Standard Operating Procedure

Modified OSU-IVG for Arsenic Laboratory Adapted (EOE)

Soil Environmental Chemistry Program, The Ohio State University, Version 4

Project/Client _____

Sample Description _____

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1.0 Scope of Method

1. Often the most important exposure pathway for arsenic (As), the risk driver, associated with human exposure is incidental soil ingestion. However, use of total soil As assuming ≥ 60% relative bioavailability (RBA) often overestimates exposure because physiochemical properties of the soil matrix can sequester As and reduces its RBA to < 60%. A more accurate and site-specific human health risk assessment accounts for RBA As in a soil matrix as part of the exposure assessment. Appropriate animal models, similar to human gastrointestinal physiology, are often used to determine bioavailability of As in contaminated soil. The most commonly used animal model for determining bioavailable As is the juvenile swine model (Basta et al. 2007; Juhasz et al. 2008; Brattin and Casteel 2013). In order to overcome some of the difficulties and expenses associated with animal dosing trials used to assess bioavailability of contaminants in soil, extensive research efforts have been directed toward development of in vitro gastrointestinal methods, that simulate the gastrointestinal environment, to predict bioavailable As. The Department of Toxic Substances Control (DTSC) was awarded a Training Research and Technical Assistance Grant to conduct an As Relative Bioavailability Study (Study) by the United States Environmental Protection Agency Region IX (USEPA). The goal of the study was to determine the range of As bioavailability that may exist in contaminated soil at former abandoned mine land (AML) sites and develop better methods to determine the human health effects caused by exposure to As at these sites. The bioaccessibility method described herein is an outcome of the DTSC study.

2.0 Definitions

3.1 Bioavailability (BA) – The fraction of an ingested dose (i.e., in vivo) that crosses the gastrointestinal epithelium and becomes available for distribution to internal target tissues and organs.

3.2 Absolute bioavailability – Bioavailability expressed as a fraction (or percentage) of a dose.

3.3 Relative bioavailability (RBA) – The ratio of the bioavailability of a metal in one exposure context (i.e., physical chemical matrix or physical chemical form of the metal) to that in another exposure context (i.e. USEPA Integrated Risk Information System, IRIS). For this method, RBA is defined as the ratio of bioavailability of As in sodium arsenate dissolved in water.

3.5 Batch – A group of analytical and control/QC samples that are extracted simultaneously and is limited to 12 environmental samples including the batch QC samples.

3.6 In vitro – outside the living body and in an artificial environment

3.7 In vivo – in the living body of an animal

3.8 In vitro bioaccessibility (IVBA) – the physiological solubility of the metal that may be available for absorption into the body

2.1 Modified OSU IVG: The Ohio State University *In Vitro* Gastrointestinal Method with modified parameters for recovery of bioaccessible As associated with iron oxides.

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

2.5 Laboratory Control Sample: The laboratory control sample is NIST SRM 2711a and goes through the same extraction/preparation procedure as the samples.

2.6 Blank Spike: A sample which contains only extraction fluid is spiked prior to incubation and run through the complete procedure in order to provide information about the effect of the extraction fluid on bioaccessibility and/or measurement methodology.

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

2.8 Duplicate sample: A duplicate of one sample per batch is processed through the same preparation procedures as the samples to determine reproducibility within each batch.

3.0 Equipment and Supplies

3.1 Incubator with temperature control of 37± 2 ºC (VWR Model 1545)

3.2 End over end rotator capable of rotating at 30±2 rotations per minute (Glas-Col Rotator model RD4512 with RD50 head)

3.3 175mL wide mouth high-density polyethylene (HDPE) bottles

3.4 Porcine pepsin powder (sigma P7125-500g)

3.5 Trace metal grade HCl

3.6 ACS grade NaCl

3.7 ACS grad L-Ascorbic Acid powder

3.8 ACS grade Na_2CO_3

3.9 Porcine bile (sigma B8631-100G)

3.10 Pancreatin (sigma P1750-500G)

3.11 12ml Luer lock syringes for filtering

3.12 0.45um nylon or cellulose acetate Luer lock syringe filters

3.13 15mL falcon tubes or other vial for ICP analysis

3.14 pH meter with combination electrode

3.15 Certified pH buffers; 1, 2, 4, and 7

3.16 NIST Montana 2711a reference material

3.17 Certified 1,000 mg/L As standard

3.18 0.45um nylon syringe filters

4.0 Sample Preparation

4.1 All test soils should be prepared by drying (<40 °C) and sieving the sample as received to <250 µm. Milling should NOT be employed to achieve the desired particle size. The <250 µm size fraction is used because this particle size is representative of that which adheres to children's hands. Stainless steel sieves are recommended.

