

**Standard Operating Procedure**  
**Modified Relative Bioaccessibility Leaching Procedure (RBALP)/EPA 9200/Solubility**  
**Bioaccessibility Research Consortium Assay (SBRC) for Lead in Soil**  
**Soil Environmental Chemistry Program, The Ohio State University**  
**Version 3**

Project/Client

---

Sample Description

---

---

---

**Standard Operating Procedure**  
**Modified Relative Bioaccessibility Leaching Procedure (RBALP)/EPA 9200/Solubility**  
**Bioaccessibility Research Consortium Assay (SBRC) for Lead in Soil**  
Soil Environmental Chemistry Program, The Ohio State University  
Version 3

## **1.0 Scope of Method**

1.1 This method is typically applicable for the characterization of lead bioaccessibility in soil. The assay may be varied or changed as required and dependent upon site conditions, equipment limitations, or limitations imposed by the procedure. Users are cautioned that deviations in the method from the assay described herein may impact the results (and the validity of the method). The *in vitro* bioaccessibility assay described in this method provides a rapid and relatively inexpensive alternative to *in vivo* assays for predicting relative bioaccessibility of lead in soils and soil-like materials. The method is based on the concept that lead solubilization in gastrointestinal fluid is likely to be an important determinant of lead bioavailability *in vivo*. The method measures the extent of lead solubilization in an extraction solvent that resembles gastric fluid. The fraction of lead which solubilizes in an *in vitro* system is referred to as *in vitro* bioaccessibility (IVBA), which may then be used as an indicator of *in vivo* RBA. Measurements of IVBA using this assay have been shown to be a reliable predictor of *in vivo* RBA of lead in a wide range of soil types and lead phases from a variety of different sites (U.S. EPA, 2007b).

At present, it appears that the relationship between IVBA and RBA is widely applicable, having been found to hold true for a wide range of different soil types and lead phases from a variety of different sites. However, the majority of the samples tested have been collected from mining and milling sites, and it is plausible that some forms of lead that do not occur at this type of site might not follow the observed correlation. Thus, whenever a sample containing an unusual and/or untested lead phase is evaluated by the IVBA protocol, this sample should be identified as a potential source of uncertainty. In the future, as additional samples with a variety of new and different lead forms are tested by both *in vivo* and *in vitro* methods, the applicability of the method will be more clearly defined. In addition, excess phosphate in the sample medium may result in interference (i.e., the assay is not suited to phosphate-amended soils).

## **2.0 Definitions**

2.1 Control Soil (CS): The laboratory control used for the RBALP is a certified reference material (NIST SRM 2711 or 2710) that goes through the same extraction/preparation procedure as the samples. The analyte composition of the laboratory control sample is certified by acid dissolution method 3051a. This SRM should be included in each batch processed.

2.2 Laboratory Control Sample (LCS): A sample which contains only extraction fluid is spiked prior to incubation and run through the complete procedure in order to

**Standard Operating Procedure**  
**Modified Relative Bioaccessibility Leaching Procedure (RBALP)/EPA 9200/Solubility**  
**Bioaccessibility Research Consortium Assay (SBRC) for Lead in Soil**  
**Soil Environmental Chemistry Program, The Ohio State University**  
**Version 3**

provide information about the effect of the extraction fluid on bioaccessibility and/or measurement methodology.

2.3 Matrix Spike: A duplicate sample is spiked prior to extraction and run through the complete procedure in order to provide information about the effect of the sample matrix on bioaccessibility and/or measurement methodology.

2.4 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.

2.4 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.

2.6 ICP-AES: Inductively Coupled Plasma-Atomic Emission Spectrometry.

### **3.0 Equipment and Supplies**

3.1 VWR Model 1545 Oven

3.2 Glas-Col Rotator Cat. No. 099A RD50

3.3 Trace metal grade hydrochloric acid.

3.4 Glycine salt

3.5  $\geq 18$  M $\Omega$  deionized water (DI).

