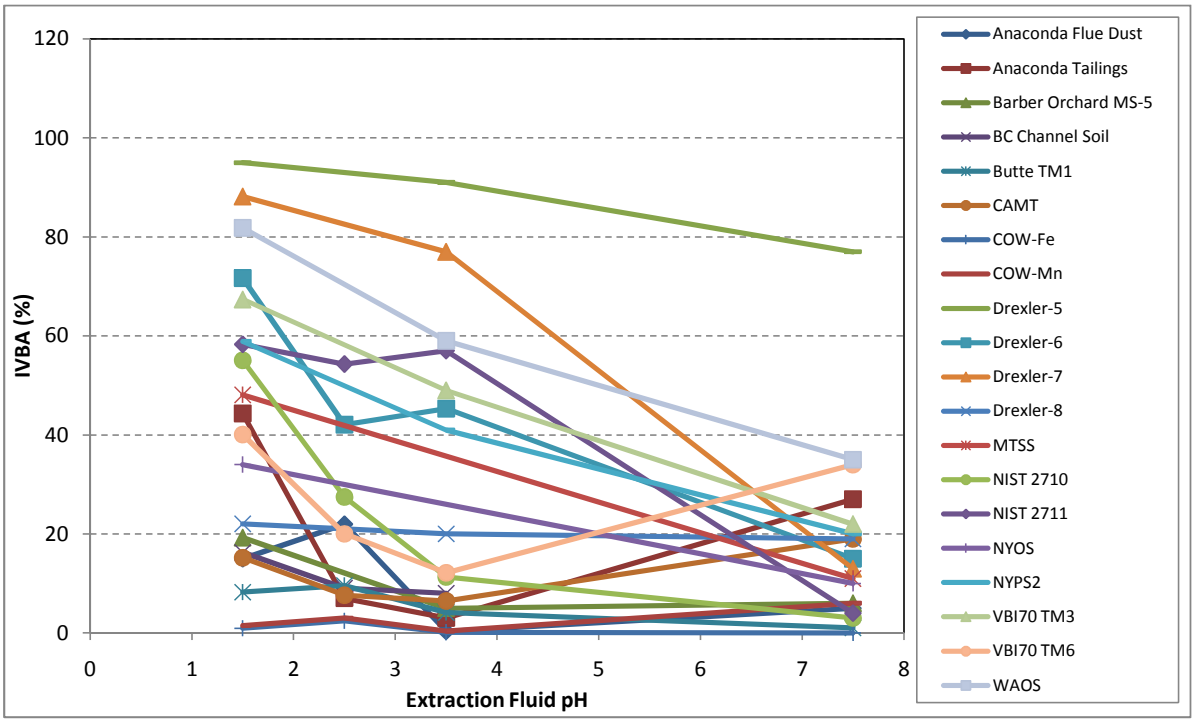
Baseline condition = 1.0 g

This page intentionally left blank for double-sided printing

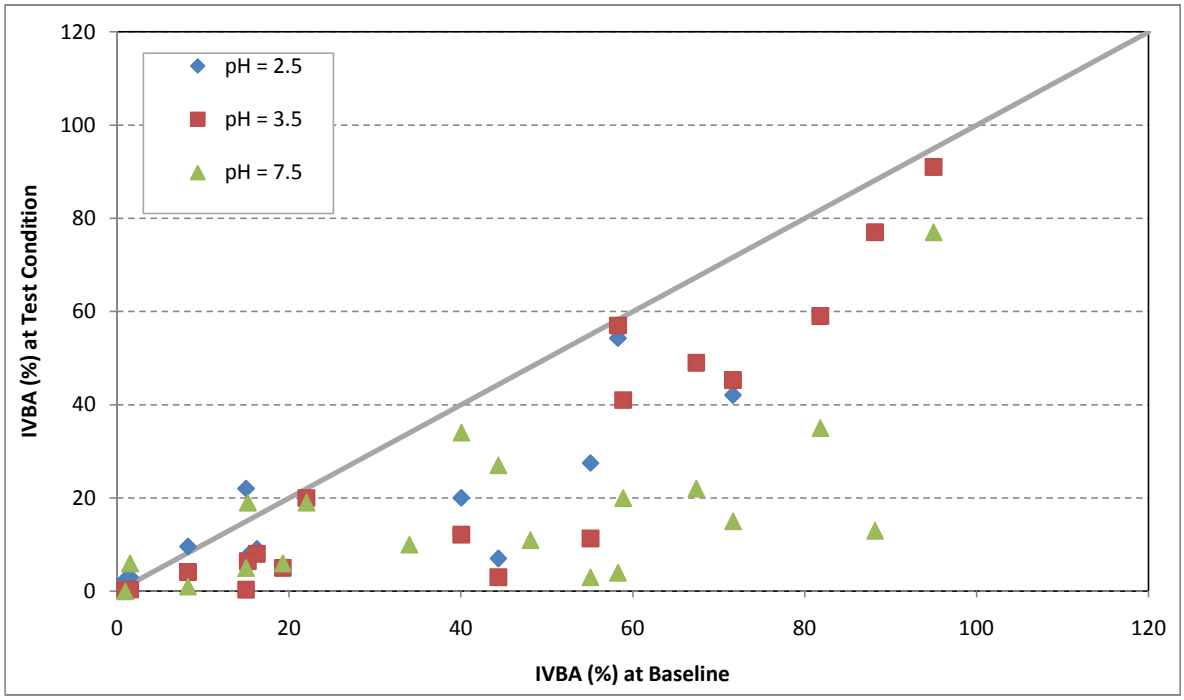
APPENDIX A
Results for All Phase II Studies and Test Materials

FIGURE A-1. EFFECT OF EXTRACTION FLUID pH ON ARSENIC IVBA

Panel A: Arsenic IVBA at Varying Extraction Fluid pH Values



Panel B: Comparison to IVBA at Baseline Condition

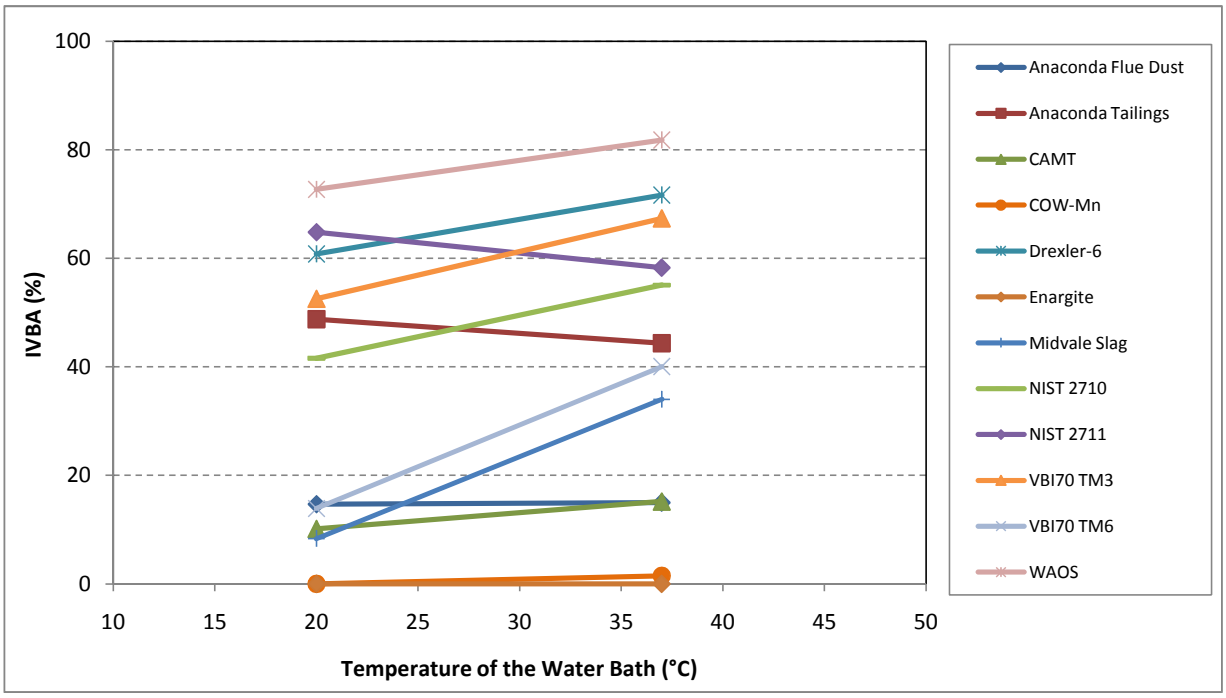


Baseline condition = pH 1.5

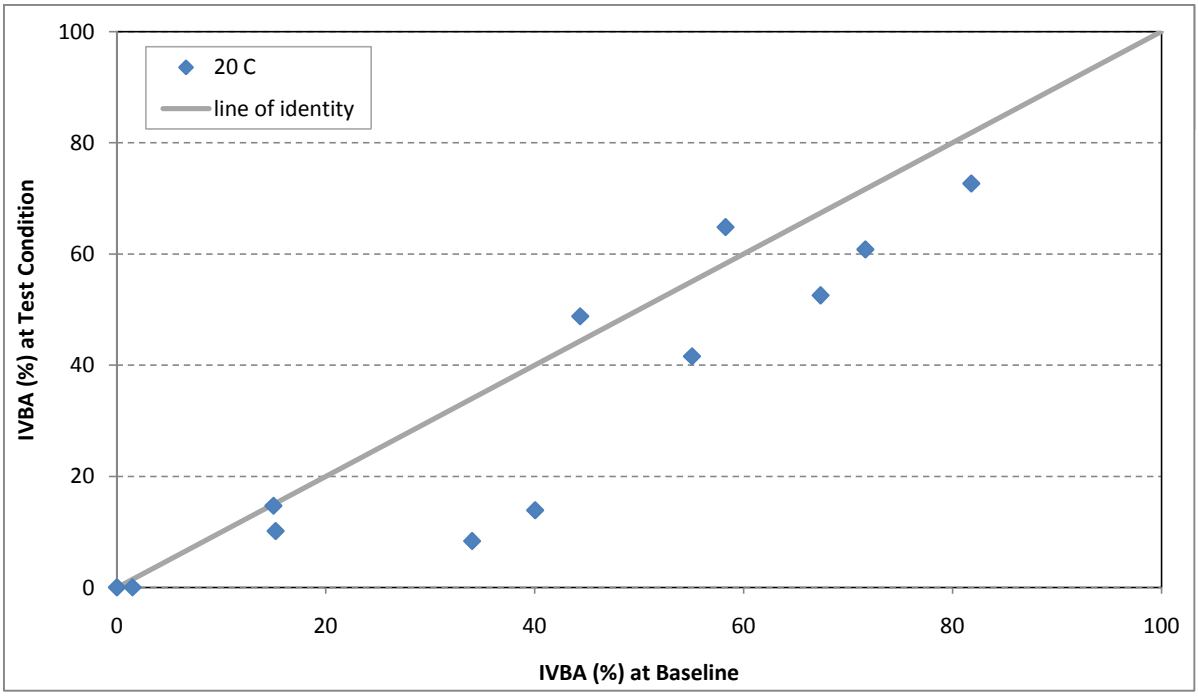
Note: Graphs include all test materials evaluated (including test materials that did not meet the Phase II data quality criteria specified in Section 2.4).

FIGURE A-2. EFFECT OF TEMPERATURE ON ARSENIC IVBA

Panel A: Arsenic IVBA at Varying Water Bath Temperatures



Panel B: Comparison to IVBA at Baseline Condition

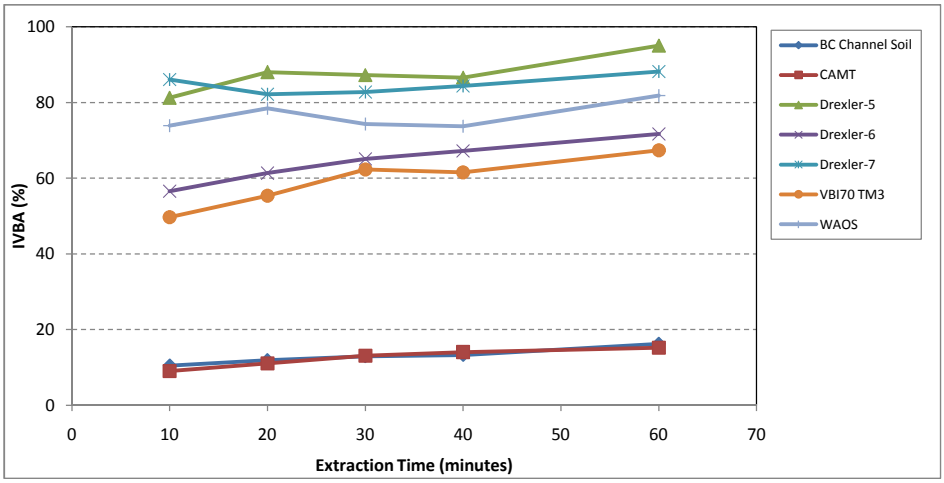


Baseline Condition = 37°C

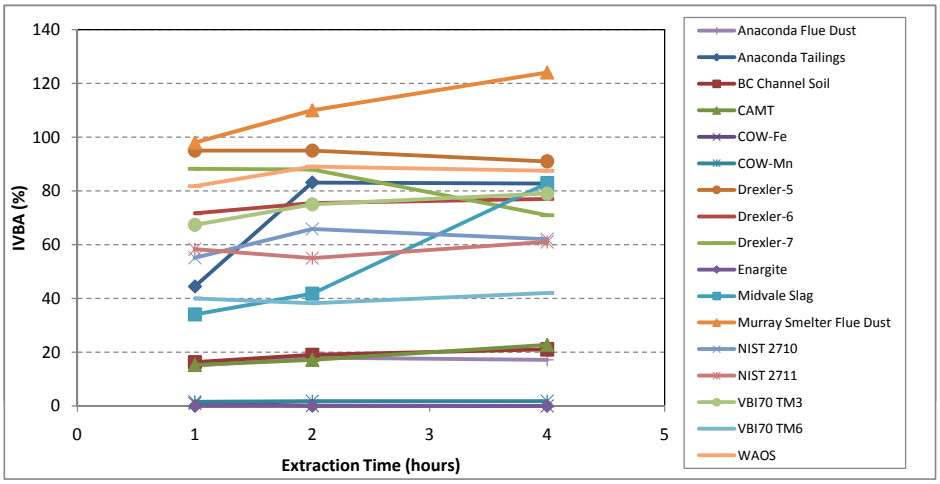
Note: Graphs include all test materials evaluated (including test materials that did not meet the Phase II data quality criteria specified in Section 2.4).

FIGURE A-3. EFFECT OF EXTRACTION TIME ON ARSENIC IVBA

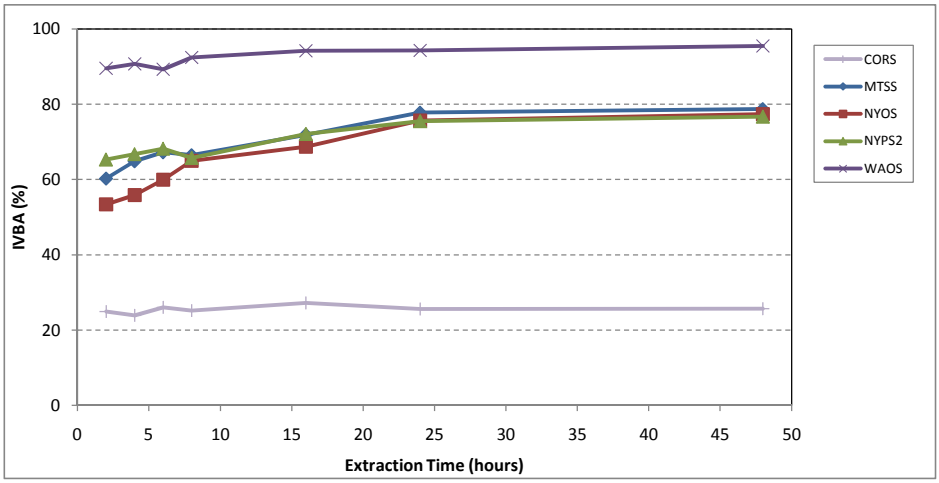
Panel A: 10-60 minutes



Panel B: 1-4 hours



Panel C: 1-48 hours



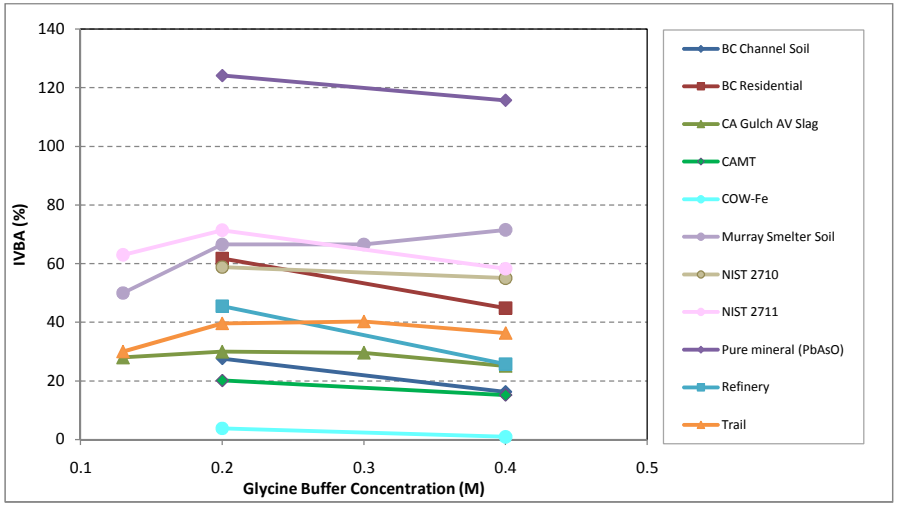
Baseline condition = 1 hour

Note: Graphs include all test materials evaluated (including test materials that did not meet the Phase II data quality criteria specified in Section 2.4).

FIGURE A-4. EFFECT OF GLYCINE BUFFER CONCENTRATION ON ARSENIC IVBA

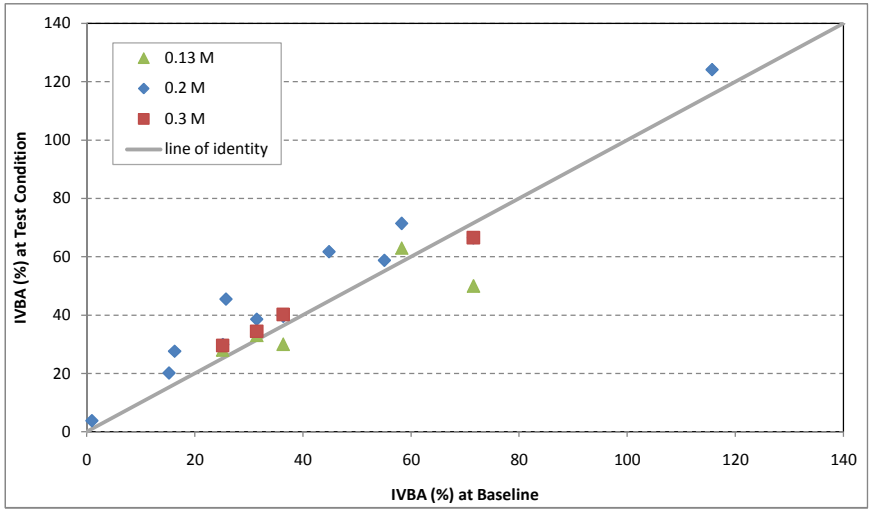
pH = 1.5

Panel A: Arsenic IVBA at Varying Glycine Buffer Concentrations



Baseline condition = pH 1.5, 0.4 M glycine

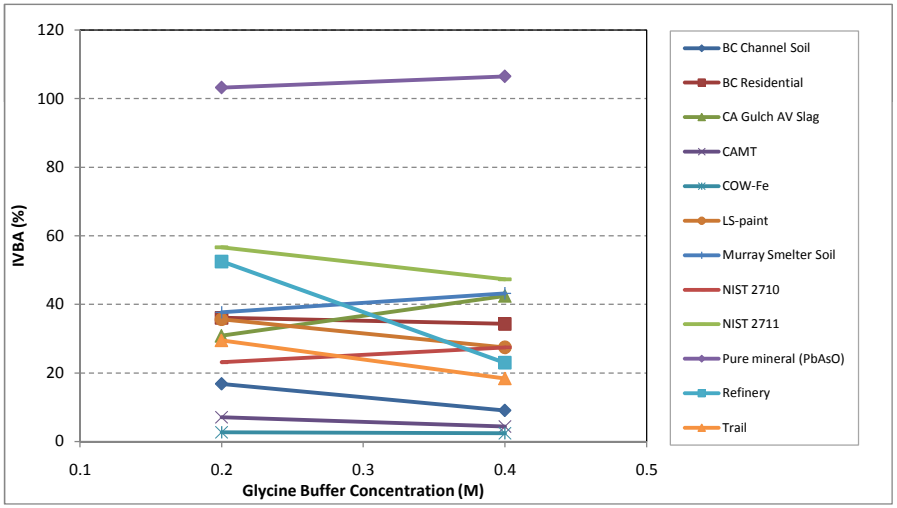
Panel B: Comparison to IVBA at Baseline Condition



Baseline condition = pH 1.5, 0.4 M glycine

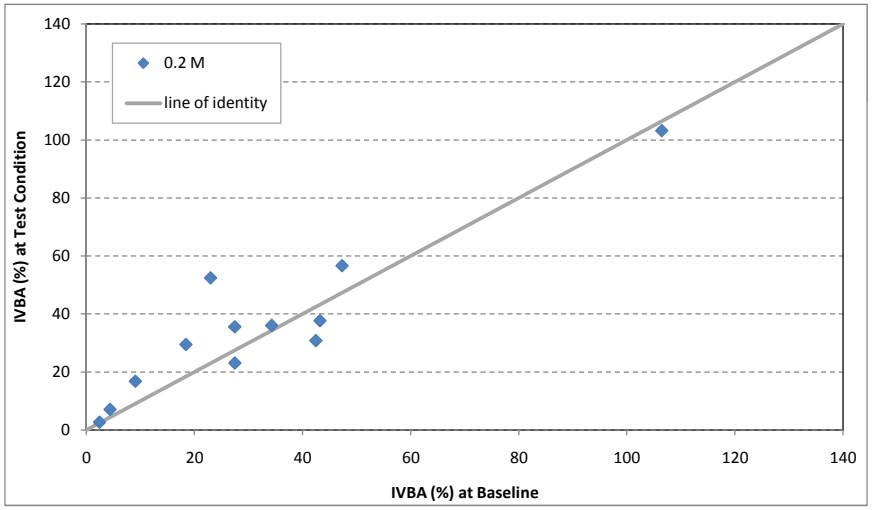
pH = 2.5

Panel A: Arsenic IVBA at Varying Glycine Buffer Concentrations



Baseline condition = pH 2.5, 0.4 M glycine

Panel B: Comparison to IVBA at Baseline Condition

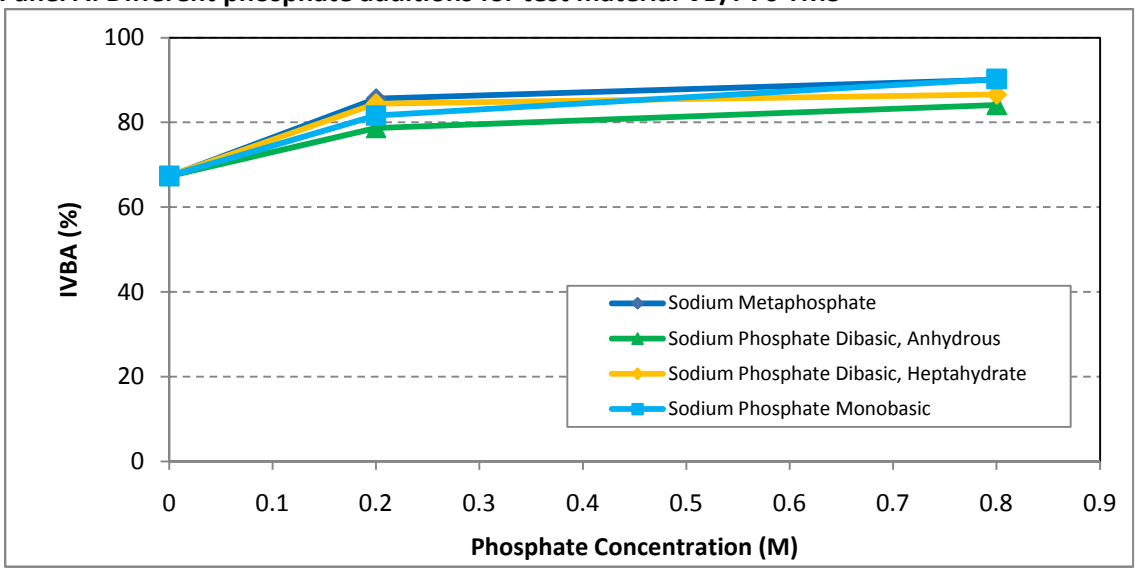


Baseline condition = pH 2.5, 0.4 M glycine

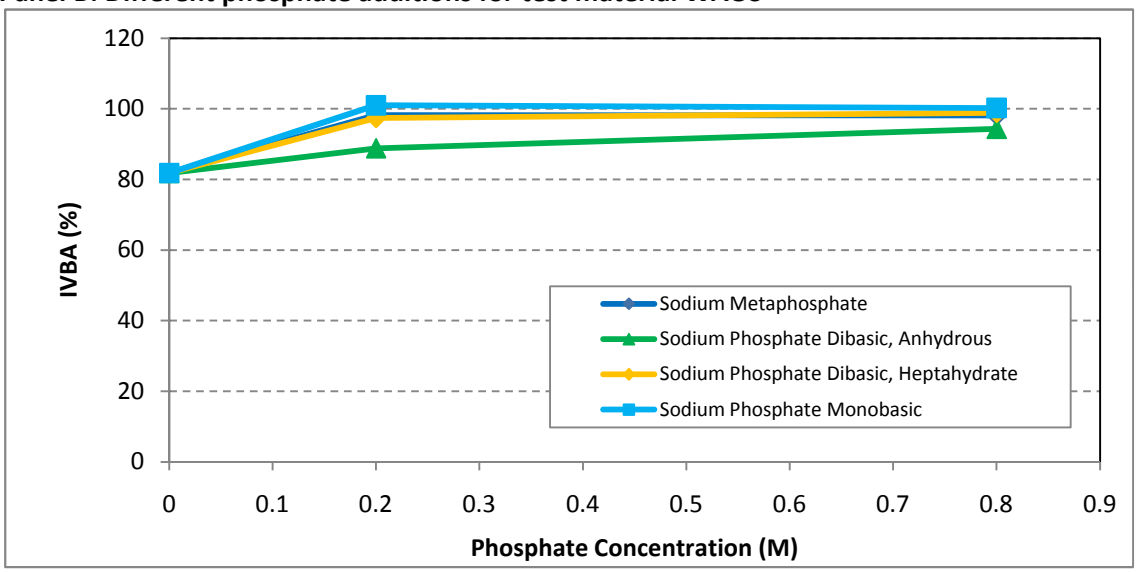
Note: Graphs include all test materials evaluated (including test materials that did not meet the Phase II data quality criteria specified in Section 2.4).

FIGURE A-5. EFFECT OF PHOSPHATE ADDITIONS ON ARSENIC IVBA

Panel A: Different phosphate additions for test material VB/I-70 TM3



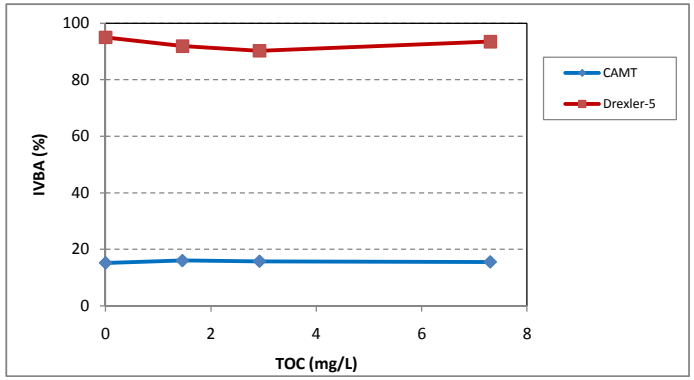
Panel B: Different phosphate additions for test material WAOS



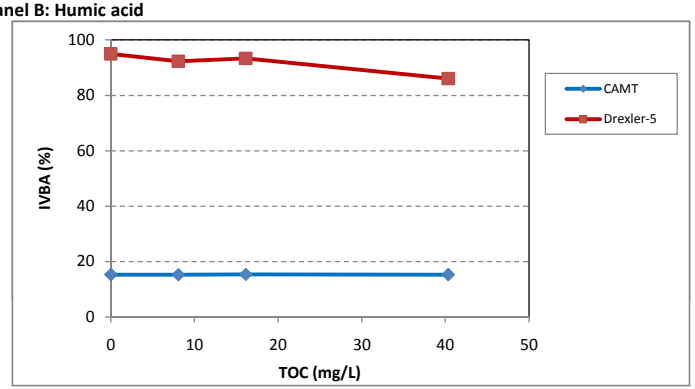
Panel A and Panel B show the effect of adding different forms of phosphate, including sodium metaphosphate, sodium phosphate dibasic (heptahydrate and anhydrous), and sodium phosphate monobasic, for two soils (VB/I70 TM3 and WAOS, respectively). As shown, there were no significant differences in the magnitude or direction of change in IVBA among the various forms of phosphate forms tested.

FIGURE A-6. EFFECT OF ORGANIC ACIDS AND SILICATE ON ARSENIC IVBA

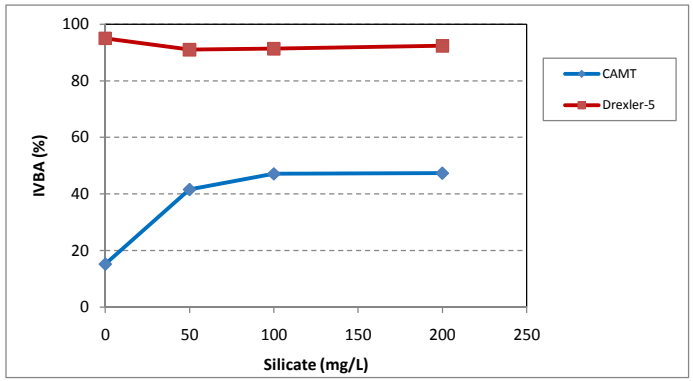
Panel A: Fulvic acid



Panel B: Humic acid



Panel C: Silicate



Baseline condition = no organic acids or silicate added

Soil organic matter (SOM) is primarily derived from the breakdown of plant matter and its residues, forming charbohydrates, amino acids and sugars. The final product is called humus. These substances are dark in color, amorphous, and hydrophilic. The vast majority of these compounds have not been identified. Because they exhibit a range in pKa values they are primarily defined based on their solubility. 1) Humic acid is soluble in alkali environments, but precipitates at acid conditions (pH <2.0), 2) Fulvic acid is soluble in both acid and alkali conditions, and 3) Humin is insoluble in either condition. Humic and fulvic acids have similar structures, however, fulvic acid is lower in molecular weight and contains more "oxygen" functional groups and less nitrogen and carbon.

Heavy metal interactions are generally considered to be a result of ion exchange, adsorption, or chelation. The extent of metal interaction is highly dependent on pH and type of competing metal cation. Based on previous studies showing a significant increase in soluble iron concentrations in natural waters do to the presence of increased humic substances, it is postulated that one could likewise increase soluble arsenic in the *in vitro* extract (increase IVBA) as a function of SOM.

Fulvic and humic acids were extracted from VB/I70 TM3 soil according to the method described in Anderson and Schoenau (1993), and varying amounts of the extracts were added to the IVBA extraction fluid. The effect of fulvic acid (Panel A) and humic acid (Panel B) addition on the arsenic IVBA result was evaluated for two test materials (CMAT and Drexler-5). As seen, the addition of humic and fulvic acid tended to decrease IVBA in the Drexler-5 test material, but not in the CMAT test material.

In a similar test, silicate (SiO_4) was added to the extraction fluid at concentrations from 50 to 200 mg Si/L. The results are shown in Panel C. The addition of silicate also tended to decrease IVBA in the Drexler-5 test material; however, IVBA increased in the CMAT test material.

Anderson, D.W. and Schoenau, J.J. 1993. Chapter 38: Soil Humus Fractions. In: M.R. Carter (Ed.), *Soil Sampling and Methods of Analysis.* Lewis Publishers.

Phase III Report:
Multi-Variate Evaluation of Key Variables Affecting IVBA Assay Results

ARSENIC IVBA OPTIMIZATION PROJECT
PHASE III REPORT
MULTI-VARIATE EVALUATION OF KEY VARIABLES AFFECTING IVBA ASSAY RESULTS

July 25, 2011

Prepared by:

U.S. Environmental Protection Agency
Region 8
15195 Wynkoop Street
Denver, CO 80202

Department of Geological Sciences
Benson Earth Science
2200 Colorado Ave.
Boulder, CO 80309

SRC, Inc.
999 18th Street, Suite 1975
Denver, CO 80202

Exponent
4141 Arapahoe Avenue, Suite 101
Boulder, CO 80303

1.0 INTRODUCTION

The overall goal of the arsenic *in vitro* bioaccessibility assay (IVBA) optimization project is to develop a method that reliably predicts the *in vivo* relative oral bioavailability (RBA) of arsenic in soil based on one or more measurements of IVBA performed *in vitro.* This project is being conducted in multiple phases, as follows (EPA 2009):

- Phase I of the project consisted of a literature review to indentify candidate test materials, and to inventory soils that were available for testing
- Phase II of the project investigated the effect of a wide range of experimental variable in the IVBA extraction protocol, and identified three key variable (pH, phosphate concentration, and hydroxylamine hydrochloride concentration) that are the most important.
- Phase III of the IVBA optimization project consisted of a series of studies to measure arsenic IVBA for a selected set of test substrates using various combinations of the three key extraction fluid variables identified in phase II. The objective of the Phase III work was to identify one or more IVBA extraction conditions that provide the best predictive relationship between IVBA and RBA (EPA 2009).

The purpose of this report is to summarize the work completed as part of Phase III of the IVBA optimization project.

2.0 PHASE III APPROACH

2.1 Baseline IVBA Procedure

The "baseline" IVBA value for each test material was established using the Relative Bioaccessability Leaching Procedure (RBALP) method, which has been validated for use in predicting RBA of lead from soil (Drexler and Brattin 2007). The baseline RBALP method involves placing 1 gram of the test substrate into a 125-mL wide-mouth high density polyethylene (HDPE) bottle to which is added 100 ± 0.5 mL of the extraction fluid (0.4 M glycine, pH 1.5). The samples are rotated end-over-end at 30 ± 2 revolutions per minute (rpm) for 1 hour while submerged in a water bath maintained at 37ºC. After 1 hour, the bottles are removed, dried, and placed upright on the bench top to allow the soil to settle to the bottom. A 15-mL sample of supernatant fluid is removed directly from the extraction bottle into a disposable 20-cc syringe, and then filtered through a 25 mm cellulose acetate filter with 0.45 um pores to remove any residual particulate matter. Filtered samples of extraction fluid are then analyzed for arsenic by inductively coupled plasma mass spectroscopy (ICP-MS) using EPA

Method 6020. IVBA is then calculated as the amount of arsenic present in the dissolved phase divided by the total amount of arsenic that was present in the test material.

2.2 Assay Variable Conditions and Test Substrates Evaluated

Based on the results of the Phase II study (EPA 2010), the following three experimental variables in the IVBA extraction were retained for multi-variate evaluation in Phase III:

1. pH of the extraction fluid
2. concentration of phosphate (PO_4) in the extraction fluid
3. concentration of hydroxylamine hydrochloride (HAH) in the extraction fluid

In the Phase III study, these key variables were evaluated in a Latin square design (i.e., one parameter was varied at a time while all other parameters were kept constant). The following 21 different assay variable conditions were evaluated:

Phosphate (M)	0	0.05		0.2		0.8	
HAH (M)	0	0.1	0.25	0.1	0.25	0.1	0.25
pH 1.5	x	x	x	x	x	x	x
pH 5.0	x	x	x	x	x	x	x
pH 7.0	x	x	x	x	x	x	x

Baseline IVBA extraction protocol

Other parameters of the baseline RBALP method remained unchanged (i.e., extraction time was maintained at 1 hour, water bath was maintained at a temperature of 37°C, glycine buffer concentration was maintained at 0.4 M, filter size was maintained at 0.45 um, and soil mass was maintained at 1 gram).

A total of 16 test substrates were selected for evaluation as part of Phase III. Table 2-1 summarizes the test substrates that were evaluated. These test substrates included several "synthetic" soils (constructed by spiking standard reference soils or soils from uncontaminated sites with specific arsenic mineral phases) and a number of contaminated site soils (obtained from various arsenic-contaminated sites across the United States). These test substrates were selected to provide:

- a range of soil arsenic concentrations and different mineralogical forms, including the most common forms encountered at Superfund sites, and
- a range of RBA results, including some test materials for which prior testing indicated that the IVBA result and the RBA result were not in good agreement.

3.0 PHASE III IVBA RESULTS

3.1 IVBA Dependence on Extraction Conditions

Table 3-1 presents the IVBA results for each test substrate for each of the 21 alternative extraction fluids tested. Figure 3-1 presents these IVBA results in a graphical format. Figure 3-1a shows IVBA as a function of pH without PO_4 or HAH additions, and Figure 3-1b shows IVBA as a function of pH with varying PO_4 and HAH additions.

As expected from Phase II studies, variation of pH generally had the most significant effect on IVBA. In nearly all samples, the highest IVBA value occurred at pH 1.5, with lower values at pH 5 and pH 7. Also as expected from Phase II results, addition of phosphate tended to increase IVBA values, often substantially, while addition of HAH usually had little additional effect.

In general, IVBA values at pH 5 were slightly higher than at pH 7, but several samples (e.g., CAMT, WAOS, Drex-7) had a minimum IVBA at pH 5, with clearly higher values at pH 7. This is presumably due to the solubility profiles of the predominant arsenic minerals in these samples (lead arsenic oxide, arsenic trioxide). Also note that soil spiked with sodium arsenate (St. Pete's, Drex-5) showed little dependence of IVBA on pH, with high values (> 90%) under all conditions. This is expected because of the generally high solubility of sodium arsenate.

3.2 Correlation between RBA and IVBA for Phase III Samples

Of the 16 test soils evaluated in Phase III, 5 have RBA values measured *in vivo* in swine, and 6 have RBA values measured in monkeys. Although not evaluated in swine, Drex-5 (sodium arsenate spiked soil) may be assumed to have an RBA value of approximately 100%, because all swine studies use sodium arsenate as the reference material.

Table 3-2 summarizes the best fit linear regression between RBA (dependent variable) and IVBA (independent variable) for each set of assay variable conditions. As shown in Panel A, when the sodium arsenate spiked test materials (St. Pete's and Drex-5) are included in the data sets, a good correlation ($R^2 > 0.7$) is apparent for swine, monkey, and the combined data under a number of conditions. These results provide good evidence that it will be possible to develop a reliable model for prediction of arsenic RBA from IVBA measurements.

However, it should be noted that these good correlations are strongly influenced by the sodium arsenate data points. This is because RBA values for most authentic test soils are clustered at the low end of the range (mainly in the 20-30% range), so addition of a data point at the high end of the range (approximately 100%) has a large effect on the regression results. Indeed, when the sodium arsenate data points are not included (Panel B), correlations are not as strong, and any high R^2 values are associated with data sets that have low slope (low dependence of RBA on

IVBA). These results emphasize the difficulty of establishing a reliable model when variation in RBA between test materials spans only a relatively narrow range.

4.0 REFERENCES

Davis, A., M.V. Ruby M. Bloom, R. Schoof, G. Freeman, P.D. Bergstrom. 1996. Mineralogic constraints on the bioavailability of arsenic in smelter-impacted soils. *Environmental Science and Technology* 30:392-399. http://pubs.acs.org/doi/pdf/10.1021/es9407857

Drexler, J.W. and Brattin, W. 2007. An *in vitro* procedure for estimation of lead relative bioavailability: with validation. *Human and Ecological Risk Assessment* 13(2):383-401.

U.S. Environmental Protection Agency (EPA). 2009. Workplan to Optimize an *in vitro* Method to Predict the Relative Oral Bioavailability of Arsenic. Prepared by EPA Region 8, Dr. John Drexler, Syracuse Research Corporation, and Exponent. March 13, 2009.

EPA. 2010. Arsenic IVBA Optimization Project - Phase II Report: Identification of Principal Variables Affecting IVBA Assay Results. Prepared by EPA Region 8, Dr. John Drexler, SRC, Inc., and Exponent. September 7, 2010.