5.0 Procedure

5.1 Solution preparation

5.1.1 Gastric Solution: Prepare 2 liters (L) of extraction fluid in a volumetric flask (Class A) using 18 mega ohm DI water. Add 20 grams of pepsin, 11.7g NaCl, and 35.2g Ascorbic acid to a flask containing 1.5 L of deionized water. Mix with magnetic stir bar until reagents are fully dissolved. Add 300mL of deionized water, cover with parafilm, place in a water bath or incubator at 37 °C and heat until the extraction fluid reaches 37 °C. At the time of the extraction, standardize the pH meter using an ATC pH electrode at 37 °C or pH buffers maintained at 37 °C. While stirring, add trace metal-grade concentrated HCl (6M) until the solution pH reaches 1.50 ± 0.01. Remove stir bar and bring the solution to a final volume of 2 L.

- Calibrate pH meter using 1,4, and 7 pH buffers held at 37± 2 ºC for each batch of extractions.
    - **Record slope of meter calibration in Appendix**
- Gastric extraction must be initiated within 24 hours of solution preparation.

5.1.2 Intestinal Reagents: Intestinal solution is prepared for each individual sample immediately following the gastric phase by the addition of 5mL of saturated Na_2CO_3 and 5ml of bile and pancreatin solution; 10.5g of bile and 1.05g of pancreatin dissolved in 100mL of water.

5.2 Gastric Phase

5.2.1 The soil or geomedia (1 g ± 0.05, < 250 µm) is weighed into a 175-mL wide-mouth HDPE bottle (record exact mass to 4 decimal places on analysis sheet).

5.2.2 Measure 150± 1.5 mL of the 37±2 °C gastric extraction fluid using a graduated automated dispenser and transfer the extraction fluid to the 175-mL wide-mouth HDPE bottle.

- **Record bottle top dispenser calibration verification in Appendix before use**

5.2.3 Add 0.750mL of 1,000 mg/L As standard to the blank spike sample.

5.2.3a When using 1,000 mg/L standard, pour a small amount into a falcon tube. DO NOT return the unused standard to the Certiprep container.

- **Record Pipette calibration verification in Appendix.**

5.2.4 Cap and properly place the bottles in the rotator and begin rotation. The rotator should be maintained at 30 ± 2 rpm for the remainder of two hours. Record the start time of rotation.

5.2.5 After one hour, the bottles should be removed from the rotator and placed upright on the bench top for pH monitoring and adjustment. Record the pH at 1hour and adjust pH using 6M HCl or saturated Na_2CO_3 to 1.5 ± 0.1. After pH adjustment, cap and return the bottles to the incubator. Ensure that bottle caps remain paired to the same bottle throughout the extraction.

5.2.6 After two hours, the bottles should be removed from the rotator, dried (if necessary), placed upright on the bench top, allowed 10 minutes for soil settling.

5.2.7 A 10-mL sample of supernatant fluid is then removed directly from the top 3 cm of solution in extraction bottle into a disposable syringe. After withdrawal of the sample into the syringe, a Luer lock attachment (equipped with a 0.45-µm nylon or cellulose acetate disk filter (25-mm diameter) is attached, and the sample is filtered through the attached disk filter to remove any particulate matter into a 15mL falcon tube.

5.2.8 Record the time that the extract is filtered (i.e., extraction is stopped). If the total time elapsed for the extraction and filtration process exceeds 145 minutes, the test must be repeated

5.2.9 Measure and record the pH of fluid remaining in the extraction bottle. If the fluid pH is not within ±0.1 pH units of the starting pH, the test must be discarded and the sample reanalyzed.

