# Solubility/Bioavailability Research Consortium

# Standard Operating Procedure:

# *In Vitro* Method for Determination of Lead and Arsenic Bioaccessibility

Doc. No.: 8601102.001 0601 1099 RN01

# Contents

# 1. Introduction

## 1.1 Synopsis

This SOP describes an *in vitro* laboratory procedure to determine a bioaccessibility value for lead or arsenic (i.e., the fraction that would be soluble in the gastrointestinal tract) for soils and solid waste materials. A recommended quality assurance program to be followed when performing this extraction procedure is also provided.

## 1.2 Purpose

An increasingly important property of materials/soils found at contaminated sites is the bioavailability of individual contaminants. Bioavailability is the fraction of a contaminant in a particular environmental matrix that is absorbed by an organism via a specific exposure route. Many animal studies have been conducted to experimentally determine the oral bioavailability of individual metals, particularly lead and arsenic. During the period 1989–1997, a juvenile swine model developed by EPA Region VIII was used to predict the relative bioavailability of lead and arsenic in approximately 20 soils/solid materials (Weis and LaVelle 1991; Weis et al. 1994; Casteel et al. 1997a,b). The bioavailability determined was relative to that of a soluble salt (i.e., lead acetate trihydrate or sodium arsenate). The tested materials had a wide range of mineralogy, and produced a range of lead and arsenic bioavailability values. In addition to the swine studies, other animal models (e.g., rats and monkeys) have been used to measure the bioavailability of lead and arsenic from soil.

Several researchers have developed *in vitro* tests to measure the fraction of a chemical solubilized from a soil sample under simulated gastrointestinal conditions. This measurement is referred to as "bioaccessibility" (Ruby et al. 1993). Bioaccessibility is thought to be an important determinant of bioavailability, and several groups have sought

to compare bioaccessibility determined in the laboratory to bioavailability determined in animal studies (Imber 1993; Ruby et al. 1996; Medlin 1997; Rodriguez et al. 1999). The *in vitro* tests consist of an aqueous fluid, into which soils containing lead and arsenic are introduced. The solution then solubilizes the soil under simulated gastric conditions. Once this procedure is complete, the solution is analyzed for lead and/or arsenic concentration. The mass of lead and/or arsenic found in the aqueous phase, as defined by filtration at the 0.45-$\mu$m pore size, is compared to the mass introduced into the test. The fraction liberated into the aqueous phase is defined as the bioaccessible fraction of lead or arsenic in that soil. To date, for lead-bearing soils tested in the EPA swine studies, this *in vitro* method has correlated well with relative bioavailability values.

# 2. Procedure

## 2.1 Sample Preparation

All soil/material samples should be prepared for testing by oven drying (<40 °C) and sieving to <250 $\mu$m. The <250-$\mu$m size fraction is used because this particle size is representative of that which adheres to children's hands. Subsamples for testing in this procedure should be obtained using a sample splitter.

## 2.2 Apparatus and Materials

### 2.2.1 Equipment

The main piece of equipment required for this procedure consists of a Toxicity Characteristic Leaching Procedure (TCLP) extractor motor that has been modified to drive a flywheel. This flywheel in turn drives a Plexiglass block situated inside a temperature-controlled water bath. The Plexiglass block contains ten 5-cm holes with stainless steel screw clamps, each of which is designed to hold a 125-mL wide-mouth high-density polyethylene (HDPE) bottle (see Figure 1). The water bath must be filled such that the extraction bottles are immersed. Temperature in the water bath is maintained at 37±2 °C using an immersion circulator heater (for example, Fisher Scientific Model 730). Additional equipment for this method includes typical laboratory supplies and reagents, as described in the following sections.

The 125-mL HDPE bottles must have an air-tight screw-cap seal (for example, Fisher Scientific 125-mL wide-mouth HDPE Cat. No. 02-893-5C), and care must be taken to ensure that the bottles do not leak during the extraction procedure.