TABLE 2-1: TEST SUBSTRATES EVALUATED IN PHASE III

Test Subtrate Identifier	Description	Soil As Conc.	Predominant Arsenic Minerology	Baseline IVBA (%)		*in vivo* RBA (%)	
				n	Mean ± StDev	Mean ± St Err	Species
BCCS	Bingham Creek Channel Soil, Salt Lake City, UT	149	FeAs sulfate, PbAsO	20	16.4 ± 2.5	39.3 ± 8.1	S
CAMT	California mine tailings	300	arsenopyrite, FeOOH	19	15.3 ± 4.7	19.0 ± 2.0	M
COSS-1	Colorado smelter soil (Smeltertown)	1,492	FeAs sulfate	8	7.7 ± 0.7	5.0 ± 4.0	M
COW-Fe	Natural soil - Cave of the Winds, CO	689	FeAsO	4	0.93 ± 1.26	---	
COW-Mn	Natural soil - Cave of the Winds, CO	490	MnOOH	2	1.5 ± 2.1	---	
St. Pete's	Florida soil spiked with sodium arsenate	514	NaAsO	2	106.1 ± 2.4	93.0	M
Drex-5	Colorado soil spiked with sodium arsenate	1,239	NaAsO	5	95.0 ± 5.1	---	
Drex-6	Natural soil - Globeville site	1,414	AsMO, CaMO, PbAsO	4	71.7 ± 8.4	---	
Drex-7	Colorado soil spiked with arsenic trioxide	1,767	As2O3	2	88.2 ± 3.1	---	
NIST 2710A	Butte soil spiked with lead oxide	1,540	FeOOH, FeAs sulfate	7	42.2 ± 1.1	41.8 ± 1.4	S
NYOS	New York orchard soil	123	MnOOH, PbAsO4	7	33.7 ± 2.3	15.0 ± 8.0	M
NYPS3	New York pesticide plant soil, T15-E4 (13B)	339	FeOOH	5	32.8 ± 6.9	28.0 ± 10.0	M
VBI70 TM1	VBI70 TM1 (CO residential soil)	312	As2O3, PbAsO	6	68.4 ± 7.8	40.3 ± 3.6	S
VBI70 TM3	VBI70 TM3 (CO residential soil)	390	As2O3	9	69.9 ± 8.2	36.7 ± 3.3	S
WAOS	Washington orchard soil	301	PbAsO4	11	81.2 ± 2.7	24.0 ± 9.0	M
Butte TM1	Silver Bow Creek/Butte Area NPL Site, Butte, MT	234	FeAs sulfate	22	8.6 ± 1.7	17.8 ± 3.2	S

--- = not evaluated *in vivo*

S = swine

M = monkey

TABLE 3-1: PHASE III IVBA RESULTS

	Arsenic IVBA (%)																				
PO_4	None			0.05M						0.2M						0.8M					
HAH	None			0.1M			0.25M			0.1M			0.25M			0.1M			0.25M		
pH	1.5	5.0	7.0	1.5	5.0	7.0	1.5	5.0	7.0	1.5	5.0	7.0	1.5	5.0	7.0	1.5	5.0	7.0	1.5	5.0	7.0
BCCS	16.4	3.0	3.3	34.1	6.6	4.6	32.4	6.6	6.0	38.4	8.8	6.0	38.7	9.4	6.7	45.9	15.1	13.3	45.9	15.0	12.9
CAMT	15.3	3.0	18.8	45.0	11.2	6.9	51.2	11.9	8.1	74.9	14.8	9.7	74.5	13.5	11.3	98.9	23.3	16.1	91.9	22.7	17.7
COSS-1	7.7	1.0	1.0	11.8	2.1	1.6	12.0	2.3	1.8	14.5	2.5	2.1	13.8	2.3	2.2	16.6	4.1	3.9	16.9	4.0	3.5
COW-Fe	0.9	0.5	0.0	2.4	1.9	1.3	2.4	2.0	1.4	2.7	1.9	1.6	2.9	1.9	1.5	2.8	2.1	2.0	2.8	2.1	2.0
COW-Mn	1.5	1.0	1.0	82.5	24.3	17.4	76.7	14.4	12.7	82.5	29.3	32.2	80.5	23.8	26.4	80.5	38.3	41.1	82.5	38.0	37.4
St. Pete's	106.0	98.0	100.0	108.0	108.0	104.0	103.0	105.0	110.0	104.0	107.0	104.0	103.0	104.0	102.0	108.0	111.0	115.0	104.0	110.0	110.0
DREX-5	95.0	73.0	76.9	102.5	89.3	91.9	98.2	97.2	87.0	97.7	94.7	89.0	98.8	89.6	94.6	95.9	98.9	96.5	89.3	94.2	94.6
DREX-6	71.7	14.0	15.0	87.4	25.9	12.7	85.9	28.2	14.4	91.1	27.4	17.0	93.9	27.5	21.1	95.1	42.9	34.4	89.1	42.7	34.1
DREX-7	88.2	7.0	13.0	99.3	3.3	30.5	98.2	6.5	83.3	100.3	2.8	92.6	86.4	2.5	92.1	98.1	13.5	98.4	95.6	10.1	94.6
NIST 2710A	42.2	1.0	1.9	82.3	16.3	13.3	81.8	17.5	14.8	86.9	20.1	18.2	85.6	19.9	20.0	86.2	36.7	34.7	87.3	38.7	36.2
NYOS	33.7	9.0	10.4	81.8	18.8	15.3	79.7	20.8	16.3	96.5	26.3	22.9	93.7	27.0	25.3	104.3	51.2	44.6	101.3	55.0	42.5
NYPS3	32.8	4.0	4.0	71.5	23.0	13.8	72.0	24.3	16.5	94.9	30.3	22.6	89.2	31.0	25.3	104.1	49.5	36.9	96.4	48.5	38.7
VBI70 TM1	68.4	31.0	30.3	84.8	40.7	35.4	86.2	41.9	36.4	88.0	47.1	41.9	91.9	51.0	45.6	91.1	60.6	57.2	87.5	63.5	56.6
VBI70 TM3	69.9	27.0	22.1	94.5	40.1	32.8	92.0	43.3	36.1	96.0	46.8	41.1	94.2	50.0	45.7	93.5	71.7	63.9	94.2	67.6	65.7
WAOS	81.2	28.0	34.1	104.2	4.3	3.1	97.5	4.6	4.8	105.1	7.4	6.3	109.9	7.0	7.8	113.6	14.8	77.4	103.1	15.3	71.9
Butte TM1	8.6	0.0	0.6	30.8	4.3	3.2	32.7	4.5	3.3	39.6	5.9	4.2	39.9	6.0	4.6	49.0	11.4	9.0	49.0	10.8	9.0

TABLE 3-2: PHASE III IVIVC RESULTS

Panel A: Including Sodium Arenate Data

Data Set	Number of samples	PO_4 / HAH / pH	None / None / 1.5	None / None / 5.0	None / None / 7.0	0.05 M / 0.1 M / 1.5	0.05 M / 0.1 M / 5.0	0.05 M / 0.1 M / 7.0	0.05 M / 0.25 M / 1.5	0.05 M / 0.25 M / 5.0	0.05 M / 0.25 M / 7.0	0.2 M / 0.1 M / 1.5	0.2 M / 0.1 M / 5.0	0.2 M / 0.1 M / 7.0	0.2 M / 0.25 M / 1.5	0.2 M / 0.25 M / 5.0	0.2 M / 0.25 M / 7.0	0.8 M / 0.1 M / 1.5	0.8 M / 0.1 M / 5.0	0.8 M / 0.1 M / 7.0	0.8 M / 0.25 M / 1.5	0.8 M / 0.25 M / 5.0	0.8 M / 0.25 M / 7.0
Swine	6	Slope	0.63	0.87	0.87	0.57	0.78	0.78	0.55	0.72	0.82	0.56	0.74	0.78	0.56	0.73	0.73	0.66	0.64	0.68	0.58	0.66	0.68
		Intercept	14.5	26.3	26.4	5.6	20.2	22.5	7.0	20.5	20.9	4.5	18.6	19.9	3.7	18.4	19.6	-4.4	14.5	14.7	2.0	13.9	14.9
		R^2	0.58	0.78	0.83	0.40	0.80	0.85	0.35	0.81	0.83	0.31	0.78	0.79	0.32	0.71	0.78	0.29	0.62	0.67	0.21	0.60	0.64
Monkey	6	Slope	0.67	0.81	0.81	0.55	0.77	0.78	0.61	0.79	0.74	0.46	0.79	0.80	0.45	0.81	0.83	0.37	0.74	0.66	0.42	0.73	0.71
		Intercept	-0.2	11.5	8.0	-8.2	9.3	11.9	-11.2	8.3	11.2	-7.0	5.8	8.3	-5.8	5.7	6.7	-2.8	-0.7	-1.6	-5.7	-0.5	-3.2
		R^2	0.68	0.92	0.91	0.41	0.95	0.95	0.42	0.95	0.95	0.26	0.94	0.94	0.25	0.93	0.94	0.18	0.82	0.74	0.21	0.79	0.76
Combined	12	Slope	0.66	0.82	0.80	0.56	0.79	0.79	0.59	0.78	0.78	0.46	0.78	0.81	0.46	0.79	0.80	0.36	0.71	0.66	0.40	0.72	0.69
		Intercept	6.4	19.2	18.1	-1.6	14.4	16.8	-2.7	13.7	16.1	2.5	11.5	13.6	2.2	11.2	12.3	8.3	5.7	7.2	6.2	5.7	6.0
		R^2	0.61	0.79	0.76	0.38	0.85	0.88	0.37	0.86	0.86	0.22	0.84	0.85	0.23	0.81	0.85	0.13	0.71	0.63	0.14	0.69	0.65

Panel B: Excluding Sodium Arenate Data

Data Set	Number of samples	PO_4 / HAH / pH	None / None / 1.5	None / None / 5.0	None / None / 7.0	0.05 M / 0.1 M / 1.5	0.05 M / 0.1 M / 5.0	0.05 M / 0.1 M / 7.0	0.05 M / 0.25 M / 1.5	0.05 M / 0.25 M / 5.0	0.05 M / 0.25 M / 7.0	0.2 M / 0.1 M / 1.5	0.2 M / 0.1 M / 5.0	0.2 M / 0.1 M / 7.0	0.2 M / 0.25 M / 1.5	0.2 M / 0.25 M / 5.0	0.2 M / 0.25 M / 7.0	0.8 M / 0.1 M / 1.5	0.8 M / 0.1 M / 5.0	0.8 M / 0.1 M / 7.0	0.8 M / 0.25 M / 1.5	0.8 M / 0.25 M / 5.0	0.8 M / 0.25 M / 7.0
Swine	5	Slope	0.20	0.23	0.27	0.20	0.26	0.30	0.19	0.24	0.30	0.20	0.24	0.26	0.20	0.21	0.24	0.23	0.18	0.21	0.23	0.20	0.20
		Intercept	26.9	32.3	32.0	22.3	29.6	29.8	22.7	29.6	29.4	21.2	29.1	29.4	21.1	29.4	29.4	18.3	28.0	27.8	18.3	27.4	27.8
		R^2	0.33	0.13	0.14	0.37	0.22	0.21	0.34	0.21	0.23	0.33	0.23	0.23	0.33	0.22	0.23	0.30	0.24	0.27	0.30	0.28	0.27
Monkey	5	Slope	0.18	0.33	0.29	0.18	0.53	0.58	0.21	0.48	0.67	0.20	0.41	0.45	0.20	0.38	0.43	0.19	0.21	0.20	0.20	0.18	0.23
		Intercept	12.0	15.2	14.2	6.7	12.0	13.5	5.1	12.1	11.8	2.5	11.5	12.4	3.1	12.0	12.0	1.7	12.2	11.2	1.9	12.8	10.2
		R^2	0.34	0.17	0.19	0.53	0.28	0.16	0.60	0.27	0.26	0.71	0.31	0.24	0.68	0.29	0.26	0.72	0.25	0.39	0.68	0.21	0.46
Combined	10	Slope	0.23	0.36	0.22	0.20	0.50	0.59	0.22	0.47	0.58	0.16	0.42	0.47	0.17	0.39	0.43	0.12	0.27	0.20	0.15	0.27	0.23
		Intercept	17.9	22.8	23.9	13.9	18.3	19.0	13.0	18.4	18.3	14.7	17.8	18.4	14.5	18.2	18.3	16.9	17.4	19.6	14.8	17.5	18.6
		R^2	0.25	0.13	0.05	0.25	0.32	0.32	0.25	0.31	0.34	0.16	0.30	0.31	0.17	0.30	0.30	0.09	0.26	0.16	0.13	0.25	0.19

$R^2 > 0.7$

FIGURE 3-1a: IVBA vs pH (without PO_4 or HAH)

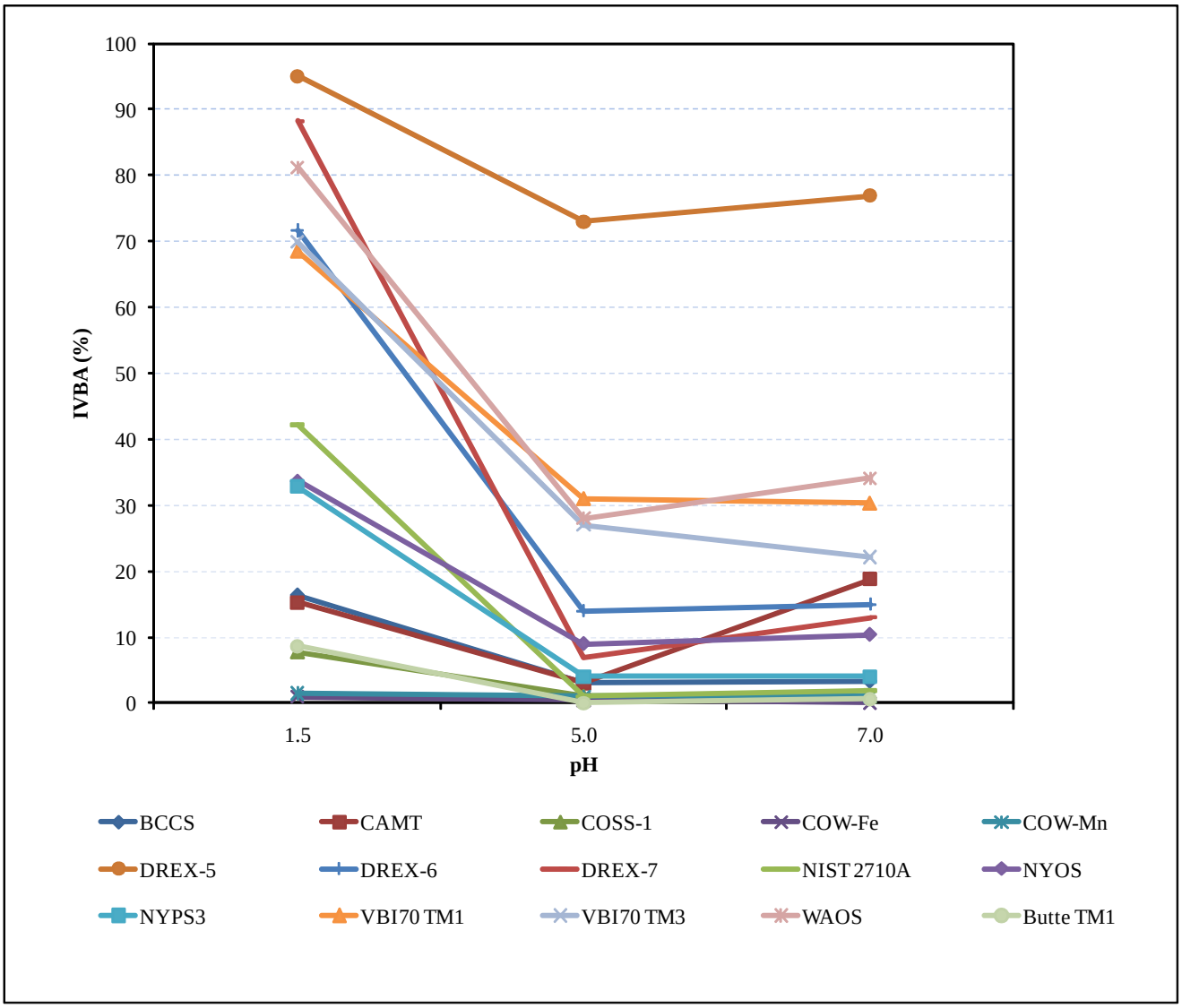
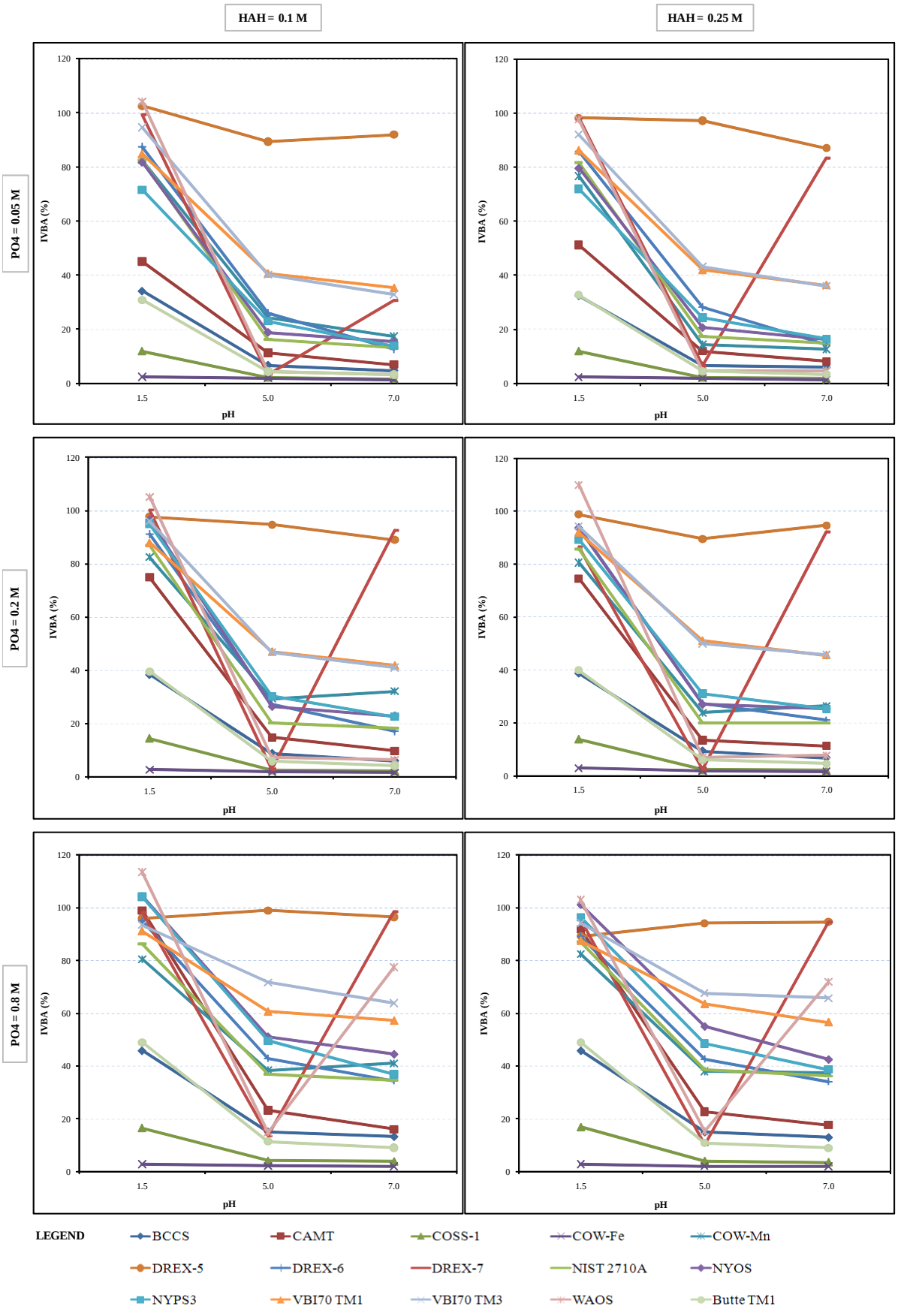


FIGURE 3-1b: IVBA vs pH (with PO_4 and HAH)



Phase IV/V Report:
Selection of Optimum RBA Prediction Methods

ARSENIC IVBA OPTIMIZATION PROJECT

PHASE IV/V REPORT
SELECTION OF OPTIMUM RBA PREDICTION METHODS

December 13, 2011

Prepared by:

U.S. Environmental Protection Agency
Region 8
15195 Wynkoop Street
Denver, CO 80202

University of Colorado
Department of Geological Sciences
Benson Earth Science
2200 Colorado Ave.
Boulder, CO 80309

SRC, Inc.
999 18th Street, Suite 1975
Denver, CO 80202

Exponent
4141 Arapahoe Avenue, Suite 101
Boulder, CO 80303

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ATTACHMENTS

1.0 INTRODUCTION

The overall goal of the arsenic *in vitro* bioaccessibility assay (IVBA) optimization project is to develop a method that reliably predicts the *in vivo* relative oral bioavailability (RBA) of arsenic in soil based on one or more measurements performed *in vitro.* This project is being conducted in multiple phases (EPA 2009):

- Phase I of the project consisted of a literature review to indentify candidate test materials, and to inventory soils that were available for testing
- Phase II of the project investigated the effect of a wide range of experimental variables in the *in vitro* extraction protocol, and identified three key variables (pH, phosphate [PO_4] concentration, and hydroxylamine hydrochloride [HAH] concentration) that are the most important.
- Phase III of the project consisted of a series of studies to measure arsenic IVBA for a selected set of test substrates using various combinations of the three key extraction fluid variables identified in phase II. The objective of the Phase III work was to select up to three alternative combinations of extraction fluid variables that provide the best predictive relationship between IVBA and RBA (EPA 2009).
- Phase IV of the project consisted of finalizing the IVBA extraction conditions. Phase V consisted of testing the final extraction protocols on an expanded set of test substrates, and assessing the degree of correlation between the IVBA values and the RBA values for the samples in the data set. The objective of this phase of the project was to develop a mathematical model that could reliably predict RBA from one or more IVBA measurements for a wide range of test materials.

The purpose of this report is to summarize the work completed as part of Phase IV and V of the IVBA optimization project, as well as some additional statistical analyses that were performed to determine whether inclusion of arsenic mineralogy data along with IVBA data would help strengthen predictive models.

2.0 PHASE IV STUDY DESIGN

2.1 Test Materials

An expanded list of 39 test substrates was evaluated in Phase IV. This expands considerably on the set of 16 test substrates evaluated in Phase III of the project that were used to identify and optimize IVBA methods. **Table 2-1** summarizes the attributes of these 39 test materials. The data set includes all soils (both site soils and soils spiked with arsenic compounds) for which reliable RBA data in swine and/or monkeys were available, and for which sufficient material was

available to support IVBA testing. Although not evaluated in swine, Drexler-5 (sodium arsenate spiked soil) may be assumed to have an RBA value of approximately 100%, because all swine studies use sodium arsenate as the reference material. Soil samples evaluated in early swine studies in which no internal sodium arsenate dose-response curve was available were excluded, because absence of an internal data set for sodium arsenic makes the RBA values less reliable than for samples evaluated with an internal standard.

2.2 Extraction Conditions

Based on the results of the Phase III study, the following three IVBA extraction conditions were selected for further evaluation in Phase IV:

1. pH 1.5, without phosphate (PO_4) or hydroxylamine hydrochloride (HAH) additions
2. pH 7.0, without PO_4 or HAH additions
3. pH 7.0, with 0.05M PO_4 and either 0.1 or 0.25M HAH[1]

Other parameters of the RBALP method remained unchanged (i.e., extraction time was maintained at 1 hour, water bath was maintained at a temperature of 37°C, glycine buffer concentration was maintained at 0.4M, filter size was maintained at 0.45 um, soil mass was maintained at 1 gram).

3.0 RBA PREDICTION FROM IVBA DATA

3.1 Data

Table 3-1 summarizes the RBA data and IVBA data collected for the 39 test materials evaluated in Phase IV.

3.2 Regression Analysis

The relationship between RBA and IVBA was evaluated using a linear model:

$$RBA = a + b \cdot IVBA$$

Most authors estimate the model coefficients (a and b) by ordinary linear regression (OLR). A potential limitation to this approach is that OLR assumes that variability (measurement error) in RBA is both normal and homoscedastic (i.e., the magnitude of the variability in RBA does not depend on RBA). However, available data indicate that variability (measurement error) in RBA

[1] There was no significant difference in the results for 0.1 or 0.25 M HAH so the results were averaged.

tends to be heteroscedastic, increasing as RBA increases (EPA 2005, Drexler and Brattin 2007). For this reason, model coefficients were estimated using the method of maximum likelihood estimation (MLE), assuming that measurement error in RBA is normal with a constant coefficient of variation (CV):

$$RBA_{obs} \sim \text{Normal}(RBA_{true}, RBA_{true} \cdot CV)$$

Table 3-2 summarizes the model coefficients estimated using MLE. For comparison to other reports that utilize OLR, the coefficients derived by that approach are also shown.

For swine, the best fit model ($R^2 = 0.723$) is obtained using IVBA measurements at pH 1.5. For monkey, the best fit ($R^2 = 0.755$) is obtained using IVBA measurements obtained at pH 7 in the presence of 0.05 M phosphate and 0.1-0.25 M HAH. These two relationships are shown graphically in **Figure 3-1**. None of the extraction conditions provided a good correlation when the data sets from swine and money were combined. **Figure 3-2** plots the observed RBA values as a function of the RBA values predicted by the best fit models.

4.0 RBA PREDICTIONS THAT INCORPORATE MINERALOGY DATA

4.1 Basic Models

Although the best-fit models described above are able to provide a good prediction of RBA based on IVBA data alone, additional modeling efforts were performed to determine if inclusion of arsenic mineralogy data along with the IVBA data could provide an improvement in model accuracy. Three basic models were evaluated, as follows:

Model 1: $$RBA = \sum_{i=1}^{b} f(i) \cdot RBA(i)$$

Model 2: $$RBA = k \cdot IVBA_{best} + \sum_{i=1}^{b} f(i) \cdot RBA(i)$$

Model 3: $$RBA = k1 \cdot IVBA_{1.5} + k2 \cdot IVBA_{7} + \sum_{i=1}^{b} f(i) \cdot RBA(i)$$

where:

RBA(i) = phase-specific RBA (estimated by fitting)
$IVBA_{best}$ = IVBA measured at pH 1.5 (no additions) for swine or
IVBA measured at pH 7 (with PO_4 and HAH) for monkeys
$IVBA_{1.5}$ = IVBA measured at pH 1.5 (no additions)
$IVBA_{7}$ = IVBA measured at pH 7.0 (with PO4 and HAH)
f(i) = fraction of sample in phase "i"

k(i) = empiric fitting constants

The basic concept behind Model 1 is that each unique mineralogical type of arsenic ("phase") has an inherent phase-specific RBA, and that the RBA of a soil sample containing a mixture of arsenic phases reflects the amount-weighted average of the phase-specific RBA values. Model 2 tests whether combining the best IVBA value (pH 1.5 for swine, pH 7 + PO4 for monkey) with the phase data provides an improved fit over Model 1. Model 3 tests whether including both IVBA values (pH 1.5 and pH 7 + PO4) yields an improved fit over Model 2. In essence, Models 2 and 3 combine two lines of evidence (IVBA data and phase data) to yield "multiple lines of evidence" models that may be more reliable than either line alone.

4.2 Speciation Data

The amount of arsenic in each phase can be expressed in two alternative ways:

- Length-weighted frequency (LWF). In this approach, the LWF of each phase is calculated as the sum of the maximum lengths of each arsenic-bearing particle assigned to each phase, divided by the sum of the lengths for all arsenic-bearing particles.

- Relative arsenic mass (RAM). In this approach, the relative mass of arsenic in each particle is calculated as the product of particle maximum length times the density of that phase times the concentration of arsenic in that phase. Then, the RAM of each phase is calculated as the sum of the relative arsenic masses in each arsenic-bearing particle assigned to each phase, divided by the sum of the relative arsenic masses for all arsenic-bearing particles.

Arsenic mineralogy in each test material was evaluated using electron microprobe analysis (EMPA). In brief, the EMPA procedure uses an electron microprobe with combined energy dispersive spectrometer (EDS) and multiple wavelength dispersive spectrometers (WDS) to evaluate the elemental composition of each arsenic-bearing particle. Based on the elemental composition, each particle is assigned to a phase. In some cases, these phases correspond to a specific mineral with fixed stoichiometry, while in other cases, the "phase" represents a range of elemental compositions with varying stoichiometry.

Mineralogy of all test materials was evaluated by Dr. John Drexler at the Laboratory for Environmental and Geological Studies at the University of Colorado in Boulder, Colorado. A detailed protocol is available online at http://www.colorado.edu/geolsci/legs/EMPASOP1.html, and a copy of this protocol is provided in **Attachment 1**. A total of 15 different mineral phases were observed in one or more samples included in the Phase IV data set of 39 samples. **Table 4-1** presents the results for LWF, and **Table 4-2** presents the results for RAM.

4.3 Fitting Protocol

Each model was fitted to each data set using MLE, assuming normal errors in RBA with a constant coefficient of variation. The quality of fit was evaluated using Akaike's Information Criterion (AIC), corrected for finite sample size (AIC_c):

$$AIC_c = 2 \cdot p - 2 \cdot LL + 2 \cdot p \cdot (p-1)/(N-p-1)$$

where:

p = number of parameters in model being fitted to the data
LL = optimum (maximum) log-likelihood value
N = Number of samples in the data set being fitted

The AIC_c statistic considers both the absolute quality of the model fit to the data (as reflected in the log-likelihood value), and also the number of fitting parameters in the model. The model with the lowest AIC_c value is identified as the preferred model (the best compromise between maximum model fit and minimum parameter number). Models with AIC_c values that are within about 2 or less of the best fit model may also be considered appropriate fits.

When testing the utility of including phase data in RBA prediction models, it is important to understand that, if different phases have similar phase-specific RBA values, then these phases may be collapsed to yield a simpler and more robust model. Accordingly, fitting was performed in a series of steps, as follows:

1. Assign each phase to a different bin (N = 15)
2. Solve for 15 phase-specific RBA(i) values using MLE
3. Rank order the 15 RBA(i) values (low to high)
4. Combine phases with similar RBA(i) values into bins. Investigate a range of different binning strategies, ranging from 1 bin (all phases are assigned to the same bin) to 15 bins (each phase is assigned to a different bin). For strategies with 2, 3 or 4 bins, systematically investigate all possible strategies to find the optimum. For 5 or more bins, use natural breaks in the rank-ordered RBA(i) values to decide how to group the phases.
5. For each different binning strategy, fit the model and record the LL and AIC_c. Plot both AIC_c and LL as a function of number of bins. Find the optimum number of bins based on minimization of AIC_c.

An example of the typical pattern that is obtained is shown in **Figure 4-1**. As expected, log-likelihood (LL) is maximum (optimal) when each of the 15 phases is treated as an individual bin,

and is minimum when all phases are assigned to the same bin. In general, the minimum AIC_c value is obtained when the 15 phases are collapsed into 3 bins (as illustrated in the example) or 4 bins.

4.4 Results

Table 4-3 presents the results of fitting each of the three data sets to each of the three models for each of the two alternative metrices of arsenic amount in each phase. As shown, the optimum fit for the swine data set is obtained using Model RAM-3, although model RAM-2 is nearly equivalent. For the monkey and the combined data sets, the best fit is obtained using model LWF-2. The best-fit coefficients and phase bins for these models are shown in **Table 4-4**. **Figure 4-2** plots the correlation between observed and predicted RBA for each data set using the best-fit models.

5.0 DISCUSSION

Data Set Used for Model Development

One of the key elements of the Phase IV investigation was the inclusion of a large number of test materials in the data set used to develop predictive mathematical models. Other authors have proposed IVBA-RBA models based on much smaller data sets (e.g., Medlin 1997, Rodriguez et al. 1999, Juhasz et al. 2007). In our experience, we have found that models that are successful for a small data set often do not work well when the data set is expanded to include test materials with a wide range of arsenic mineralogy. We have restricted our analysis to test materials for which we have high confidence in the results of the *in vivo* RBA assays. Therefore, the models proposed here (based on fitting 20-39 samples) are considered to be the most robust that is possible at the present time.

Inclusion of Soils Spiked with Sodium Arsenate

The best-fit regression models described above are strongly influenced by the inclusion of sodium arsenate (NaAs) spiked soil (Drexler-5 for swine and St. Pete's for monkey). The effect of excluding these soils is to decrease the strength of the correlation, as shown below:

Data set	R^2 value for IVBA Only Regression Models		R^2 value for IVBA Plus Phase Regression Models	
	With NaAs	Without NaAs	With NaAs	Without NaAs
Swine	0.723	0.532	0.906	0.776
Monkey	0.755	0.057	0.816	0.300

The effect is most significant for the monkey data set. This is because the data for all but one of the test materials evaluated in monkeys (NYPS 2) have IVBA values clustered at the low end of the range (IVBA = 0-20%). This makes it very difficult to fit a reliable model without additional data points that fall outside of this narrow data range.

Because of the substantial effect of including the sodium arsenate test materials on the results of the model fitting, it is important to consider the degree to which the inclusion of these samples is appropriate or not. Arguments for and against inclusion include the following:

Arguments for inclusion:

- Inclusion of the samples helps constrain the slope of the IVBA-RBA regression model line by providing a data point at the high end of the range.
- Although high arsenic RBA values are not common, values of 75% or higher have been observed in a few authentic soil samples (e.g., a soil from the Ruston Tacoma site [Lorenzana et al. 1996] and from an Australian railway corridor soil [Juhasz et al. 2007]), so inclusion of this value is not unreasonable.
- The RBA of the St. Pete's sample has been assessed in monkeys, and the soil is not inherently different than any other sample and represents a test of the IVBA method to accurately predict RBA of samples having a single arsenic phase that is highly bioaccessible (essentially 100%).
- The Drexler-5 sample, although not tested in swine, is expected to have an RBA of 100%, since sodium arsenate is used as the reference material for estimation of RBA. This expectation is supported by the outcome of the RBA assay of the St. Pete's sample.

Arguments against inclusion:

- The Drexler-5 sample has not been tested in swine or monkeys and the assignment of a value of 100% for the RBA is an assumption, not an actual observation of the relationship between IVBA and RBA. The value of 100% assumes that the soil has no effect on the RBA of sodium arsenate. While plausible and perhaps even likely, this is not known.
- A soil sample spiked with sodium arsenate is expected to have IVBA and RBA values of 100%. Therefore, measurements that confirm this expectation (e.g., as were obtained for the St. Pete's sample) are essentially a quality control test for both assays, and the results do not inform the predictive value of the IVBA test for predicting RBA in soils encountered at sites of interest.
- In general, any single data point that has a large effect on the parameters of a regression and/or the strength of the relationship between IVBA and RBA should be treated with caution in a statistical analysis that is intended to evaluate the strength of this relationship for the purpose deciding if the method can be used to predict arsenic RBA in soils at sites of interest.

- In general, estimation of RBA from IVBA measurements will be of greatest practical significance when RBA is low, since this is most likely to influence estimates of risk and clean-up decisions. Therefore, the determination of whether or not an IVBA assay is useful for risk assessment should not be overly influenced by its ability to predict extremely high RBAs.

Although there is merit in both arguments, we conclude that inclusion of the sodium arsenate samples is appropriate in the regression modeling, and the models we recommend are based on the data fits with these samples included. It should be noted that if sodium arsenate samples were excluded, the model fit for the swine data set would still be reliable and useful, although the model for the monkey data set would not be reliable.

IVBA Values for VBI70 Samples

Soil samples from the VBI70 site displayed unusual IVBA behavior. In most cases, IVBA values are reproducible and stable when measured repeatedly over time (see **Figure 5-1, Panel A**). However, IVBA values measured at pH 1.5 for all six VBI70 samples have tended to increase over time (see **Figure 5-1, Panel B**). The cause of this increase is not known, but because the RBA value was measured at the same time as the original pH 1.5 IVBA measurements, these original IVBA values are retained as the most appropriate match to the RBA values. IVBA values for other extraction conditions (pH 7 with and without PO_4 and HAH additions) were not measured at the time of the RBA study, so only "new" data are available for these results. It is not known whether these "new" IVBA values differ from what would have been measure at the time of the RBA study.

Statistical Model Choice

Two basic statistical modeling approaches were evaluated in Phase IV; the first utilizes IVBA data only, and the second utilizes both IVBA data and arsenic phase data. **Table 5-1** compares the best fit models for each approach. As seen, models that incorporate phase data along with IVBA data are more successful (higher R^2 values and lower AIC_c values) than models that are based on IVBA alone. Consequently, models that utilize both types of data are likely to yield the most accurate predictions of RBA than models that utilize IVBA data only.

Note, however, that while the collection of IVBA data is relatively fast and inexpensive, collection of phase data is relatively slow and costly, and requires special equipment and considerable technical expertise and experience. Consequently, it is expected that, for most sites, predictions of RBA will be based on statistical models that utilize IVBA data only, and that collection of phase data to improve the accuracy of RBA predictions will be implemented more selectively.

Choice of Animal Data Set

Based on the data that are presently available, it appears that no single statistical model (that is, the same equation with the same parameters) provides a good fit to both the monkey and swine data (see Table 3-2 and Table 4-3). If it is assumed that the data for each animal species (swine and monkey) are reliable, then it follows that the apparent differences must be due to authentic differences in RBA estimated from the swine and monkey bioassays. These differences could be related to differences in the assay protocols (e.g., dosing regimen) and/or differences in gastrointestinal physiology that determine bioaccessibility and absorption of arsenic in the two animal species.

The best way to test this conclusion is by measuring arsenic RBA of matched soil samples in both species, and comparing the results. **Figure 5-2** shows the data for seven samples for which paired RBA results are presently available. As shown, there is little apparent correlation, with RBA values measured in swine tending to be higher than those measured in monkeys. Although this data set is too limited to support a firm conclusion, these data suggest that RBA results from swine and monkeys are not likely to be equivalent.

If future data collection efforts confirm the conclusion that the monkey and swine bioassays do not yield equivalent RBAs for the same test materials, risk assessors will need to determine which animal species is a more useful predictor of RBA in humans, and use the mathematical model based on data from that species. At present, an empirical basis for determining which bioassay best predicts bioavailability of arsenic in humans does not exist, since this would require measuring arsenic RBA in human subjects. If the apparent differences between the species are ultimately concluded to be unimportant, then using a model that combines the data sets is likely to be the best approach.

Until it is clear whether RBA values based on measurements in swine and monkey are similar or dissimilar, it is suggested that the statistical models based on the swine data be used as the preferred method for estimating site-specific RBA values. This is because the data set based on measurements in swine is currently more extensive than the data set based on monkey, and because the models fit to the swine data are much less dependent on the effect of the sodium arsenate sample that the models based on monkey data.

RBA Predictions at Low IVBA

One potential shortcoming of the current IVBA-based models is that the minimum RBA (obtained when IVBA = 0) is 20% (swine) or 14% (monkey). Intuitively, if IVBA is very low, it is expected that RBA should also be quite low. It is expected that collection of additional data

pairs (especially in the low RBA/IVBA range) might lead to refined models in which the intercept term is somewhat decreased.

Effect of HAH

As noted above, the best fit models for the monkey data set utilize IVBA measured at pH 7 in the presence of 0.05 M PO_4 and HAH (0.1 or 0.25 M). Although earlier studies revealed that HAH (alone) can have a significant impact on IVBA, the data from Phase IV studies suggest that at pH 7, and in the presence of PO_4, the addition of HAH has only a small effect. For 26 samples for which paired data were available (pH 7 + PO_4 vs. pH 7 + PO_4 + HAH), the IVBA with HAH was higher than without HAH by an average of only 4%. Based on this, it is expected that IVBA measured at pH 7 in the presence of 0.05 M phosphate will provide results that are nearly equivalent to those that utilize HAH. Consequently, if the regression model based on data from monkeys is utilized (we are not recommending this at this time), IVBA data may be collected by extraction at pH 7 with PO_4 alone (no HAH), both for convenience and laboratory safety reasons.

Averaging Data for Samples from the Same Site

Inspection of the list of test materials evaluated in Phase IV (see Table 2-1) reveals that while most sites are represented by only one sample, several sites are represented by two or more samples. This raises a question as to whether the samples from these sites should be treated as independent samples, or should be collapsed (averaged). If it is thought that the characteristics of the arsenic contamination are approximately constant within a site, then multiple samples are expected to behave as replicates, and the data should be averaged. If it is suspected that the nature of the arsenic may vary from location to location within a site, then each sample should be evaluated independently and should not be averaged.

In order to investigate the effect of combining samples within a site, the following data sets were averaged because it was judged the samples were more nearly similar than dissimilar:

- Barber Orchard samples 1, 4, 5 and 8
- VBI70 samples TM1, TM2, TM3, TM4 and TM5. Sample TM6 was not included because samples 1 to 5 are authentic site samples, while sample 6 is a “clean” site soil spiked with an arsenical pesticide
- NYPS samples 1, 2 and 3

This reduced the sample number for the swine data set from 20 to 13, and reduced the monkey data set from 17 to 12. Note that Aberjona River samples TM1 and TM2 and Iron King samples TM1 and TM2 were not combined because it was judged that these samples were clearly distinct from each other.

These reduced data sets were re-fit using both the IVBA-only and the IVBA plus phase data approaches. The results are summarized in **Table 5-2**. As seen, there was relatively little impact on the model coefficients, and there was a small increase in the R^2 values. Based on this, it is concluded that model fitting is relatively insensitive to whether similar samples are kept separate or are averaged.

6.0 RECOMMENDATIONS

Based on the findings presented above, the following approach is recommended for selecting RBA values for arsenic in soil:

Step 1: Perform risk calculations assuming the RBA for arsenic in soil is equal to the default value recommended by the Regional toxicologist at the site. If risks are below a level of concern, and it is not anticipated that a more refined estimate of RBA would change risk management decisions or influence soil cleanup strategies, then no further effort is necessary.