5.3 I**ntestinal Phase**

5.3.1 Valid gastric samples (end pH 1.5 ± 0.1) immediately receive 5mL of saturated Na_2CO_3 and 5ml of bile and pancreatin solution (Sec. 5.1.2).

- **Record Pipette calibration verification in Appendix.**

5.3.2 Cap and properly place the bottles in the rotator and begin rotation. The rotator should be maintained at 30 ± 2 rpm for the remainder of four hours. Record the start time of rotation.

5.3.3 After two hours, the bottles should be removed from the rotator and placed upright on the bench top for pH monitoring and adjustment. Record the pH at 2hours and adjust pH using 6M HCl or saturated Na_2CO_3 to 7.5 ± 0.5.

5.3.4 After four hours, the bottles should be removed from the rotator, placed upright on the bench top, and allowed 10 minutes for soil settling.

5.3.5 A 10-mL sample of supernatant fluid is then removed directly from the top 3 cm of solution in extraction bottle into a disposable syringe. After withdrawal of the sample into the syringe, a Luer lock attachment (equipped with a 0.45-µm nylon or cellulose acetate disk filter (25-mm diameter)) is attached, and the sample is filtered through the attached disk filter to remove any particulate matter into a 15mL falcon tube.

5.3.6 Record the time that the extract is filtered (i.e., extraction is stopped). If the total time elapsed for the extraction and filtration process exceeds 270 minutes, the test must be repeated.

5.3.7 Measure and record the pH of fluid remaining in the extraction bottle. If the fluid pH is not within ±0.5 pH units of the starting pH, the test must be discarded and the sample reanalyzed.

NOTE: In some cases (mainly silica slag contaminated media), the test material can increase the pH of the extraction buffer and this could influence the results of the bioaccessibility measurement. To guard against this, the pH of the fluid should be measured at the end of the extraction step (just after a sample was withdrawn for filtration and analysis). If the pH is not within 0.1 pH units of the starting pH (1.5), the sample should be re-extracted. If the second test also results in an increase in pH of >0.1 units, it is reasonable to conclude that the test material is buffering the solution. In these cases, the test should be repeated using additional manual pH adjustment during the extraction process, stopping the extraction at 15, 30, 60, and 75 minutes and adjusting the pH down to pH 1.5 at each interval by drop-wise addition of trace-metal grade 6M HCl.

5.3.8 Store filtered sample(s) in a refrigerator at 4±2 °C until they are analyzed. This filtered sample of extraction fluid is then analyzed for arsenic by an appropriate method.

NOTE: Calibration standards should be prepared in a matrix similar to the samples (0.1M NaCl + 0.4M HCl). Dilution of the extracts (minimum of 5 fold) is recommended before analysis. Dilution of the samples with 0.1M NaCl + 0.4M HCl can cause precipitation to occur, particularly in the intestinal extractions. As a result, dilutions should be performed using only 0.1M NaCl). Correct for any dilutions in the calculations. Samples that fall below detection limit

with dilution may be run undiluted, but nebulizer and torch stability will be limited. Alternately, a high solids nebulizer may be used without dilution.

6.0 Quality Control

The laboratory should establish the LLOQ as the lowest point of quantitation. The LLOQ should be verified by the analysis of at least seven replicate samples, which are spiked at the LLOQ and processed through all preparation and analysis steps of the method. The mean recovery and relative standard deviation of these samples provide an initial statement of precision and accuracy at the LLOQ. In most cases, the mean recovery should be ±35% of the true value and the RSD should be ≤20%.

6.1 Reagent blank – Unprocessed (not run through the extraction procedure) extraction fluid should be analyzed for each new batch of extraction fluid. The reagent blank is considered within control limits if its result is less than the lower limit of quantitation (LLOQ). The corrective action for a blank hit above LLOQ should include preparing a new batch of extraction fluid and reprocessing any samples that were prepared with the failing reagent fluid.

6.2 Method blank – Extraction fluid only (i.e., no test soil) is carried through all steps of the method at a frequency of 1 per batch. The method blank is considered within control limits if its result is less than the LLOQ. The corrective action for a recovery above the LLOQ should include making a new extraction fluid and reprocessing any samples that were prepared with the failing method blank.

