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.

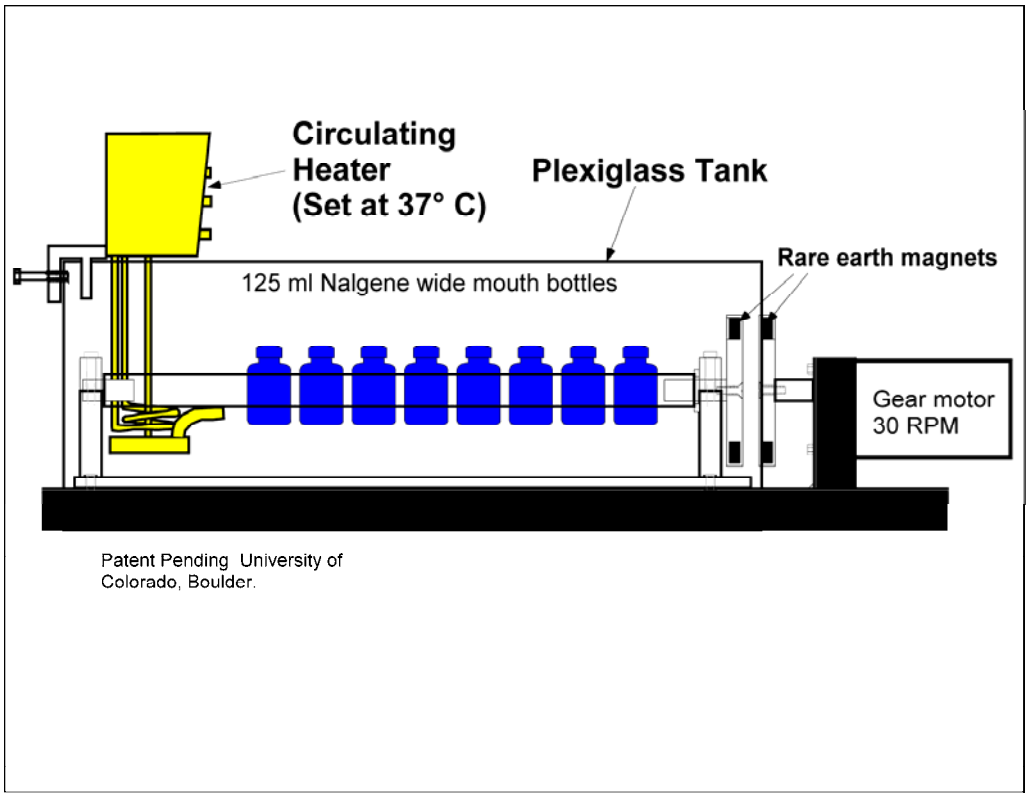

Figure 1. Schematic Diagram of IVBA Extraction Device

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

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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.

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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	
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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%	
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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.