Step 2: If risks from arsenic (assuming a default RBA) are sufficient to influence soil cleanup decisions, then it is usually appropriate to measure the IVBA of arsenic in several soil samples from the site to obtain an improved estimate of RBA. As noted above, until more data become available on the relation between RBA values measured in swine and monkey, it is recommended that the IVBA data be collected at pH 1.5, and the value utilized to predict RBA based on the swine model:

$$RBA = 19.7 + 0.62 \cdot IVBA(pH\ 1.5)$$

Step 3: If risks from arsenic (assuming a site-specific RBA value based on IVBA measurements) are still sufficient to influence soil cleanup decisions, and if it is suspected that a more accurate estimate of RBA might have significant impacts on the extent or cost of cleanup, then it may be appropriate to collect data on the arsenic phases present in several soil samples from the site to allow estimation of RBA using models that utilize both IVBA and phase data. This could be done using either of the acceptable swine models (RAM-2 or RAM-3). Because the RAM-2 model only requires the measurement of IVBA at pH 1.5, this approach is recommended as the most useful at present:

$$RBA = 0.573 \cdot IVBA(pH\ 1.5) + 0.081 \cdot RAM_{Bin\ 1} + 0.236 \cdot RAM_{Bin\ 2} + 0.346 \cdot RAM_{Bin\ 3}$$

An example exercise, based on hypothetical data, is provided in **Attachment 2** to illustrate how this process might proceed.

7.0 REFERENCES

Drexler, J.W. and Brattin, W. 2007. An *in vitro* procedure for estimation of lead relative bioavailability: with validation. *Human and Ecological Risk Assessment* 13(2):383-401.

EPA 2005. Estimation of Relative Bioavailability of Arsenic in Soil and Soil-Like Materials by In Vivo and In Vitro Methods - USEPA Review Draft. U.S. Environmental Protection Agency, Region 8, Denver, CO. March 2005. http://www.epa.gov/region8/r8risk/hh_rba-as.html

EPA. 2009. Workplan to Optimize an *in vitro* Method to Predict the Relative Oral Bioavailability of Arsenic. Prepared by EPA Region 8, Dr. John Drexler, Syracuse Research Corporation, and Exponent. March 13, 2009.

EPA. 2010. Arsenic IVBA Optimization Project - Phase II Report: Identification of Principal Variables Affecting IVBA Assay Results. Prepared by EPA Region 8, Dr. John Drexler, SRC, Inc., and Exponent. September 7, 2010.

EPA. 2011. Arsenic IVBA Optimization Project - Phase III Report: Multi-Variate Evaluation of Key Variables Affecting IVBA Assay Results. Report prepared by U.S. Environmental Protection Agency Region 8, University of Colorado Department of Geological Sciences, SRC, Inc., and Exponent. July 25, 2011.

Juhasz, A.L., Smith, E., Weber, J., Rees, M., Rofe,A., Kuchel,T., Sansom, L., and Naidu, R. 2007. Comparison of in vivo and in vitro methodologies for the assessment of arsenic bioavailability in contaminated soil. Chemosphere 69: 961-966.

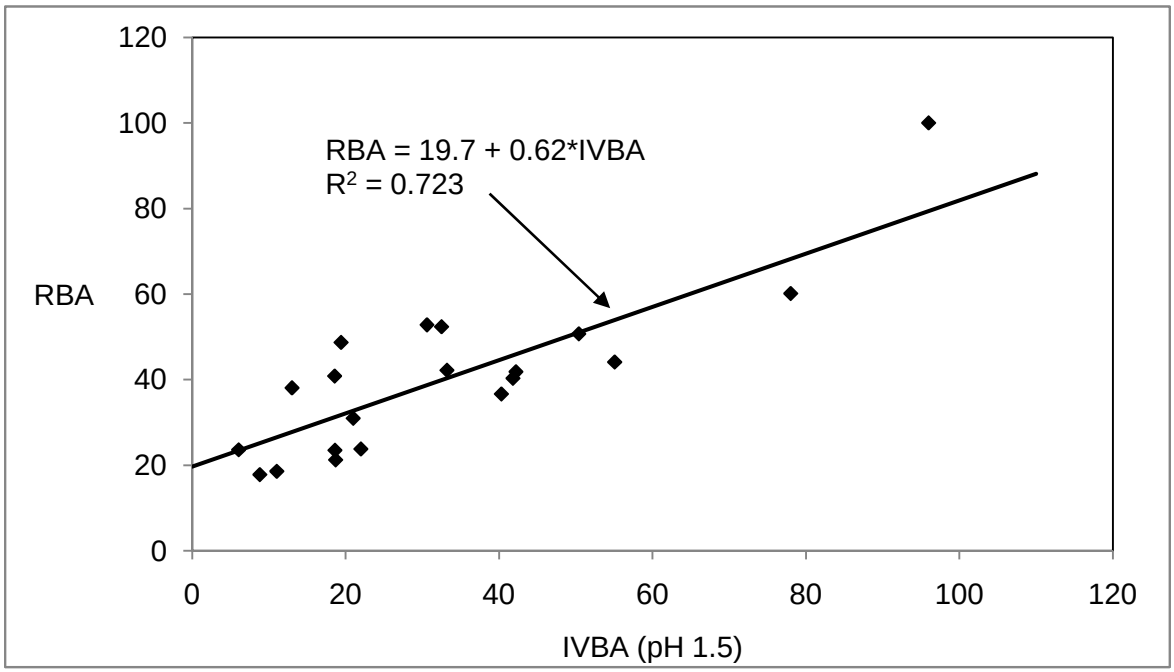
Lorenzana RM, Duncan B, Ketterer M, Lowry J, Simon J, Dawson M, Poppenga R. 1996. Bioavailability of Arsenic and Lead in Environmental Substrates. EPA 910/R-96-002. February 1996.

Medlin EA. 1997. An In Vitro Method for Estimating the Relative Bioavailability of Lead in Humans. Masters Thesis, University of Colorado, Boulder.

Rodriguez, R.R., Basta, N.T., Casteel, S.W., and Pace, L.W. 1999. An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and soil media. Environ. Sci. Tech. 33(4):642-649.

FIGURE 3-1. BEST FIT MLE LINEAR REGRESSION MODELS BASED ON IVBA

PANEL A: SWINE



PANEL B: MONKEY

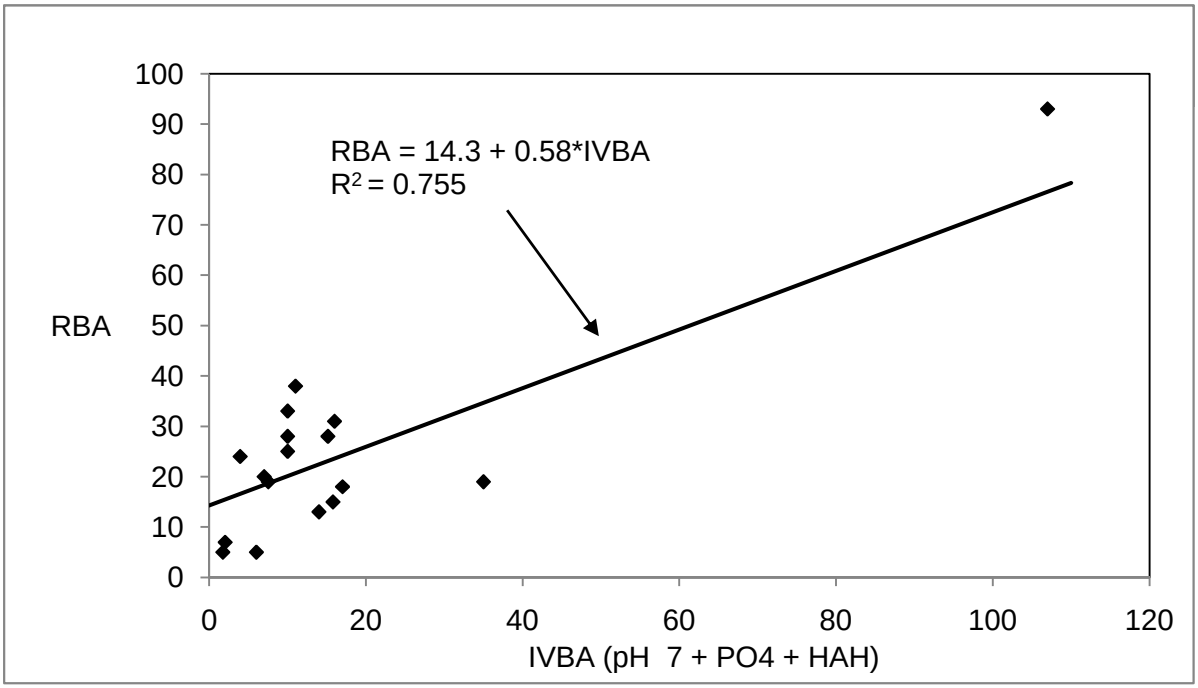
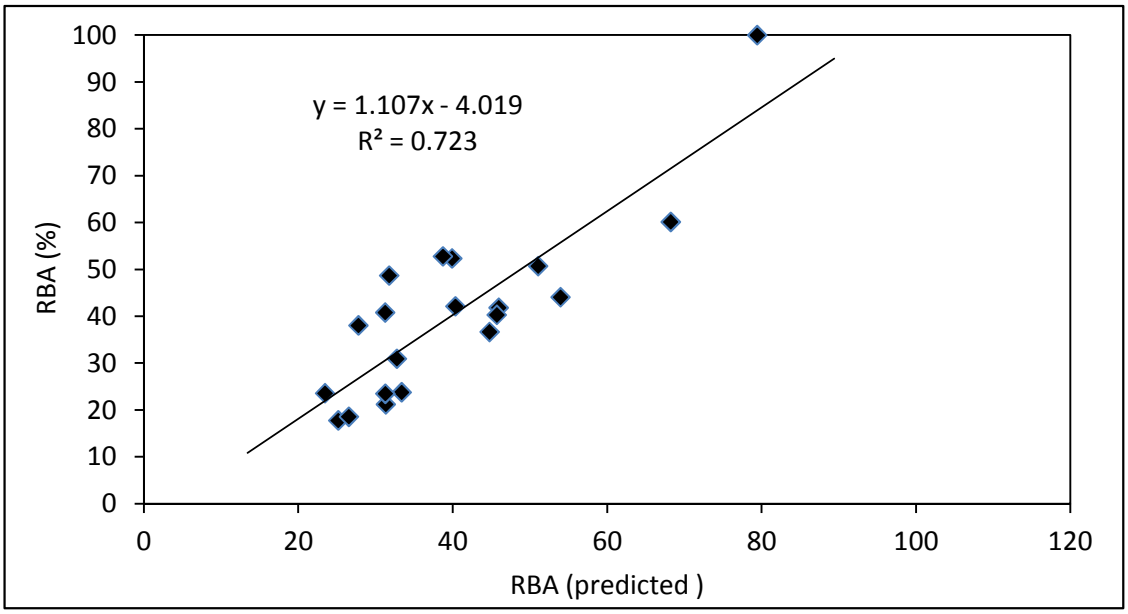


FIGURE 3-2. OBSERVED vs PREDICTED RBA BASED ON IVBA

PANEL A: SWINE (pH 1.5)



PANEL B: MONKEY (pH 7 + PO4 + HAH)

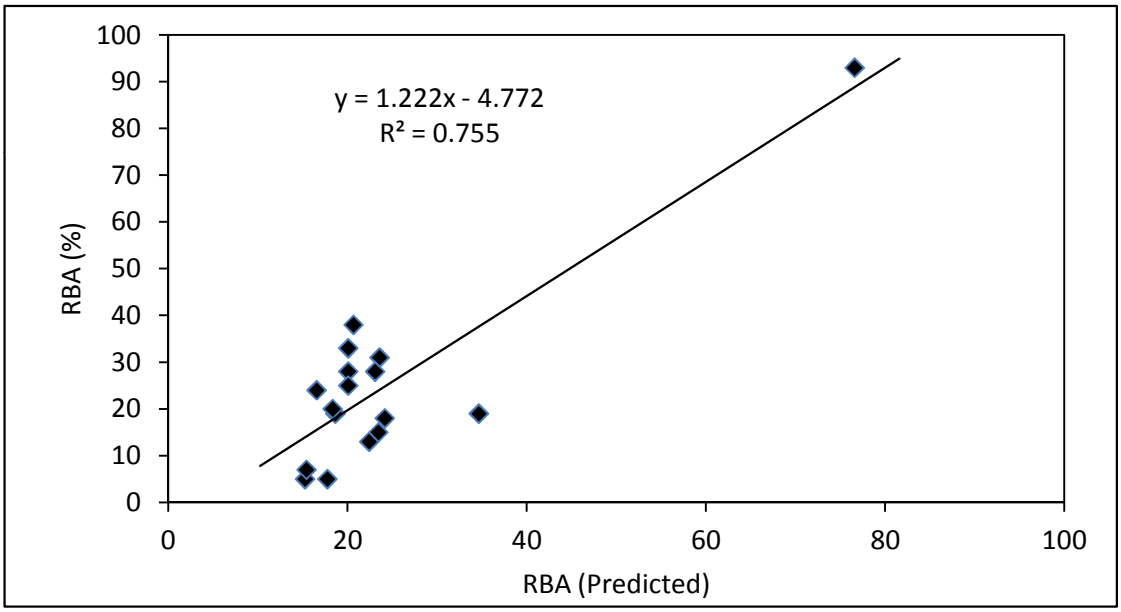


FIGURE 4-1. EXAMPLE FITTING RESULTS AS A FUNCTION OF NUMBER OF PHASE BINS

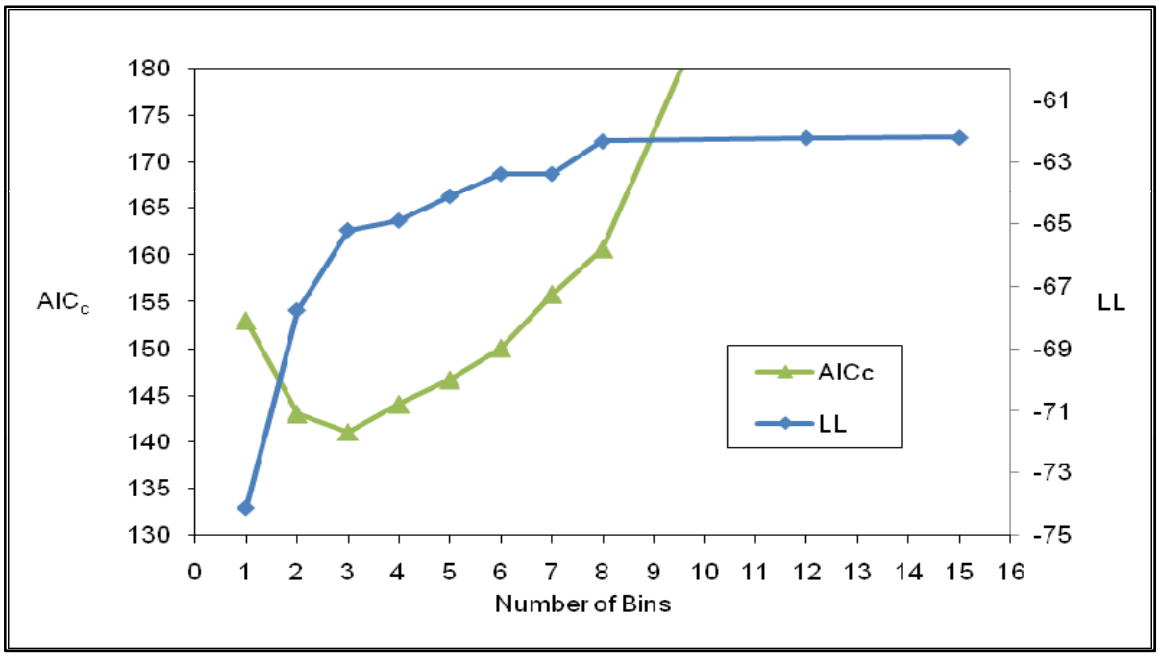
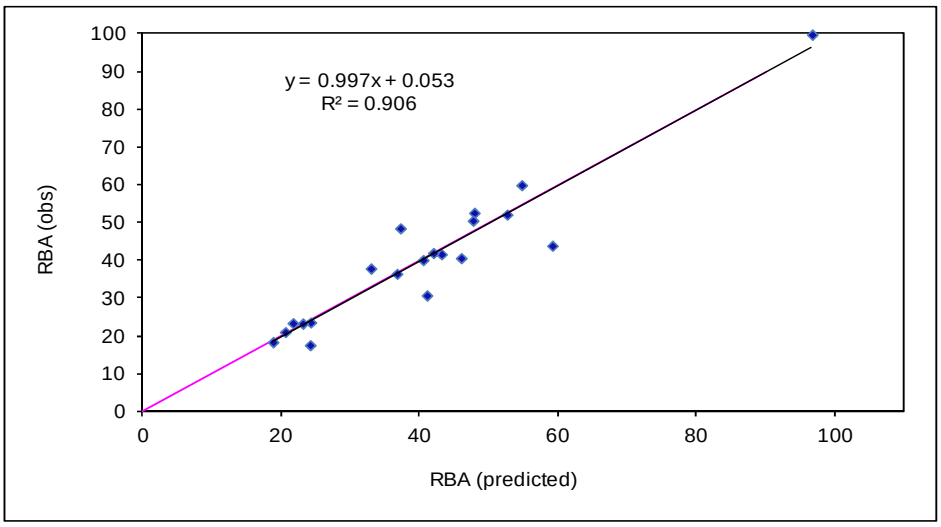


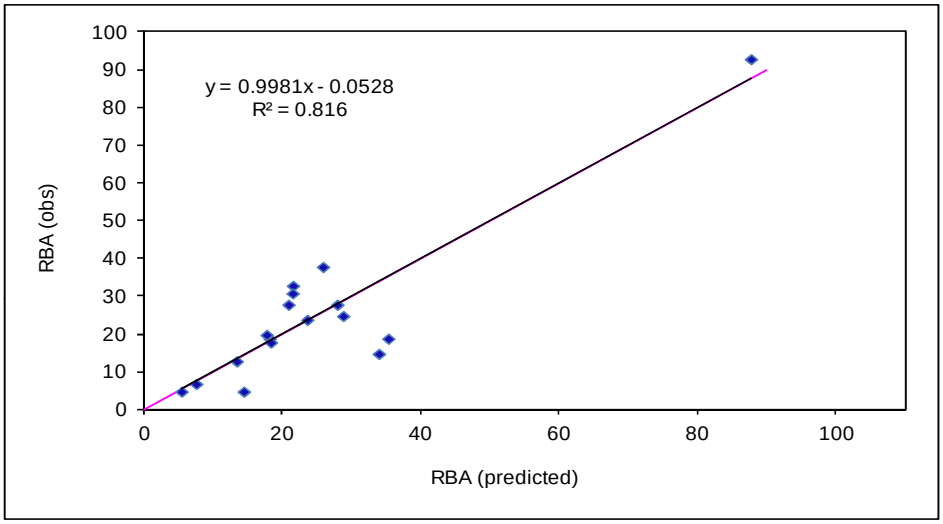
Figure 4-1 Example Fitting.xlsx

FIGURE 4-2. BEST-FIT MODELS USING IVBA AND PHASE DATA

Panel A: Best-Fit Swine Model



Panel B: Best-Fit Monkey Model



Panel C: Combined Model

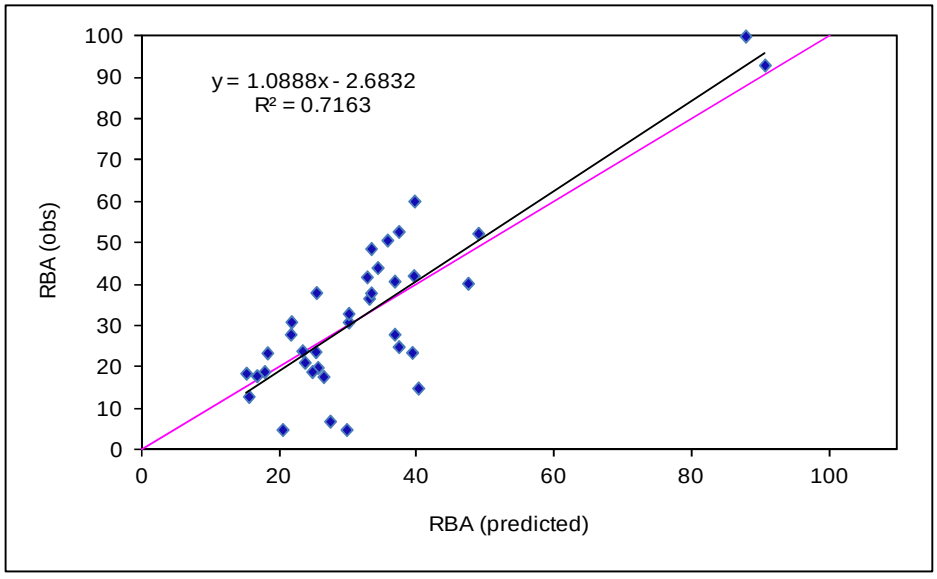
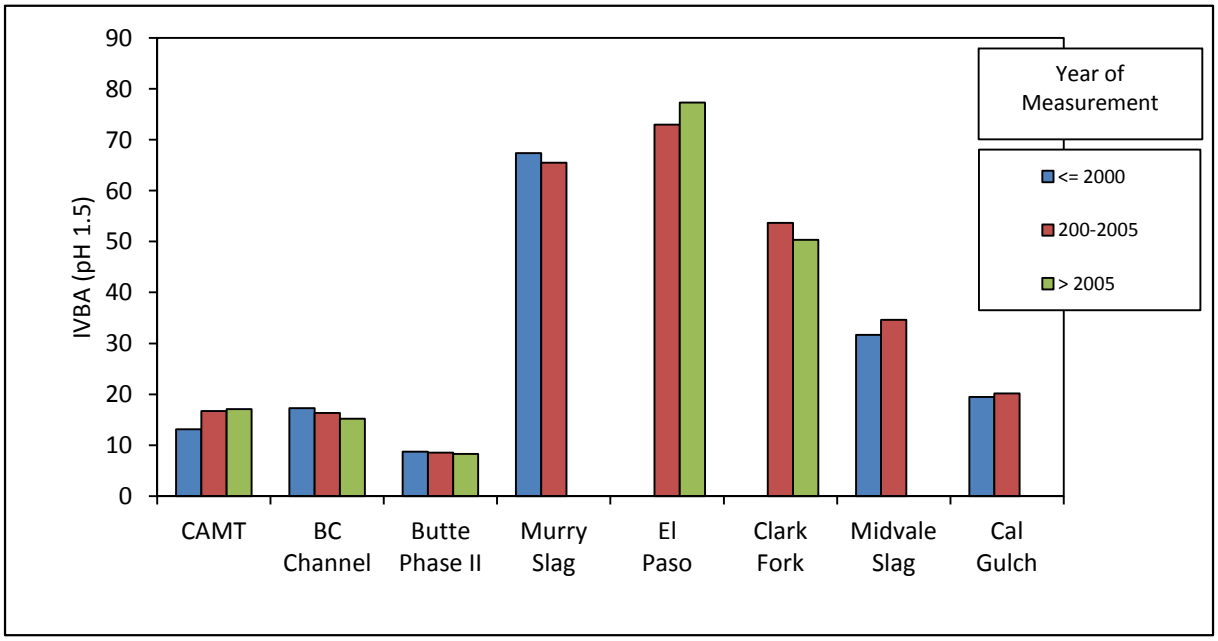


Fig 4-2 and Fig 4-3 Best Fit IVBA-Phase Models.xls

FIGURE 5-1. IVBA vs TIME

Panel A: Typical Data



Panel B: VBI70 Data

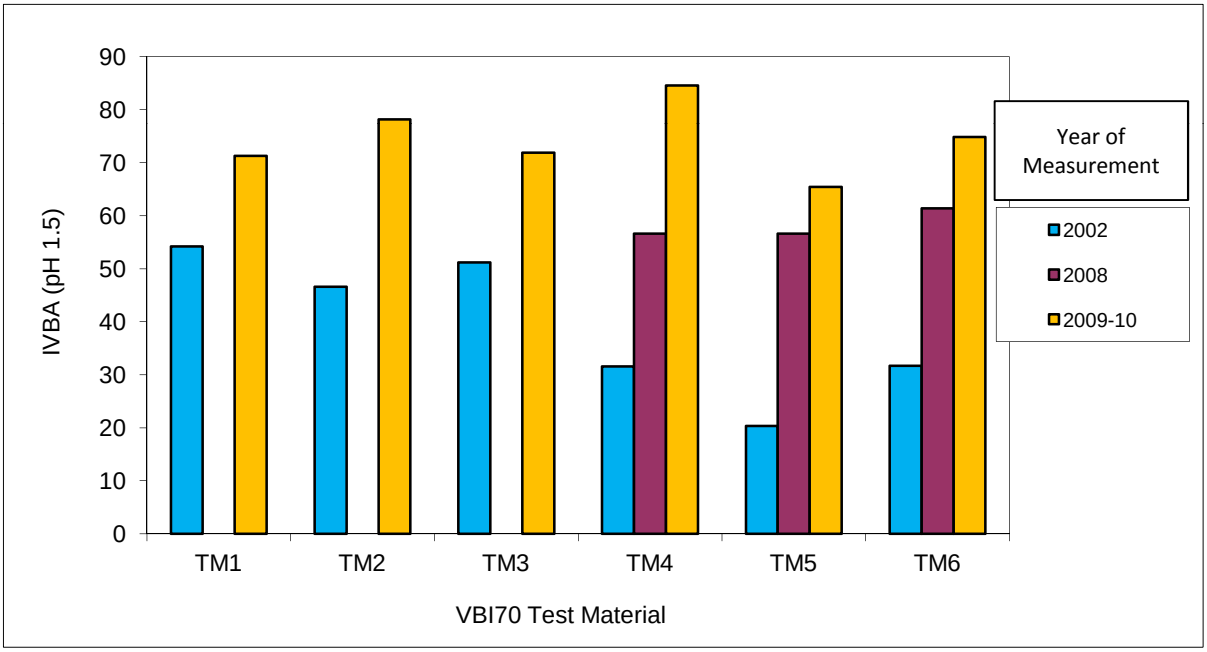
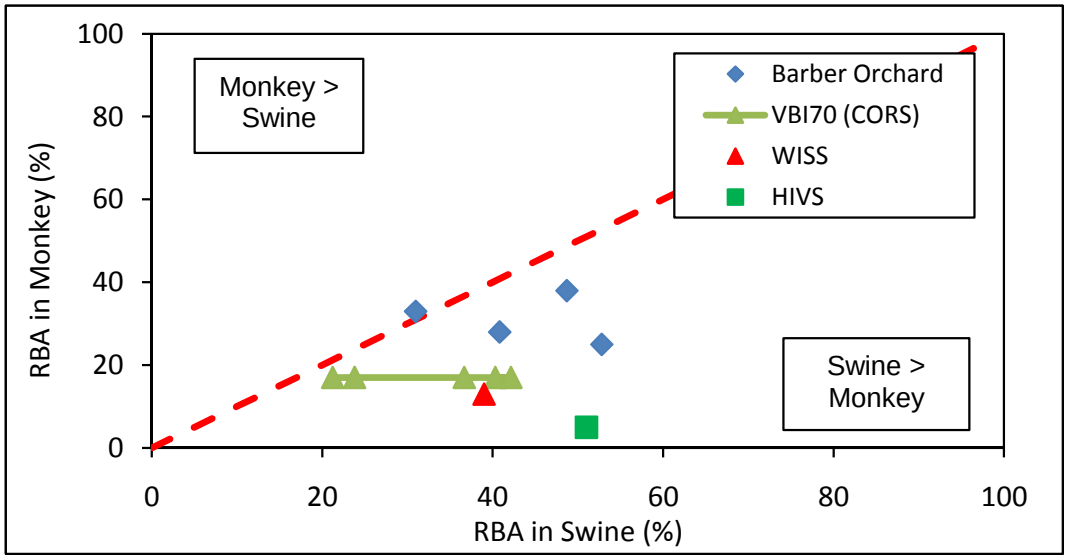


Fig 5-1 VBI70 vs time.xls

FIGURE 5-2 RBA IN SWINE vs MONKEY



Sample	RBA	
	Swine	Monkey
Barber Orchard MS-1	31	33
Barber Orchard MS-4	41	28
Barber Orchard MS-5	49	38
Barber Orchard MS-8	53	25
WISS	39	13
HIVS	51	5
CORS (a)	21-42	17

(a) Five RBA values in swine are shown for this sample because the sample provided to monkeys was not identical to any of the five tested in swine.

TABLE 2-1
TEST MATERIALS EVALUATED IN PHASE IV

Test Material	Descrription	Arsenic Conc. (ppm)	Primary Arsenic Minerology	RBA Tested in	
				Swine	Monkey
Drexler-5	Colorado soil spiked with sodium arsenate	1,239	NaAsO	X	
Aberjona River TM1	Sediment from Aberjona River, MA	676	FeOOH	X	
Aberjona River TM2	Sediment from Aberjona River, MA	313	FeOOH,Fe-SULFATE,ZnSiO4	X	
Butte TM1	Silver Bow Creek/Butte Area NPL Site, Butte, MT	234	SULFOSALT,FeOOH	X	
Butte TM2	Silver Bow Creek/Butte Area NPL Site, Butte, MT	367	Fe-SULFATE,FeOOH	X	
Clark Fork Tailings	Overbank tailings from Clark Fork River, MT	181	FeOOH,Fe-SULFATE,SULFOSALT	X	
Iron King TM1	Iron King, smelter soil Humbolt AZ	200	Fe-SULFATE,SULFOSALT	X	
Iron King TM2	Iron King, smelter soil Humbolt AZ	3,957	ARSENOPYRITE,PYRITE,Fe-SULFATE,SULFOSALT	X	
NIST 2710	NIST standard soil (obtained from Butte MT site)	590	FeOOH,SULFOSALT	X	
NIST 2710A	NIST stardard soil (Butte MT soil spiked with lead oxide)	1,400	FEOOH,SULFOSALT	X	
VBI70 TM1	Residential yard soil 1 from VBI70 site, CO	312	As2O3,PbAsO	X	
VBI70 TM2	Residential yard soil 2 from VBI70 site, CO	983	PbAsO,As2O3	X	
VBI70 TM3	Residential yard soil 3 from VBI70 site, CO	390	As2O3,PbAsO,FeOOH	X	
VBI70 TM4	Residential yard soil 4 from VBI70 site, CO	813	As2O3,PbAsO	X	
VBI70 TM5	Residential yard soil 5 from VBI70 site, CO	368	As2O3	X	
VBI70 TM6	Yard soil from VBI70 site, spiked with PAX pesticide	516	As2O3,PbAsO	X	
CAMT	California mine tailings, Mesa de Oro	300	ARSENOPYRITE,FeOOH		X
CORS	Colorado residential soil (VBI-70 Site)	1,230	As2O3,PbAsO		X
COSCS	Colorado smelter composite soil (Globeville)	394	AsMO,PbAsO		X
COSS	Colorado smelter soil (Smeltertown)	1,492	Fe-SULFATE,FeOOH		X
FLCDV	Soil from FL cattle dip site	150	CLAY,FeOOH		X
FLCPS	Florida Inglis	268	Fe-SULFATE,FeOOH		X
HIVS	Hawaii Volcanic soil	724	CLAY,FeOOH		X
MTSS	Montana smelter soil	647	FeOOH,SULFOSALT		X
NYOS	New York orchard soil	123	MnOOH,PbAsO		X
NYPS1	New York pesticide plant soil	1,000	FeOOH,PbAsO		X
NYPS2	New York pesticide plant soil	549	FeOOH		X
NYPS3	New York pesticide plant soil	339	FeOOH		X
WAOS	Washington orchard soil	301	PbAsO		X
WISS	Western iron slag soil (Rodriguez #8 soil)	1,412	PbAsO,FeOOH		X
St. Pete's	Florida nursery soil spiked with sodium arsenate	514	NaAsO		X
Barber Orchard MS-1	Soil from barber Orchard site, NC	290	PbAsO	X	X
Barber Orchard MS-4	Soil from barber Orchard site, NC	388	PbAsO,MnOOH	X	X
Barber Orchard MS-5	Soil from barber Orchard site, NC	382	PbAsO,SULFOSALT	X	X
Barber Orchard MS-8	Soil from barber Orchard site, NC	364	PbAsO	X	X

TABLE 3-1
RBA AND IVBA DATA

Animal Species	Test Material	RBA (%)	IVBA (%)		
			pH 1.5 (no additions)	pH 7 (no additions)	pH 7 + PO4 + HAH
Swine	Drexler-5	[100]*	96.0	76.9	89.4
	Aberjona River TM1	38.1	13.0	1.0	7.0
	Aberjona River TM2	52.4	32.5	3.0	14.0
	Barber Orchard MS-1	31.0	21.0	4.3	10.0
	Barber Orchard MS-4	40.8	18.6	10.4	10.0
	Barber Orchard MS-5	48.7	19.4	6.5	11.0
	Barber Orchard MS-8	52.8	30.6	6.0	10.0
	Butte TM1	17.8	8.8	0.6	3.3
	Butte TM2	23.6	6.0	2.0	4.0
	Clark Fork Tailings	50.7	50.4	5.0	9.0
	Iron King TM1	60.2	78.0	1.0	14.0
	Iron King TM2	18.6	11.0	1.0	1.0
	NIST 2710	44.1	55.1	5.8	14.0
	NIST 2710A	41.8	42.2	1.9	14.1
	VBI70 TM1	40.3	41.8	30.3	35.9
	VBI70 TM2	42.2	33.2	33.9	42.0
	VBI70 TM3	36.7	40.3	22.1	34.5
	VBI70 TM4	23.8	22.0	32.1	43.0
	VBI70 TM5	21.2	18.7	32.0	43.0
	VBI70 TM6	23.5	18.6	48.0	54.0
Monkey	Barber Orchard MS-1	33.0	21.0	4.3	10.0
	Barber Orchard MS-4	28.0	18.6	10.4	10.0
	Barber Orchard MS-5	38.0	19.4	6.5	11.0
	Barber Orchard MS-8	25.0	30.6	6.0	10.0
	CAMT	19.0	15.7	18.8	7.5
	CORS	17.0	38.0	--	--
	COSCS	18.0	76.0	22.3	17.0
	COSS	5.0	8.5	1.0	1.7
	FLCDV	31.0	39.7	9.0	16.0
	FLCPS	7.0	5.7	1.0	2.0
	HIVS	5.0	10.4	1.0	6.0
	MTSS	13.0	49.8	10.6	14.0
	NYOS	15.0	34.1	10.4	15.8
	NYPS1	20.0	48.2	3.0	7.0
	NYPS2	19.0	58.3	19.9	35.0
	NYPS3	28.0	32.8	4.0	15.1
	WAOS	24.0	81.0	34.1	3.9
	WISS	13.0	48.3	--	--
	St. Pete's	93.0	106.0	100.0	107.0

* Assumed

TABLE 3-2. LINEAR REGRESSION PARAMETERS

Fitting Method	Data Set	Fitting Parameter	IVBA Extraction Fluid		
			pH 1.5	pH 7	pH 7 + PO4
MLE	Swine	N	20	20	20
		Slope	0.62	0.31	0.35
		Intercept	19.68	35.45	32.55
		R2	0.723	0.143	0.178
	Monkey	N	19	17	17
		Slope	0.32	0.43	0.58
		Intercept	11.07	17.10	14.26
		R2	0.336	0.706	0.755
	Combined	N	39	37	37
		Slope	0.44	0.33	0.44
		Intercept	16.42	27.61	23.90
		R2	0.345	0.328	0.409
OLR	Swine	N	20	20	20
		Slope	0.69	0.35	0.36
		Intercept	17.77	34.75	32.18
		R2	0.723	0.143	0.178
	Monkey	N	19	17	17
		Slope	0.41	0.71	0.71
		Intercept	7.57	13.76	12.65
		R2	0.336	0.706	0.755
	Combined	N	39	37	37
		Slope	0.48	0.55	0.57
		Intercept	14.91	24.53	21.63
		R2	0.345	0.328	0.409

TABLE 4-1 LENGTH-WEIGHTED FREQENCY VALUES

Test Material	AsMO	FeOOH	PbAsO	PbMO	Slag	FeSO4	MnOOH	Phos-phate	Arseno-pyrite	Pyrite	Sulfo-Salt	Clay	ZnSiO4	As2O3	NaAsO4
Aberjona River TM1		76.70				20.78				0.88			1.63		
Aberjona River TM2		22.67				26.31				8.10			42.92		
Barber Orchard MS-1		67.34	12.78			0.71	3.75	15.42							
Barber Orchard MS-4		22.31	0.80			0.40	76.49								
Barber Orchard MS-5		42.30	2.34				52.68	2.34			0.35				
Barber Orchard MS-8		17.02	6.59				74.32	2.07							
Butte TM1	0.18	61.97			1.90	27.47				3.11	3.36	2.01			
Butte TM2		11.66				48.51	36.46	3.36							
Clark Fork Tailings		43.26			9.08	24.07	3.88	18.46			1.25				
Iron King TM1		13.48		2.91		79.73					3.88				
Iron King TM2	0.20	4.00				26.91			1.24	66.07	1.58				
NIST 2710		43.49				1.03	49.86	3.00			1.22	1.41			
NIST 2710A		59.93				23.89	7.28	3.85			5.05				
VBI70 TM1		25.65	7.89	0.25		0.50	21.55	34.70				3.87		5.60	
VBI70 TM2		32.95	24.95	0.18	5.44	0.45	3.63	26.14				2.99		3.27	
VBI70 TM3		36.93	0.87	0.32	9.26		41.18	3.92				2.28		5.23	
VBI70 TM4		25.87	4.56	1.30	28.99	2.32	9.85	7.47				3.05		16.59	
VBI70 TM5		59.60		0.33	0.99	0.55	10.37							28.17	
VBI70 TM6	4.56	14.18	16.33		32.54			1.88		0.50		0.25		29.76	
CAMT		68.23				6.73			8.97	16.07					
CORS	0.14	31.39	15.43			9.13	4.33	3.32						36.27	
COSCS	20.20	37.97	7.71	4.79	20.34	7.28	1.50	0.21							
COSS		42.68		4.35		52.98									
FLCDV		5.39										94.61			
FLCPS		62.06				37.94									
HIVS		17.89	0.05				1.55					80.51			
MTSS	0.23	29.33			57.48	9.90	1.50				1.56				
NYOS		9.45	1.06	0.46			88.83	0.20							
NYPS1		77.35	0.92			0.92	17.27	2.34				0.78			0.42
NYPS2		100.00													
NYPS3		99.79					0.21								
WAOS		22.53	62.98				12.91	1.58							
WISS	3.00	51.50	12.24	1.52		28.50		0.49		1.82			0.93		
Drexler-5															100.00
St. Pete's															100.00

TABLE 4-2. RELATIVE ARSENIC MASS VALUES

Test Material	AsMO	FeOOH	PbAsO	PbMO	Slag	FeSO4	MnOOH	Phos-phate	Arseno-pyrite	Pyrite	Sulfo-Salt	Clay	ZnSiO4	As2O3	NaAsO4
Aberjona River TM1		69.62				27.52				0.24			2.62		
Aberjona River TM2		16.26				27.52				1.76			54.46		
Barber Orchard MS-1		9.40	85.99			0.04	0.34	4.22							
Barber Orchard MS-4		25.13	45.85			0.06	28.97								
Barber Orchard MS-5		17.39	47.60				7.72	0.73			26.55				
Barber Orchard MS-8		7.58	85.40				5.92	1.10							
Butte TM1	0.33	39.08			0.04	18.70				0.13	41.66	0.07			
Butte TM2		17.17				61.93	8.39	12.38							
Clark Fork Tailings		42.72			0.12	25.65	2.07	5.13			24.32				
Iron King TM1		6.50				51.80					41.59		0.12		
Iron King TM2	1.99	3.43				18.73			36.32	25.37	14.16				
NIST 2710		64.24				0.54	15.83	0.36			18.91	0.12			
NIST 2710A		45.36				8.42	1.13	0.56			44.54				
VBI70 TM1		3.27	34.34	0.14		0.06	1.70	8.15				0.14		52.18	
VBI70 TM2		2.80	72.40	0.07	0.01	0.04	0.19	4.10				0.07		20.33	
VBI70 TM3		7.63	6.14	0.31	0.03		5.26	1.49				0.14		79.00	
VBI70 TM4	0.83	1.81	10.88		0.03	0.15	0.43	0.96				0.06		84.84	
VBI70 TM5		2.80		0.07		0.02	0.30							96.80	
VBI70 TM6	1.12	0.51	19.99	0.01	0.02			0.12		0.05				78.18	
CAMT		27.20				2.30			70.40	0.20					
CORS	0.13	0.63	16.09			0.61	0.41	1.02						81.12	
COSCS	65.99	6.96	20.66	2.99	0.02	3.27	0.10								
COSS		26.82		0.97		72.20									
FLCDV		15.32										84.66			
FLCPS		26.20				73.80									
HIVS		14.69	13.13				1.79					70.39			
MTSS	6.36	70.00			1.93	4.66	0.42				18.40				
NYOS		5.48	49.99	1.10			43.41	0.02							
NYPS1		65.67	14.50			0.02	12.47	0.33				0.01			7.00
NYPS2		100.00													
NYPS3		99.91					0.09								
WAOS		0.55	99.28				0.16	0.02							
WISS	7.20	17.54	60.11	2.55		10.91		0.06		1.44			0.16		
Drexler 5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
St. Pete's	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00

TABLE 4-3. PHASE-BASED MODEL FITTING RESULTS

Phase Metric	Model Number	Model Equation	Swine			Monkey			Combined		
			AIC_c	Bins	R^2	AIC_c	Bins	R^2	AIC_c	Bins	R^2
LWF	LWF-1	$RBA = \Sigma (RBA_i * LWF_i)$	148.68	4	0.781	135.26	3	0.871	305.40	4	0.705
	LWF-2	$RBA = k1*IVBA + \Sigma (RBA_i * LWF_i)$	141.09	3	0.874	124.64	3	0.816	291.86	4	0.716
	LWF-3	$RBA = k1*IVBA1 + k2*IVBA2 + \Sigma (RBA_i * LWF_i)$	137.69	3	0.904	128.43	3	0.798	294.37	4	0.732
RAM	RAM-1	$RBA = \Sigma (RBA_i * RAM_i)$	162.95	3	0.690	140.30	3	0.836	308.37	4	0.692
	RAM-2	$RBA = k1*IVBA + \Sigma (RBA_i * RAM_i)$	136.52	3	0.903	127.01	3	0.864	293.23	4	0.675
	RAM-3	$RBA = k1*IVBA1 + k2*IVBA2 + \Sigma (RBA_i * RAM_i)$	135.59	3	0.906	127.01	3	0.864	292.70	4	0.719

TABLE 4-4. BEST-FIT IVBA/PHASE-BASED MODEL PARAMETERS

SWINE

Model RAM-3

RBA = k1*IVBA1 + k2*IVBA2 + Σ (RBAi * RAMi)

Parameter	Value
Bins	3
k1	0.613
k2	-0.113
RBA(1)	0.048
RBA(2)	0.137
RBA(3)	0.338
RBA(4)	--

Phase Groupings

Bin 1	AsMO Slag Phosphate Pyrite Clay Sulfo Salt As2O3 Arsenopyrite FeSO4
Bin 2	FeOOH PbAsO
Bin 3	MnOOH PbMO ZnSiO4 NaAsO4

SWINE

Model RAM-2

RBA = k1*IVBA + Σ (RBAi * RAMi)

Parameter	Value
Bins	3
k1	0.573
k2	--
RBA(1)	0.081
RBA(2)	0.236
RBA(3)	0.346
RBA(4)	--

Phase Groupings

Bin 1	AsMO Slag Phosphate Pyrite Clay Sulfo Salt As2O3 Arsenopyrite
Bin 2	FeSO4 FeOOH
Bin 3	PbAsO MnOOH ZnSiO4 NaAsO4 PbMO

MONKEY

Model LWF-2

RBA = k1*IVBA + Σ (RBAi * LWFi)

Parameter	Value
Bins	3
k1	0.728
k2	--
RBA(1)	0.000
RBA(2)	0.099
RBA(3)	0.239
RBA(4)	--

Phase Groupings

Bin 1	AsMO PbMO Slag FeSO4 Sulfo Salt
Bin 2	FeOOH NaAsO4 Clay
Bin 3	Pyrite PbAsO MnOOH Arsenopyrite As2O3 ZnSiO4 Phosphate

COMBINED

Model LWF-2

RBA = k1*IVBA + Σ (RBAi * LWFi)

Parameter	Value
Bins	4
k1	0.160
k2	--
RBA(1)	0.034
RBA(2)	0.191
RBA(3)	0.399
RBA(4)	0.734

Phase Groupings

Bin 1	PbMO Slag Arsenopyrite Pyrite As2O3 AsMO
Bin 2	PbAsO FeOOH Clay
Bin 3	MnOOH FeSO4
Bin 4	NaAsO4 ZnSiO4 Phosphate Sulfo Salt

TABLE 5-1. MODEL COMPARISON

Data Set	AIC_c		R^2	
	IVBA Alone	IVBA + Phase Data	IVBA Alone	IVBA + Phase Data
Swine	152.53	135.59	0.723	0.906
Monkey	131.30	124.64	0.755	0.816
Combined	311.17	291.86	0.409	0.716

TABLE 5-2. EFFECT OF COMBINING SIMILAR SAMPLES WITHIN A SITE

Model Variables	Parameter	Swine		Monkey	
		Separate (a)	Combined (b)	Separate (c)	Combined (d)
IVBA only	Slope	0.62	0.65	0.58	0.61
	Intercept	19.7	18.4	14.3	11.1
	R^2	0.723	0.81	0.755	0.891
IVBA + Phase data	Bins	3	3	3	3
	k1	0.613	0.564	0.728	0.685
	k2	-0.113	0.098	--	--
	RBA(1)	0.048	0.000	0.000	0.000
	RBA(2)	0.137	0.265	0.099	0.104
	RBA(3)	0.338	0.383	0.239	0.200
	R^2	0.906	0.988	0.816	0.923

(a) N = 20
(b) N = 13; Combine Barber Orchard 1, 3, 5 and 8; Combine VBI70 TM1, 2, 3, 4 and 5
(c) N = 17
(d) N = 12; Combine Barber Orchard 1, 3, 5 and 8; Combine NYPS 1, 2 and 3

ATTACHMENT 1
STANDARD OPERATING PROCEDURE FOR ARSENIC SPECIATION BY ELECTRON MICROPROBE ANALYSIS

1.0 OBJECTIVES

The objectives of this Standard Operating Procedure (SOP) are to specify the proper methodologies and protocols to be used during metal speciation of various solid samples including; tailings, slags, sediments, dross, bag house dusts, wipes, paint, soils, and dusts for metals. The metal speciation data generated from this SOP may be used to assess the solid samples as each phase relates to risk. Parameters to be characterized during the speciation analyses include particle size, associations, stoichiometry, frequency of occurrence of metal-bearing forms and relative mass of metal-bearing forms. This electron microprobe analysis (EMPA) technique, instrument operation protocols and sample preparation to be used during implementation of the Metals Speciation SOP are discussed in the following sections.