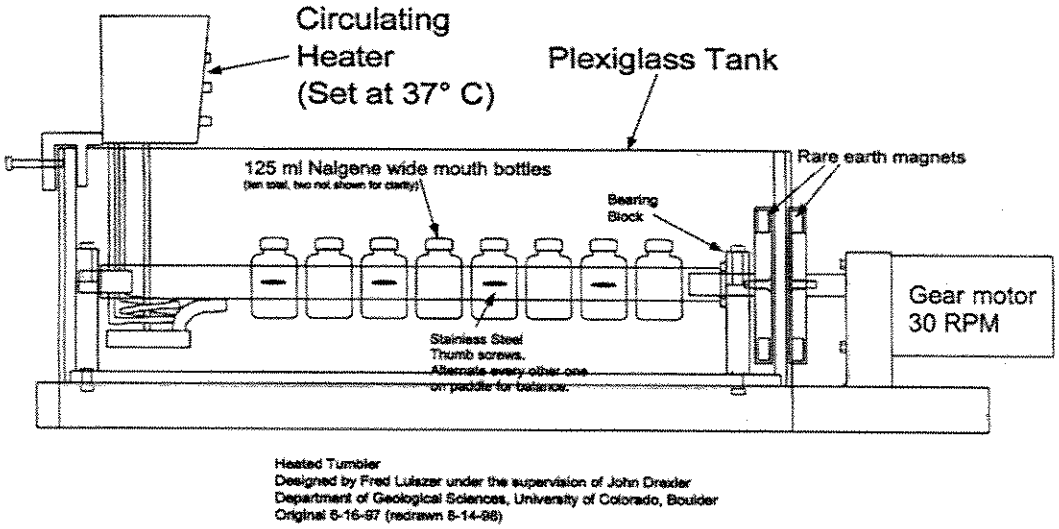


Figure 1. Extraction device for performing the SBRC *in vitro* extraction

### 2.2.2 Standards and Reagents

The leaching procedure for this method uses a buffered extraction fluid at a pH of 1.5. The extraction fluid is prepared as described below.

The extraction fluid should be prepared using ASTM Type II deionized (DI) water. To 1.9 L of DI water, add 60.06 g glycine (free base, Sigma Ultra or equivalent). Place the mixture in a water bath at 37 °C until the extraction fluid reaches 37 °C. Standardize the pH meter using temperature compensation at 37 °C or buffers maintained at 37 °C in the water bath. Add concentrated hydrochloric acid (12.1 N, Trace Metal grade) until the solution pH reaches a value of 1.50 ±0.05 (approximately 120 mL). Bring the solution to a final volume of 2 L (0.4 M glycine).

Cleanliness of all reagents and equipment used to prepare and/or store the extraction fluid is essential. All glassware and equipment used to prepare standards and reagents must be properly cleaned, acid washed, and finally, rinsed with DI water prior to use. All

reagents must be free of lead and arsenic, and the final fluid should be tested to confirm that lead and arsenic concentrations are less than 25 and 5 $\mu$g/L, respectively.

## 2.3 Leaching Procedure

Measure 100 ±0.5 mL of the extraction fluid, using a graduated cylinder, and transfer to a 125-mL wide-mouth HDPE bottle. Add 1.00 ±0.05 g of test substrate (<250 $\mu$m) to the 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 soil. Record the volume of solution and mass of soil added to the bottle on the extraction test checklist (see Attachment A for example checklists). Hand-tighten each bottle top, and shake/invert to ensure that no leakage occurs, and that no soil is caked on the bottom of the bottle.

Place the bottle into the modified TCLP extractor, 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 Control samples.

The temperature of the water bath must be 37±2 °C. Record the temperature of the water bath at the beginning and end of each extraction batch on the appropriate extraction test checklist sheet (see Attachment A).

Rotate the extractor end over end at 30±2 rpm for 1 hour. Record start time of rotation.

When extraction (rotation) is complete, immediately remove bottles, wipe them dry, and place them upright on the bench top.

Draw extract directly from reaction vessel into a disposable 20-cc syringe with a Luer-Lok attachment. Attach a 0.45-$\mu$m cellulose acetate disk filter (25 mm diameter) to the syringe, and filter the extract into a clean 15-mL polypropylene centrifuge tube or other

appropriate sample vial for analysis. Store filtered sample(s) in a refrigerator at 4 °C until they are analyzed.