3.6 175mL high-density polyethylene (HDPE) bottles

3.7 15ml Falcon tubes

3.8 12 ml syringes

3.9 Fisher brand 0.45 $\mu$ m nylon syringe filters

3.10 Spex Certiprep 1000mg/L ICP standard

### **4.0 Procedure**

Review SOP for handling acids prior to beginning the procedure.

**Standard Operating Procedure**  
**Modified Relative Bioaccessibility Leaching Procedure (RBALP)/EPA 9200/Solubility**  
**Bioaccessibility Research Consortium Assay (SBRC) for Lead in Soil**  
**Soil Environmental Chemistry Program, The Ohio State University**  
**Version 3**

- 4.1 Weigh 1.0 g from each sample to the nearest 0.01 g into a labeled 175 mL acid washed HDPE bottle and record sample mass on analysis sheet.
- 4.2 Prepare 0.4M glycine extraction solution at 37°C, adjusting pH to 1.50 +/- 0.5 with trace metal HCl. For a 2L solution, add 60.06g of glycine to a 2L volumetric and fill halfway with lab grade deionized water. To adjust pH to 1.5, start by adding 55 mL of concentrated HCl. Continue to add 1 mL increments of concentrated HCl until the desired pH is met. Before preparing solution, calibrate the pH meter with buffers (2.0, 4.0, and 7.0) that have been heated to 37°C.
  - 4.2.1 Extraction solution can also be prepared at pH 2.5 for project specific objectives. Note that CS reference values have not been established for pH 2.5.
- 4.3 Add 100 ± 0.5mL extraction solution with a bottle pipette checked for accuracy (Appendix) to each bottle.
- 4.4 Add 1mL of 1000 mg/L Pb standard to the blank spike sample and to the matrix spike sample.
  - 4.4a Make a Reagent blank spike with 1mL of 1000 mg/L Pb.
  - 4.4b Add to the Matrix Spike 1mL of 1000 mg/L Pb.
  - 4.4c Check pipette accuracy and record results in appendix prior to spiking the sample.
  - 4.4d When using 1000 mg/L standard, pour a small amount into a dixie cup and pipette from the dixie cup. DO NOT return the unused standard to the Certiprep container. Dispose of the unused standard in one of the inorganic waste tubs in the lab.
- 4.5 Cap the bottle. Properly place the bottles in the rotator and begin rotation. The rotator should be maintained at 30 ± 2 rpm for one hour. If the total time elapsed for the extraction process exceeds 90 minutes (from the time the extraction fluid is added to the final aliquot removal), the test must be repeated
- 4.6 After the one hour rotation remove a 10mL aliquot of suspension. Syringe filter samples into labeled falcon tubes using dry acid washed syringes and 0.45um nylon syringe filters.
- 4.7 Measure the pH of the remaining fluid in the extraction bottle and record in analysis sheet. If the fluid pH was not within pH 1.5±0.5, the extraction should be repeated with manual adjustment during the extraction.

**Standard Operating Procedure**  
**Modified Relative Bioaccessibility Leaching Procedure (RBALP)/EPA 9200/Solubility**  
**Bioaccessibility Research Consortium Assay (SBRC) for Lead in Soil**  
**Soil Environmental Chemistry Program, The Ohio State University**  
**Version 3**

- 4.8 To manually adjust the extraction stop the rotator at 5, 10, 15 and 30 minutes into the extraction and adjust the suspension pH to pH  $1.5 \pm 0.5$  with trace metal grade hydrochloric acid. Discontinue the manual adjustment when the suspension pH remains consistent between adjustment time points.
- 4.9 Filtered extracts should be stored in the refrigerator at 4°C for preservation until analysis (within one week of extraction). The samples should be analyzed for lead by ICP-AES or ICP-MS (U.S. EPA Method 6010 or 6020, U.S. EPA, 1986).