2.0 BACKGROUND

To date, numerous metal-bearing forms of soils have been identified from various environments within western mining districts (Emmons et al., 1927; Drexler, 1991 per. comm.; Drexler, 1992; Davis et al., 1993; Ruby et al., 1994; CDM, 1994; WESTON, 1995). This listing does not preclude the identification of other metal-bearing forms, but only serves as an initial point of reference. Many of these forms are minerals with varying metal concentrations (e.g., lead phosphate, iron-lead oxide, and slag). Since limited thermodynamic information is available for many of these phases and equilibrium conditions are rarely found in soil environments, the identity of the mineral class (e.g., lead phosphate) will be sufficient and exact stoichiometry is not necessary.

It may be important to know the particle-size distribution of metal-bearing forms in order to assess potential risk. It is believed that particles less than 250 microns (μm) are most available for human ingestion and/or inhalation (Bornschein, et al., 1987). For this study, the largest dimension of any one metal-bearing form will be measured and the frequency of occurrence weighted by that dimension. Although not routinely performed, particle area can be determined, it has been shown (CDM, 1994) that data collected on particle area produces similar results. These measurements add a considerable amount of time to the procedure, introduce new sources of potential error and limit the total number of particles or samples that can be observed in a study.

Mineral association may have profound effects on the ability for solubilization. For example, if a lead-bearing form in one sample is predominantly found within quartz grains while in another

sample it is free in the sample matrix, the two samples are likely to pose significantly different risk levels to human health. Therefore, associations of concern include the following:

1. free or liberated
2. inclusions within a second phase
3. cementing rimming

3.0 SAMPLE SELECTION

Samples should be selected and handled according to the procedure described in the Project Plan. Unless help in determination of sample selection and frequency is requested by the client it is their responsibility to provide a "representative" number of samples to the laboratory for analyses.

4.0 SCHEDULE

A schedule for completion of projects performed under this Metals Speciation SOP will be provided in writing or verbally to the contractor along with monthly reporting requirements if large projects are performed. These schedules are based on an aggressive analytical program designed to ensure that the metals speciation analyses are completed in a timely period. Monthly reports are expected to reflect schedule status.

5.0 INSTRUMENTATION

Speciation analyses will be conducted at the Laboratory for Environmental and Geological Studies (LEGS) at the University of Colorado, Boulder or other comparable facilities. Primary equipment used for this work will include:

Electron Microprobe (JEOL 8600) equipped with four wavelength spectrometers, energy dispersive spectrometer (EDS), BEI detector and the Geller, dQuant data processing system. Geller dPict hardware may be used for image storage and processing. An LEDC spectrometer crystal for carbon and LDE-1 crystal for oxygen analyses are essential.

6.0 PRECISION AND ACCURACY

The precision of the EMPA speciation will be evaluated based on sample duplicates analyzed at a frequency of 10% as selected by the laboratory. However the client may also submit "blind" duplicates for analyses. The precision of the data generated by the "EMPA point count" will be evaluated by calculating RPD values for all major (>20% frequency) phases, comparing the original result with the duplicate result. If the duplicate analyses are from samples that have produced at least 100 total particles it is expected that all (100%) of the dominant species (representing 60% of frequency) be found in both, and that their individual frequency of occurrence not vary by more than 30%, relative. In the evaluation of the method precision it is

most important to consider the variation in results among all samples studied for a particular media, since the overall particle count is very large. Data generated by the "EMPA point count" will be further evaluated statistically based on the methods of Mosimann (1965) at the 95% confidence level on the frequency data following Equation 1.

$$E_{0.95} = 2P(100-P)/N$$

where: $E_{0.95}$ = Probable error at the 95% confidence level

P = Percentage of N of an individual metal-bearing phase based on percent length frequency

N = Total number of metal-bearing grains counted

Accuracy of quantitative metal analyses on non-stoichiometric metal phases is based on established EMPA procedures, and data reduction, Heinrich, 1981 and is generally 1-2% relative. All quantitative analyses will be performed using a series of certified mineral standards. In general, site-specific concentrations for these variable, metal-bearing forms will be determined by performing "peak counts" on the appropriate wavelength spectrometer. Average concentrations will then be used for further calculations. Data on specific gravity will be collected from referenced databases or estimated based on similar compounds.

7.0 PERSONNEL RESPONSIBILITY

The analysts will carefully read this SOP prior to any sample examination.

It is the responsibility of the laboratory supervisor and designates to ensure that these procedures are followed, to examine quality assurance (QA) samples and replicate standards, and to check EDS and WDS calibrations. The laboratory supervisor will collect results, ensure they are in proper format, and deliver them to the contractor.

Monthly reports summarizing all progress, with a list of samples speciated to date with data analyses sheets (DAS), will be submitted each month.

It is also the responsibility of the laboratory supervisor to notify the contractor representative of any problems encountered in the sample analysis process.

8.0 SAMPLE PREPARATION

Grain mounts (1.5 inches in diameter) of each sample will be prepared using air-cured epoxy. Previous work (CDM, 1994, Weston, 1995) found that neither using mono-layer mounts nor repetitive exposure of deeper layers within the epoxy puck produced speciation results outside those errors observed from single sample duplicates. Once a sample is well stirred within the

epoxy minimal settling was observed. This grain mounting technique is appropriate for most speciation projects, however polished thin-sections, paint chips, dust wipes, or filters may be prepared in a similar manner. The grain mounting is performed as follows:

1. Log the samples for which polished mounts will be prepared.
2. Inspect all disposable plastic cups, making sure each is clean and dry.
3. Label each "mold" with its corresponding sample number.
4. All samples will be split to produce a homogeneous 1-4 gram sample.
5. Mix epoxy resin and hardener according to manufacturer's directions.
6. Pour 1 gram of sample into mold. Double check to make sure sample numbers on mold and the original sample container match. Pour epoxy into mold to just cover sample grains.
7. Use a new wood stirring stick with each sample, carefully blend epoxy and grains so as to coat all grains with epoxy.
8. Set molds to cure at ROOM TEMPERATURE in a clean restricted area. Add labels with sample numbers and cover with more epoxy resin. Leave to cure completely at room temperature.
9. One at a time remove each sample from its mold and grind flat the back side of the mount.
10. Use 600 grit wet abrasive paper stretched across a grinding wheel to remove the bottom layer and expose as many mineral grains as possible. Follow with 1000 grit paper.
11. Polish with 15 um oil-based diamond paste on a polishing paper fixed to a lap. Use of paper instead of cloth minimizes relief.
12. Next use 6um diamond polish on a similar lap.
13. Finally polish the sample with 1um oil-based diamond paste on polishing paper, followed by 0.05 um alumina in water suspension. The quality should be checked after each step. Typical polishing times are 30 minutes for 15 um, 20 minutes for 6 um, 15 minutes for 1 um, and 10 minutes for 0.05 um.
    NOTE: use low speed on the polishing laps to avoid "plucking" of sample grains.
14. Samples should be completely cleaned in an ultrasonic cleaner with isopropyl alcohol or similar solvent to remove oil and fingerprints.
15. To ensure that no particles of any metal are being cross-contaminated during sample preparation procedures, a blank (epoxy only) mold will be made every 20th sample (5% of samples) following all of the above procedures. This mold will then be speciated along with the other samples.
16. Each sample must be carbon coated. Once coated, the samples should be stored in a clean, dry environment with the carbon surface protected from scratches or handling.

9.0 GEOCHEMICAL SPECIATION USING ELECTRON MICROPROBE

All investigative samples will also be characterized using EMPA analysis to determine the chemical speciation, particle size distribution and frequency for several target metals.

9.1 Concentration Prescreening

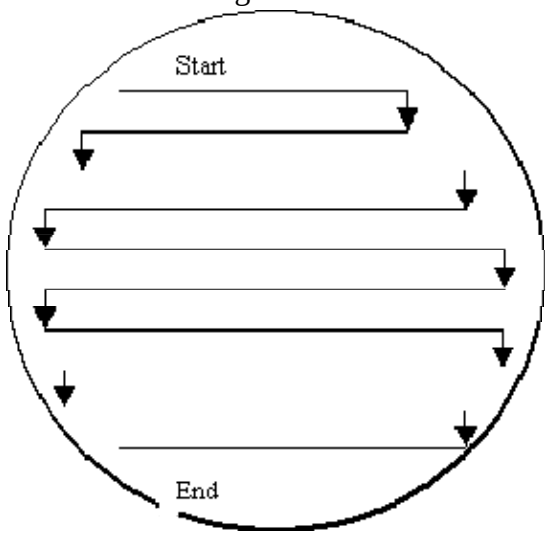
All samples will be initially examined using the electron microprobe to determine if the number of particles are too great to obtain a representative count. The particle counting will be considered representative if the entire sample (puck) has been traversed about the same time in which the counting criteria are achieved.

If this examination reveals that one metal is highly abundant (> 10,000 mg/kg in concentration), clean quartz sand (SiO2) will be mixed with the sample material. The sand should be certified to be free of target analytes. The quartz sand should be added to an aliquot of the investigative sample, then mixed by turning the sample for a minimum of one hour, or until the sample is fully homogenized. The initial mass of the investigative sample aliquot and the mass of the quartz addition must be recorded on the Data Analysis Sheet (DAS).

9.2 Point Counting

Counts are made by traversing each sample from left-to-right and top-to-bottom as illustrated in Figure 9-1. The amount of vertical movement for each traverse would depend on magnification and CRT (cathode-ray tube) size. This movement should be minimized so that NO portion of the sample is missed when the end of a traverse is reached. Two magnification settings generally are used, one ranging from 40-100X and a second from 300-500X. The last setting will allow one to find the smallest identifiable (1-2 micron) phases.

Figure 9-1



The portion of the sample examined in the second pass, under the higher magnification, will depend on the time available, the number of metal-bearing particles, and the complexity of metal mineralogy. A maximum of 8 hours will be spent on each analysis or a total particle count of 100.

The point counting procedure in petrography is a well established technique as outlined by Chayes, 1949. For our procedure we have simply substituted the electron microprobe for a simple petrographic microscope as a means of visually observing a particle and identifying its composition using the attached x-ray analyzers. The operator error (identification of phase and sizing) is generally negligible. However the particle counting error can be significant depending on the total number of particles counted and the individual component (species) percent. Based on studies in El-Hinnawi, 1966, it was shown that the relative error of a point count based on 100 total particles versus one of 300 total particles is only 10% and 6% , respectively (for a species representing 30% of the count). It is our belief that this small decrease in error is not justified when cost and time of analysis are considered, and that it is much more beneficial to increase your total sample population and address representativeness.

9.3 Data Reduction

Analysts will record data as they are acquired from each sample using the LEGS software (see Figure 9-2), which places all data in a spreadsheet file format (see Figure 9-3). Columns have been established for numbering the metal-bearing phase particles, their identity, size of longest dimension in microns, along with their association (L = liberated, C= cementing, R = rimming, I = included). The analyst may also summarize his/her observations in the formatted data summary files.

The frequency of occurrence and relative metal mass of each metal-bearing form as it is distributed in each sample will be depicted graphically as a frequency bar-graph (see Figure 9-5). The particle size distribution of metal-bearing forms will be depicted in a histogram. Size-histograms of each metal-bearing form can be constructed from data in the file.

Data from EMPA will be summarized using two methods. The first method is the determination of FREQUENCY OF OCCURRENCE. This is calculated by summing the longest dimension of all the metal-bearing phases observed and then dividing each phase by the total.

Equation 2 will serve as an example of the calculation.

$$F_M \text{ in phase-1} = \frac{\Sigma(PLD)_{\text{phase 1}}}{\Sigma(PLD)_{\text{phase-1}} + \Sigma(PLD)_{\text{phase-2}} + \Sigma(PLD)_{\text{phase-n}}}$$

Where:

F_M = Frequency of occurrence of metal in a single phase.

PLD = An individual particle's longest dimension.

$\%F_M$ in phase-1 = F_M in phase-1 * 100

These data thus illustrate which metal-bearing phase(s) are the most commonly observed in the sample or relative volume percent.

The second calculation used in this report is the determination of RELATIVE METAL MASS. These data are calculated by substituting the PLD term in the equation above with the value of MM. This term is calculated as defined below.

$$M_M = FM * SG * ppm_M$$

where:

M_M = Mass of metal in a phase

SG = Specific Gravity of a phase

ppm_M = Concentration in ppm of metal in a phase

The advantage in reviewing the RELATIVE METAL MASS determination is that it gives one information as to which metal-bearing phase(s) in a sample are likely to control the total bulk concentration for a metal of interest. For example, PHASE-1 may comprise 98% relative volume of the sample; however, it has a low specific gravity and contains only 1,000 parts per million (ppm) arsenic. PHASE-2 comprised 2% of the sample, has a high specific gravity, and contains 850,000 ppm of arsenic. In this example it is PHASE-2 that is the dominant source of arsenic to the sample.

Finally, a concentration for each phase is calculated. This quantifies the concentration of each metal-bearing phase. This term is calculated as defined below (Eq. 4).

$$ppm_M = M_M * \text{Bulk metal concentration in ppm}$$

9.4 Analytical Procedure

A brief visual examination of each sample will be made, prior to EMPA examination. This examination may help the operator by noting the occurrence of slag and/or organic matter. Standard operating conditions for quantitative and qualitative analyses of most metal-bearing forms are given in Figure 9-4. However, it is the responsibility of the operator to select the appropriate analytical line (crystal/KeV range) to eliminate peak overlaps and ensure proper identification/quantification of each analyte. Quality control will be maintained by analyzing duplicates at regular intervals (Section 6).

The backscattered electron threshold will be adjusted so that all particles in a sample are seen. This procedure will minimize the possibility that low metal-bearing minerals may be overlooked during the scanning of the polished grain mount. The scanning will be done manually in a

manner similar to that depicted in Figure 9-1. Typically, the magnification used for scanning all samples except for airborne samples will be 40-100X and 300-600X. The last setting will allow the smallest identifiable (1-2 um) phases to be found. Once a candidate particle is identified, then the backscatter image will be optimized to discriminate any different phases that may be making up the particle or defining its association. Identification of the metal-bearing phases will be done using both EDS and WDS on an EMPA, with spectrometers typically peaked at sulfur, oxygen, carbon and the metal(s) of concern (M). The size of each metal-bearing phase will be determined by measuring in microns the longest dimension.

As stated previously, a maximum of 8 hours will be spent in scanning and analyzing each mount. For most speciation projects the goal is to count between 100-300 particles. In the event that these goals are achieved in less than 8 hours, particle counting may be stopped so the analyst may move to another sample in order to increase the sample population.

Quantitative Analyses

Quantitative analyses are required to establish the average metal content of the metal-bearing minerals, which have variable metal contents as: Iron-(M) sulfate, Iron-(M) oxide, Manganese-(M) oxide, organic, and slag. These determinations are important, especially in the case of slag, which is expected to have considerable variation in their dissolved metal content.

Results will be analyzed statistically to establish mean values. They may also be depicted as histograms to show the range of metal concentrations measured as well as the presence of one or more populations in terms of metal content. In the later case, non-parametric statistics may have to be used or the median value has to be established.

Associations

The association of the metal-bearing forms will be established from the backscattered electron images. Particular attention will be paid in establishing whether the grains are totally enclosed, encapsulated or liberated. The rinds of metal-bearing grains will be identified. Representative photomicrographs of backscatter electron images establishing the association of the principal metal-bearing forms will be obtained for illustration purposes.

9.5 Instrument Calibration and Standardization

The WDS will have spectrometers calibrated for the metal of concern, carbon, oxygen and sulfur on the appropriate crystals using mineral standards. The EDS will have multi-channel analyzer (MCA) calibrated for known peak energy centroids. Calibration will be performed so as to have both low (1.0-3.0 KeV) and high (6.0-9.0 KeV) energy peaks fall within 0.05 KeV of its known centroid.

The magnification marker on the instrument will be checked once a week. This will be performed by following manufacturer instructions or by measurement of commercially available grids or leucite spheres. Size measurements must be within 4 microns of certified values.

Initial calibration verification standards (ICVs) must be analyzed at the beginning of each analytical batch or once every 48 hours, whichever is more frequent. A set of mineral or glass standards will be run quantitatively for the metal of concern, sulfur, oxygen and carbon. If elemental quantities of the ICVs do not fall within +/- 5% of certified values for each element, the instrument must be recalibrated prior to analysis of investigative samples.

The metal-bearing forms in these samples will be identified using a combination of EDS, WDS and BEI. Once a particle is isolated with the backscatter detector, a 5-second EDS spectra is collected and peaks identified. The count rates for the metal(s) of concern, sulfur, carbon and oxygen can be either visually observed on the wavelength spectrometers or K-ratios calculated.

9.6 Documentation

Photomicrographs along with EDS x-ray spectra should be taken for each sample, at a rate of 5% (1 photograph per 20 particles counted), or a minimum of 10 per sample and submitted with the results. Particles selected for photography must be recorded on the EMPA graph, as well as in the Electron Micrograph Logbook. Any additional photographs should be labeled as "opportunistic".

A 128x128 (minimum) binary image in ".tif" format may be stored. Recorded on each photomicrograph will be a scale bar, magnification, sample identification, date and phase identification. Abbreviations for the identified phases can be used. A final list must be submitted with the laboratory report.

10.0 PERSONAL HEALTH AND SAFETY

Each individual operating the electron microprobe instruments will have read the "Radiation Safety Handbook" prepared by the University and follow all State guidelines for operation of X-ray equipment.

Latex gloves and particulate masks will be worn during preparation of sample cups. All material that comes in contact with the samples or used to clean work surface areas will be placed in poly-bags for disposal.

11.0 FINAL REPORT

A final laboratory report will be provided to the Contractor. The report will include all EMPA data including summary tables and figures. Individual sample data will be provided on disk.

Speciation results will include: 1) a series of tables summarizing frequency of occurrence for each metal phase identified along with a confidence limit; 2) summary histograms of metal phases identified for each waste type; 3) a summary histogram of particle size distribution in each waste type; and 4) a summary of metal phase associations. Representative .tif images and EDS x-ray spectra will also be included in the final report.

12.0 REFERENCES

Bornschein, R.L., P.A. Succop, K.M. Kraft, and C.S. Clark. 1987. Exterior surface lead dust, interior lead house dust and childhood lead exposure in an urban environment. In D.D. Hemphil, Ed., Trace Substances in Environmental Health XX Proceedings of the University of Missouri's 20th Annual Conference. June 1986, pp 322-332. University of Missouri, Columbia, MO.

CDM (Camp Dresser and McKee). 1994. Metal Speciation Data Report, Leadville, CO. CERCLA Site. September, 1994.

Chayes, F., 1949, A simple point counter for thin section analysis. Am. Mineralogist, V. 34, p. 1.

Drexler, J.W. 1992. Speciation Report on the Smuggler Mine, Aspen CO., Prepared for EPA.

Davis, A., J.W. Drexler, M.V. Ruby, and A. Nicholson. 1993. The micromineralogy of mine wastes in relation to lead bioavailability, Butte, Montana. Environ. Sci. Technol. (In Press).

El-Hinnawi, E.E., 1966, Methods in chemical and mineral microscopy, Elsevier Publishing Co., New York, 222p.

Emmons, S.F., J.D. Irving, and G.F. Loughlin. 1927. Geology and Ore Deposits of the Leadville Mining District, Colorado. USGS Professional Paper 148.

Heinrich, K.F.J., 1981, Electron beam x-ray microanalysis. Van Nostrand-Reinhold Co., Dallas, 578p.

Mosimann, J.E. 1965. Statistical methods for the Pollen Analyst. In: B. Kummel and D. Raup (EDS.). Handbook of Paleontological Techniques. Freeman and Co., San Francisco, pp. 636-673.

Ruby, M.V., A. Davis, J.H. Kempton, J.W. Drexler, and P.D. Bergstrom. 1992. Lead bioavailability: Dissolution kinetics under simulated gastric conditions. Environ. Sci. Technol. 26(6): pp 1242-1248.

WESTON (Roy F. Weston, Inc.). 1995. Metal Speciation Interpretive Report, Leadville, CO. CERCLA Site. March, 1995.

Figure 9-2

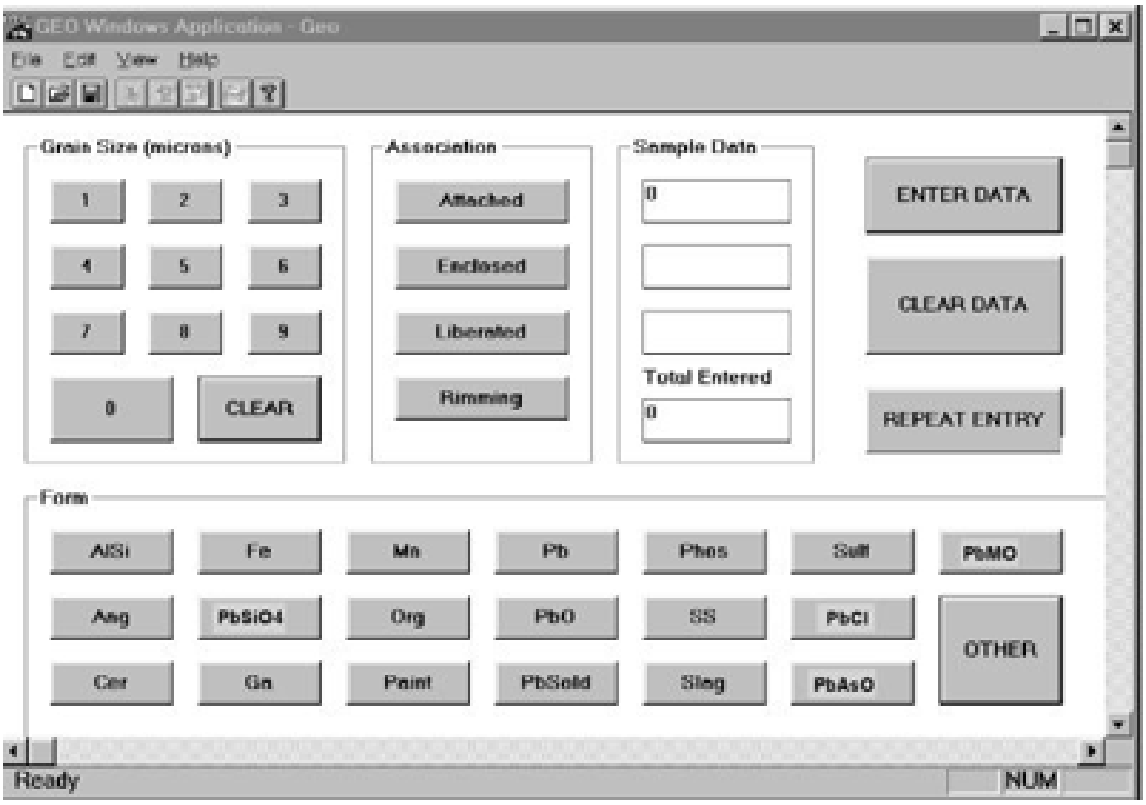

GEO Windows Application - Geo
File Edit View Help
Grain Size (microns)
1
2
3
4
5
6
7
8
9
0
CLEAR
Association
Attached
Enclosed
Liberated
Rimming
Sample Data
0
Total Entered
0
ENTER DATA
CLEAR DATA
REPEAT ENTRY
Form
AlSi
Fe
Mn
Pb
Phos
Sulf
PbMO
Asg
PbSiO4
Org
PbO
SS
PbCl
OTHER
Cer
Ga
Paint
PbSolid
Slag
PbAsO
Ready
NUM

Figure 9-3

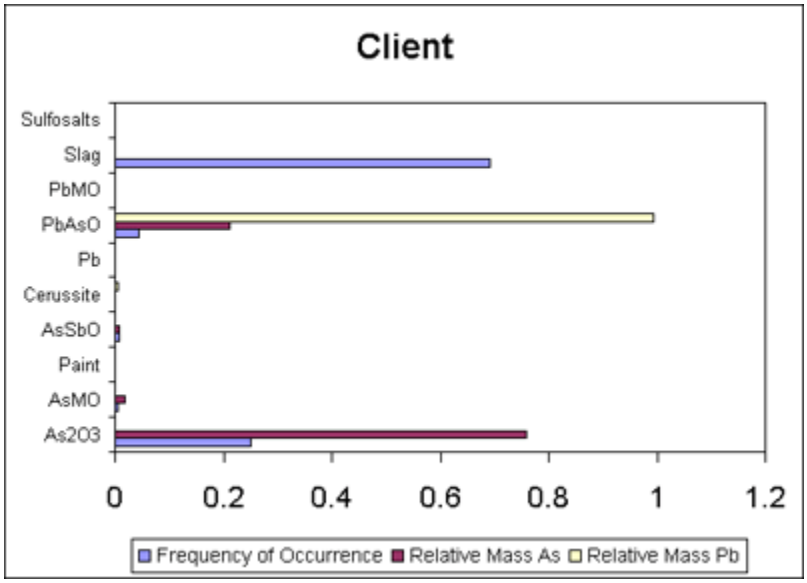
Particle	Notes	Phase	Length (a)	Phase Association			
				Liberated	Included	Cemented	Rimming
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
21							
22							

(a) Longest dimension, µm

Figure 9-4

Parameter	WDS	EDS
Accelerating Voltage	15 KV	15-20 KV
Beam Size	1-2 microns	1-2 microns
Cup Current	10-30 NanoAmps	10-30 NanoAmps
Ev/Channel	N/A	10 or 20
Stage Tilt	N/A	Fixed
Working Distance	N/A	Fixed
MCA Time Constant	N/A	7.5-12 microseconds
X-Ray Lines	S K-alpha PET O K-alpha LDE1 C K-alpha LDEC Zn K-alpha PET As L-alpha TAP Cu K-alpha LIF Cd L-alpha PET Pb M-alpha PET Pb L-alpha LIF In L-alpha PET Tl L-alpha LIF Hg L-alpha LIF Se L-alpha LIF Sb L-alpha PET	S K-alpha 2.31 KeV O K-alpha 0.52 KeV C K-alpha 0.28 KeV Pb M-alpha 2.34 KeV Pb L-alpha 10.5 KeV Zn K-alpha 8.63 KeV Cu K-alpha 8.04 KeV As K-alpha 10.5 KeV As L-alpha 1.28 KeV Cd L-alpha 3.13 KeV In L-alpha 3.28 KeV Tl M-alpha 2.27 KeV Tl L-alpha 10.26 KeV Hg L-alpha 9.98 KeV Hg M-alpha 2.19 KeV Se L-alpha 1.37 KeV Sb L-alpha 3.60 KeV

Figure 9-5



Site Created by Ben Drexler

ATTACHMENT 2
EXAMPLE DATA AND CALCULATIONS

1.0 OVERVIEW

This Attachment presents a hypothetical example of how measurements of IVBA and/or phase might be used in a site-specific human health risk assessment for arsenic.

At this hypothetical site, the land use is residential. Human health risk from arsenic is based on the reasonable maximum exposure (RME) cancer risk. RME exposure parameters are EPA defaults:

Parameter	Units	Child	Adult
Soil ingestion rate	mg/day	200	100
Body weight	kg	15	70
Exposure frequency	days/yr	350	350
Exposure duration	yrs	6	24

The exposure point concentration for arsenic is 125 mg/kg.

The risk manager has determined that if the RME cancer risk estimate from arsenic exceeds a value of 1E-04, then soil cleanup will be required.

2.0 EXAMPLE RESULTS

Step 1: Evaluate Risk using the National Default RBA

The national default RBA for arsenic in soil is 55%. Based on this assumption, the RME cancer risk from soil ingestion is 1.6E-04. If this value were used, soil cleanup for arsenic would be required.

However, it is suspected that the RBA of arsenic at this site might be lower than 55%. Therefore, the risk manager and the risk assessor determined that it would be valuable to measure the IVBA of arsenic is a sample of soil from the site in order to derive a site-specific estimate of RBA.

Step 2: Estimate RBA Based on IVBA Measurements

Two composite samples of soil were collected from the site and submitted for IVBA analysis at pH 1.5. The resulting values were 17% and 26%.

Based on these values, the site-specific estimates of RBA were calculated as follows:

RBA(sample 1) = 19.7 + 0.62 · 17 = 30%
RBA(sample 2) = 19.7 + 0.62 · 26 = 36%

Based on these two site-specific RBA estimates, the excess cancer risk estimates were as follows:

Sample	RBA	Risk
1	30%	8.9E-05
2	36%	1.1E-04

These results indicated that risks are close to and potentially below the 1E-04 decision criterion for soil cleanup. However, the risk manager determined that the results were not sufficient to be certain whether action was needed or not. Therefore, the risk manager and the risk assessor determined that measurement of the mineral phases in the sample would be helpful to strengthen the RBA estimate and the risk management decision.

Step 3: Estimate RBA Based on IVBA and Phase Measurements

The phase content of the samples was determined by electron microprobe analysis. The results, expressed as relative arsenic mass (RAM), are shown in Table A2-1. For each sample, the RAM values were summed within each of the three bins, yielding the following:

Bin	Sample 1	Sample 2
1	82	74
2	14	18
3	4	8

Utilizing the IVBA values measured previously (see Step 2), the RBA estimates were calculated as follows using the swine-based RAM-2 model:

RBA(sample 1) = 0.573·17+ 0.081·82 + 0.236·14 + 0.346·4 = 29%
RBA(sample 2) = 0.573·26+ 0.081·74 + 0.236·18 + 0.346·8 = 34%

Based on these RBA values, risk estimates were calculated as follows:

Sample	RBA	Risk
1	31%	8.4E-05
2	33%	9.8E-05

Based on these measurements and calculations, the risk manager determined that there was sufficient evidence to conclude that risks from arsenic in soil did not exceed the risk-based criterion for soil cleanup.

TABLE A2-1
RELATIVE ARSENIC MASS ESTIMATES
MEASURED BY ELECTRON MICROPROBE

Bin (a)	Phase	Sample 1	Sample 2
1	AsMO	26	17
	Slag	1	2
	Phosphate	41	22
	Pyrite		
	Clay		
	Sulfo Salt	14	29
	As2O3		
	Arsenopyrite		4
2	FeSO4	11	3
	FeOOH	3	15
3	PbAsO		
	MnOOH	4	8
	ZnSiO4		
	NaAsO4		
	PbMO		

(a) Bins are based on the swine RAM-2 model (see Table 4-4)

Phase VI Report:
Results of Round-Robin Evaluation of IVBA Testing and Arsenic Speciation

FINAL

ARSENIC IVBA OPTIMIZATION PROJECT

PHASE VI REPORT
RESULTS OF ROUND-ROBIN EVALUATION OF IVBA TESTING AND ARSENIC SPECIATION

March 29, 2012

Prepared by:

U.S. Environmental Protection Agency
Region 8
15195 Wynkoop Street
Denver, CO 80202

University of Colorado
Department of Geological Sciences
Benson Earth Science
2200 Colorado Ave.
Boulder, CO 80309

SRC, Inc.
999 18th Street, Suite 1975
Denver, CO 80202

Exponent
4141 Arapahoe Avenue, Suite 101
Boulder, CO 80303

CDM Smith
555 17th Street, Suite 1100
Denver, CO 80202

TABLE OF CONTENTS

LIST OF APPENDICES

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

The overall goal of the arsenic *in vitro* bioaccessibility assay (IVBA) optimization project is to develop a method that reliably predicts the *in vivo* relative oral bioavailability (RBA) of arsenic in soil based on one or more measurements performed *in vitro.* This project has been conducted in multiple phases (EPA 2009):

- Phase I of the project consisted of a literature review to indentify candidate test materials and to inventory soils that were available for testing.
- Phase II of the project investigated the effect of a wide range of experimental variables in the *in vitro* extraction protocol and identified three variables (pH, phosphate [PO_4] concentration, and hydroxylamine hydrochloride [HAH] concentration) that impact IVBA results the most.
- Phase III of the project consisted of a series of studies to measure arsenic IVBA for a selected set of test substrates using various combinations of the three key extraction fluid variables identified in Phase II. The objective of the Phase III work was to select up to three alternative combinations of extraction fluid variables that provide the best predictive relationship between IVBA and RBA (EPA 2009).
- Phase IV and Phase V of the project consisted of finalizing the IVBA extraction conditions and testing the final extraction protocols on an expanded set of test substrates. The outcome of this effort was a set of mathematical models to predict RBA from IVBA measurements, with or without supplemental arsenic speciation data, for a wide range of test materials.

The purpose of this report is to summarize the results of Phase VI of the overall project. Phase VI consisted of an inter-laboratory ("round-robin") testing of several IVBA extraction methods, and also the results of inter-laboratory testing of arsenic speciation by electron microprobe analysis. The purpose of Phase VI was to evaluate the reliability and reproducibility of the laboratory protocols for obtaining IVBA and speciation data needed for RBA prediction.

2.0 PHASE VI STUDY OF IVBA EXTRACTIONS

2.1 Participating Laboratories

Three laboratories participated in the Phase VI round-robin evaluation of IVBA extractions:

- ACZ Laboratories, Inc. (ACZ), a commercial analytical laboratory located in Steamboat Springs, CO
- EPA Region 7 (R7) Regional Laboratory, located in Kansas City, KS

- EPA Region 8 (R8) Regional Laboratory, located in Golden, CO

2.2 Test Materials

A set of 12 test soils was evaluated in the Phase VI assessment of IVBA extraction. This set of test soils was selected based on a consideration of the amount of soil available (a minimum of 10 grams per laboratory was needed), and with the goal of providing samples with a wide range of observed IVBA values and good variation in the nature (concentration and phase composition) of the arsenic contamination. These test soils selected for use are listed in Table 2-1, along with the IVBA values and predominant arsenic phases identified by the reference laboratory (University of Colorado, Boulder [UCB]).

2.3 Extraction Conditions

Three IVBA extraction conditions were evaluated in Phase VI:

1. pH 1.5, with 0.05 M PO_4 and 0.1 M HAH
2. pH 1.5, without PO_4 or HAH additions
3. pH 7.0, with 0.05 M PO_4

Other parameters of the Relative Bioaccessibility Leaching Procedure (RBALP) method remained unchanged (i.e., extraction time was maintained at 1 hour, water bath was maintained at a temperature of 37°C, glycine buffer concentration was maintained at 0.4 M, filter size was maintained at 0.45 um, soil mass was maintained at 1 gram).

2.4 Round-Robin Design

Each laboratory was provided with one 10-g bottle of each of the 12 test soils. Each laboratory performed triplicate extractions of each soil for each of the three different extraction conditions. The detailed protocol provided to each laboratory is attached as Appendix A.

2.5 Round-Robin Results for IVBA

QC Samples

Each laboratory included one laboratory blank and one spiked laboratory standard in each set of extraction bottles. This resulted in a total of 12 blanks and 12 spikes per laboratory. The results are summarized below:

Participating Laboratory	Blank (ug/L)		Spike (% Recovery)	
	Mean	Stdev	Mean	Stdev
UCB	-0.01	0.03	107.6	1.8
ACZ	2.0	0.7	105.9	5.0
R7 (unadj.)	-140.7	25.9	93.2	6.3
R7 (adj.)	0.0	0.0	98.2	5.9
R8	< 5	--	107.0	1.5

As seen, for UCB, ACZ and R8, concentrations of arsenic measured in laboratory blanks were very low, and were sufficiently small compared to concentrations in test material extracts (typically < 1%) that no background subtraction was performed. For R7, the raw data for laboratory blanks were biased low by a significant amount (about 141 ug/L), resulting in an overall low bias in the results for laboratory spikes and test materials. Consequently, R7 adjusted each batch of results by subtracting the laboratory blank associated with that batch.

In all cases, the recovery of laboratory spikes was close to 100%.

These data indicate that the analytical measurements of arsenic concentration in IVBA extraction fluids performed by each laboratory were of good quality and are appropriate for use in this study.

Test Materials

Tables 2-2 to 2-5 present the IVBA data reported for each test material by each laboratory, along with the mean and standard deviation for each test material for each extraction condition.

Within-laboratory Precision

Within-laboratory precision was evaluated by examining the magnitude of the standard deviation for each set of three replicate values. As seen, within-laboratory precision was typically less than 3%, with an average of 0.8% for all three laboratories. This variability is well within the acceptable limits of ± 20% for pure analytical variation by Method 6020.

Between-Laboratory Precision

Between-laboratory precision was evaluated by comparing the mean IVBA result for each soil for each extraction condition between each pair of laboratories, including the reference laboratory.