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

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 as follows.

If the pH has dropped by 0.5 or more pH units, the test will be re-run in an identical fashion. If the second test also results in a decrease in pH of greater than 0.5 s.u., the pH will be recorded, and the extract filtered for analysis. If the pH has increased by 0.5 or more units, the test must be repeated, but the extractor must be stopped at specific intervals and the pH manually adjusted down to pH 1.5 with dropwise addition of HCl (adjustments at 5, 10, 15, and 30 minutes into the extraction, and upon final removal from the water bath [60 minutes]). Samples with rising pH values must be run in a separate extraction, and must not be combined with samples being extracted by the standard method (continuous extraction).

Extracts are to be analyzed for lead and arsenic concentration using analytical procedures taken from the U.S. EPA publication, *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. SW-846.* (current revisions). Inductively coupled plasma (ICP) analysis, method 6010B (December 1996 revision) will be the method of choice. This method should be adequate for determination of lead concentrations in sample extracts, at a project-required detection limit (PRDL) of 100 $\mu$g/L. The PRDL of 20 $\mu$g/L for arsenic may be too low for ICP analysis for some samples. For extracts that have arsenic concentrations less than five times the PRDL (e.g., <100 $\mu$g/L arsenic), analysis by ICP-hydride generation (method 7061A, July 1992 revision) or ICP-MS (method 6020, September 1994 revision) will be required.

## 2.4 Calculation of the Bioaccessibility Value

A split of each solid material (<250 $\mu$m) that has been subjected to this extraction procedure should be analyzed for total lead and/or arsenic concentration using analytical procedures taken from the U.S. EPA publication, *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. SW-846.* (current revisions). The solid material should be acid digested according to method 3050A (July 1992 revision) or method 3051 (microwave-assisted digestion, September 1994 revision), and the digestate analyzed for lead and/or arsenic concentration by ICP analysis (method 6010B). For samples that have arsenic concentrations below ICP detection limits, analysis by ICP-hydride generation (method 7061A, July 1992 revision) or ICP-MS (method 6020, September 1994 revision) will be required.

The bioaccessibility of lead or arsenic is calculated in the following manner:

$$Bioaccessibility\ value = \frac{(concentration\ in\ in\ vitro\ extract,\ mg/L)\ (0.1L)}{(concentration\ in\ solid,\ mg/kg)\ (0.001\ kg)} \times 100$$

## 2.5 Chain-of-Custody/Good Laboratory Practices

All laboratories that use this SOP should receive test materials with chain-of-custody documentation. When materials are received, each laboratory will maintain and record custody of samples at all times. All laboratories that perform this procedure should follow good laboratory practices as defined in 40 CFR Part 792 to the extent practical and possible.

## 2.6 Data Handling and Verification

All sample and fluid preparation calculations and operations should be recorded in bound and numbered laboratory notebooks, and on extraction test checklist sheets. Each page must be dated and initialed by the person who performs any operations. Extraction and filtration times must be recorded, along with pH measurements, adjustments, and buffer preparation. Copies of the extraction test checklist sheets should accompany the data package.

# 3. Quality Control Procedures

## 3.1 Elements of Quality Assurance and Quality Control (QA/QC)

A standard method for the *in vitro* extraction of soils/solid materials, and the calculation of an associated bioaccessibility value, are specified above. Associated QC procedures to ensure production of high-quality data are as follows (see Table 1 for summary of QC procedures, frequency, and control limits):

- Reagent blank—Extraction fluid analyzed once per batch.
- Bottle blank—Extraction fluid only run through the complete extraction procedure at a frequency of no less than 1 per 20 samples or one per extraction batch, whichever is more frequent.
- Blank spikes—Extraction fluid spiked at 10 mg/L lead and/or 1 mg/L arsenic and run through the extraction procedure at a frequency of no less than every 20 samples or one per extraction batch, whichever is more frequent. Blank spikes should be prepared using traceable 1,000-mg/L lead and arsenic standards in 2 percent nitric acid.
- Duplicate—duplicate extractions are required at a frequency of 1 for every 10 samples. At least one duplicate must be performed on each day that extractions are conducted.
- Standard Reference Material (SRM)—National Institute of Standards and Technology (NIST) material 2711 (Montana Soil) should be used as a laboratory control sample (LCS).

