SOP Title: *In Vitro* Bioaccessibility (IVBA) Procedure for Arsenic

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Revision Log:

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0	9/25/2012	---

1.0 PURPOSE

This standard operating procedure (SOP) describes a method for measuring the *in vitro* bioaccessibility (IVBA) of arsenic in soil or soil-like matrices.

Background on the development and validation of this *in vitro* test system for estimating IVBA of arsenic, lead and other metals in soil can be found in Brattin et al. (2012).

2.0 TEST MATERIALS

This SOP is intended for application to soil or other soil-like media (sediment, tailings, flue dust, waste rock, etc.).

Sample Preparation

All test materials are prepared for the *in vitro* assay by drying (< 40 °C) followed by sieving to < 250 μm. Samples should not be ground, since altering particle size may alter IVBA.

Sample Analysis

If the concentration of arsenic in the test material (prepared as described above) has not previously been measured and provided to the laboratory (e.g., on the chain-of-custody form or other technical directive), two subsamples of the prepared test material shall be removed and digested in accordance with EPA Method 3050 followed by analysis for arsenic by EPA Method 6020. Calculations of IVBA will be based on the mean of the duplicate analyses.

3.0 APPARATUS AND MATERIALS

3.1 Equipment

The extraction device used in the IVBA procedure is illustrated in **Figure 1**. The device holds ten 125-mL wide-mouth high-density polyethylene (HDPE) bottles that are rotated within a water bath maintained at 37 ± 2 °C. The bottles must have a watertight 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

Required reagents include:

- Glycine, Tissue Grade. CASRN 56-40-6.
- Hydrochloric Acid (HCl), Trace-Metal Grade. CASRN 7647-01-0.

All solutions are prepared utilizing American Society for Testing and Materials (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 (< 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 triple-rinsed with DI water prior to use. Disposable labware is recommended whenever possible.

Extraction Fluid
The IVBA extraction fluid consists of 0.4 M glycine pH 1.5, and is 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 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 standard buffers using temperature compensation at 37 °C or buffers maintained at 37 °C. Add, dropwise, trace-metal grade concentrated HCl (12.1N) until the solution pH reaches a value of 1.50 ± 0.05.

If prepared ahead, the extraction fluids must be kept cool (2-4 °C) until needed, but not longer than 7 days.

4.0 EXTRACTION PROCEDURE

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

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

The extraction solution 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 8).

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 ± 0.05 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 control device 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 extraction fluid using a graduated cylinder or calibrated dispenser, and transfer to the 125-mL wide-mouth HPDE bottle containing the test substrate. 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) remain tightly fastened. Fill the extractor with 125-mL bottles containing test materials or quality control (QC) samples.

Turn on the extractor and rotate end-over-end at 28 ± 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. After filling the syringe, 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 a unique sample identifier [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 each extraction bottle. If the fluid pH is not within ± 0.5 pH units of the starting pH, the test must be repeated. If the same pH outcome is obtained in the repeated test, the results should be qualified.

If the pH in the post-extraction fluid exceeds 2, preserve the filtered sample by adding 2 drops of trace-metal grade nitric acid (HNO_3) to labeled 15-mL polypropylene centrifuge tube. Store the filtered and preserved 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.

5.0 EXTRACTION FLUID ANALYSIS

Extracts are analyzed for arsenic using EPA Methods 6010B, 6020, or 7061A (to be specified by Study Director). For EPA Method 6020, dilute each sample 50:1 (200 µL extract in 10 mL DI water) for analysis. This is needed to reduce the inductively coupled plasma/mass spectrometry (ICP/MS) interference caused by chlorine from the extraction fluid plus argon. Alternatively,

dilution may not be needed if the dynamic reaction cell (DRC) is used along with a non-argon carrier gas.

To date, no evidence has been observed that arsenic may become saturated in IVBA extraction fluid (this is a potential concern for lead IVBA studies). Consequently, this is not expected to be a limitation of this method.

6.0 QUALITY CONTROL/QUALITY ASSURANCE

Quality assurance for the extraction procedure will consist of the following QC samples:

- A Laboratory Blank [LB] 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 [BS] is a bottle containing 2.5 ppm (2.5 µg/mL) arsenic, prepared by adding 250 µL of 1,000 ppm National Institute of Standards and Technology (NIST) Traceable ICP arsenic standard solution to 100 mL of extraction fluid. This sample should be put through the entire extraction process but with no added soil or test substrate.
- A Matrix Spike [MS] is a bottle containing one gram of a test substrate plus 2.5 ppm (2.5 µg/mL) of added arsenic, prepared by adding 250 µL of 1,000 ppm NIST Traceable ICP arsenic standard solution to 100 mL of extraction fluid.
- A Laboratory Duplicate [LD] is a bottle containing a one gram sample of the same test substrate as prepared in another bottle.
- A Standard Soil [SS] is a one gram sample of a NIST Standard Reference Material (SRM). This may be any of the following: 2710, 2710A, 2711 or 2711A.

Unless otherwise specified by the Study Director, recommended minimum QC sample frequencies and control limits are listed below:

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
Matrix Spike	10%	75-125% recovery
Laboratory Duplicate	10%	RPD < 20%
Standard Soil	5%	RPD < 20%

RPD = relative percent difference

A typical pattern for incorporating these samples into groups of 10 (the number of bottles held by the extraction device) is as follows:

Table 2: Typical Pattern of Test Materials and QC Samples
This "typical pattern" is not consistent with the "analysis frequency" specified above for any of the QC samples

Bottle	Content
1-10	10 Test Materials
11-14	Laboratory Blank, Blank Spike, Matrix Spike, Laboratory Duplicate
15-20	6 Test Materials
21-25	Laboratory Blank, Blank Spike, Matrix Spike, Laboratory Duplicate,
26-30	Standard Soil
	5 Test Material

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

Attachment 2 provides an electronic file template ("IVBA Bench Sheet and EDD v1.xlsx") that contains a laboratory bench sheet and electronic data deliverable (EDD) for recording the data from IVBA studies.