Figure 2-1 presents these data in "scattergram" format. If there were perfect agreement between each pair of laboratories, all of the data points would lie on the line of identity (indicated by the red diagonal line in each panel). As seen, there is good agreement between laboratories in most cases, although some systematic differences are apparent. For example, for extraction fluid 1 (pH 1.5 with 0.05 M PO_4 and 0.1 M HAH), the values reported by R7 and R8 tend to be slightly low compared to the values reported by UCB and ACZ.

Figure 2-2 shows these same data presented in bar graph format. In this figure, the bar shows the mean IVBA and the error bars represent ± one standard deviation.

A second way to evaluate between-laboratory precision is to examine the between-laboratory variability in the mean IVBA values for each test soil for each extraction condition. Results are shown in Table 2-6. As seen, for most test materials, between-laboratory variation in mean values was generally less than 7%, with an overall average of 3%. As noted above, this variation is substantially smaller than that associated with pure analytical variability considered acceptable for Method 6020, indicating that the IVBA extraction protocol itself is highly reproducible, both within and between laboratories..

2.6 Conclusions for IVBA

Based on the results of the Phase VI investigation, it is concluded that IVBA extractions for arsenic can be implemented by laboratories with high within-laboratory precision and good between-laboratory precision, well within limits that are generally considered to be acceptable.

3.0 PHASE VI STUDY OF ARSENIC SPECIATION

3.1 Participating Laboratories

Analysts from two organizations participated in the Phase VI round-robin evaluation of arsenic speciation:

- The Denver office of CDM Smith Inc., a national environmental consulting and engineering firm. The electron microprobe work was performed in the UCB laboratory by CDM Smith personnel.
- Geller Environmental, a commercial analytical laboratory located near Fairfax City, VA.

3.2 Test Materials

A set of 3 test substrates was evaluated in the Phase VI assessment of arsenic speciation by electron microprobe analysis. This set of test soils was selected based on a consideration of the phase composition of the arsenic contamination. These test soils are listed in Table 3-1, along with the speciation results obtained by the reference laboratory (UCB).

For each test soil, a single puck was prepared for analysis by UCB, and the same puck was used for analysis by each participant. All pucks were polished between analyses.

3.3 Speciation Protocol

The detailed protocol that was provided to each participating laboratory is provided in Appendix B. In brief, the protocol specified the arsenic count rate needed to select a grain as arsenic bearing, the number of grains needed per sample, as well as text descriptions and example energy-dispersive spectrometry (EDS) spectrograms of each of 16 phases.

During implementation of the analysis by the participants, it became apparent that the time required to analyze each sample was much higher than for the reference laboratory (UCB). The reason for the slow throughput was likely due to a lower level of experience in analyzing soil samples by the participating individuals, as well as a tendency to misidentify numerous clay particles as arsenic-bearing grains. This misidentification arises because clay particles are typically high in magnesium, and the EDS peak for magnesium K-alpha overlaps the peak for arsenic L-alpha, giving the appearance of elevated arsenic concentration. In addition, the WDS spectrometer count rate for arsenic is elevated because of interferences from both magnesium and iron peaks.

Because of these difficulties, the design of the speciation study was changed from a true round-robin design to a design where each result was used to identify and correct problems in subsequent analyses. For example, once the problem with misidentification of clay particles was recognized, the count rate needed to identify a particle as arsenic-bearing was increased to help minimize the selection of clay particles. In addition, frequent consultations between the participating individuals and the reference laboratory were performed to help answer questions and increase the speed and reliability of particle phase identification.

3.4 Speciation Results

Table 3-1 summarizes the arsenic speciation results for the three test soils, both for length-weighted frequency (Panel A) and for relative arsenic mass (Panel B). Inspection of the data in this table reveals that agreement is generally poor.

For example, in the table of results based on length-weighted frequency table (Table 3-1, Panel A), both CDM Smith and Geller tended to assign a much larger number of particles to "Clay" than did UCB. Conversely, CDM Smith and Geller tended to identify far fewer slag particles than UCB, even in Sample 2 (MTSS), which is collected from a location known to be contaminated with slag.

With regard to relative arsenic mass (Panel B), for Sample 1 (VBI70 TM-1), CDM Smith estimated that most of the arsenic was present as FeOOH and none was present as As2O3, while both UCB and Geller estimated that most of the arsenic in this sample was present as As2O3, with only low amounts of FeOOH. For Sample 3 (Barber Orchard MS-5), UCB estimated that most of the arsenic mass was present as PbAsO and sulfosalts, while CDM Smith and Geller did not report any arsenic in these phases.

3.5 Discussion of Speciation Results

The poor agreement between laboratories for the speciation data is likely the result of two main factors.

Counting Variability

Random counting variability in the frequency of particles observed is likely to be a substantial factor in the between-laboratory variation. For example, in a sample in which the true fraction of particles of type "x" is 5%, in an examination of 200 particles, the expected count of such particles would be 10. However, due to random Poisson counting variability, the actual number of type "x" particles observed could vary from about 5 to 18 (close to a four-fold range). This problem is especially important for estimating

relative arsenic mass in samples that are characterized by low-frequency particles that have high concentrations of arsenic. The variability due to low particle counts can be reduced by increasing the number of particles counted, but this would increase both the time and cost per sample.

Phase Assignment

The second factor that appears to be contributing to the high between-laboratory variability is differences in the phase assignments for a given particle. For example, Geller provided EDS spectra for all particles that were evaluated. Based on a random selection of 30 of these spectra, UCB assigned a different phase in 9 out of 30 cases. This problem is probably related to differences in operator experience and/or to inadequate direction from EPA on how to make phase assignments. Hence, this problem could likely be minimized by increased detail in the Speciation SOW, coupled with increased training.

3.6 Conclusions for Speciation

Although it is likely that additional efforts to identify and minimize sources of variability could lead to decreased between-laboratory differences in speciation results, such an effort would almost certainly require a substantial investment of time and money, well beyond the scope that was planned and budgeted for this project. In addition, even if agreement were good, the time and cost of speciation appears to be too high to be feasible in most cases. Based on this, it is concluded that, under present conditions, speciation results are too costly and too variable to support the use of mathematical models that require speciation data to improve estimates of RBA.

TABLE 2-1 SOILS SELECTED FOR ROUND ROBIN TESTING

	Test material	Bulk As mg/kg	RBA %	Reference IVBA (%)			Predominant Arsenic Phases (a)
				Fluid 1	Fluid 2	Fluid 3	
1	WAOS	300	24.0	96.8	84.1	2.8	PbAsO
2	VBI70 TM1	312	40.3	83.4	73.3	29.8	As2O3, PbAsO
3	NYPS2	339	19.0	80.5	56.2	27.6	FeOOH
4	COSS	1492	5.0	10.8	7.6	1.5	FeSO4, FeOOH
5	MTSS	647	13.0	67.6	46.6	10.3	FeOOH, Sulfosalt
6	CAMT	325	19.0	35.9	14.6	4.2	Arsenopyrite, FeOOH
7	NYOS	123	15.0	74.5	32.0	11.0	PbAsO, MnOOH
8	Barber MS-5	382	48.7	64.5	17.6	9.9	PbAso, Sulfosalt, FeOOH
9	Bingham Cr.	149	39.3	30.3	15.6	3.7	Arsenopyrite, FeOOH,FeSO4
10	Butte TM1	234	17.8	27.4	8.0	2.3	Sulfosalt, FeOOH
11	VBI70 TM3	390	36.7	81.4	72.6	25.9	As2O2
12	NYPS3	549	28.0	59.0	25.2	8.9	FeOOH

(a) based on relative arsenic mass

TABLE 2-2 IVBA RESULTS FOR UCB

Extraction Fluid	Test Soil		IVBA				
	Number	Name	Rep. 1	Rep. 2	Rep. 3	Mean	Stdev
pH 1.5 +PO4+HAH	1	WAOS	98%	94%	99%	97%	2.7%
	2	VBI70 TM1	80%	86%	84%	83%	2.9%
	3	NYPS2	96%	97%	93%	96%	2.1%
	4	COSS	11%	11%	11%	11%	0.2%
	5	MTSS	68%	67%	68%	68%	0.4%
	6	CAMT	37%	36%	35%	36%	1.4%
	7	NYOS	75%	75%	73%	74%	1.1%
	8	Barber MS-5	65%	64%	64%	64%	0.1%
	9	Bingham Cr.	30%	30%	30%	30%	0.1%
	10	Butte TM1	28%	28%	27%	27%	0.7%
	11	VBI70 TM3	83%	79%	82%	81%	2.4%
	12	NYPS3	51%	49%	49%	50%	0.7%
pH 1.5 No additions	1	WAOS	87%	86%	79%	84%	4.7%
	2	VBI70 TM1	80%	72%	68%	73%	5.9%
	3	NYPS2	40%	42%	41%	41%	0.7%
	4	COSS	7.6%	7.5%	7.6%	7.6%	0.0%
	5	MTSS	47%	46%	47%	47%	0.6%
	6	CAMT	15%	14%	15%	15%	0.4%
	7	NYOS	32%	32%	32%	32%	0.1%
	8	Barber MS-5	18%	17%	17%	18%	0.4%
	9	Bingham Cr.	16%	16%	15%	16%	0.5%
	10	Butte TM1	8%	8%	8%	8%	0.3%
	11	VBI70 TM3	73%	69%	75%	73%	3.0%
	12	NYPS3	36%	34%	34%	35%	0.8%
pH 7.0 + PO4	1	WAOS	2.9%	2.8%	2.7%	2.8%	0.1%
	2	VBI70 TM1	30%	30%	30%	30%	0.3%
	3	NYPS2	14%	14%	15%	14%	0.2%
	4	COSS	1.5%	1.5%	1.4%	1.5%	0.0%
	5	MTSS	11%	10%	10%	10%	0.3%
	6	CAMT	4.1%	4.2%	4.3%	4.2%	0.1%
	7	NYOS	11%	11%	11%	11%	0.3%
	8	Barber MS-5	10.5%	9.7%	9.5%	9.9%	0.6%
	9	Bingham Cr.	4.0%	3.5%	3.7%	3.7%	0.2%
	10	Butte TM1	2.4%	2.3%	2.1%	2.3%	0.1%
	11	VBI70 TM3	26%	26%	26%	26%	0.3%
	12	NYPS3	17%	17%	17%	17%	0.3%

TABLE 2-3 IVBA RESULTS FOR ACZ LABORATORIES, INC.

Extraction Fluid	Test Soil		IVBA				
	Number	Name	Rep. 1	Rep. 2	Rep. 3	Mean	Stdev
pH 1.5 +PO4+HAH	1	WAOS	94%	94%	87%	92%	4.1%
	2	VBI70 TM1	76%	78%	82%	79%	2.8%
	3	NYPS2	111%	106%	102%	106%	4.7%
	4	COSS	11%	11%	11%	11%	0.0%
	5	MTSS	68%	68%	70%	69%	1.3%
	6	CAMT	40%	38%	38%	39%	1.1%
	7	NYOS	76%	76%	76%	76%	0.5%
	8	Barber MS-5	64%	65%	65%	64%	0.5%
	9	Bingham Cr.	34%	32%	34%	34%	1.1%
	10	Butte TM1	31%	30%	30%	31%	0.7%
	11	VBI70 TM3	90%	83%	82%	85%	4.3%
	12	NYPS3	48%	48%	48%	48%	0.3%
pH 1.5 No additions	1	WAOS	85%	79%	81%	82%	2.8%
	2	VBI70 TM1	71%	71%	72%	71%	0.5%
	3	NYPS2	51%	50%	50%	50%	0.6%
	4	COSS	8.4%	8.4%	8.5%	8.5%	0.1%
	5	MTSS	50%	49%	49%	50%	0.3%
	6	CAMT	19%	19%	19%	19%	0.1%
	7	NYOS	39%	38%	39%	39%	0.4%
	8	Barber MS-5	17%	17%	17%	17%	0.0%
	9	Bingham Cr.	18%	19%	19%	19%	0.6%
	10	Butte TM1	10%	10%	10%	10%	0.1%
	11	VBI70 TM3	69%	68%	68%	68%	1.0%
	12	NYPS3	32%	33%	33%	33%	0.6%
pH 7.0 + PO4	1	WAOS	2.7%	2.7%	2.7%	2.7%	0.0%
	2	VBI70 TM1	27%	27%	28%	27%	0.6%
	3	NYPS2	15%	16%	16%	16%	0.3%
	4	COSS	1.5%	1.6%	1.6%	1.6%	0.1%
	5	MTSS	14%	13%	12%	13%	1.2%
	6	CAMT	5.1%	4.6%	4.7%	4.8%	0.3%
	7	NYOS	12%	11%	12%	12%	0.5%
	8	Barber MS-5	9.1%	9.1%	9.4%	9.2%	0.2%
	9	Bingham Cr.	3.9%	4.0%	4.0%	4.0%	0.1%
	10	Butte TM1	2.6%	2.5%	2.9%	2.7%	0.2%
	11	VBI70 TM3	30%	27%	28%	28%	1.3%
	12	NYPS3	20%	18%	16%	18%	1.6%

TABLE 2-4 IVBA RESULTS FOR EPA REGION 7 LABORATORY

Extraction Fluid	Test Soil		IVBA				
	Number	Name	Rep 1	Rep 2	Rep 3	Mean	Stdev
pH 1.5 +PO4+HAH	1	WAOS	81%	81%	83%	81%	1.0%
	2	VBI70 TM1	69%	70%	71%	70%	1.2%
	3	NYPS2	77%	77%	85%	80%	4.8%
	4	COSS	9%	9%	9%	9.3%	0.1%
	5	MTSS	57%	58%	58%	58%	0.3%
	6	CAMT	31%	30%	31%	31%	0.6%
	7	NYOS	63%	62%	61%	62%	0.8%
	8	Barber MS-5	54%	54%	55%	54%	0.8%
	9	Bingham Cr.	26%	26%	26%	26%	0.2%
	10	Butte TM1	23%	22%	23%	23%	0.4%
	11	VBI70 TM3	69%	68%	67%	68%	1.2%
	12	NYPS3	42%	43%	43%	42%	0.5%
pH 1.5 No additions	1	WAOS	75%	74%	73%	74%	1.1%
	2	VBI70 TM1	64%	69%	67%	66%	2.4%
	3	NYPS2	36%	37%	40%	38%	1.7%
	4	COSS	7%	7%	8%	7.2%	0.3%
	5	MTSS	48%	51%	45%	48%	2.7%
	6	CAMT	15%	12%	12%	13%	1.6%
	7	NYOS	30%	29%	29%	29%	0.6%
	8	Barber MS-5	16%	16%	16%	16%	0.2%
	9	Bingham Cr.	15%	14%	14%	15%	0.6%
	10	Butte TM1	7%	7%	7%	7.1%	0.3%
	11	VBI70 TM3	62%	62%	62%	62%	0.4%
	12	NYPS3	31%	32%	32%	32%	0.3%
pH 7.0 + PO4	1	WAOS	3%	3%	3%	3.2%	0.6%
	2	VBI70 TM1	24%	26%	24%	25%	1.0%
	3	NYPS2	14%	14%	16%	14%	1.3%
	4	COSS	1%	2%	1%	1.5%	0.0%
	5	MTSS	12%	11%	10%	11%	1.1%
	6	CAMT	4%	5%	5%	4.5%	0.8%
	7	NYOS	9%	9%	10%	10%	0.4%
	8	Barber MS-5	8%	8%	8%	8.1%	0.4%
	9	Bingham Cr.	3%	3%	3%	2.9%	0.0%
	10	Butte TM1	3%	3%	2%	2.6%	0.1%
	11	VBI70 TM3	26%	27%	27%	27%	0.6%
	12	NYPS3	17%	16%	16%	17%	0.4%

TABLE 2-5 IVBA RESULTS FOR EPA REGION 8 LABORATORY

Extraction Fluid	Test Soil		IVBA				
	Number	Name	Rep. 1	Rep. 2	Rep. 3	Mean	Stdev
pH 1.5 +PO4+HAH	1	WAOS	90%	96%	91%	92%	2.8%
	2	VBI70 TM1	77%	76%	76%	76%	0.5%
	3	NYPS2	86%	85%	86%	86%	0.6%
	4	COSS	9%	9%	9%	9%	0.2%
	5	MTSS	59%	60%	59%	59%	0.5%
	6	CAMT	29%	32%	31%	31%	1.4%
	7	NYOS	72%	67%	72%	70%	3.1%
	8	Barber MS-5	61%	59%	59%	59%	1.1%
	9	Bingham Cr.	24%	25%	26%	25%	0.8%
	10	Butte TM1	22%	21%	22%	21%	0.6%
	11	VBI70 TM3	79%	80%	79%	79%	0.9%
	12	NYPS3	47%	47%	47%	47%	0.1%
pH 1.5 No additions	1	WAOS	80%	80%	79%	80%	0.9%
	2	VBI70 TM1	69%	71%	68%	69%	1.5%
	3	NYPS2	47%	50%	47%	48%	1.8%
	4	COSS	8.5%	8.2%	8.0%	8.2%	0.2%
	5	MTSS	48%	47%	47%	47%	0.4%
	6	CAMT	16%	17%	16%	17%	0.1%
	7	NYOS	30%	31%	31%	31%	0.9%
	8	Barber MS-5	17%	17%	17%	17%	0.2%
	9	Bingham Cr.	15%	16%	17%	16%	0.8%
	10	Butte TM1	8.0%	7.5%	7.2%	7.5%	0.4%
	11	VBI70 TM3	65%	67%	67%	66%	1.0%
	12	NYPS3	33%	34%	32%	33%	1.0%
pH 7.0 + PO4	1	WAOS	2.4%	2.4%	2.5%	2.5%	0.1%
	2	VBI70 TM1	26%	27%	26%	27%	0.8%
	3	NYPS2	14.6%	14.9%	14.4%	14.6%	0.3%
	4	COSS	1.4%	1.5%	1.4%	1.4%	0.1%
	5	MTSS	11%	11%	11%	11%	0.2%
	6	CAMT	4.7%	4.2%	4.3%	4.4%	0.2%
	7	NYOS	9.4%	10%	10%	10%	0.4%
	8	Barber MS-5	7.6%	7.4%	7.8%	7.6%	0.2%
	9	Bingham Cr.	3.5%	3.5%	3.6%	3.5%	0.1%
	10	Butte TM1	2.3%	2.3%	2.4%	2.3%	0.0%
	11	VBI70 TM3	22%	23%	23%	23%	0.5%
	12	NYPS3	16%	16%	16%	16%	0.4%

TABLE 2-6 BETWEEN-LABORATORY VARIABILITY

Test material		Fluid 1		Fluid 2		Fluid 3	
		Mean (a)	Stdev (b)	Mean (a)	Stdev (b)	Mean (a)	Stdev (b)
1	WAOS	90.6	6.5	79.9	4.2	2.8	0.3
2	VBI70 TM1	77.1	5.6	70.0	3.0	27.1	2.1
3	NYPS2	91.9	11.7	44.1	5.8	14.8	0.6
4	COSS	10.2	1.1	7.9	0.6	1.5	0.1
5	MTSS	63.3	5.8	47.9	1.2	11.4	1.2
6	CAMT	34.0	4.1	15.8	2.6	4.5	0.3
7	NYOS	70.7	6.3	32.5	4.2	10.5	1.0
8	Barber MS-5	60.7	4.8	16.9	0.7	8.7	1.0
9	Bingham Cr.	28.7	3.9	16.2	1.8	3.5	0.5
10	Butte TM1	25.5	4.2	8.2	1.4	2.5	0.2
11	VBI70 TM3	78.5	7.2	67.3	4.4	25.8	2.4
12	NYPS3	46.8	3.1	33.1	1.3	16.9	1.0

(a) mean of means
(b) stdev of means

TABLE 3-1. SPECIATION DATA

Panel A: Length-Weighted Frequency

		VBI70 TM-1			MTSS			Barber Orchard MS-5		
Index	Phase	UCB	CDM	Geller	UCB	CDM	Geller	UCB	CDM	Geller
1	AsMO	1.3		1.4	0.2	5.3	7.7			
2	FeOOH	25.9	47.5	16.9	29.3	54.1	48.9	42.3	13.9	3.0
3	PbAsO	4.6	0.1	0.6			0.5	2.3		
4	PbMO		0.1			2.9	0.1			
5	Slag	29.0		1.2	57.5	0.5				
6	FeSO4	2.3	1.4		9.9	24.4	0.5			
7	MnOOH	9.9	13.1	3.3	1.5	0.5	1.0	52.7	4.7	
8	Phosphate	7.5	5.3	7.1		3.3	1.5	2.3		
9	Arsenopyrite									
10	Pyrite					2.9				
11	Sulfosalt				1.6	3.8	5.6	0.4		
12	Clay	3.0	29.5	53.0		1.0	24.8		73.9	80.0
13	ZnSiO4									
14	As2O3	16.6		5.1						
15	NaAsO4			5.5			1.6			
16	Zn		0.6							
17	Other		2.5	6.0		1.4	7.7		7.5	17.0

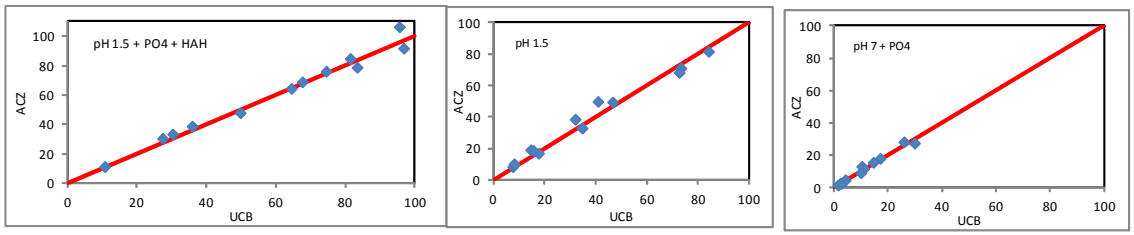
Panel B: Relative Arsenic Mass

		VBI70 TM-1			MTSS			Barber Orchard MS-5		
Index	Phase	UCB	CDM	Geller	UCB	CDM	Geller	UCB	CDM	Geller
1	AsMO	0.8		2.7	6.4	28.2	53.7			
2	FeOOH	1.8	61.6	3.6	68.2	50.3	25.9	17.4	78.7	100.0
3	PbAsO	10.9	2.2	4.5			2.1	47.6		
4	PbMO		1.4				0.2			
5	Slag				1.9	0.01				
6	FeSO4	0.2	1.5		4.7	4.0	0.1			
7	MnOOH	0.4	9.9	0.4	0.4	0.04	Tr	7.7	4.3	
8	Phosphate	1.0	12.0	2.8		1.1	0.1	0.7		
9	Arsenopyrite									
10	Pyrite					0.03				
11	Sulfosalt				18.4	16.1	16.6	28.6		
12	Clay	0.1	10.9	3.3		0.1	0.9		16.9	
13	ZnSiO4									
14	As2O3	84.8		82.0						
15	NaAsO4						Tr			
16	Zn		0.1							
17	Other			0.7			Tr			

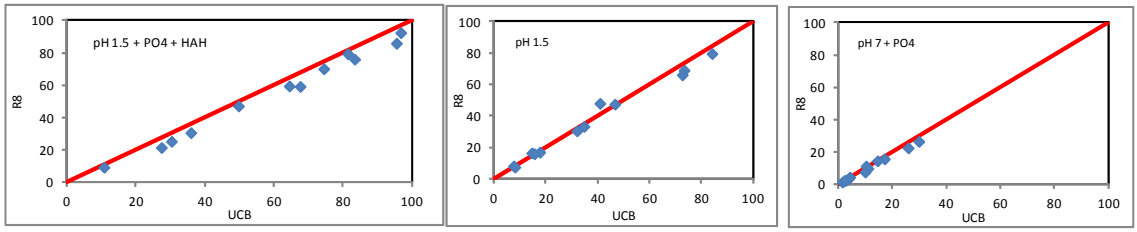
Tr = Trace

FIGURE 2-1 EVALUATION OF BETWEEN LABORATORY PRECISION

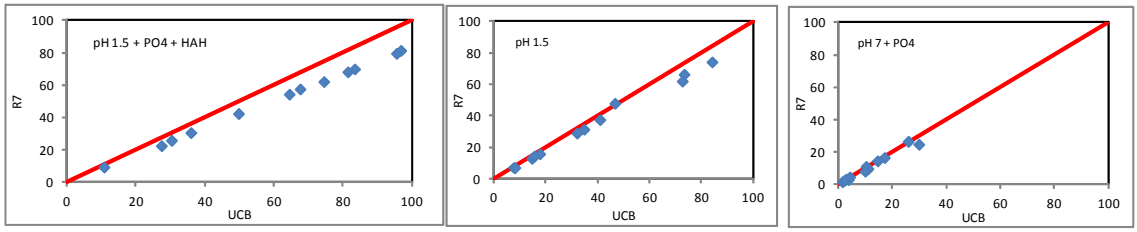
UCB vs ACZ



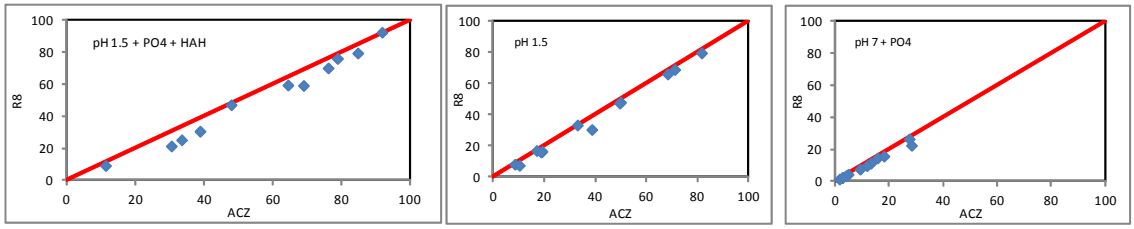
UCB vs R8



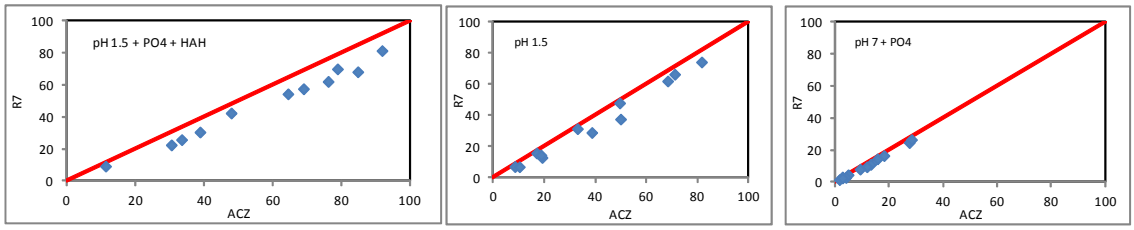
UCB vs R7



ACZ vs R8



ACZ vs R7



R8 vs R7

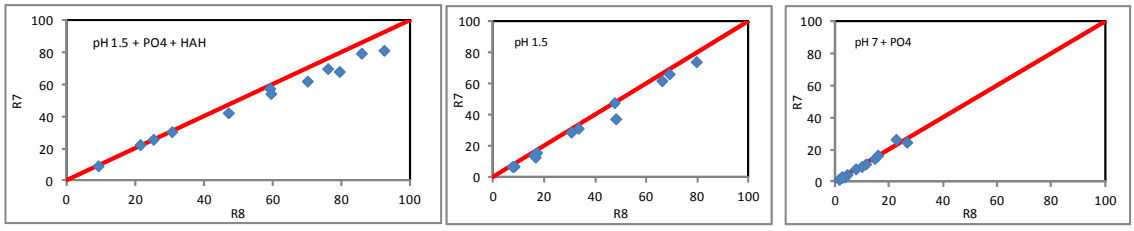
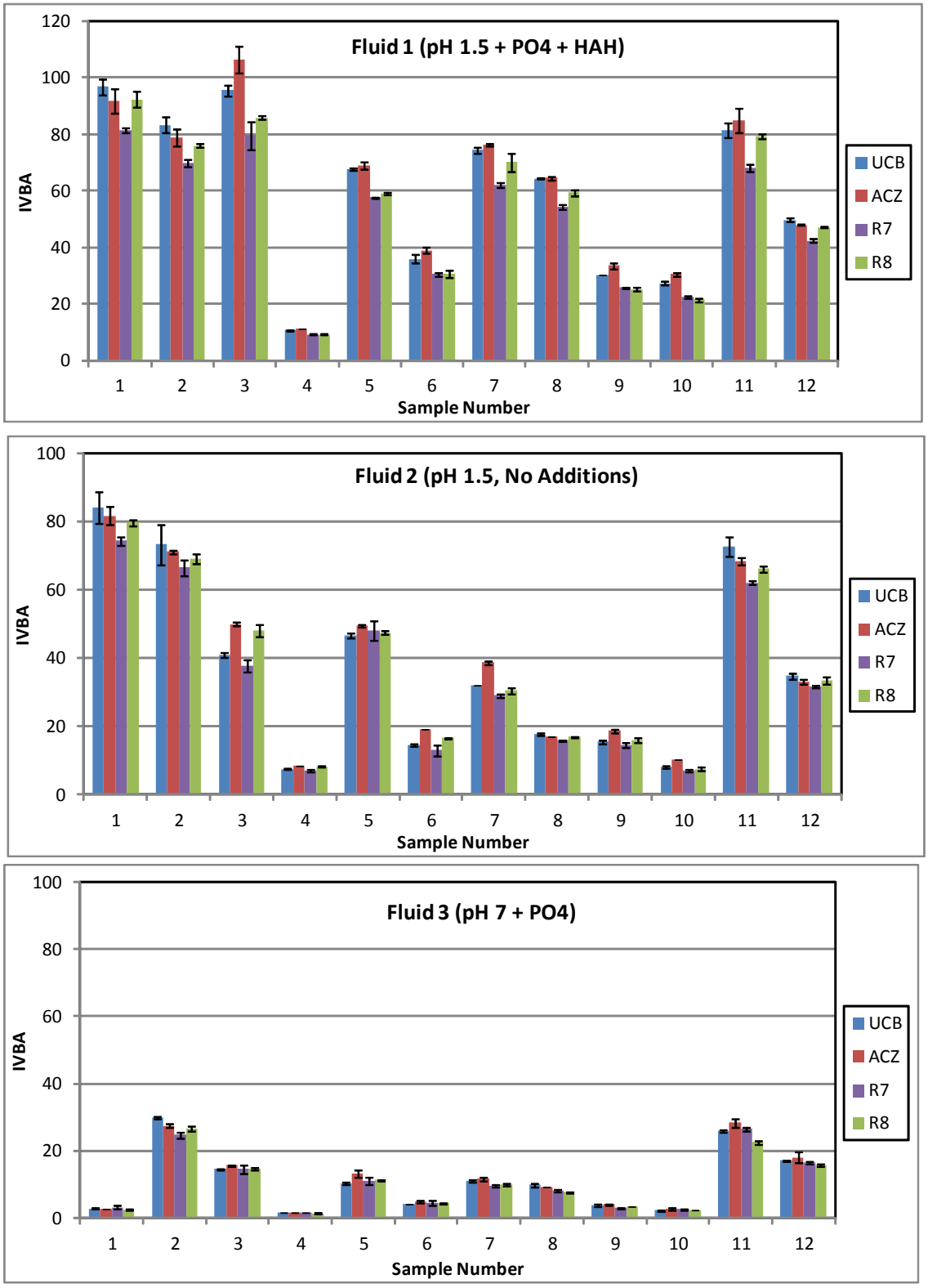


FIGURE 2-2 BETWEEN-LABORATORY PRECISION FOR IVBA



APPENDIX A

SOP FOR IVBA

Standard Operating Procedure

In Vitro Bioacessibility (IVBA) Procedure for Arsenic

June 2011

1.0 PURPOSE

This Standard Operating procedure (SOP) describes a method for measuring the *in vitro* bioacessibility (IVBA) of arsenic under several different *in vitro* extraction conditions. These extraction conditions are being investigated to determine if IVBA values can be used to reliably predict the relative bioavailability (RBA) of arsenic measured *in vivo*.

Background on the development and validation of *in vitro* test systems for estimating IVBA of arsenic, lead and other metals in soil metals can be found in Ruby et al. (1993, 1996), Medlin (1972), Medlin and Drexler (1997), Drexler (1998), Casteel et al. (2006), USEPA (2006), and Drexler and Brattin (2007).

2.0 SAMPLE PREPARATION

Directions for soil preparation steps (if any) by the analytical laboratory will be provided by the study director.

In general, all test materials are prepared for the *in vitro* assay by drying (< 40 °C) followed by sieving to < 250 μm.

3.0 APPARATUS AND MATERIALS

3.1 Equipment

The extraction device used in the IVBA procedure is illustrated in Figure 1. For further information on the design, contact Dr. John W. Drexler, at 303-492-5251 or drexlerj@colorado.edu.

The device holds ten 125 mL wide-mouth high-density polyethylene (HDPE) bottles. These are rotated within a Plexiglas tank by a TCLP extractor motor with a modified flywheel. The water bath must be filled such that the extraction bottles remained immersed. Temperature in the water bath is maintained at 37 ± 2 °C using an immersion circulator heater.

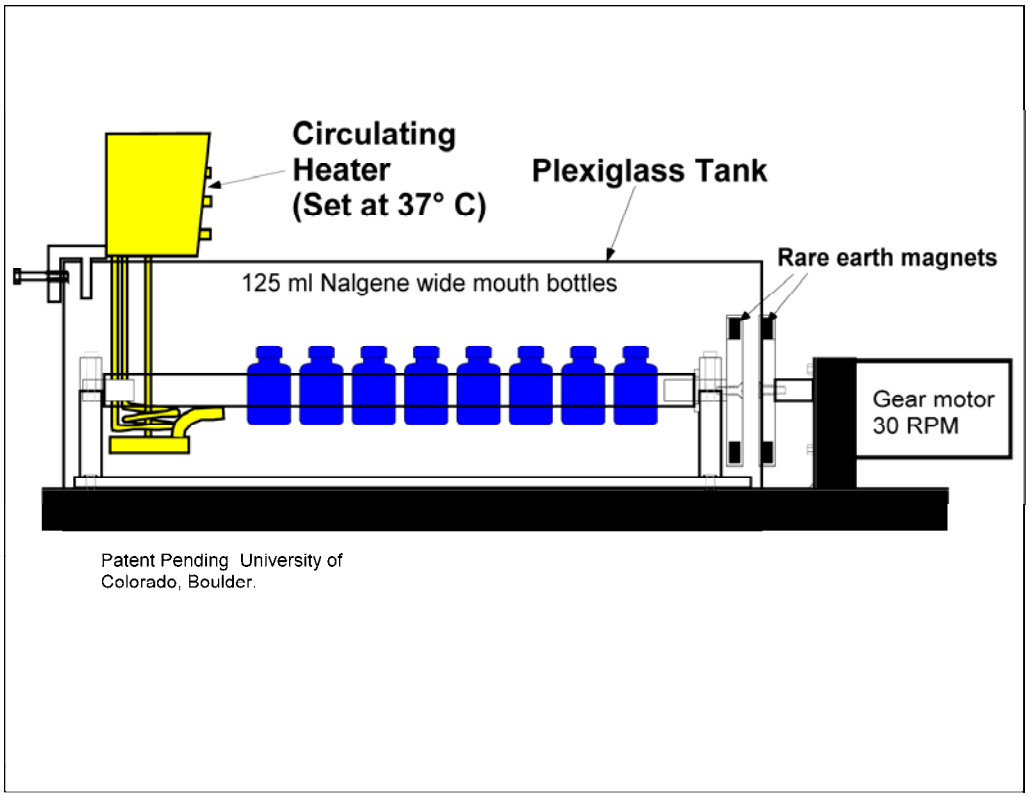


Figure 1. Schematic Diagram of IVBA Extraction Device

The 125-mL HDPE bottles must have an airtight screw-cap seal, and care must be taken to ensure that the bottles do not leak during the extraction procedure.

Other equipment required is listed below:

- Disposable 15 mL polypropylene centrifuge tubes.
- Disposable 25 mm 0.45 µm surfactant- free cellulose acetate syringe filters.
- Disposable 10 mL polypropylene syringes with Luer-Lok fittings

3.2 Solutions and Reagents

Depending on the specific test requested, three extraction solutions may be required. Required reagents include:

- Sodium Hydroxide, 50% w/w. CAS 1310-73-2.
- Glycine, Tissue Grade. CAS 56-40-6.
- Sodium Phosphate Dibasic Anhydrous, ACS Grade. CAS 7558-79-4.
- Hydrochloric Acid, Trace Metal Grade. CAS 7647-01-0.
- Nitric Acid, Trace Metal Grade. CAS 7697-37-2.

- Hydroxylamine Hydrochloride, ACS Grade. CAS 5470-11-1

All solutions are prepared utilizing ASTM Type II de-ionized (DI) water. All reagents and water must be free of arsenic, and the final fluid must be tested to confirm that arsenic concentrations are less than one-fourth of the project required detection limits (PRDLs) of 20 µg/L (e.g., 5 µg/L arsenic in the final fluid).

Cleanliness of all materials used to prepare and/or store the extraction fluid and buffer is essential. All non-disposable glassware and equipment used to prepare standards and reagents must be properly cleaned, acid washed, and finally, triple-rinsed with de-ionized water prior to use. Disposable labware is recommended whenever possible.

Extraction Fluid 1

Extraction Fluid 1 consists of 0.4 M glycine pH 1.5 supplemented with 0.05 M phosphate and 0.1 M hydroxylamine hydrochloride (HAH), prepared as follows:

To 1.937 L of DI water, add 60.6 g glycine (free base, reagent grade), 14.196 g anhydrous dibasic sodium phosphate and 13.90 g hydroxylamine hydrochloride. Add 63 ml of Trace-Metal grade hydrochloric acid (HCl) bringing the final solution volume to 2 L. Place the mixture in the water bath at 37 °C until the extraction fluid reaches 37 °C. Standardize the pH meter using both pH 2.0 and a pH 4.0 pH standards buffers using temperature compensation at 37 °C or buffers maintained at 37 °C. Add, dropwise, trace metal grade, concentrated hydrochloric acid (12.1N) until the solution pH reaches a value of 1.50 +/- 0.05.

Extraction Fluid 2

Extraction Fluid 2 consists of 0.4 M glycine pH 1.5 without phosphate or HAH, prepared as follows:

To 1.937 L of DI water, add 60.6 g glycine (free base, reagent grade). Add 63 ml of Trace-Metal Grade hydrochloric acid (HCl) bringing the final solution volume to 2 L. Place the mixture in the water bath at 37 °C until the extraction fluid reaches 37 °C. Standardize the pH meter using both pH 2.0 and a pH 4.0 pH standards buffers using temperature compensation at 37 °C or buffers maintained at 37 °C. Add, dropwise, trace metal grade, concentrated hydrochloric acid (12.1N) until the solution pH reaches a value of 1.50 +/- 0.05.

Extraction Fluid 3

Extraction Fluid 3 consists of 0.4 M glycine pH 7.0 supplemented with 0.05 M phosphate, prepared as follows:

To 2.0L of DI water, add 60.06 g glycine and 14.196 g anhydrous dibasic sodium phosphate. Place the mixture in the water bath at 37 °C until the extraction fluid reaches 37 °C. Standardize the pH meter using both pH 4.0 and pH 7.0 pH standard buffers using temperature compensation at 37 °C or buffers maintained at 37 °C. Add, dropwise, sodium hydroxide solution 50% w/w or concentrated, Trace-Metal Grade hydrochloric acid (HCl) until the solution pH reaches a value of 7.00 +/- 0.05.

4.0 EXTRACTION PROCEDURE

Attachment 1 provides a checklist that shall be followed when performing an IVBA extraction. Key steps are described below.

Extraction solution(s) must be placed in heated water bath prior to use and allowed to achieve operating temperature of 37 +/- 2 °C. The final pH is then adjusted (if necessary) and recorded as “starting pH” on the laboratory worksheet (see Section 7).

All test substances must be thoroughly mixed prior to use in the IVBA test to ensure homogeneity. This mixing may be achieved using a roller mixer (several minutes) or by end-over-end mixing for about 30 seconds.

After mixing, measure 1.00 +/- 0.5 g of test substrate and place in a clean 125 mL Nalgene bottle, ensuring that static electricity does not cause soil particles to adhere to the lip or outside threads of the bottle. If necessary, use an antistatic brush to eliminate static electricity prior to adding the media. Record the mass of substrate added to the bottle on the laboratory worksheet.

Measure 100 ± 0.5 mL of the designated extraction fluid, using a graduated cylinder, and transfer to a 125 mL wide-mouth HPDE bottle. Hand-tighten each bottle top and shake/invert to ensure that no leakage occurs, and that no media is caked on the bottom of the bottle.

Place the bottle into the extraction device (Figure 1), making sure each bottle is secure and the lid(s) are tightly fastened. Fill the extractor with 125 mL bottles containing test materials or QA samples.

The temperature of the water bath must be 37 ± 2 °C.

Turn on the extractor and rotate end-over-end at 30 ± 2 rpm for 1 hour. Record the start time of rotation on the laboratory worksheet.

After one hour, stop the extractor rotation and remove the bottles. Wipe them dry and place upright on the bench top.

Draw extract directly from the top portion of the extraction bottle into a disposable 10 mL syringe with a Luer-Lok attachment. Attach a 0.45 µm cellulose acetate disk filter (25 mm diameter) to the syringe, and filter the extract into a clean 15 mL polypropylene centrifuge tube (labeled with sample ID) or other appropriate sample vial for analysis.

Record the time on the laboratory worksheet that the extract is filtered (i.e., extraction is stopped). If the total extraction time elapsed is greater than 1 hour 30 minutes, the test must be repeated.

Measure and record on the worksheet the pH (Final pH) of the remaining fluid in the extraction bottle. If the fluid pH is not within ± 1.0 pH units of the starting pH, the test must be discarded and the sample reanalyzed.