Control limits for these QC samples are delineated in Table 1, and in the following discussion.

**Table 1. Summary of QC samples, frequency of analysis, and control limits**

| QC Sample | Minimum Frequency of Analysis | Control Limits |
|---|---|---|
| Reagent Blank | Once per batch (min. 5%) | <25 µg/L lead<br><5 µg/L arsenic |
| Bottle Blank | Once per batch (min. 5%) | <50 µg/L lead<br><10 µg/L arsenic |
| Blank Spike | Once per batch (min. 5%) | 85–115% recovery |
| Duplicate | 10% | ±20% RPD |
| SRM (NIST 2711) | 2% | 9.22 ±1.50 mg/L Pb<br>0.59 ±0.09 mg/L As |

## 3.2 QA/QC Procedures

Specific laboratory procedures and QC steps are described in the analytical methods cited in Section 2.3, and should be followed when using this SOP.

### 3.2.1 Laboratory Control Sample (LCS)

The NIST SRM 2711 should be used as a laboratory control sample for the *in vitro* extraction procedure. Analysis of 18 blind splits of NIST SRM 2711 (105 mg/kg arsenic and 1,162 mg/kg lead) in four independent laboratories resulted in arithmetic means ± standard deviations of 9.22 ±1.50 mg/L lead and 0.59 ±0.09 mg/L arsenic. This SRM is available from the National Institute of Standards and Technology, Standard Reference Materials Program, Room 204, Building 202, Gaithersburg, Maryland 20899 (301/975-6776).

### 3.2.2 Reagent Blanks/Bottle Blanks/Blank Spikes

Reagent blanks must not contain more than 5 µg/L arsenic or 25 µg/L lead. Bottle blanks must not contain arsenic and/or lead concentrations greater than 10 and 50 µg/L,

respectively. If either the reagent blank or a bottle blank exceeds these values, contamination of reagents, water, or equipment should be suspected. In this case, the laboratory must investigate possible sources of contamination and mitigate the problem before continuing with sample analysis. Blank spikes should be within 15% of their true value. If recovery of any blank spike is outside this range, possible errors in preparation, contamination, or instrument problems should be suspected. In the case of a blank spike outside specified limits, the problems must be investigated and corrected before continuing sample analysis.

# 4. References


Casteel, S.W., R.P. Cowart, C.P. Weis, G.M. Henningsen, E. Hoffman, et al. 1997a. Bioavailability of lead in soil from the Smuggler Mountain site of Aspen, Colorado. Fund. Appl. Toxicol. 36:177–187.

Casteel, S.W., L.D. Brown, M.E. Dunsmore, C.P. Weis, G.M.Henningsen, E. Hoffman, W.J. Brattin, and T.L. Hammon. 1997b. Relative bioavailability of arsenic in mining waste. U.S. Environmental Protection Agency, Region VIII, Denver, CO.

Imber, B.D. 1993. Development of a physiologically relevant extraction procedure. Prepared for BC Ministry of Environment, Lands and Parks, Environmental Protection Division, Victoria, BC. CB Research International Corporation, Sidney, BC.

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.

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

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 bioavailability using a physiologically based extraction test. Environ. Sci. Technol. 30(2):422–430.

Weis, C.P., and J.M. LaVelle. 1991. Characteristics to consider when choosing an animal model for the study of lead bioavailability. In: Proceedings of the International Symposium on the Bioavailability and Dietary Uptake of Lead. Sci. Technol. Let. 3:113–119.

Weis, C.P., R.H. Poppenga, B.J. Thacker, and G.M. Henningsen. 1994. Design of phamacokinetic and bioavailability studies of lead in an immature swine model. In: Lead in paint, soil, and dust: Health risks, exposure studies, control measures, measurement methods, and quality assurance, ASTM STP 1226, M.E. Beard and S.A. Iske (Eds.). American Society for Testing and Materials, Philadelphia, PA, 19103-1187.