6.3 Laboratory Control Sample (LCS) – A LCS consisting of a spiked blank must be run once per batch. The LCS may be spiked with the same source as the calibration standards and needs to be carried through all steps of the rotation procedure. The control limits are 85-115% recovery. The corrective action for outliers should include an analyst review that all dilutions and spike concentrations were performed correctly. If no error is found, either re-extract the samples or flag and narrate the defect and possible bias in the data.

6.4 Matrix Spike (MS) – A MS should be run once per ICP method batch (See 6010 SOP). The MS should be prepared after extraction and filtration of the supernatant. The control limits are 75-125% recovery. The corrective action for outliers should include an analyst review that all dilutions and spike concentrations were performed correctly. If no error is found, either re-extract the samples or flag and narrate the defect and possible bias in the data.

6.5 Blank Spike (BS) - A BS should be run once per extraction batch. The control limits are 85-115% recovery. The corrective action for outliers should include an analyst review that all dilutions and spike concentrations were performed correctly. If no error is found, either re-extract the samples or flag and narrate the defect and possible bias in the data.

6.5 Duplicate sample – A duplicate sample should be run once per batch and carried through all steps of the method. The relative percent difference (RPD) should be less than 20%. The

corrective action for outliers should include either re-extraction of the samples or flagging the data.

6.6 The reference material shall be carried through all steps of the method and analyzed at a frequency of 1 per batch. The NIST SRM 2711a should yield a mean GE IVBA result of 80.5% ± 15% (68.4% – 92.5%) and a mean IE IVBA result of 79.5% ± 15% (67.6% – 91.4%). For the As concentration in the SRM, the median As concentration presented in the Addendum to the NIST certificate for leachable concentrations determined using Method 3050 (89 mg/kg) should be used.

7.0 Data Calculation and Assessment

7.1 If the IVBA factor is to be determined, a split of each solid material (<250 μm) that has been subjected to this extraction procedure should be analyzed for total As concentration using analytical procedures taken from USEPA SW-846 or a non-destructive method such as Instrumental Neutron Activation Analysis. If USEPA SW-846 methods are used, the solid material should be acid digested according to an appropriate preparation method (e.g., Method 3050 or Method 3051). The digestate should be analyzed for As concentration by an appropriate analytical method.

7.2 If dilutions were performed, apply the appropriate corrections to the sample values.

7.3 In vitro bioaccessibility (IVBA)
7.3.1 The IVBA is calculated on a mass basis (mg/kg) using the following equations:

mg/kg Gastric bioaccessible = [(mg/L As * dilution factor)*(150/soil mass(g)]
mg/kg Intestinal bioaccessible = [(mg/L As * dilution factor)*(150/soil mass(g)]

7.3.2 The IVBA is calculated and expressed on a percentage basis using the following equation:

% Bioaccessible = [bioaccessible As (mg/kg)]/[total As (mg/kg)] *100

7.3.3 In order for an in vitro bioaccessibility test system to be useful in predicting the in vivo RBA of a test material, it is necessary to empirically establish that a strong correlation exists between the in vivo and the in vitro results across many different samples (see Reference 10). At present the linear regression model for predicting RBA is under evaluation and will be incorporated into this SOP when it becomes available.

8.0 References

Basta, N. T., J. N. Foster, et al. (2007). "The effect of dosing vehicle on As bioaccessibility in smelter-contaminated soils." J Environ Sci Health A Tox Hazard Subst Environ Eng 42(9): 1275-1281.

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Nelson, C.M., T. M. Gilmore, J. M. Harrington, K. G. Scheckel, B. W. Miller, and K. D. Bradham, "Evaluation of a low-cost commercially available extraction device for assessing lead bioaccessibility in contaminated soils," *Environ. Sci.: Processes Impacts*, Volume 15, pages 573-578, 2013.

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Extraction Solution pH day of extraction _____

Batch _____

Initials/Date _____

Pipette Calibration

Volume	g DI	g DI	g DI	g DI	g DI	date	initials

Volume	g DI	g DI	g DI	g DI	g DI	date	initials

Volume	g DI	g DI	g DI	g DI	g DI	date	initials
Volume	g DI	g DI	g DI	g DI	g DI	date	initials

pH Calibration

%Slope	

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