Store filtered samples in a refrigerator at 4 °C until they are analyzed. Analysis for arsenic concentrations must occur within 1 week of extraction for each sample. To preserve the sample add 2 drops of trace-metal grade nitric acid (HNO_3) to labeled 15mL polypropylene centrifuge tube.

5.0 SAMPLE ANALYSIS

Extracts are analyzed for arsenic using USEPA Methods 6010B, 6020, or 7061A, as specified by the Study Director. For Method 6020, dilute each sample 50:1 (200 µL extract in 10 mL DI water) for analysis. This is needed to reduce interference from chlorine plus argon.

6.0 QUALITY CONTROL/QUALITY ASSURANCE

Quality Assurance for the extraction procedure will consist of the following quality control samples:

- A Laboratory Blank is a bottle containing 100 mL of extraction fluid put through the entire extraction process but with no added soil or test substrate
- A Blank-Spike is a bottle containing 2.5 ppm (2.5 µg/mL) arsenic, prepared by adding 250 µL of 1000 ppm NIST Traceable ICP arsenic standard solution to 100 mL of extraction fluid.

Unless otherwise specified by the study director, both QC samples types will be collected at a rate of 10%. Control limits are listed in Table 1. These values may be revised as additional data are collected.

Table 1. IVBA QC Sample Requirements

QC Sample Type	Analysis Frequency	Control Limits
Laboratory Blank	10%	<10 µg/L arsenic
Blank spike	10%	85-115% recovery

7.0 CHAIN-OF-CUSTODY PROCEDURES

Once received by the Laboratory, all test substances must be maintained under standard chain-of-custody.

8.0 DATA RECORDING, VALIDATION AND TRANSMITTAL

Data Recording

Figure 2 provides a worksheet for recording raw laboratory data. All raw data will be reported by hand by the individual performing the IVBA tests.

After the test is complete, the laboratory data and the analytical results will be recorded in the most recent version of a Microsoft Excel Electronic Data Deliverable (EDD), provided as Attachment 2. Figure 3 illustrates the structure of this EDD.

Data Validation

After data entry is complete, the laboratory director shall review the EDD compared to the laboratory worksheet and the analytical data package and ensure that all data have been entered correctly.

Data Transmittal

After validation, all data, including laboratory worksheets, analytical reports, and EDDs, shall be transmitted Laboratory Director to the Study Director.

9.0 REFERENCES

Casteel SW, Weis CP, Henningsen GM, Brattin WJ. 2006. Estimation of Relative Bioavailability of Lead in Soil and Soil-Like Materials Using Young Swine. Environ Health Perspect. 114:1162-1171. doi:10.1289/ehp.8852

Drexler, J.W. 1998. An in vitro method that works! A simple, rapid and accurate method for determination of lead bioavailability. EPA Workshop, Durham, NC.

Drexler,J. and Brattin,W. 2007. An *In Vitro* Procedure for Estimation of Lead Relative Bioavailability: With Validation. Human and Ecological Risk Assessment. 13(2), pp. 383-401.

Medlin, E., and Drexler, J.W. 1995. Development of an in vitro technique for the determination of bioavalability from metal-bearing solids. International Conference on the Biogeochemistry of Trace Elements, Paris, France.

Medlin, E.A. 1997. An In Vitro method for estimating the relative bioavailability of lead in humans. Masters thesis. Department of Geological Sciences, University of Colorado, Boulder.

Ruby, M.W., A. Davis, T.E. Link, R. Schoof, R.L. Chaney, G.B. Freeman, and P. Bergstrom. 1993. Development of an in vitro screening test to evaluate the in vivo bioaccessibility of ingested mine-waste lead. Environ. Sci. Technol. 27(13): 2870-2877.

Ruby, M.W., A. Davis, R. Schoof, S. Eberle, and C.M. Sellstone. 1996. Estimation of lead and arsenic bioavailbilty using a physiologically based extraction test. Environ. Sci. Technol. 30(2): 422-430.

USEPA. 2006. Estimation of Relative Bioavailability of Lead in Soil and Soil-Like Materials Using In Vivo and In Vitro Methods. U.S. Environmental Protection Agency: Washington, DC. Available online at http://www.epa.gov/superfund/health/contaminants/bioavailability/lead_tsdmain.pdf.

FIGURE 2 EXAMPLE LABORATORY WORKSHEET

ARSENIC IVBA MEASURMENTS

Lab Name: XYZ Labs
Date: 7/21/2011
Analyst: J. Smith

Index	Sample ID	Laboratory ID	Arsenic Conc. (ug/g)	Extraction Fluid Type	Extraction Fluid Vol. (mL)	Sample Mass (g)	pH Start	pH End	Time Start	Time Filter	Notes
1	12-34137	AB-10001	847	pH 1.5	100.4	0.997	1.48	1.56	10:12	11:21	
2	12-34137	AB-10002	847	pH 7.0	99.6	1.021	7.05	7.11	10:12	11:24	
3	12-34137	AB-10003	847	pH 7+PO4	100.3	1.035	6.98	7.08	10:12	11:29	
4	15-5123	AB-10004	451	pH 1.5	99.81	0.991	1.48	1.55	10:12	11:33	
5											
6											
7											
8											
9											
10											
11											
12											
13											
14											
15											
16											
17											
18											
19											
20											
21											
22											
23											
24											
25											

General Comments:
Water bath = 37.5

FIGURE 3 IVBA EDD FORMAT

ELECTRONIC DATA DELIVERABLE FOR ARSENIC IVBA MEASURMENTS

	Lab	Client	Laboratory	Arsenic	Extraction Fluid		Sample	pH		Time		Analysis	Conc in		
Index	Name	ID	ID	Conc. (ug/g)	Type	Vol. (mL)	Mass (g)	Start	End	Start	Filter	Method	Fluid (ug/L)	IVBA	Comments
1	XYZ labs	12-34137	AB-10001	847	pH 1.5	100.4	0.997	1.48	1.56	10:12	11:21	6020	257	30.6%	
2	XYZ labs	12-34137	AB-10002	847	pH 7.0	99.6	1.021	7.05	7.11	10:12	11:24	6020	184	21.2%	
3	XYZ labs	12-34137	AB-10003	847	pH 7+PO4	100.3	1.035	6.98	7.08	10:12	11:29	6020	192	22.0%	
4	XYZ labs	15-5123	AB-10004	451	pH 1.5	99.81	0.991	1.48	1.55	10:12	11:33	6020	38.4	8.6%	
5															
6															
7															
8															
9															
10															
11															
12															
13															
14															
15															
16															
17															
18															
19															
20															
21															
22															
23															
24															
25															
26															
27															
28															
29															
30															
31															
32															
33															
34															
35															
36															
37															
38															
39															
40															
41															
42															

ATTACHMENT 1
IVBA Procedure Checklist

1 Verify sample identification.
2 Label (black, permanent Sharpie) a NEW 125 mL Nalgene wide-mouth bottle with the sample identification.
3 Mix the sample thoroughly. Weigh 1.0 ± 0.05 g of sample (dried, <250 micron) onto NEW weighing paper.
4 Record the weight (± 0.0001 g) on the laboratory worksheet.
5 Place weighed sample into labeled 125 mL Nalgene bottle and tighten the bottle cap.
6 Heat water in the extraction apparatus to 37 ± 2 °C.
7 Prepare extraction fluid(s) as directed. If prepared ahead, the extraction fluids must be kept cool (2-4 °C) until needed.
8 Allow the extraction fluid to come to equilibrium with extraction apparatus at 37 ± 2 °C.

Steps 8-18 must be completed in < 90 minutes from the start of extraction or repeat the process

9 Calibrate the pH meter. Adjust (if necessary) and record the pH of the extraction fluid at 37±2°C.
10 Add 100 ± 0.5 mL of the designated extraction fluid to a labeled 125mL Nalgene bottle containing the test material.
11 Secure the labeled 125 mL Nalgene bottles in the extraction apparatus and rotate end-over-end for 1 hour.
12 Record the start time of rotation and initial extraction fluid pH.
13 After 1 hour, remove the labeled 125mL Nalgene bottles from the extraction apparatus, place upright, and wipe dry.
14 Using a NEW 10 mL disposable syringe with a Luer-Lok, remove an aliquot of un-filtered extract directly from the upper portion of the labeled 125 mL Nalgene bottle.
15 Attach a NEW 0.45 µm cellulose acetate filter to the Luer-Lok of the 10 mL syringe and filter the extract into a labeled 15 mL polypropylene centrifuge tube.
16 To preserve the sample add 2 drops of trace-metal grade nitric acid (HNO_3) to labeled 15mL polypropylene centrifuge tube.
17 Measure and record the final pH of the extraction fluid directly from the labeled 125mL Nalgene bottle.
18 The final pH must be within ± 1.0 of the initial extraction fluid pH or repeat the test.
19 Refrigerate labeled 15 mL polypropylene centrifuge tubes until analysis.

ATTACHMENT 2

IVBA EDD

See attached electronic file ("IVBA EDD v2.xls")

APPENDIX B

SOP FOR ARSENIC SPECIATION

Standard Operating Procedure

Arsenic Speciation

September 2011

1.0 INTRODUCTION

This Standard Operating Procedure (SOP) specifies methodologies and protocols that may be used to characterize (speciate) the chemical and physical nature of arsenic in arsenic-bearing particles that are present in a wide range of solid samples including soils, dusts, sediments, tailings, slags, dross, and other industrial wastes. Parameters characterized during the speciation analyses include mineral type (phase), particle size, and frequency of occurrence of arsenic-bearing forms.

2.0 INSTRUMENTATION

Arsenic speciation may be performed using either electron microprobe analysis (EMPA) or scanning electron microscopy (SEM) with instruments equipped with both wavelength dispersive spectroscopy (WDS) and energy dispersive spectroscopy (EDS) systems. However, because of the many x-ray limitations imposed on most SEM instruments, EMPA is preferred.

The WDS will have spectrometers calibrated for arsenic, oxygen and sulfur on the appropriate crystals using mineral standards. The EDS will have a multi-channel analyzer (MCA) calibrated for known peak energy centroids. Calibration will be performed so as to have both low (1.0-3.0 KeV) and high (6.0-15.0 KeV) energy peaks fall within 0.05 KeV of its known centroid.

Standard operating conditions for quantitative and qualitative analyses for arsenic-bearing forms are given in Table 1. However, it is the responsibility of the operator to select the appropriate analytical line (crystal/KeV range) to eliminate peak overlaps and ensure proper identification/quantification of arsenic and other identifying elements.

The magnification marker on the instrument will be checked once a week. This will be performed by following manufacturer instructions or by measurement of commercially available grids or Lucite spheres. Size measurements must be within 4 microns of certified values.

3.0 ANALYTICAL PROCEDURE

3.1 Sample Preparation

Directions for sample preparation steps (if any) by the analytical laboratory will be provided by the Study Director.

In general, all test materials are prepared for speciation analysis by drying (< 40 °C) followed by sieving to < 250 μm.

Samples of test material for this project have been prepared for speciation analysis by embedding approximately 1-2 grams of sample in a 1.25 inch round epoxy “puck” which is then polished to provide a smooth surface for examination.

Each laboratory should lightly clean the polished surface with alcohol prior to carbon coating.

3.2 Scanning Procedure

1. Set the initial magnification to 400X. This may be adjusted downward if small arsenic-bearing particles are absent or rare, and may be adjusted upward if small arsenic-bearing particles are common and cannot be adequately identified at 400X.
2. Adjust the electron backscatter detector threshold so that all particles in the sample are seen. This procedure will minimize the possibility that arsenic-bearing minerals with low average atomic number are overlooked during the scanning of the polished mount.
3. Peak the WDS spectrometer(s) for arsenic, oxygen and sulfur. Calibrate arsenic using a certified standard and provide the standard composition in final report. Using the accelerating voltage and beam current used during the point counting procedure, determine the background count rate (counts/second) for arsenic on a particle of quartz or feldspar.
4. Using the same accelerating voltage and beam current used during the analysis, determine the background count rate (counts/second) for arsenic on a particle of quartz or feldspar.
5. Begin scanning the sample manually using the scanning pattern depicted in Figure 1. That is, begin in the upper left portion of the puck, and traverse from left-to-right and top-to-bottom. The amount of vertical movement for each traverse depends on magnification and CRT (cathode-ray tube) size. This movement shall be adjusted so that each traverse lies just below the previous and that NO portion of the sample has been missed when the end of a traverse is reached

6. Arsenic-bearing particles are defined as particles where the count rate for arsenic is 2.0 times or more greater than the background count rate identified in Step 4[1]. Once an arsenic-bearing particle is identified, check for optical focus and make sure your magnification and beam location are optimized to provide a "clean" spectrum. Then record the data specified in Section 3.3.
7. Continue scanning until a minimum of 200 arsenic-bearing particles have been identified and characterized.

3.3 Particle Characterization

Particle Length

For each arsenic-bearing particle identified, adjust the backscatter image to clearly define the size and appearance of the particle. Measure and record the longest dimension (um) of the particle.

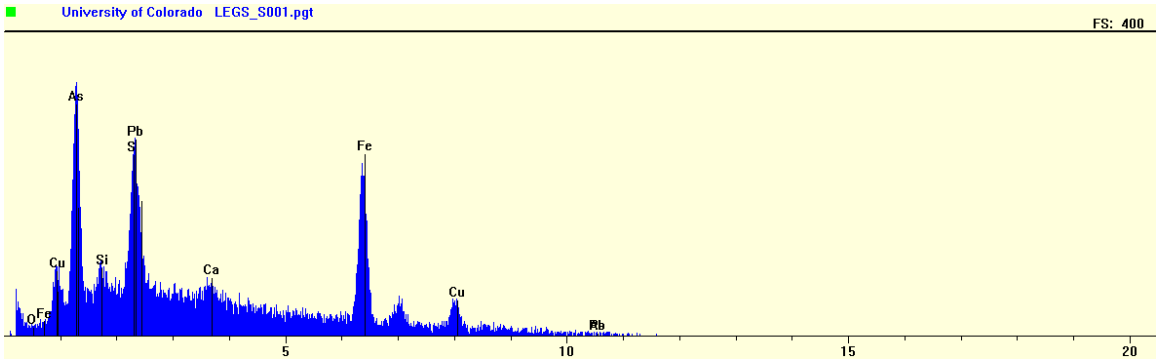
Phase Identification

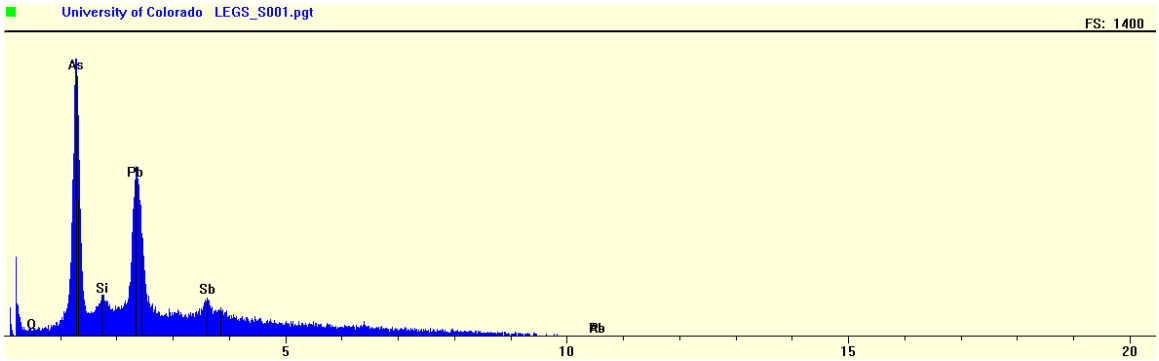
The form of arsenic in an arsenic bearing particle will be identified using a combination of EDS, WDS and electron backscatter intensity (BEI). Once the particle is clearly defined in the backscatter image, observe the count rates for arsenic, sulfur, and oxygen on the WDS rate meters and collect a 10-20 second EDS spectrum and identify the peaks. As noted above, check for optical focus and make sure your magnification and beam location are optimized to provide a "clean" spectrum. The presence/absence of oxygen may be determined by WDS or EDS (assuming the instrument has a light-element, thin-window detector). Sulfur must be verified with WDS because of the complete overlaps from Pb M-alpha and Mo L-alpha on EDS.

It is important to emphasize that not all arsenic bearing particles will consist of pure mineral phase with a fixed stoichiometry. Rather, many particles will contain arsenic with elemental ratios that do not define a unique mineral phase. Therefore, each particle will be classified into one of 16 alternative generic phase categories that are only semi-quantitative with regard to elemental composition. These 16 categories are described below, along with example EDS spectra.

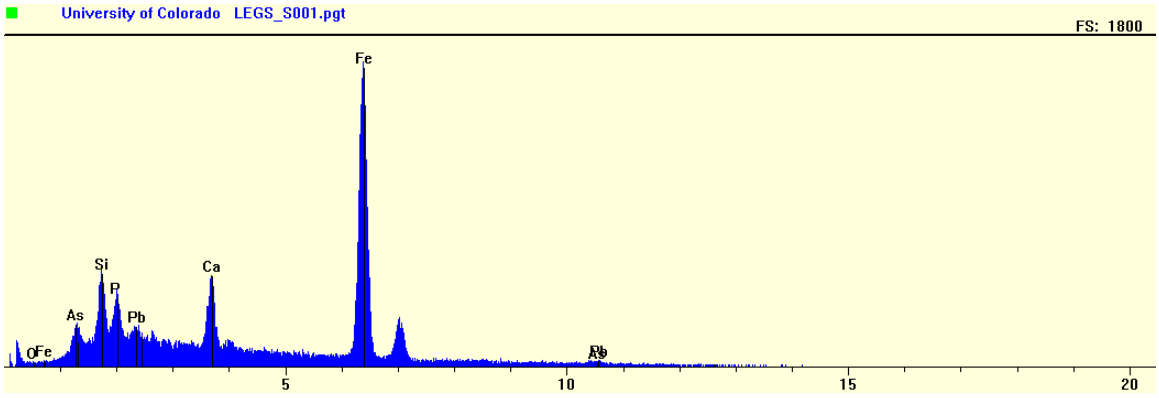
[1] NOTE. There may be instances when elevated background counts are observed and no arsenic is present. These are likely to occur in particles with high concentrations of heavy elements (barium, lead, rare earth elements, etc.) or high concentrations of magnesium (Mg). If the analyst cannot rectify this by comparing WDS peak counts with EDS spectra, then a WDS "measure" of arsenic may be necessary to determine if the k-ratio is positive, or if the background count rate is unusually high on either side of the peak.

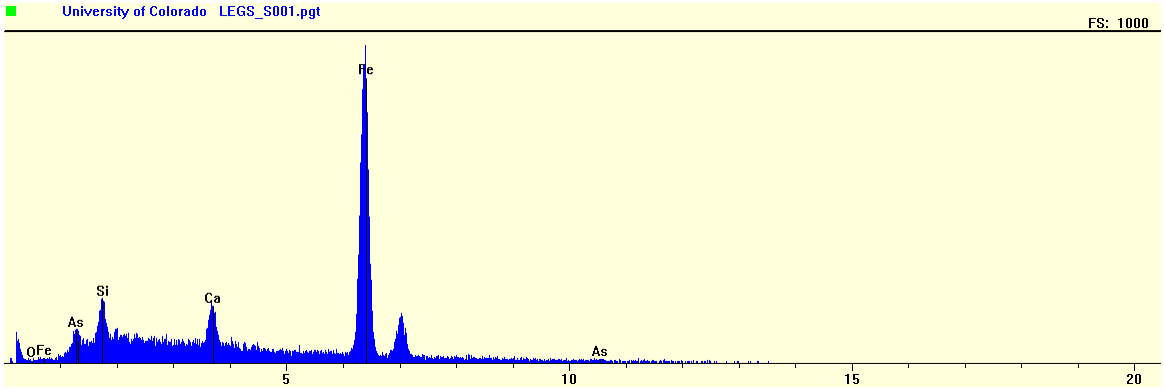
1. **AsMO** - This phase is generally associated with smelting waste, and is characterized by high concentrations of arsenic (5-25 wt%) and oxygen, with other metals (abbreviated "M") such as Pb, Cd, Fe, Cu, Sb and/or Zn in lesser concentrations. Some sulfur may also be present, but quantities are generally very low.



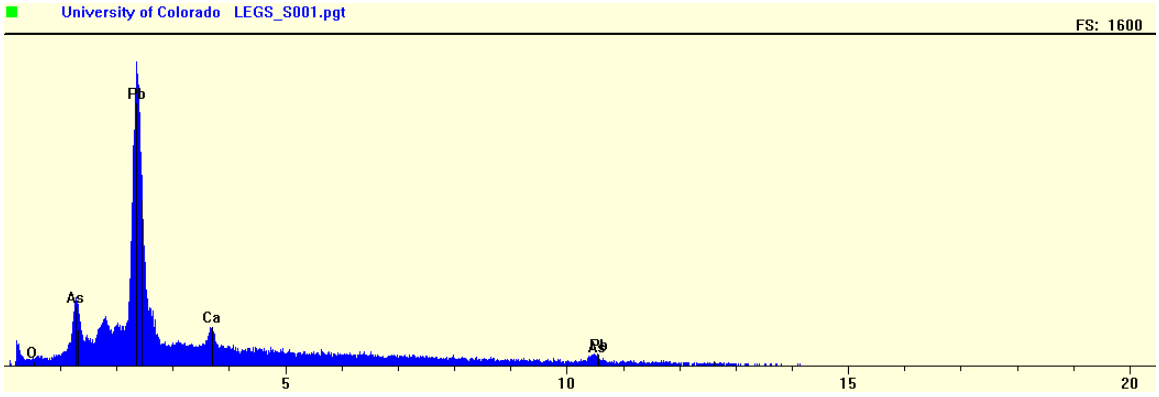


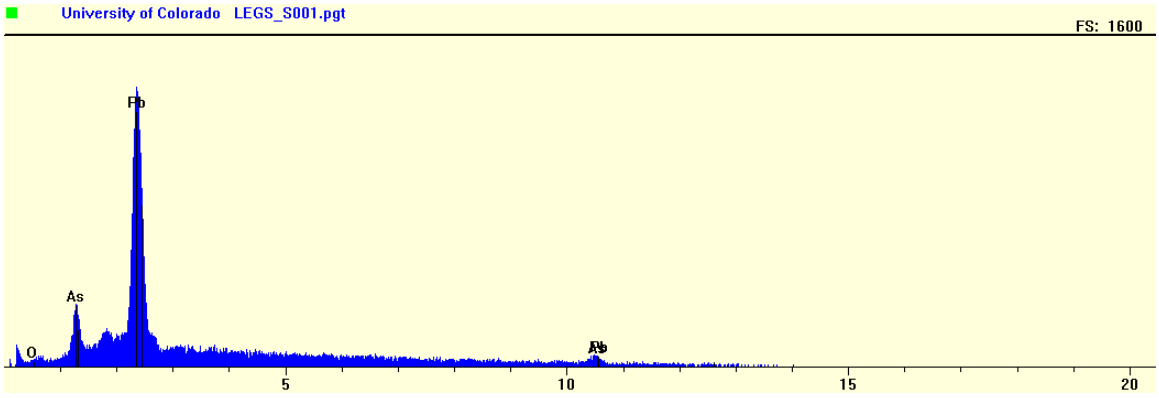
2. **FeOOH**- This phase is characterized by iron oxides in soil that have sorbed arsenic. Arsenic concentrations generally range from 1000 ppm to 6 wt%. Iron and oxygen are the major components, but other elements may be observed (Si, Al, Ca, P, Mn) in low quantities.



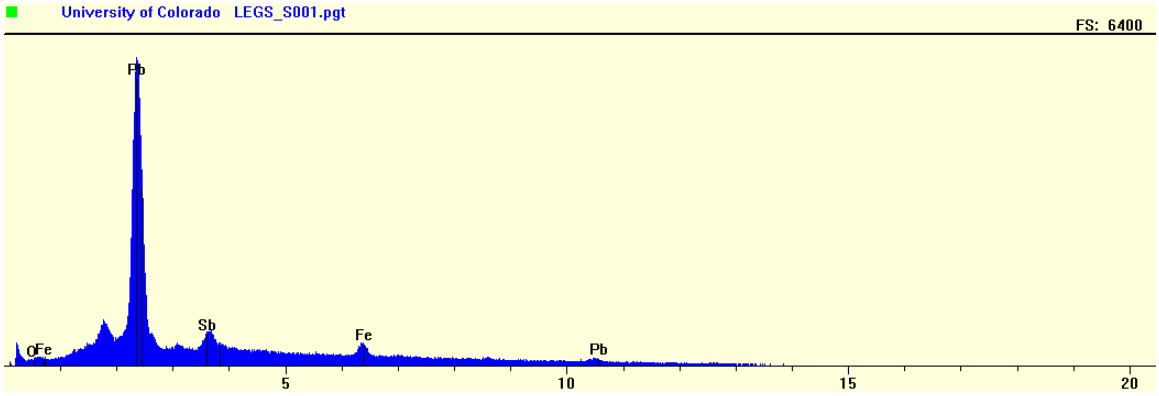


3. **PbAsO**- This phase can be observed in both smelting waste and some pesticide products. It contains high concentrations of arsenic (15-25 wt%), lead (40-55 wt%) and oxygen.

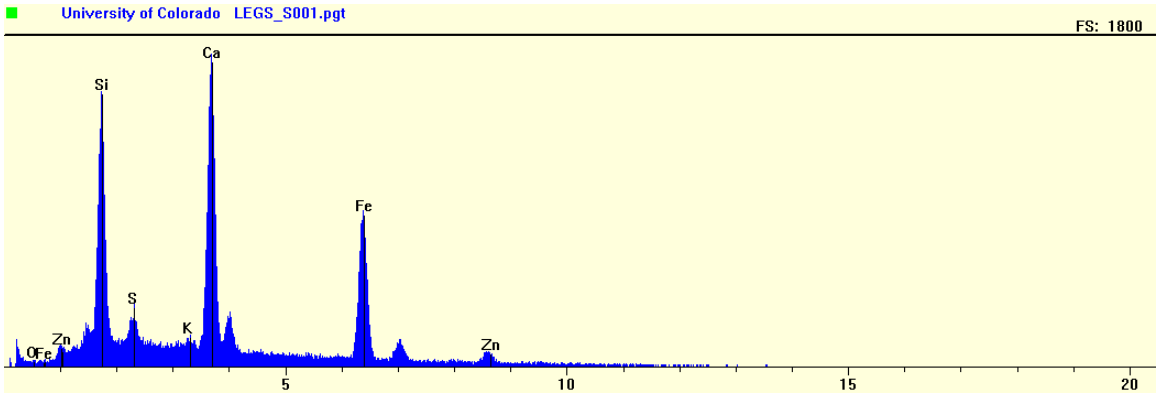


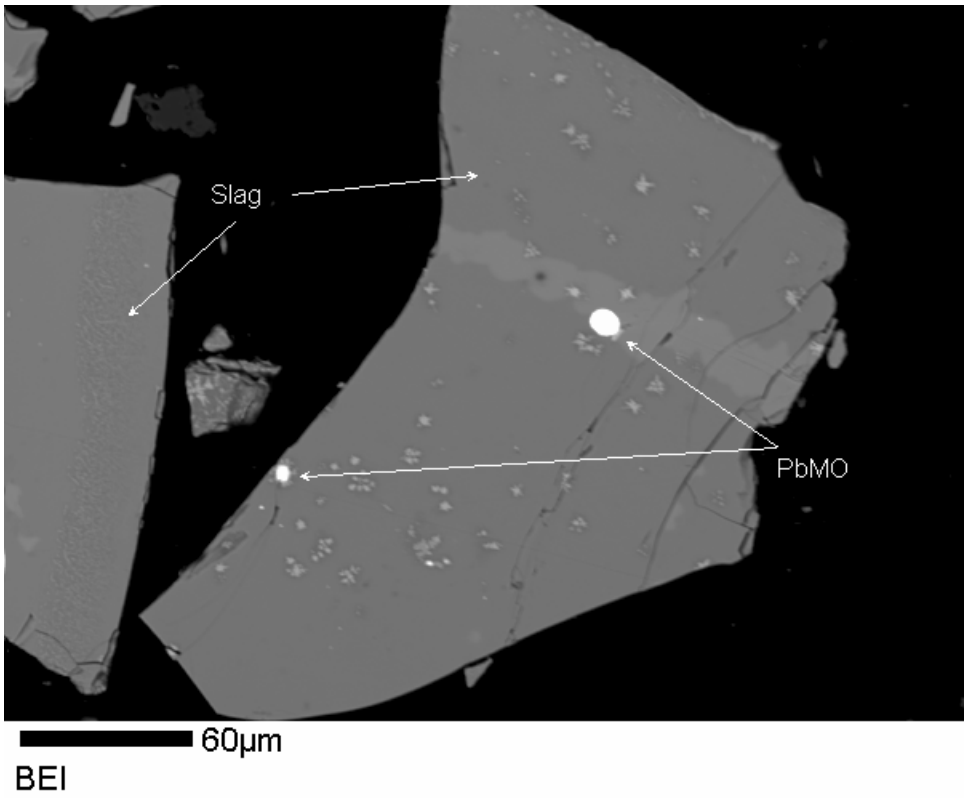


4. **PbMO**- This phase is generally associated with smelting waste. It contains high concentrations of lead (40-55 wt%) and arsenic (3-10 wt%) and oxygen, with other metals (M) such as Cd or Sb in lesser concentrations. Some sulfur may also be present, but quantities are generally very low.

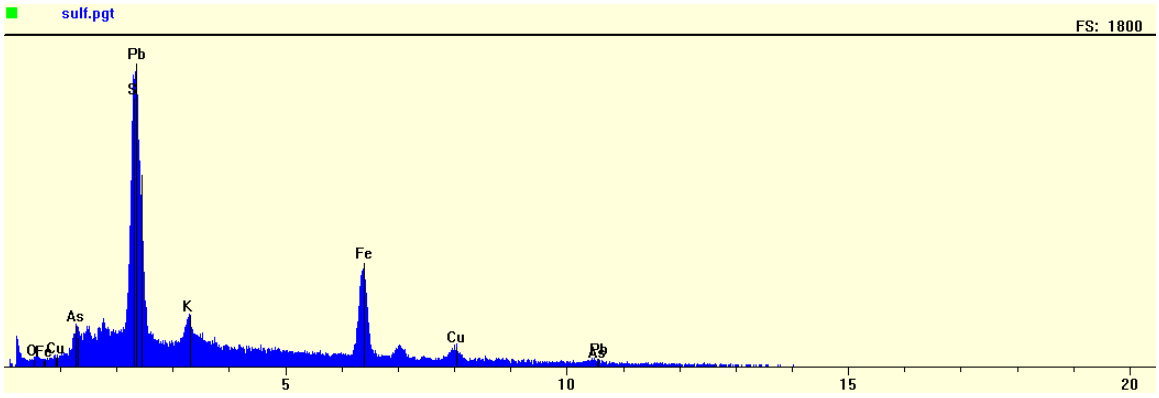


5. **Slag**- This phase is a vitreous-type particle generally associated with smelting waste. It contains low concentrations of lead (100 ppm-5 wt%) and arsenic (100 ppm- 5000 ppm). It is predominantly composed of Si-Ca-Fe-O with some sulfur and zinc, along with a characteristic morphology (see example photomicrograph). [Note: grains of arsenic-rich phases that are included in the slag are ranked as such, not as slag.]

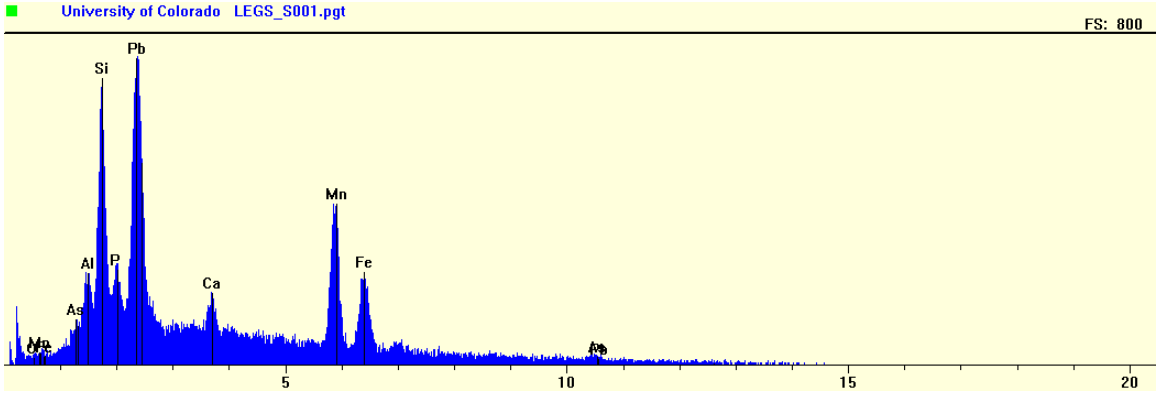


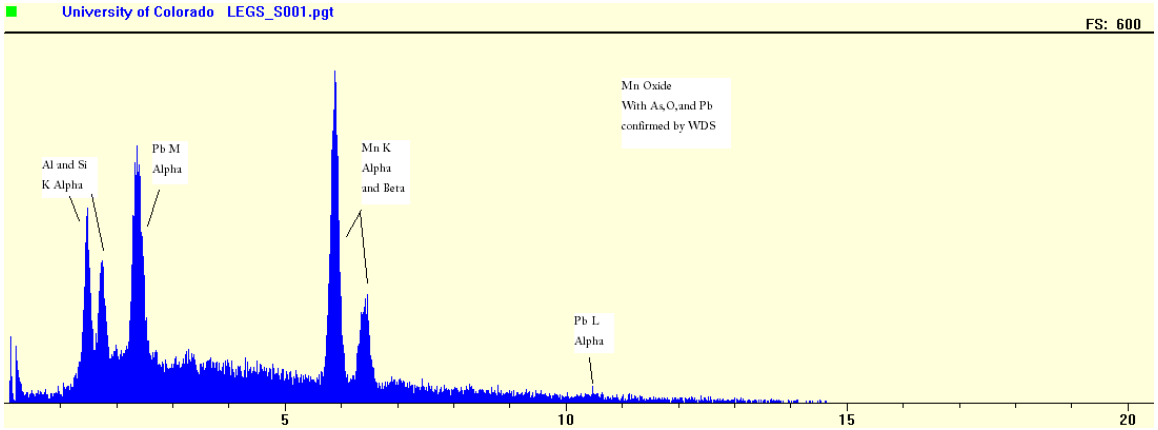


6. **FeSO4**- This phase may include both mining/smelting waste as well as iron sulfate-containing soil particles that have sorbed arsenic. Arsenic concentrations generally range from 1 to 6 wt%. Iron, sulfur, and oxygen are the major components, but other elements may be observed (Si, Al, K) in low quantities.

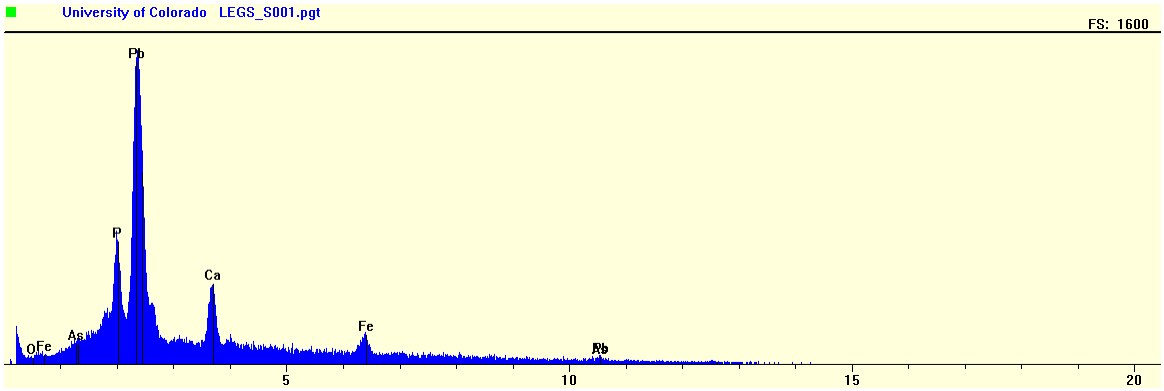


7. **MnOOH**- This phase is characterized by soil-forming manganese oxides that have sorbed arsenic. Arsenic concentrations generally range from 1000 ppm to 10 wt%. Manganese and oxygen are the major components, but other elements may be observed (Si, Al, Fe and Pb) in significant quantities.

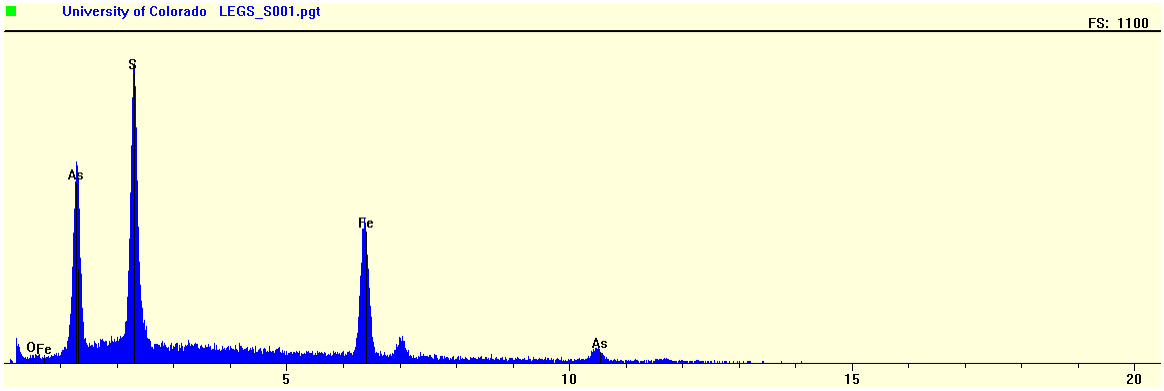




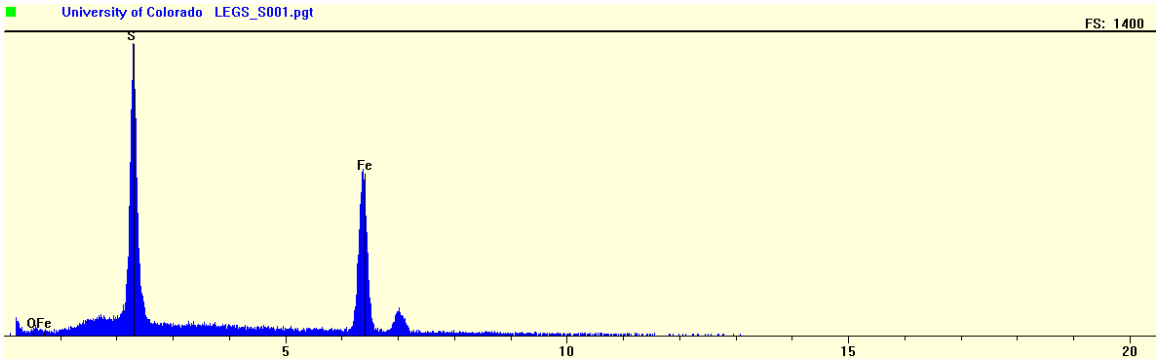
8. **Phosphate**- This phase represents soil-forming phosphate that has sorbed arsenic. Arsenic concentrations are generally low, ranging from 1000 ppm to 5 wt%. Phosphorous, calcium and oxygen are the major components, but other elements may be observed (Si, Al, Fe and Pb) in significant quantities.



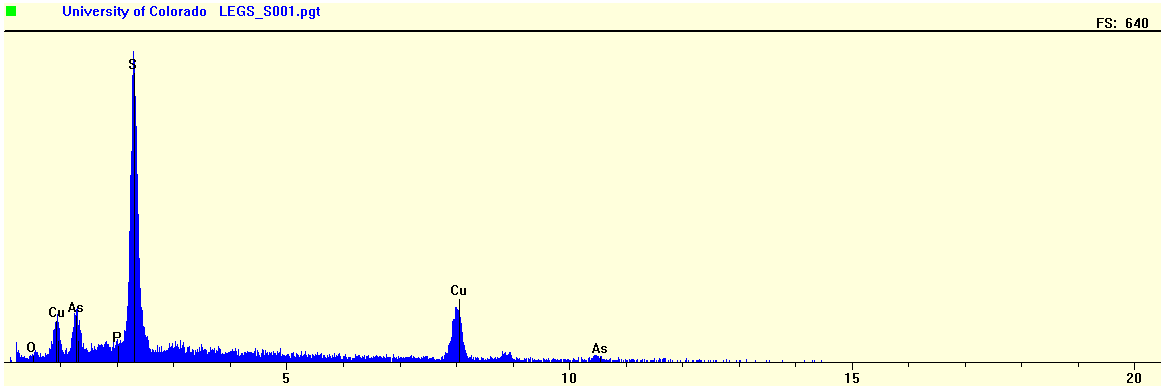
9. **Arsenopyrite**- This phase is generally associated with mining waste. It contains high concentrations of arsenic (46.0 wt%) along with iron and sulfur.