# Attachment A:

# Extraction Test Checklist Sheets

**Extraction Fluid Preparation**

Date of Extraction Fluid Preparation:_____ Prepared by:_____

Extraction Fluid Lot #:_____

| Component | Lot Number | Fluid Preparation 1L | Fluid Preparation 2L | Acceptance Range | Actual Quantity | Comments |
|---|---|---|---|---|---|---|
| Deionized Water | | 0.95 L (approx.) | 1.9 L (approx.) | --- | | |
| Glycine | | 30.03±0.05 g | 60.06±0.05g | --- | | |
| HCl [a] | | 60 mL (approx.) | 120 mL (approx.) | --- | | |
| Final Volume | --- | 1 L (Class A, vol.) | 2 L (Class A, vol.) | --- | | |
| Extraction Fluid pH value (@ 37°C) | --- | 1.50±0.05 | 1.50±0.05 | 1.45–1.55 | | |

[a] Concentrated hydrochloric acid (12.1 N)

**INVITRO PROCEDURE REQUIRED PARAMETERS:**

Volume of extraction fluid (V) = 100 ±0.5 mL
Mass of test substrate (M) = 1.00 ±0.05 g
Temperature of water bath = 37 ±2 °C
Extraction time = 60 ±5 min

Extractor rotation speed = 30 ±2 rpm
Maximum elapsed time from extraction to filtration = 90 minutes
Maximum pH difference from start to finish (ΔpH)= 0.5 pH units
Spike solution concentrations: As = 1 mg/L; Pb = 10 mg/L

Date of Extraction:_____
Extraction Fluid Lot #:_____
Extracted by:_____

As Spike Solution Lot #:_____
Pb Spike Solution Lot #:_____

**EXTRACTION LOG (Page 1 of 2)**
**[Complete 1 log for every batch of 20 samples]**

| | Sample Preparation | | Extraction | | | | | | | | Filtration | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample ID | V (mL) | M (g) | Start Time[a] | End Time[a] | Elapsed Time (min) | Start pH | End pH | ΔpH | Start Temp (°C) | End Temp (°C) | Time[a] | Time Elasped from extraction (min) |
| Acceptance Range | (95.5–100.5) | (0.95–1.05) | --- | --- | (55–65 min) | --- | --- | (≤ 0.5) | (35–39) | (35–39) | | (Max = 90 min) |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |

**EXTRACTION LOG (Page 2 of 2)**
**[Complete 1 log for every batch of 20 samples]**

| | Sample Preparation | | Extraction | | | | | | | | Filtration | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample ID | V (mL) | M (g) | Start Time[a] | End Time[a] | Elapsed Time (min) | Start pH | End pH | ΔpH | Start Temp (°C) | End Temp (°C) | Time[a] | Time Elasped from extraction (min) |
| Acceptance Range | (95.5–100.5) | (0.95–1.05) | --- | --- | (55–65 min) | --- | --- | (≤ 0.5) | (35–39) | (35–39) | | (Max = 90 min) |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |

[a] 24-hour time scale

NOTES:

## Analytical Procedures

QC Requirements:

| QC Sample | Minimum Analysis Frequency | Control Limits | Corrective Action[a] |
|---|---|---|---|
| Reagent blank | once per batch (min. 5%) | < 25 µg/L Pb <5 µg/L As | Investigate possible sources of target analytes. Mitigate contamination problem before continuing analysis. |
| Bottle blank | once per batch (min. 5%) | < 50 µg/L Pb <10 µg/L As | Investigate possible sources of target analytes. Mitigate contamination problem before continuing analysis. |
| Blank spike | once per batch (min. 5%) | 85–115% | Re-extract and reanalyze sample batch |
| Duplicate | 10% (min. once/day) | ±20% RPD | Re-homongenize, re-extract and reanalyze |

RPD – Relative percent difference
a – Action required if control limits are not met