**5.0 Quality Control**

5.1 Control Soil (CS): The laboratory control sample must fall within  $\pm 10\%$  of the known value or within the %. The laboratory control sample must be run with each batch of extractions.

NIST SRM 2710a: Analysis of the NIST SRM 2710a standard should yield a mean IVBA result of 67.5% (acceptable IVBA range 60.7-74.2%). For the lead concentration (Pb soil) in the SRM, the median lead concentration presented in the Addendum to the NIST certificate for leachable concentrations determined using Method 3050 (5,100 mg/kg) should be used

NIST SRM 2711a: The NIST SRM 2711a should yield a mean IVBA result of 85.7% (acceptable IVBA range 75.2-96.2%). For the lead concentration (Pb soil) in the SRM, the median lead concentration presented in the Addendum to the NIST certificate for leachable concentrations determined using Method 3050 (1,300 mg/kg) should be used.

5.2 Sample Duplicates: The relative percent difference (RPD) must be no more than  $\pm 20\%$ . One sample duplicate must be run with every extraction batch.

$$RPD = 100 \times \frac{(S - D)}{\text{Avg. (S,D)}}$$

5.3 Laboratory Control Sample (LCS): Spike recoveries must fall within the limits of 85-115%. At least one spike analyses (matrix spikes) shall be performed on each batch of extractions. Blank spikes are to be done at the following levels for elements of interest.

Final Spike concentration	mg/L spike solution	mL spike prior to digest
Pb – 10 mg/L	1000	1

5.4 Matrix Spike: Spike recoveries must fall within the limits of 75-125%. At least one spike analyses (matrix spikes) shall be performed on each batch of extractions. Matrix spikes are to be done at the following levels for elements of interest.

**Standard Operating Procedure**  
**Modified Relative Bioaccessibility Leaching Procedure (RBALP)/EPA 9200/Solubility**  
**Bioaccessibility Research Consortium Assay (SBRC) for Lead in Soil**  
**Soil Environmental Chemistry Program, The Ohio State University**  
**Version 3**

Final Spike concentration	mg/L spike solution	mL spike prior to digest
Pb – 10 mg/L	1000	1

5.5 Preparation Blank: If any analyte concentration is above the method detection limit in the preparation blank, the lowest concentration of the analyte reported in associated samples must be  $\geq 10$  times the preparation blank concentration. A preparation blank must be performed with each for each new preparation of extraction solution.

## 7.0 Reporting

7.1 If any of the QC actions fail, the data shall be flagged indicating which QC check failed and determination will be made by the Laboratory Manager if corrective actions should be taken.

## 8.0 References

- 8.1 United States Environmental Protection Agency. Standard Operating Procedure for an *In Vitro* Bioaccessibility Assay for Lead in Soil. In EPA 9200. 1-86; U.S. EPA: Washington, DC, 2008.
- 8.2 United States Environmental Protection Agency. Method 6010C. Inductively Coupled Plasma-Atomic Emission Spectrometry. In SW-846; U.S. EPA: Washington, DC, 2007.
- 8.3 United States Environmental Protection Agency. Method 6020A. Inductively Coupled Plasma-Atomic Mass Spectrometry. In SW-846; U.S. EPA: Washington, DC, 2007.
- 8.4 Drexler, J.W. and Brattin, W. J. *An In Vitro Procedure for Estimation of Lead Relative Bioavailability: With Validation*. Human and Ecological Risk Assessment (2007, 13, 383-401.

**Standard Operating Procedure**  
**Modified Relative Bioaccessibility Leaching Procedure (RBALP) for Lead in Soil**  
**Soil Environmental Chemistry Program, The Ohio State University**

**Appendix**

Pipette Calibration  
Verification

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

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

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

**Standard Operating Procedure**  
**Modified Relative Bioaccessibility Leaching Procedure (RBALP) for Lead in Soil**  
**Soil Environmental Chemistry Program, The Ohio State University**

**Appendix**

---