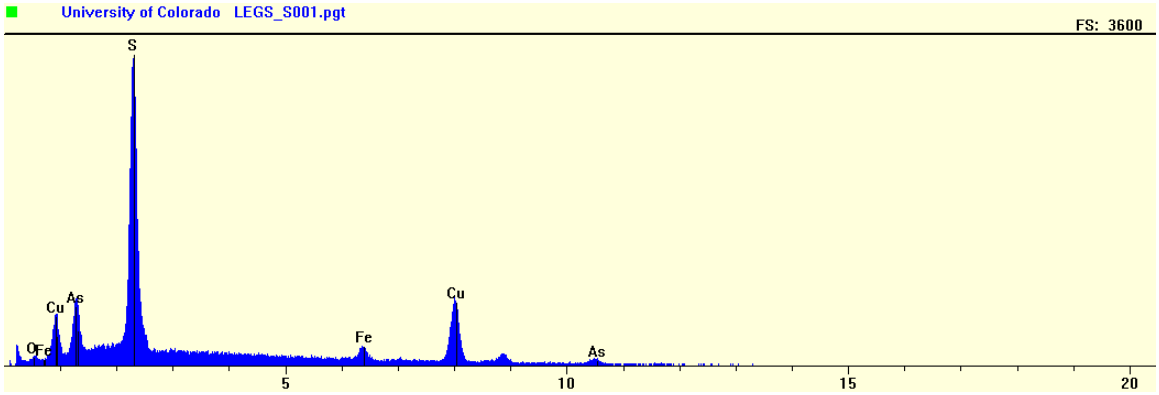


10. **Pyrite**- This phase is generally associated with mining waste. It contains low concentrations of arsenic (100-1000 ppm) along with high levels of iron and sulfur.

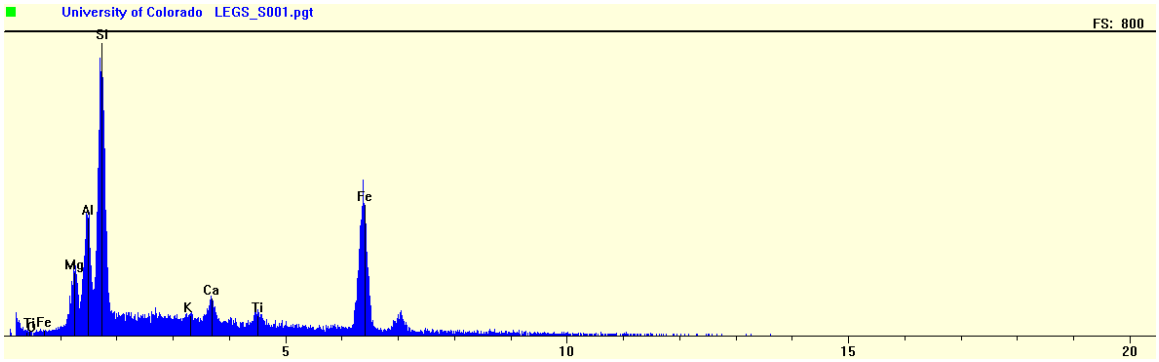


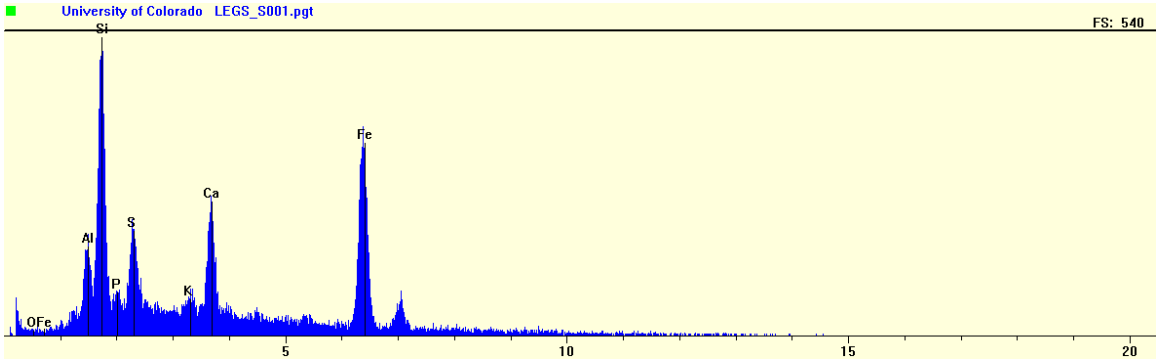
11. Sulfosalts- This phase is generally associated with mining waste. It contains high concentrations of arsenic (10-25 wt%) along with iron, antimony, copper, lead and sulfur.



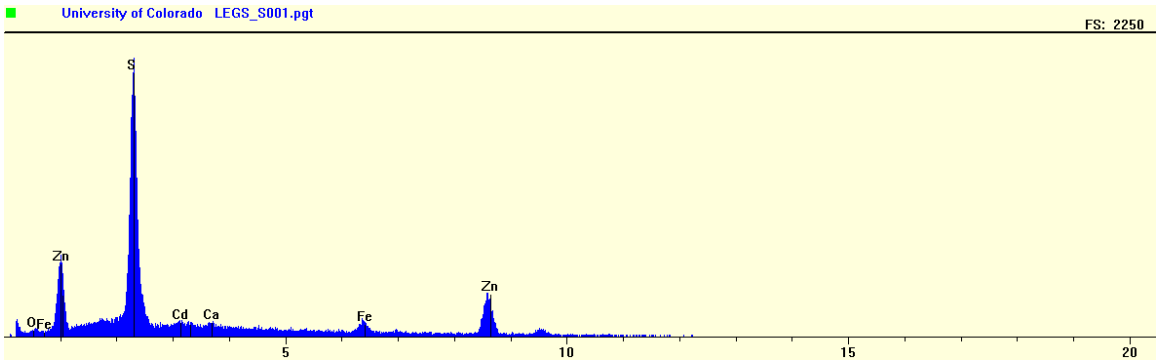


12. Clays- This phase represents soil-forming alumino silicates that have sorbed arsenic. Arsenic concentrations are generally low, ranging from 1000 ppm to 5 wt%. Alumina, silica, and oxygen are the dominant with lesser quantities of Ca,K,Mg, and Fe.

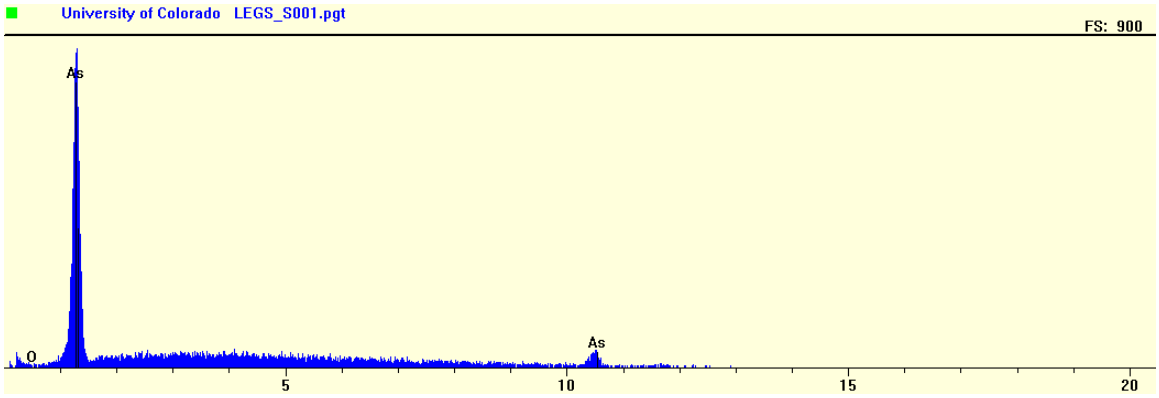




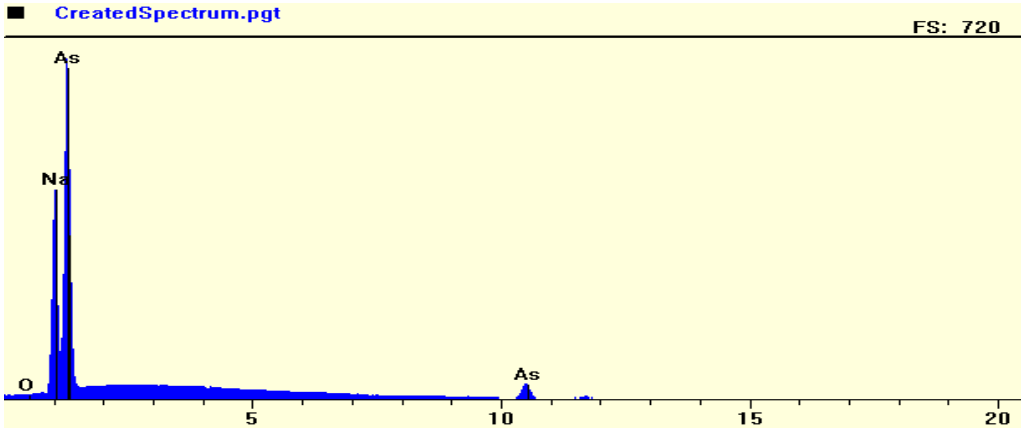
13. Zn- This phase is generally associated with smelting waste. It contains low concentrations of arsenic (100 ppm- 4000 ppm). It is predominantly composed of zinc, with some silica, sulfur and/or oxygen.

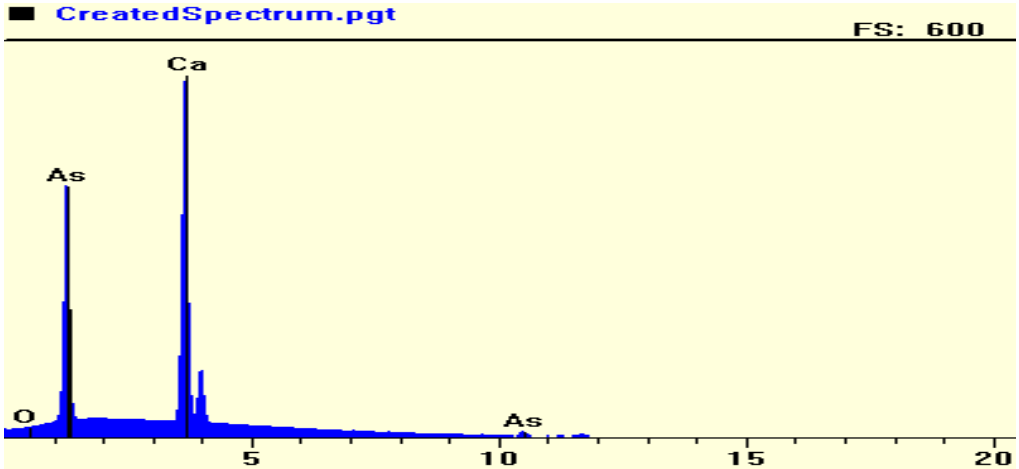


14. As2O3- This phase may occur in both smelting waste and in some pesticide products. It contains high concentrations of arsenic (~70 wt%) and oxygen.



15. Na-Ca AsO4- This phase is usually associated with pesticide/rodenticide products. It contains high concentrations of arsenic (~50-60 wt%) along with sodium or calcium and oxygen.





16. **Other**- This category should be used for arsenic-bearing particles that are not well characterized by any of the 15 phase groups described above. For example, some types of brass, solder, babbit, and paint pigments may contain low levels of arsenic, and should be recorded. If a particle is classified as "Other", use the comment field to indicate the nature of the particle (e.g., "brass"), and provide an image, the arsenic K-ratio, and the EDS spectrum to allow reviewers to reclassify if necessary.

4.0 DATA RECORDING AND DOCUMENTATION

Analysts will record data on each arsenic-bearing particle as they are acquired from each sample using either the Excel Electronic Data Deliverable (EDD) spreadsheet provided as Attachment 1, or a paper laboratory worksheet of similar design. If data are originally recorded on paper forms, the data must subsequently be transferred to the Excel EDD provided in Attachment 1.

Table 2 lists standard "shorthand" phase abbreviations to use when completing the EDD. When entering phase identifiers, be certain to use one of these standard abbreviations. In the EDD, these are available as a "pick list", or may be entered by hand if that is more convenient.

Figure 2 provides an example of the data recording sheet. Columns are established for numbering the arsenic-bearing phase particles, size of longest dimension (um), and the phase category assigned. Any relevant comments may be entered to the right of the data for each arsenic-bearing particle.

Photomicrographs and EDS x-ray spectra must be recorded for particles from each sample at a rate of 1 photograph per 20 particles counted.

5.0 FINAL REPORT

A final laboratory report will be provided that includes the following:

1) A list of samples received and the laboratory ID assigned to each
2) All EDDs on disk or CD.
3) All photomicrographs and EDS spectra recorded. These shall be submitted as a 128x128 (minimum) binary image in ".tif" or ".bmp" format. Recorded on each photomicrograph will be a scale bar, magnification, sample identification, date and phase identification using the standard abbreviations listed in Table 2. EDS spectra must have all major peaks labeled.
4) A narrative statement that identifies any issues or difficulties encountered, and any deviations from the methods specified in this SOP..

Figure 1
Scanning pattern

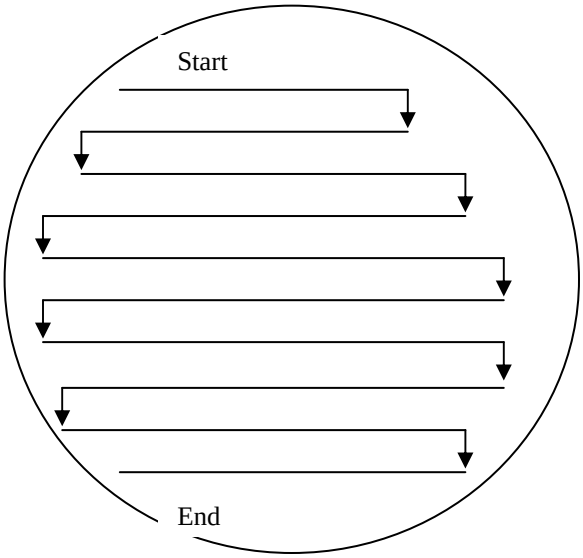


Figure 2
Example Data Recording Format

ELECTRONIC DATA DELIVERABLE FOR ARSENIC PHASE DATA

Lab name:	XYZ Labs
Client ID:	ABC TM2
Laboratory ID:	X-123
Analysis date:	6/12/2011
Analyst :	J Smith

Index	Phase	Length (um)	Comments
1	FeOOH	90	
2	FeOOH	2	
3	FeSO4	22	
4	FeOOH	6	
5	AsMO	11	
6	FeOOH	2	
7	SS	9	
8	MnOOH	8	
9	FeOOH	35	
10	PbAsO	10	
11	FeOOH	3	
12	FeOOH	70	
13	FeOOH	22	
14	FeOOH	4	
15	AsMO	2	
16	Py	33	
17	PbAsO	2	
18	FeOOH	10	
19	FeSO4	3	
20	AsMO	16	
21	FeOOH	8	
22	Py	4	
23	FeOOH	26	
24	PbAsO	3	
25	SS	2	
26	FeOOH	98	
27	FeSO4	36	
28	Phos	10	
29	PbAsO	18	
30	PbAsO	13	
31			
32			
33			
34			
35			

Table 1
EMPA Standard Operating Conditions

Parameter	WDS	EDS
Accelerating Voltage	15-20 KV	15-20 KV
Beam Size	1-2 microns	1-2 microns
Cup Current	10-30 NanoAmps	10-30 NanoAmps
Ev/Channel	NA	20
Stage Tilt	NA	Fixed
Working Distance	NA	Fixed
MCA time Constant	NA	7.5-12 microseconds
X-ray lines	S K-alpha PET O K-alpha LDE1 As L-alpha TAP	S K-alpha 2.31 KeV O K-alpha 0.52 KeV As K-alpha 10.5 KeV As K-beta 11.72 KeV As L-alpha 1.28 KeV

Table 2
Standard Phase Abbreviations

Phase Description	Valid Abbreviation
AsMO	AsMO
FeOOH	FeOOH
PbAsO	PbAsO
PbMO	PbMO
Slag	Slag
FeSO4	FeSO4
MnOOH	MnOOH
Phosphate	Phos
Arsenopyrite	Aspy
Pyrite	Py
Sulfosalts	SS
Clay	Clay
Zn	Zn
As2O3	As2O3
Na-Ca AsO4	NaCa
Other	Other

Appendix C: Standard Operating Procedures

Standard Operating Procedure:

In Vitro Bioacessibility (IVBA) Procedure for Arsenic

Standard Operating Procedure

In Vitro Bioacessibility (IVBA) Procedure for Arsenic

June 2011

1.0 PURPOSE

This Standard Operating procedure (SOP) describes a method for measuring the *in vitro* bioacessibility (IVBA) of arsenic under several different *in vitro* extraction conditions. These extraction conditions are being investigated to determine if IVBA values can be used to reliably predict the relative bioavailability (RBA) of arsenic measured *in vivo*.

Background on the development and validation of *in vitro* test systems for estimating IVBA of arsenic, lead and other metals in soil metals can be found in Ruby et al. (1993, 1996), Medlin (1972), Medlin and Drexler (1997), Drexler (1998), Casteel et al. (2006), USEPA (2006), and Drexler and Brattin (2007).

2.0 SAMPLE PREPARATION

Directions for soil preparation steps (if any) by the analytical laboratory will be provided by the study director.

In general, all test materials are prepared for the *in vitro* assay by drying (< 40 °C) followed by sieving to < 250 μm.

3.0 APPARATUS AND MATERIALS

3.1 Equipment

The extraction device used in the IVBA procedure is illustrated in Figure 1. For further information on the design, contact Dr. John W. Drexler, at 303-492-5251 or drexlerj@colorado.edu.

The device holds ten 125 mL wide-mouth high-density polyethylene (HDPE) bottles. These are rotated within a Plexiglas tank by a TCLP extractor motor with a modified flywheel. The water bath must be filled such that the extraction bottles remained immersed. Temperature in the water bath is maintained at 37 ± 2 °C using an immersion circulator heater.

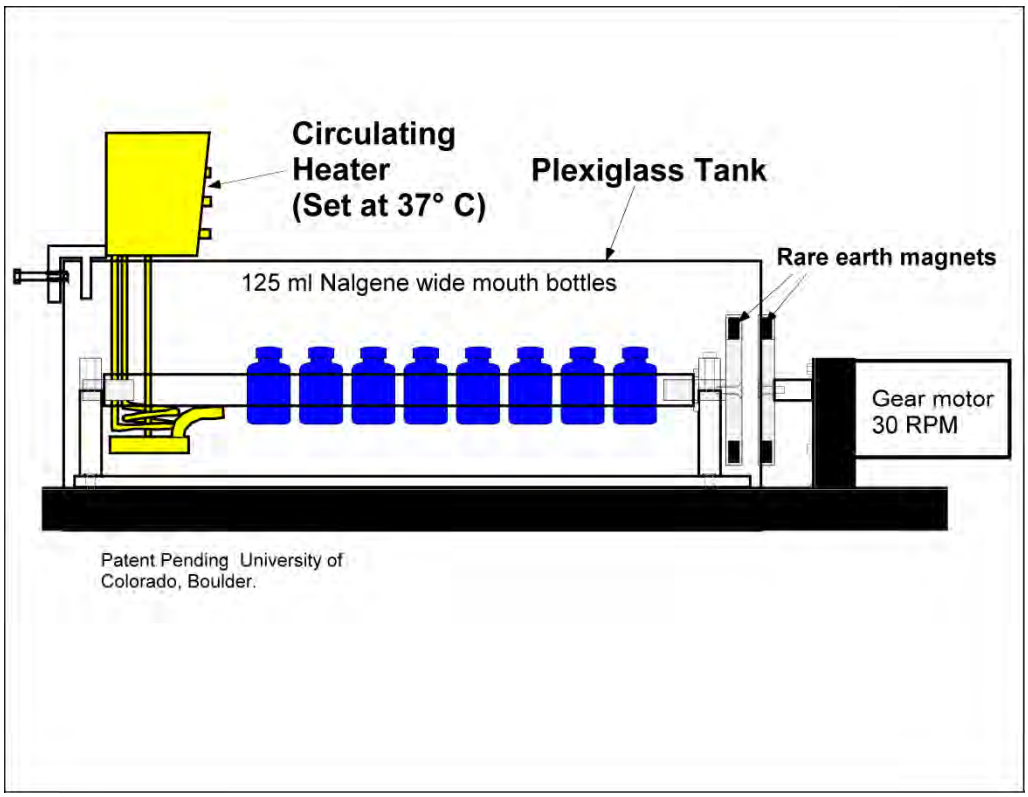


Figure 1. Schematic Diagram of IVBA Extraction Device

The 125-mL HDPE bottles must have an airtight screw-cap seal, and care must be taken to ensure that the bottles do not leak during the extraction procedure.

Other equipment required is listed below:

- Disposable 15 mL polypropylene centrifuge tubes.
- Disposable 25 mm 0.45 µm surfactant- free cellulose acetate syringe filters.
- Disposable 10 mL polypropylene syringes with Luer-Lok fittings

3.2 Solutions and Reagents

Depending on the specific test requested, three extraction solutions may be required. Required reagents include:

- Sodium Hydroxide, 50% w/w. CAS 1310-73-2.
- Glycine, Tissue Grade. CAS 56-40-6.
- Sodium Phosphate Dibasic Anhydrous, ACS Grade. CAS 7558-79-4.
- Hydrochloric Acid, Trace Metal Grade. CAS 7647-01-0.
- Nitric Acid, Trace Metal Grade. CAS 7697-37-2.

- Hydroxylamine Hydrochloride, ACS Grade. CAS 5470-11-1

All solutions are prepared utilizing ASTM Type II de-ionized (DI) water. All reagents and water must be free of arsenic, and the final fluid must be tested to confirm that arsenic concentrations are less than one-fourth of the project required detection limits (PRDLs) of 20 µg/L (e.g., 5 µg/L arsenic in the final fluid).

Cleanliness of all materials used to prepare and/or store the extraction fluid and buffer is essential. All non-disposable glassware and equipment used to prepare standards and reagents must be properly cleaned, acid washed, and finally, triple-rinsed with de-ionized water prior to use. Disposable labware is recommended whenever possible.

Extraction Fluid 1

Extraction Fluid 1 consists of 0.4 M glycine pH 1.5 supplemented with 0.05 M phosphate and 0.1 M hydroxylamine hydrochloride (HAH), prepared as follows:

To 1.937 L of DI water, add 60.6 g glycine (free base, reagent grade), 14.196 g anhydrous dibasic sodium phosphate and 13.90 g hydroxylamine hydrochloride. Add 63 ml of Trace-Metal grade hydrochloric acid (HCl) bringing the final solution volume to 2 L. Place the mixture in the water bath at 37 °C until the extraction fluid reaches 37 °C. Standardize the pH meter using both pH 2.0 and a pH 4.0 pH standards buffers using temperature compensation at 37 °C or buffers maintained at 37 °C. Add, dropwise, trace metal grade, concentrated hydrochloric acid (12.1N) until the solution pH reaches a value of 1.50 +/- 0.05.

Extraction Fluid 2

Extraction Fluid 2 consists of 0.4 M glycine pH 1.5 without phosphate or HAH, prepared as follows:

To 1.937 L of DI water, add 60.6 g glycine (free base, reagent grade). Add 63 ml of Trace-Metal Grade hydrochloric acid (HCl) bringing the final solution volume to 2 L. Place the mixture in the water bath at 37 °C until the extraction fluid reaches 37 °C. Standardize the pH meter using both pH 2.0 and a pH 4.0 pH standards buffers using temperature compensation at 37 °C or buffers maintained at 37 °C. Add, dropwise, trace metal grade, concentrated hydrochloric acid (12.1N) until the solution pH reaches a value of 1.50 +/- 0.05.

Extraction Fluid 3

Extraction Fluid 3 consists of 0.4 M glycine pH 7.0 supplemented with 0.05 M phosphate, prepared as follows:

To 2.0L of DI water, add 60.06 g glycine and 14.196 g anhydrous dibasic sodium phosphate. Place the mixture in the water bath at 37 °C until the extraction fluid reaches 37 °C. Standardize the pH meter using both pH 4.0 and pH 7.0 pH standard buffers using temperature compensation at 37 °C or buffers maintained at 37 °C. Add, dropwise, sodium hydroxide solution 50% w/w or concentrated, Trace-Metal Grade hydrochloric acid (HCl) until the solution pH reaches a value of 7.00 +/- 0.05.

4.0 EXTRACTION PROCEDURE

Attachment 1 provides a checklist that shall be followed when performing an IVBA extraction. Key steps are described below.

Extraction solution(s) must be placed in heated water bath prior to use and allowed to achieve operating temperature of 37 +/- 2 °C. The final pH is then adjusted (if necessary) and recorded as “starting pH” on the laboratory worksheet (see Section 7).

All test substances must be thoroughly mixed prior to use in the IVBA test to ensure homogeneity. This mixing may be achieved using a roller mixer (several minutes) or by end-over-end mixing for about 30 seconds.

After mixing, measure 1.00 +/- 0.5 g of test substrate and place in a clean 125 mL Nalgene bottle, ensuring that static electricity does not cause soil particles to adhere to the lip or outside threads of the bottle. If necessary, use an antistatic brush to eliminate static electricity prior to adding the media. Record the mass of substrate added to the bottle on the laboratory worksheet.

Measure 100 ± 0.5 mL of the designated extraction fluid, using a graduated cylinder, and transfer to a 125 mL wide-mouth HPDE bottle. Hand-tighten each bottle top and shake/invert to ensure that no leakage occurs, and that no media is caked on the bottom of the bottle.

Place the bottle into the extraction device (Figure 1), making sure each bottle is secure and the lid(s) are tightly fastened. Fill the extractor with 125 mL bottles containing test materials or QA samples.

The temperature of the water bath must be 37 ± 2 °C.

Turn on the extractor and rotate end-over-end at 30 ± 2 rpm for 1 hour. Record the start time of rotation on the laboratory worksheet.

After one hour, stop the extractor rotation and remove the bottles. Wipe them dry and place upright on the bench top.

Draw extract directly from the top portion of the extraction bottle into a disposable 10 mL syringe with a Luer-Lok attachment. Attach a 0.45 µm cellulose acetate disk filter (25 mm diameter) to the syringe, and filter the extract into a clean 15 mL polypropylene centrifuge tube (labeled with sample ID) or other appropriate sample vial for analysis.

Record the time on the laboratory worksheet that the extract is filtered (i.e., extraction is stopped). If the total extraction time elapsed is greater than 1 hour 30 minutes, the test must be repeated.

Measure and record on the worksheet the pH (Final pH) of the remaining fluid in the extraction bottle. If the fluid pH is not within ± 1.0 pH units of the starting pH, the test must be discarded and the sample reanalyzed.

Store filtered samples in a refrigerator at 4 °C until they are analyzed. Analysis for arsenic concentrations must occur within 1 week of extraction for each sample. To preserve the sample add 2 drops of trace-metal grade nitric acid (HNO_3) to labeled 15mL polypropylene centrifuge tube.

5.0 SAMPLE ANALYSIS

Extracts are analyzed for arsenic using USEPA Methods 6010B, 6020, or 7061A, as specified by the Study Director. For Method 6020, dilute each sample 50:1 (200 µL extract in 10 mL DI water) for analysis. This is needed to reduce interference from chlorine plus argon.

6.0 QUALITY CONTROL/QUALITY ASSURANCE

Quality Assurance for the extraction procedure will consist of the following quality control samples:

- A Laboratory Blank is a bottle containing 100 mL of extraction fluid put through the entire extraction process but with no added soil or test substrate
- A Blank-Spike is a bottle containing 2.5 ppm (2.5 µg/mL) arsenic, prepared by adding 250 µL of 1000 ppm NIST Traceable ICP arsenic standard solution to 100 mL of extraction fluid.

Unless otherwise specified by the study director, both QC samples types will be collected at a rate of 10%. Control limits are listed in Table 1. These values may be revised as additional data are collected.

Table 1. IVBA QC Sample Requirements

QC Sample Type	Analysis Frequency	Control Limits
Laboratory Blank	10%	<10 µg/L arsenic
Blank spike	10%	85-115% recovery

7.0 CHAIN-OF-CUSTODY PROCEDURES

Once received by the Laboratory, all test substances must be maintained under standard chain-of-custody.

8.0 DATA RECORDING, VALIDATION AND TRANSMITTAL

Data Recording

Figure 2 provides a worksheet for recording raw laboratory data. All raw data will be reported by hand by the individual performing the IVBA tests.

After the test is complete, the laboratory data and the analytical results will be recorded in an Electronic Data Deliverable (EDD), using the format illustrated in Figure 3.

Data Validation

After data entry is complete, the laboratory director shall review the EDD compared to the laboratory worksheet and the analytical data package and ensure that all data have been entered correctly.

Data Transmittal

After validation, all data, including laboratory worksheets, analytical reports, and EDDs, shall be transmitted Laboratory Director to the Study Director.

9.0 REFERENCES

Casteel SW, Weis CP, Henningsen GM, Brattin WJ. 2006. Estimation of Relative Bioavailability of Lead in Soil and Soil-Like Materials Using Young Swine. Environ Health Perspect. 114:1162-1171. doi:10.1289/ehp.8852

Drexler, J.W. 1998. An in vitro method that works! A simple, rapid and accurate method for determination of lead bioavailability. EPA Workshop, Durham, NC.

Drexler,J. and Brattin,W. 2007. An *In Vitro* Procedure for Estimation of Lead Relative Bioavailability: With Validation. Human and Ecological Risk Assessment. 13(2), pp. 383-401.

Medlin, E., and Drexler, J.W. 1995. Development of an in vitro technique for the determination of bioavalability from metal-bearing solids. International Conference on the Biogeochemistry of Trace Elements, Paris, France.

Medlin, E.A. 1997. An In Vitro method for estimating the relative bioavailability of lead in humans. Masters thesis. Department of Geological Sciences, University of Colorado, Boulder.

Ruby, M.W., A. Davis, T.E. Link, R. Schoof, R.L. Chaney, G.B. Freeman, and P. Bergstrom. 1993. Development of an in vitro screening test to evaluate the in vivo bioaccessibility of ingested mine-waste lead. Environ. Sci. Technol. 27(13): 2870-2877.

Ruby, M.W., A. Davis, R. Schoof, S. Eberle, and C.M. Sellstone. 1996. Estimation of lead and arsenic bioavailbilty using a physiologically based extraction test. Environ. Sci. Technol. 30(2): 422-430.

USEPA. 2006. Estimation of Relative Bioavailability of Lead in Soil and Soil-Like Materials Using In Vivo and In Vitro Methods. U.S. Environmental Protection Agency: Washington, DC. Available online at http://www.epa.gov/superfund/health/contaminants/bioavailability/lead_tsdmain.pdf.

FIGURE 2 EXAMPLE LABORATORY WORKSHEET

ARSENIC IVBA MEASURMENTS

Lab Name: XYZ Labs
Date: 7/21/2011
Analyst: J. Smith

Index	Sample ID	Laboratory ID	Arsenic Conc. (ug/g)	Extraction Fluid Type	Extraction Fluid Vol. (mL)	Sample Mass (g)	pH Start	pH End	Time Start	Time Filter	Notes
1	12-34137	AB-10001	847	pH 1.5	100.4	0.997	1.48	1.56	10:12	11:21	
2	12-34137	AB-10002	847	pH 7.0	99.6	1.021	7.05	7.11	10:12	11:24	
3	12-34137	AB-10003	847	pH 7+PO4	100.3	1.035	6.98	7.08	10:12	11:29	
4	15-5123	AB-10004	451	pH 1.5	99.81	0.991	1.48	1.55	10:12	11:33	
5											
6											
7											
8											
9											
10											
11											
12											
13											
14											
15											
16											
17											
18											
19											
20											
21											
22											
23											
24											
25											

General Comments:
Water bath = 37.5

FIGURE 3 IVBA EDD FORMAT

ELECTRONIC DATA DELIVERABLE FOR ARSENIC IVBA MEASURMENTS

	Lab	Client	Laboratory	Arsenic	Extraction Fluid		Sample	pH		Time		Analysis	Conc in		
Index	Name	ID	ID	Conc. (ug/g)	Type	Vol. (mL)	Mass (g)	Start	End	Start	Filter	Method	Fluid (ug/L)	IVBA	Comments
1	XYZ labs	12-34137	AB-10001	847	pH 1.5	100.4	0.997	1.48	1.56	10:12	11:21	6020	257	30.6%	
2	XYZ labs	12-34137	AB-10002	847	pH 7.0	99.6	1.021	7.05	7.11	10:12	11:24	6020	184	21.2%	
3	XYZ labs	12-34137	AB-10003	847	pH 7+PO4	100.3	1.035	6.98	7.08	10:12	11:29	6020	192	22.0%	
4	XYZ labs	15-5123	AB-10004	451	pH 1.5	99.81	0.991	1.48	1.55	10:12	11:33	6020	38.4	8.6%	
5															
6															
7															
8															
9															
10															
11															
12															
13															
14															
15															
16															
17															
18															
19															
20															
21															
22															
23															
24															
25															
26															
27															
28															
29															
30															
31															
32															
33															
34															
35															
36															
37															
38															
39															
40															
41															
42															

ATTACHMENT 1
IVBA Procedure Checklist

1 Verify sample identification.
2 Label (black, permanent Sharpie) a NEW 125 mL Nalgene wide-mouth bottle with the sample identification.
3 Mix the sample thoroughly. Weigh 1.0 ± 0.05 g of sample (dried, <250 micron) onto NEW weighing paper.
4 Record the weight (± 0.0001 g) on the laboratory worksheet.
5 Place weighed sample into labeled 125 mL Nalgene bottle and tighten the bottle cap.
6 Heat water in the extraction apparatus to 37 ± 2 °C.
7 Prepare extraction fluid(s) as directed. If prepared ahead, the extraction fluids must be kept cool (2-4 °C) until needed.
8 Allow the extraction fluid to come to equilibrium with extraction apparatus at 37 ± 2 °C.

Steps 8-18 must be completed in < 90 minutes from the start of extraction or repeat the process

9 Calibrate the pH meter. Adjust (if necessary) and record the pH of the extraction fluid at 37±2°C.
10 Add 100 ± 0.5 mL of the designated extraction fluid to a labeled 125mL Nalgene bottle containing the test material.
11 Secure the labeled 125 mL Nalgene bottles in the extraction apparatus and rotate end-over-end for 1 hour.
12 Record the start time of rotation and initial extraction fluid pH.
13 After 1 hour, remove the labeled 125mL Nalgene bottles from the extraction apparatus, place upright, and wipe dry.
14 Using a NEW 10 mL disposable syringe with a Luer-Lok, remove an aliquot of un-filtered extract directly from the upper portion of the labeled 125 mL Nalgene bottle.
15 Attach a NEW 0.45 µm cellulose acetate filter to the Luer-Lok of the 10 mL syringe and filter the extract into a labeled 15 mL polypropylene centrifuge tube.
16 To preserve the sample add 2 drops of trace-metal grade nitric acid (HNO_3) to labeled 15mL polypropylene centrifuge tube.
17 Measure and record the final pH of the extraction fluid directly from the labeled 125mL Nalgene bottle.
18 The final pH must be within ± 1.0 of the initial extraction fluid pH or repeat the test.
19 Refrigerate labeled 15 mL polypropylene centrifuge tubes until analysis.

Standard Operating Procedure:

Arsenic Speciation

Standard Operating Procedure

Arsenic Speciation

September 2011

1.0 INTRODUCTION

This Standard Operating Procedure (SOP) specifies methodologies and protocols that may be used to characterize (speciate) the chemical and physical nature of arsenic in arsenic-bearing particles that are present in a wide range of solid samples including soils, dusts, sediments, tailings, slags, dross, and other industrial wastes. Parameters characterized during the speciation analyses include mineral type (phase), particle size, and frequency of occurrence of arsenic-bearing forms.

2.0 INSTRUMENTATION

Arsenic speciation may be performed using either electron microprobe analysis (EMPA) or scanning electron microscopy (SEM) with instruments equipped with both wavelength dispersive spectroscopy (WDS) and energy dispersive spectroscopy (EDS) systems. However, because of the many x-ray limitations imposed on most SEM instruments, EMPA is preferred.

The WDS will have spectrometers calibrated for arsenic, oxygen and sulfur on the appropriate crystals using mineral standards. The EDS will have a multi-channel analyzer (MCA) calibrated for known peak energy centroids. Calibration will be performed so as to have both low (1.0-3.0 KeV) and high (6.0-15.0 KeV) energy peaks fall within 0.05 KeV of its known centroid.

Standard operating conditions for quantitative and qualitative analyses for arsenic-bearing forms are given in Table 1. However, it is the responsibility of the operator to select the appropriate analytical line (crystal/KeV range) to eliminate peak overlaps and ensure proper identification/quantification of arsenic and other identifying elements.

The magnification marker on the instrument will be checked once a week. This will be performed by following manufacturer instructions or by measurement of commercially available grids or Lucite spheres. Size measurements must be within 4 microns of certified values.

3.0 ANALYTICAL PROCEDURE

3.1 Sample Preparation

Directions for sample preparation steps (if any) by the analytical laboratory will be provided by the Study Director.

In general, all test materials are prepared for speciation analysis by drying (< 40 °C) followed by sieving to < 250 μm.

Samples of test material for this project have been prepared for speciation analysis by embedding approximately 1-2 grams of sample in a 1.25 inch round epoxy “puck” which is then polished to provide a smooth surface for examination.

Each laboratory should lightly clean the polished surface with alcohol prior to carbon coating.

3.2 Scanning Procedure

1. Set the initial magnification to 400X. This may be adjusted downward if small arsenic-bearing particles are absent or rare, and may be adjusted upward if small arsenic-bearing particles are common and cannot be adequately identified at 400X.
2. Adjust the electron backscatter detector threshold so that all particles in the sample are seen. This procedure will minimize the possibility that arsenic-bearing minerals with low average atomic number are overlooked during the scanning of the polished mount.
3. Peak the WDS spectrometer(s) for arsenic, oxygen and sulfur. Calibrate arsenic using a certified standard and provide the standard composition in final report. Using the accelerating voltage and beam current used during the point counting procedure, determine the background count rate (counts/second) for arsenic on a particle of quartz or feldspar.
4. Using the same accelerating voltage and beam current used during the analysis, determine the background count rate (counts/second) for arsenic on a particle of quartz or feldspar.
5. Begin scanning the sample manually using the scanning pattern depicted in Figure 1. That is, begin in the upper left portion of the puck, and traverse from left-to-right and top-to-bottom. The amount of vertical movement for each traverse depends on magnification and CRT (cathode-ray tube) size. This movement shall be adjusted so that each traverse lies just below the previous and that NO portion of the sample has been missed when the end of a traverse is reached

6. Arsenic-bearing particles are defined as particles where the count rate for arsenic is 2.0 times or more greater than the background count rate identified in Step 4[1]. Once an arsenic-bearing particle is identified, check for optical focus and make sure your magnification and beam location are optimized to provide a "clean" spectrum. Then record the data specified in Section 3.3.
7. Continue scanning until a minimum of 200 arsenic-bearing particles have been identified and characterized.

3.3 Particle Characterization

Particle Length

For each arsenic-bearing particle identified, adjust the backscatter image to clearly define the size and appearance of the particle. Measure and record the longest dimension (um) of the particle.

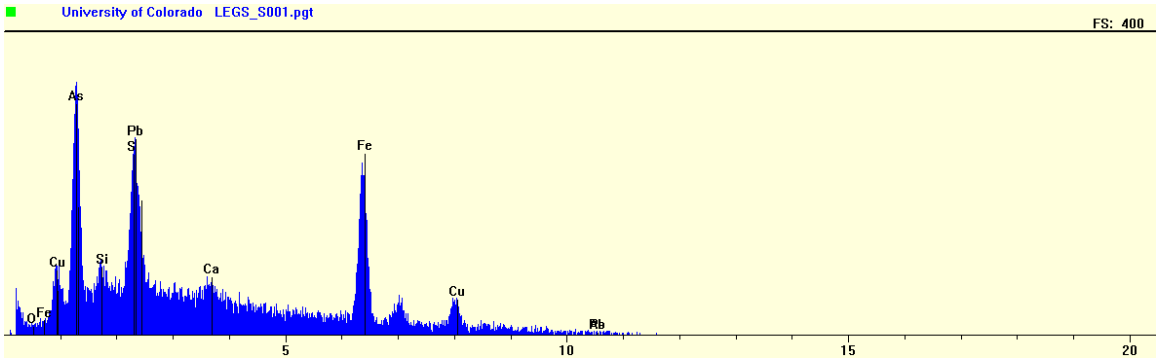
Phase Identification

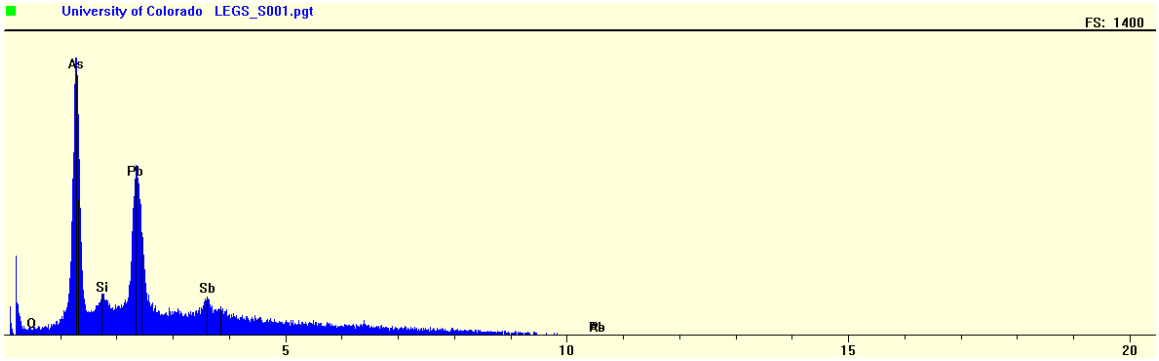
The form of arsenic in an arsenic bearing particle will be identified using a combination of EDS, WDS and electron backscatter intensity (BEI). Once the particle is clearly defined in the backscatter image, observe the count rates for arsenic, sulfur, and oxygen on the WDS rate meters and collect a 10-20 second EDS spectrum and identify the peaks. As noted above, check for optical focus and make sure your magnification and beam location are optimized to provide a "clean" spectrum. The presence/absence of oxygen may be determined by WDS or EDS (assuming the instrument has a light-element, thin-window detector). Sulfur must be verified with WDS because of the complete overlaps from Pb M-alpha and Mo L-alpha on EDS.