Figure 2 provides an example of the bench sheet for recording raw laboratory data. All raw data will be recorded 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 the EDD. **Figure 3** illustrates the structure of this EDD.

Data Verification

After data entry is complete, the Laboratory Director shall review the EDD for omissions and errors, and compare the recorded data to the laboratory worksheet and the analytical data package and ensure that all data have been entered correctly.

Data Transmittal

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

9.0 REFERENCES

Brattin W, Drexler J, Lowney Y, Griffin S, Diamond G, Woodbury L. 2012. An *In Vitro* Method for Estimation of Arsenic Relative Bioavailability in Soil. *J. Toxicol. Environ. Health* (submitted for publication).

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

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EPA (U.S. Environmental Protection Agency). 2007. 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.

Medlin, EA and Drexler, JW. 1995. Development of an *in vitro* technique for the determination of bioavailability from metal-bearing solids. International Conference on the Biogeochemistry of Trace Elements, Paris, France.

Medlin, EA. 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 MW, Davis A, Link TE, Schoof R, Chaney RL, Freeman GB, and Bergstrom P. 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 MW, Davis A, Schoof R, Eberle S, and Sellstone CM. 1996. Estimation of lead and arsenic bioavailability using a physiologically based extraction test. *Environ. Sci. Technol.* 30(2): 422-430.

FIGURE 1
SCHEMATIC DIAGRAM OF IVBA EXTRACTION DEVICE

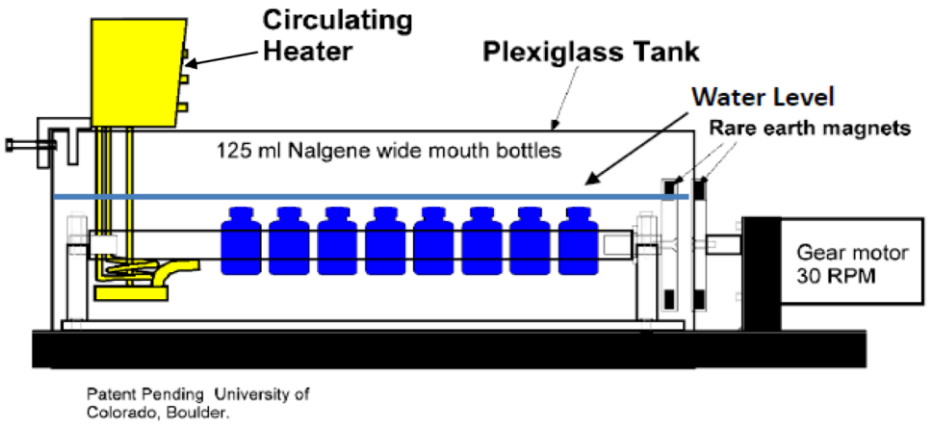

Figure 2

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FIGURE 3
ELECTRONIC DATA DELIVERABLE FOR ARSENIC IVBA MEASUREMENTS

Index	Labor Nar	…	Comments
1	XYZ		
2	XYZ		
3	XYZ		
4	XYZ		
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LABORATORY BENCH SHEET FOR ARSENIC IVBA MEASUREMENTS

Laboratory Name: XYZ LABS
Date: 10/12/2012
Analyst: J. SMITH
Bath Temp. (°C): 37.1

Index	Sample ID	Laboratory ID	Sample Type*	Fluid Vol. (mL)	Sample Mass (g)	pH		Time		Comments
						Start	End	Start	Filter	
1	12-31474	AB-10001	TM	100	0.997	1.48	1.51	11:21	12:30	
2	75	02	↓	↓	1.021	↓	1.50	↓	12:34	
3	76	03	↓	↓	1.035	↓	1.52	↓	12:38	
4	77	04	↓	↓	0.991	↓	1.50	↓	12:40	
5										
6										
7										
8										
9										
10										

General Comments:

*Valid Sample Types: TM - Test Material, LB - Laboratory Blank, BS - Blank Spike, MS - Matrix Spike, LD - Laboratory Duplicate, SS - Standard Soil

Note: For MS and LD samples, ensure that the recorded Sample ID reflects the parent sample ID.

ATTACHMENT 1
IVBA Procedure Checklist

1 Verify sample identification.
2 Using a black permanent marker, label 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 µm) 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.
8 Allow the extraction fluid to come to equilibrium with extraction apparatus at 37 ± 2 °C.

****Steps 8-19 must be completed within 90 minutes from the start of extraction or repeat the process****

9 Calibrate the pH meter. Adjust the pH of the extraction fluid at 37 ± 2 °C (if necessary) and record the pH.
10 Add 100 ± 0.5 mL of the designated extraction fluid to labeled 125-mL Nalgene bottles 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 125-mL 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 15-mL polypropylene centrifuge tube.
17 Measure and record the final pH of the extraction fluid directly from the labeled 125-mL Nalgene bottle.

18 The final pH must be within ± 0.5 of the initial extraction fluid pH or repeat the test.

19 Refrigerate labeled 15-mL polypropylene centrifuge tubes until analysis. All analyses must be complete within one week of extraction.

ATTACHMENT 2

IVBA Bench Sheet and EDD

See attached electronic file ("IVBA Bench Sheet and EDD v1.xls")