It is important to emphasize that not all arsenic bearing particles will consist of pure mineral phase with a fixed stoichiometry. Rather, many particles will contain arsenic with elemental ratios that do not define a unique mineral phase. Therefore, each particle will be classified into one of 16 alternative generic phase categories that are only semi-quantitative with regard to elemental composition. These 16 categories are described below, along with example EDS spectra.

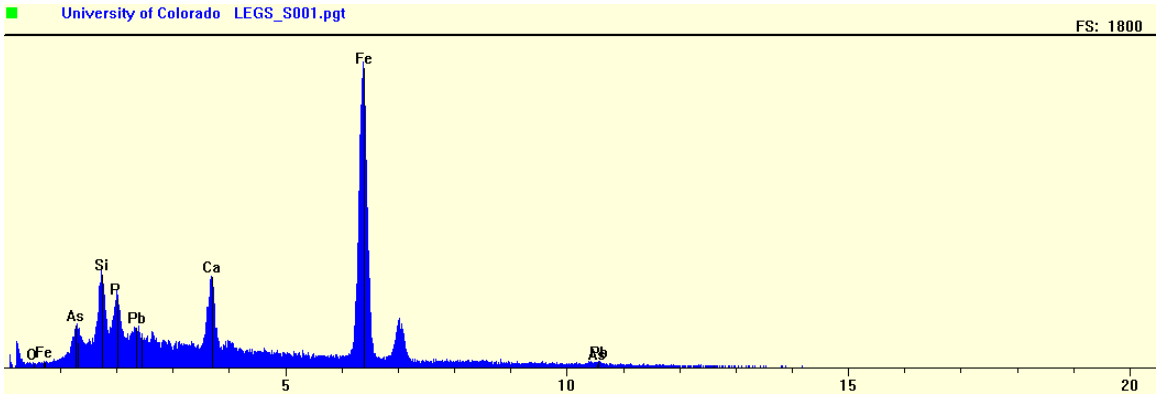
[1] NOTE. There may be instances when elevated background counts are observed and no arsenic is present. These are likely to occur in particles with high concentrations of heavy elements (barium, lead, rare earth elements, etc.) or high concentrations of magnesium (Mg). If the analyst cannot rectify this by comparing WDS peak counts with EDS spectra, then a WDS "measure" of arsenic may be necessary to determine if the k-ratio is positive, or if the background count rate is unusually high on either side of the peak.

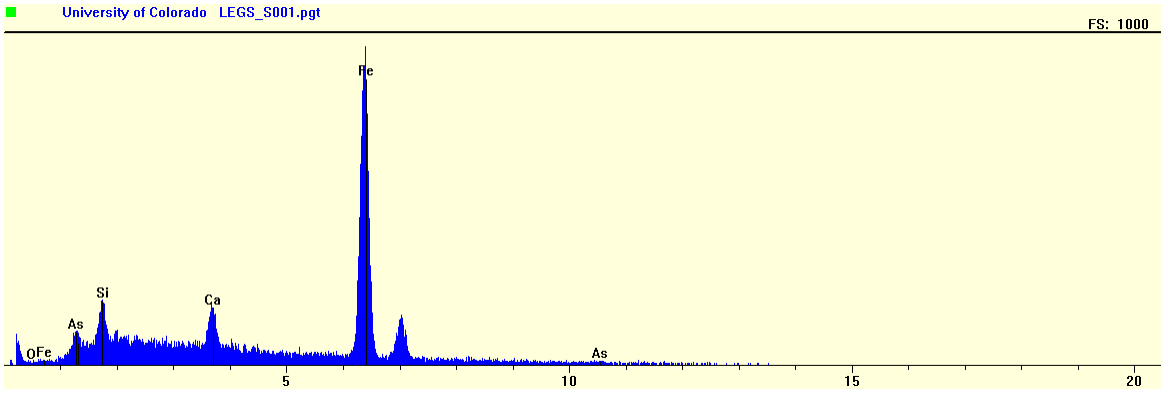
1. **AsMO** - This phase is generally associated with smelting waste, and is characterized by high concentrations of arsenic (5-25 wt%) and oxygen, with other metals (abbreviated "M") such as Pb, Cd, Fe, Cu, Sb and/or Zn in lesser concentrations. Some sulfur may also be present, but quantities are generally very low.



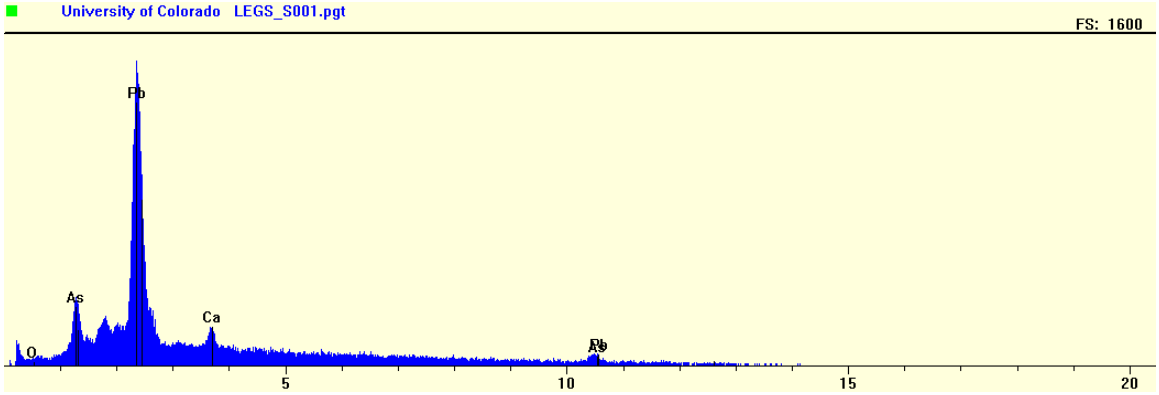


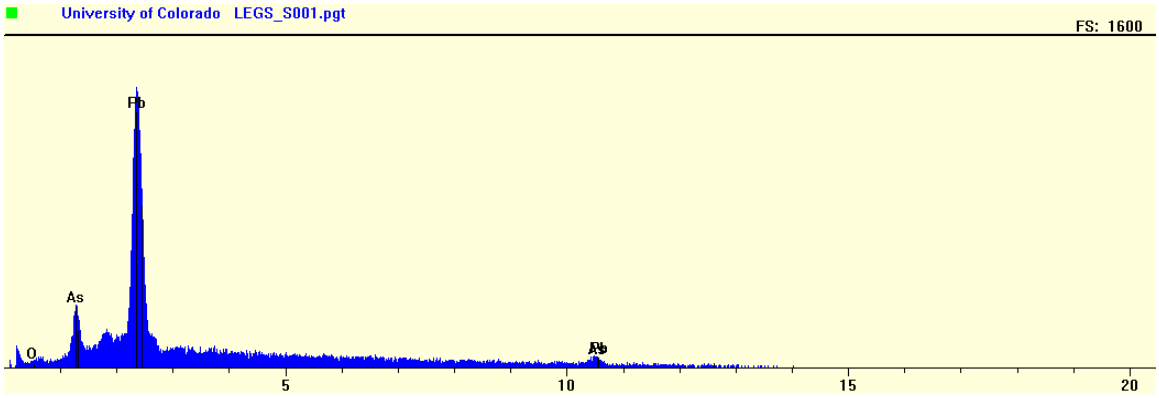
2. **FeOOH**- This phase is characterized by iron oxides in soil that have sorbed arsenic. Arsenic concentrations generally range from 1000 ppm to 6 wt%. Iron and oxygen are the major components, but other elements may be observed (Si, Al, Ca, P, Mn) in low quantities.



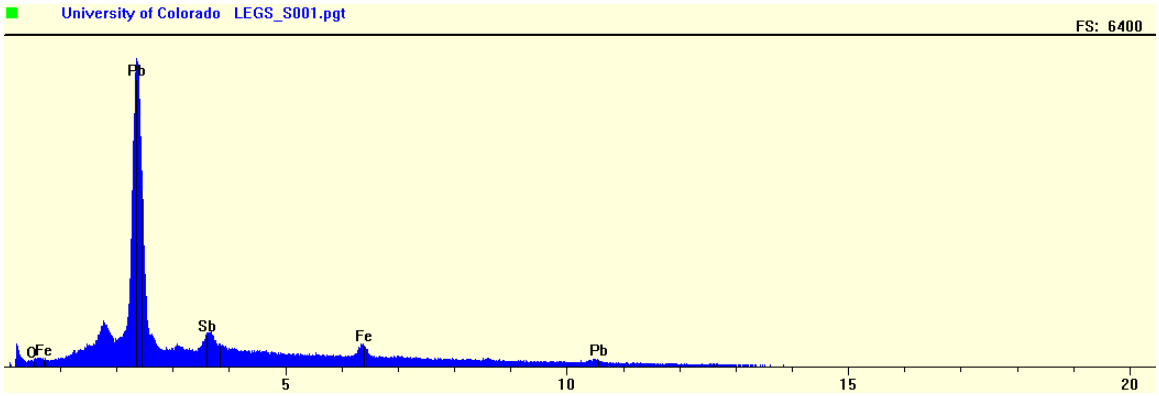


3. **PbAsO**- This phase can be observed in both smelting waste and some pesticide products. It contains high concentrations of arsenic (15-25 wt%), lead (40-55 wt%) and oxygen.

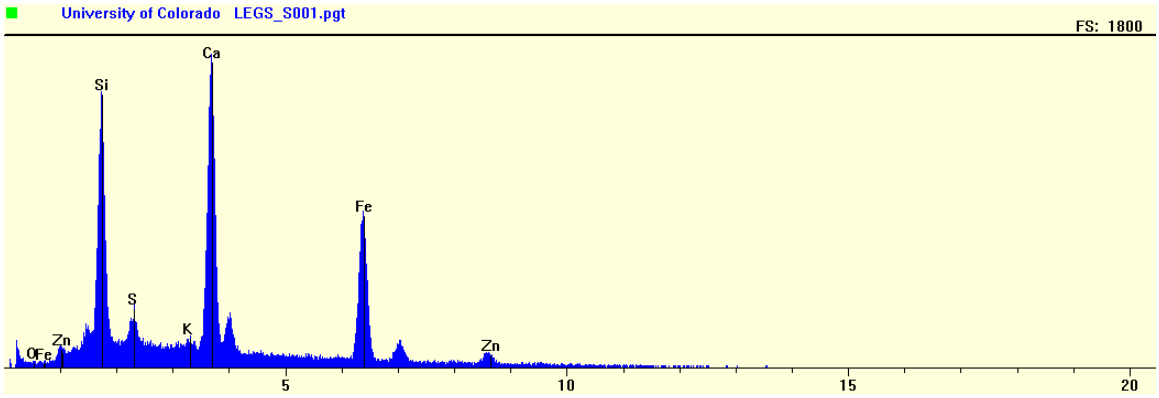


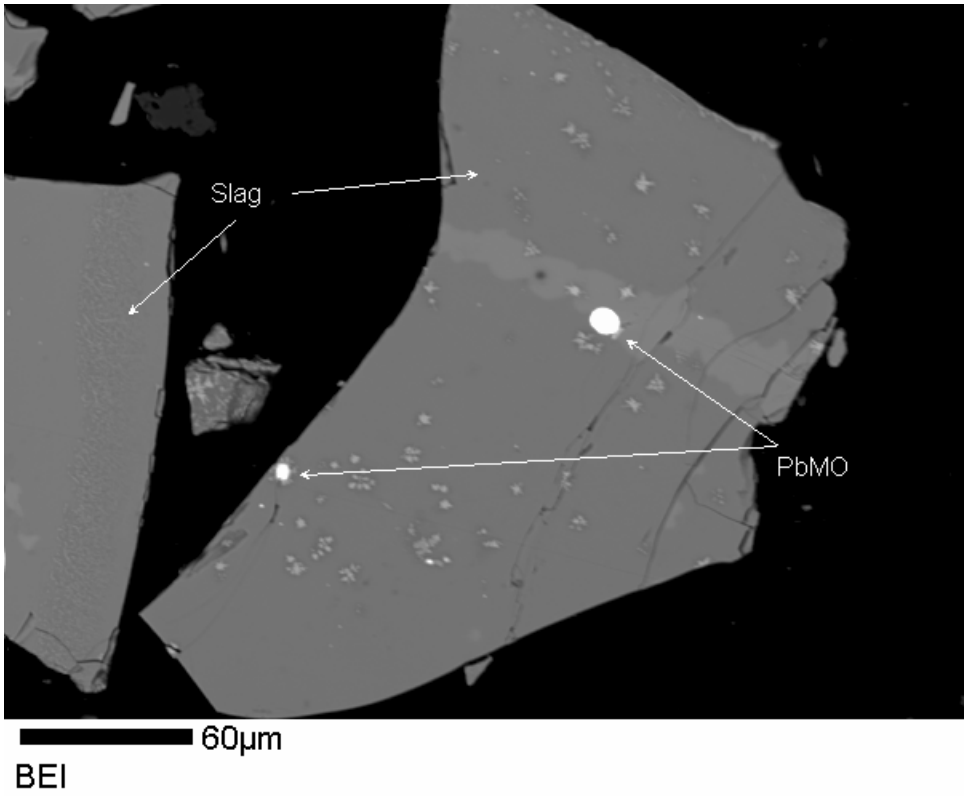


4. **PbMO**- This phase is generally associated with smelting waste. It contains high concentrations of lead (40-55 wt%) and arsenic (3-10 wt%) and oxygen, with other metals (M) such as Cd or Sb in lesser concentrations. Some sulfur may also be present, but quantities are generally very low.

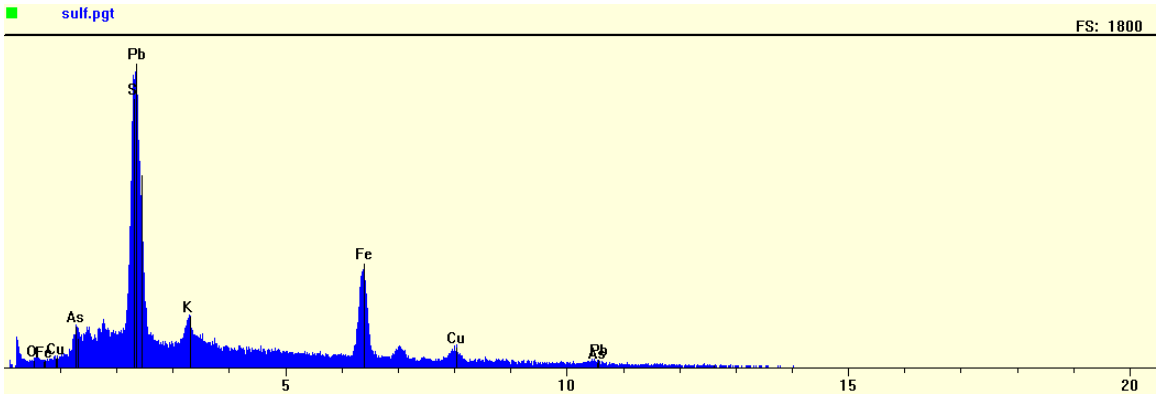


5. **Slag**- This phase is a vitreous-type particle generally associated with smelting waste. It contains low concentrations of lead (100 ppm-5 wt%) and arsenic (100 ppm- 5000 ppm). It is predominantly composed of Si-Ca-Fe-O with some sulfur and zinc, along with a characteristic morphology (see example photomicrograph). [Note: grains of arsenic-rich phases that are included in the slag are ranked as such, not as slag.]

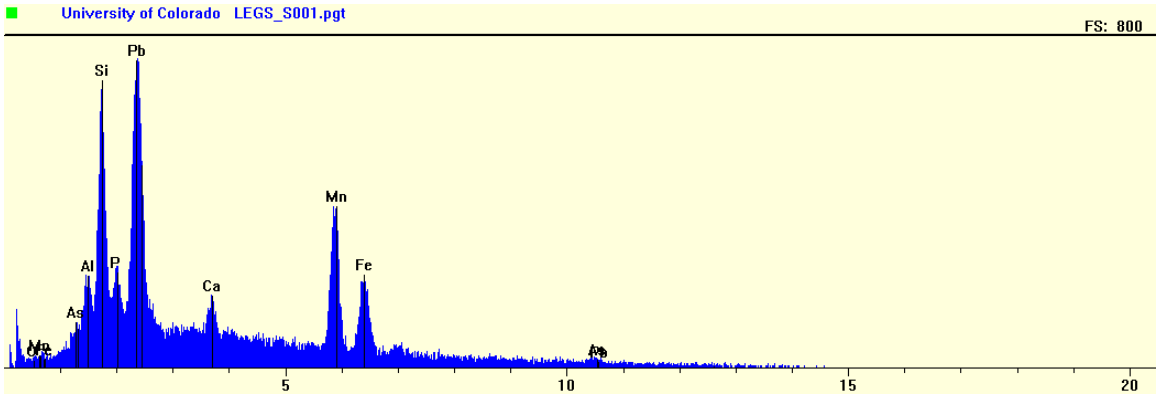


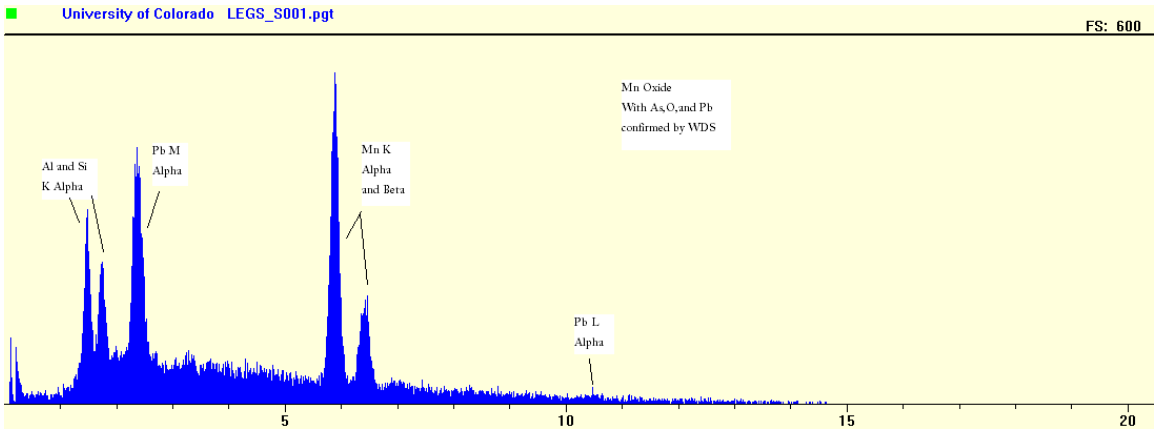


6. **FeSO4-** This phase may include both mining/smelting waste as well as iron sulfate-containing soil particles that have sorbed arsenic. Arsenic concentrations generally range from 1 to 6 wt%. Iron, sulfur, and oxygen are the major components, but other elements may be observed (Si, Al, K) in low quantities.

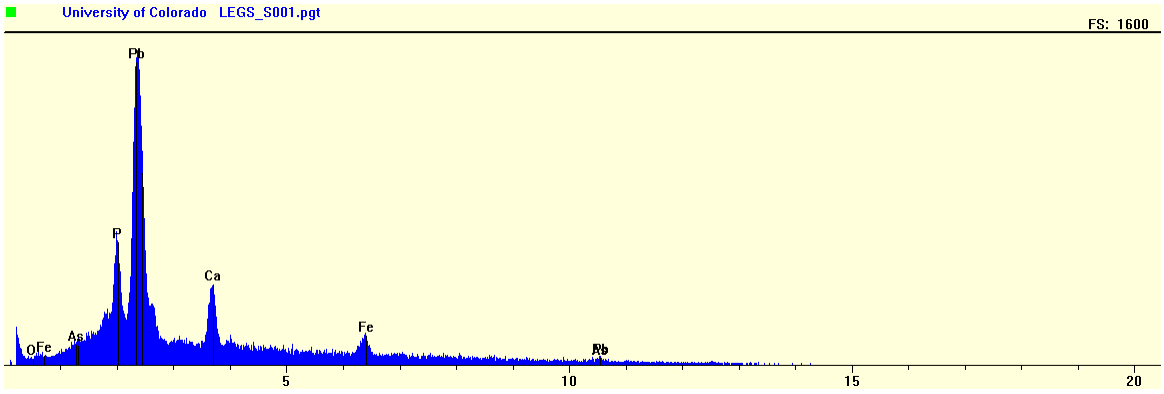


7. **MnOOH**- This phase is characterized by soil-forming manganese oxides that have sorbed arsenic. Arsenic concentrations generally range from 1000 ppm to 10 wt%. Manganese and oxygen are the major components, but other elements may be observed (Si, Al, Fe and Pb) in significant quantities.

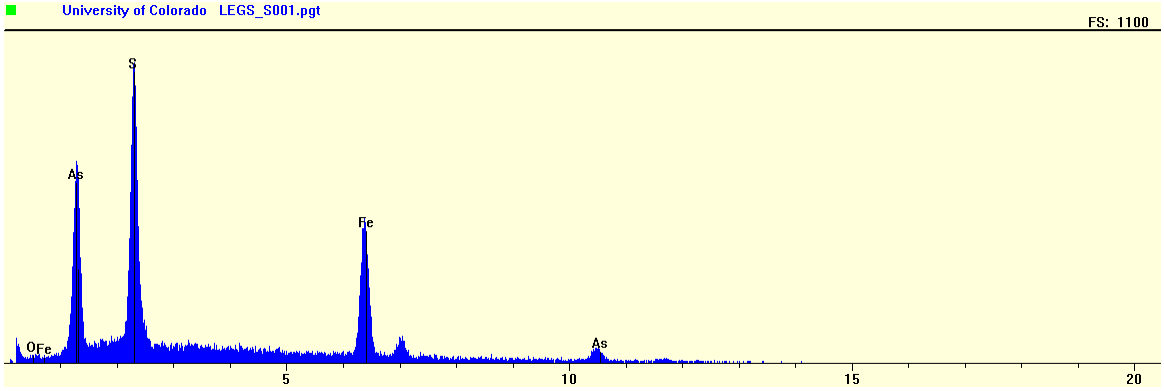




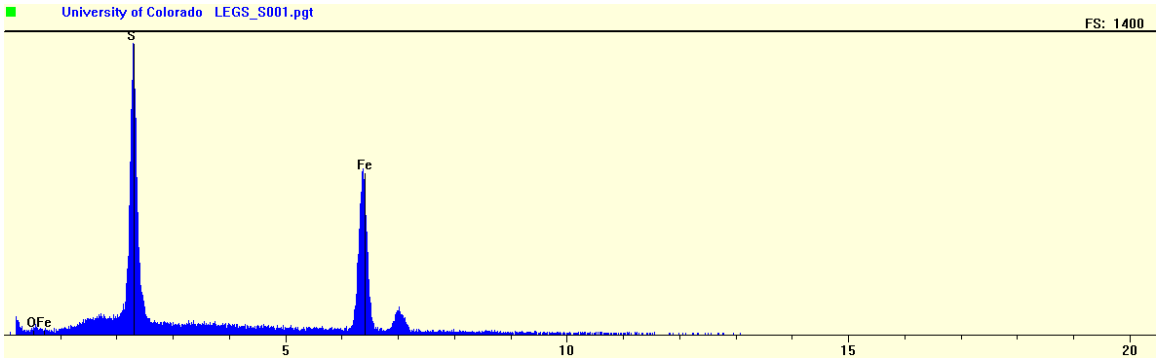
8. **Phosphate**- This phase represents soil-forming phosphate that has sorbed arsenic. Arsenic concentrations are generally low, ranging from 1000 ppm to 5 wt%. Phosphorous, calcium and oxygen are the major components, but other elements may be observed (Si, Al, Fe and Pb) in significant quantities.



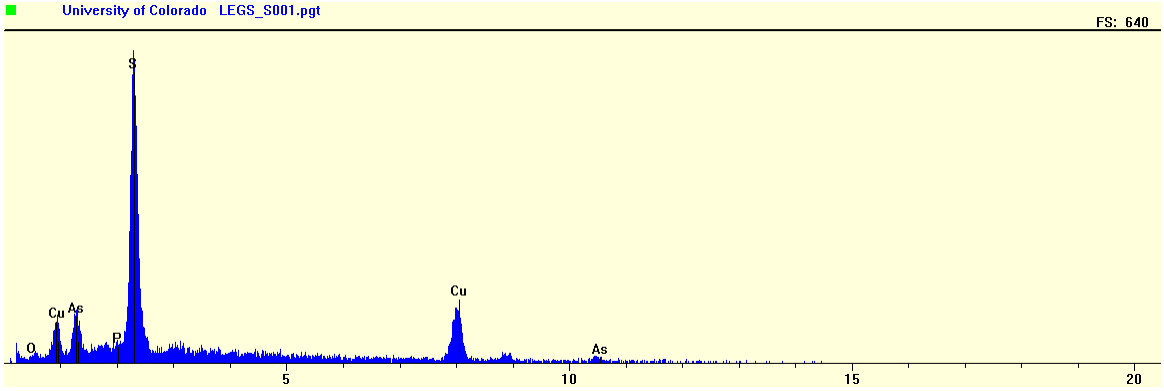
9. **Arsenopyrite**- This phase is generally associated with mining waste. It contains high concentrations of arsenic (46.0 wt%) along with iron and sulfur.

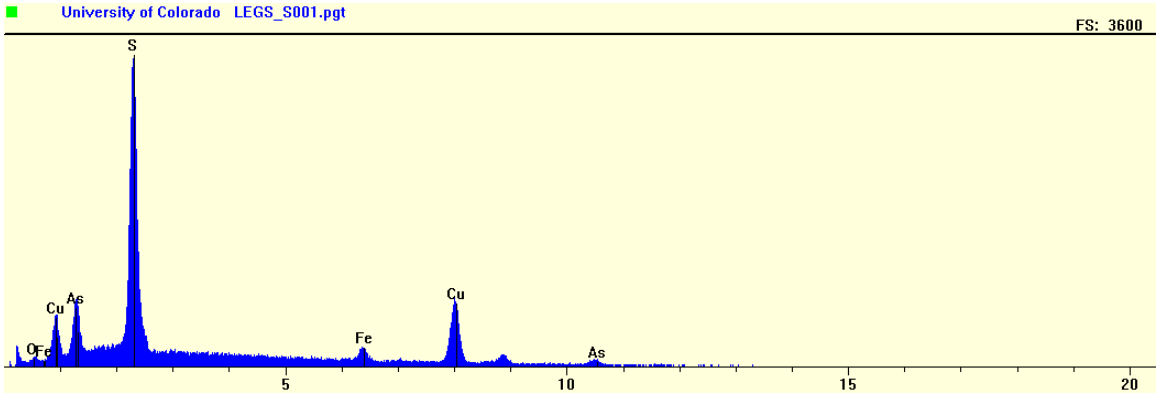


10. **Pyrite**- This phase is generally associated with mining waste. It contains low concentrations of arsenic (100-1000 ppm) along with high levels of iron and sulfur.

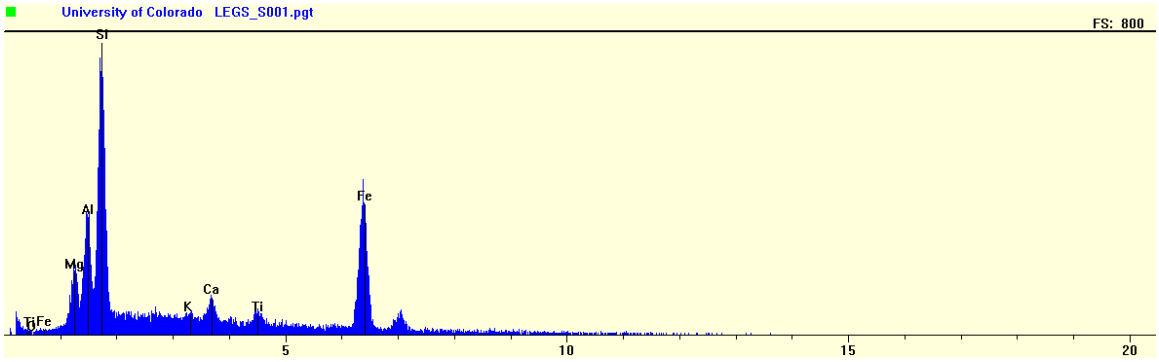


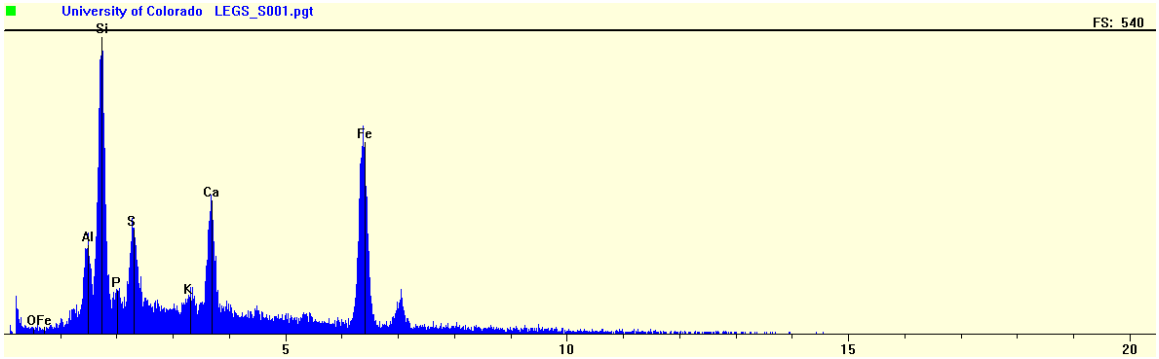
11. Sulfosalts- This phase is generally associated with mining waste. It contains high concentrations of arsenic (10-25 wt%) along with iron, antimony, copper, lead and sulfur.



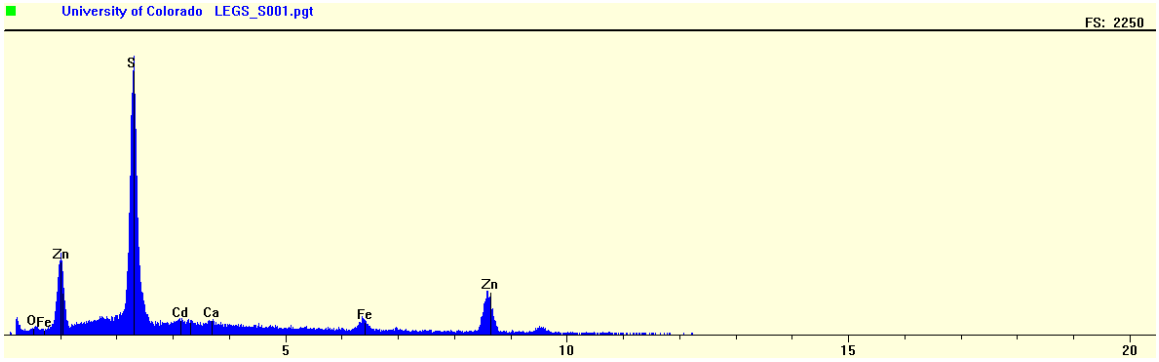


12. Clays- This phase represents soil-forming alumino silicates that have sorbed arsenic. Arsenic concentrations are generally low, ranging from 1000 ppm to 5 wt%. Alumina, silica, and oxygen are the dominant with lesser quantities of Ca,K,Mg, and Fe.

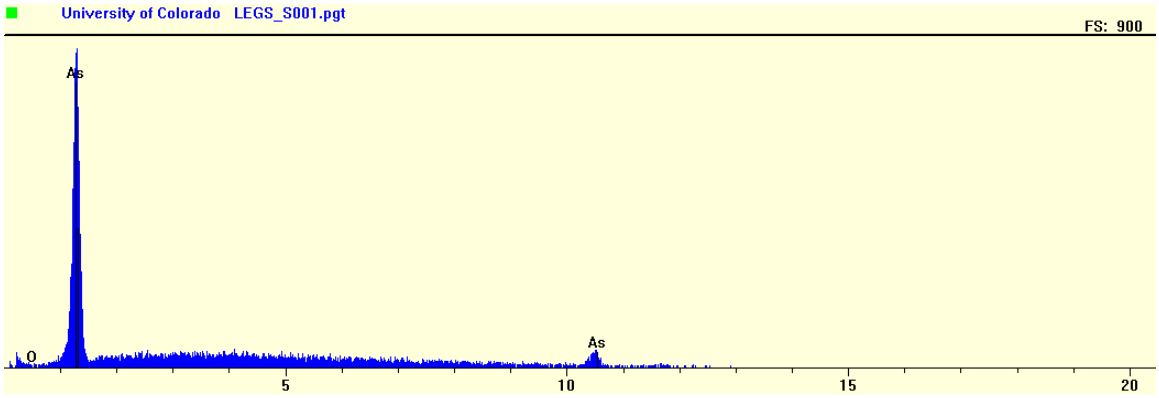




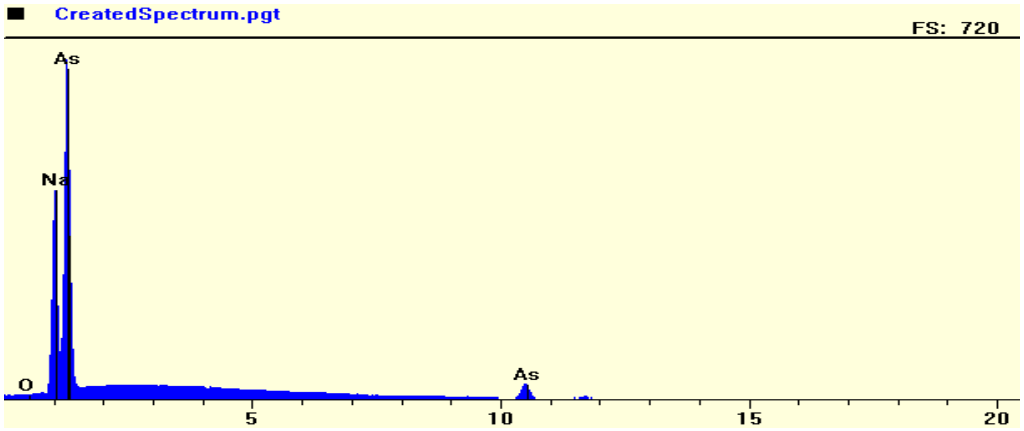
13. Zn- This phase is generally associated with smelting waste. It contains low concentrations of arsenic (100 ppm- 4000 ppm). It is predominantly composed of zinc, with some silica, sulfur and/or oxygen.

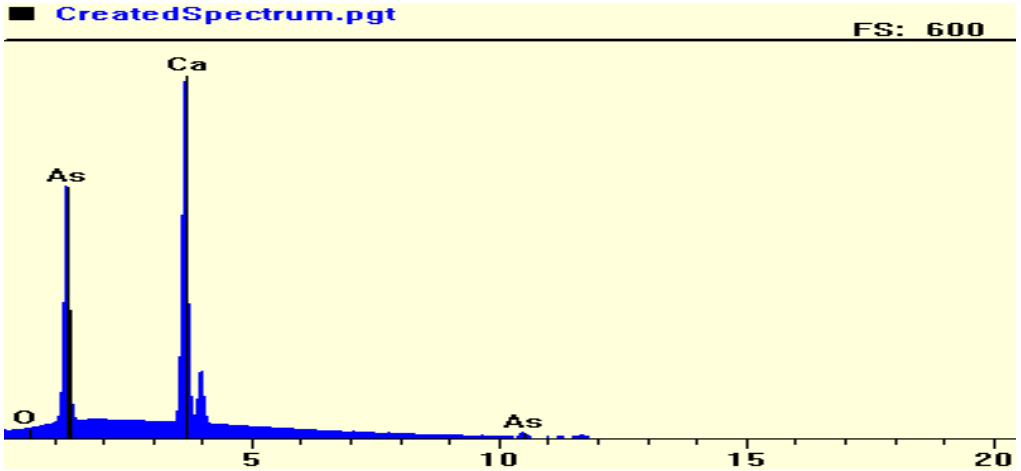


14. As2O3- This phase may occur in both smelting waste and in some pesticide products. It contains high concentrations of arsenic (~70 wt%) and oxygen.



15. Na-Ca AsO4- This phase is usually associated with pesticide/rodenticide products. It contains high concentrations of arsenic (~50-60 wt%) along with sodium or calcium and oxygen.





16. **Other**- This category should be used for arsenic-bearing particles that are not well characterized by any of the 15 phase groups described above. For example, some types of brass, solder, babbit, and paint pigments may contain low levels of arsenic, and should be recorded. If a particle is classified as "Other", use the comment field to indicate the nature of the particle (e.g., "brass"), and provide an image, the arsenic K-ratio, and the EDS spectrum to allow reviewers to reclassify if necessary.

4.0 DATA RECORDING AND DOCUMENTATION

Analysts will record data on each arsenic-bearing particle as they are acquired from each sample using a data recording sheet as illustrated in Figure 2. Table 2 lists standard "shorthand" phase abbreviations to use when completing the data sheet. When entering phase identifiers, be certain to use one of these standard abbreviations. Columns are established for numbering the arsenic-bearing phase particles, size of longest dimension (um), and the phase category assigned. Any relevant comments may be entered to the right of the data for each arsenic-bearing particle.

Photomicrographs and EDS x-ray spectra must be recorded for particles from each sample at a rate of 1 photograph per 20 particles counted.

5.0 FINAL REPORT

A final laboratory report will be provided that includes the following:

1) A list of samples received and the laboratory ID assigned to each
2) All laboratory data sheets.
3) All photomicrographs and EDS spectra recorded. These shall be submitted as a 128x128 (minimum) binary image in ".tif" or ".bmp" format. Recorded on each photomicrograph will be a scale bar, magnification, sample identification, date and phase identification using the standard abbreviations listed in Table 2. EDS spectra must have all major peaks labeled.
4) A narrative statement that identifies any issues or difficulties encountered, and any deviations from the methods specified in this SOP.

.

Figure 1
Scanning pattern

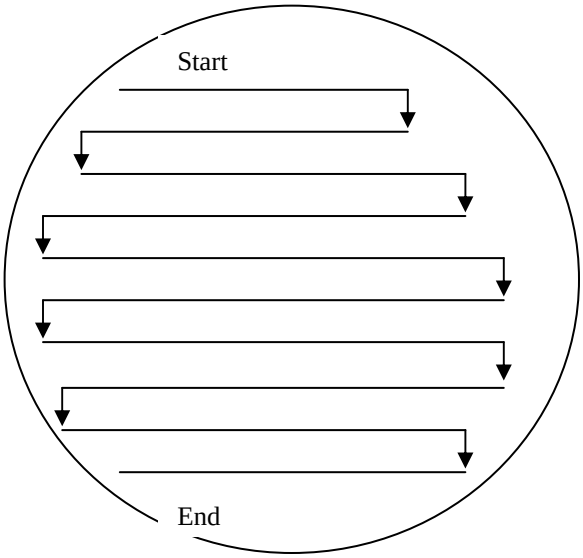


Figure 2
Example Data Recording Format

ELECTRONIC DATA DELIVERABLE FOR ARSENIC PHASE DATA

Lab name:	XYZ Labs
Client ID:	ABC TM2
Laboratory ID:	X-123
Analysis date:	6/12/2011
Analyst :	J Smith

Index	Phase	Length (um)	Comments
1	FeOOH	90	
2	FeOOH	2	
3	FeSO4	22	
4	FeOOH	6	
5	AsMO	11	
6	FeOOH	2	
7	SS	9	
8	MnOOH	8	
9	FeOOH	35	
10	PbAsO	10	
11	FeOOH	3	
12	FeOOH	70	
13	FeOOH	22	
14	FeOOH	4	
15	AsMO	2	
16	Py	33	
17	PbAsO	2	
18	FeOOH	10	
19	FeSO4	3	
20	AsMO	16	
21	FeOOH	8	
22	Py	4	
23	FeOOH	26	
24	PbAsO	3	
25	SS	2	
26	FeOOH	98	
27	FeSO4	36	
28	Phos	10	
29	PbAsO	18	
30	PbAsO	13	
31			
32			
33			
34			
35			

Table 1
EMPA Standard Operating Conditions

Parameter	WDS	EDS
Accelerating Voltage	15-20 KV	15-20 KV
Beam Size	1-2 microns	1-2 microns
Cup Current	10-30 NanoAmps	10-30 NanoAmps
Ev/Channel	NA	20
Stage Tilt	NA	Fixed
Working Distance	NA	Fixed
MCA time Constant	NA	7.5-12 microseconds
X-ray lines	S K-alpha PET O K-alpha LDE1 As L-alpha TAP	S K-alpha 2.31 KeV O K-alpha 0.52 KeV As K-alpha 10.5 KeV As K-beta 11.72 KeV As L-alpha 1.28 KeV

Table 2
Standard Phase Abbreviations

Phase Description	Valid Abbreviation
AsMO	AsMO
FeOOH	FeOOH
PbAsO	PbAsO
PbMO	PbMO
Slag	Slag
FeSO4	FeSO4
MnOOH	MnOOH
Phosphate	Phos
Arsenopyrite	Aspy
Pyrite	Py
Sulfosalts	SS
Clay	Clay
Zn	Zn
As2O3	As2O3
Na-Ca AsO4	NaCa
Other	Other