

IN VITRO BIOACCESSIBILITY ASSAY FOR LEAD IN SOIL

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts formally trained in the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique, which a laboratory can use as a basic starting point for generating its own detailed standard operating procedure (SOP), either for its own general use or for a specific project application. Performance data included in this method are for guidance purposes only and must not be used as absolute quality control (QC) acceptance criteria for the purposes of laboratory QC or accreditation.

1.0 SCOPE AND APPLICATION

1.1 The purpose of this method is to define the proper analytical procedure for the validated *in vitro* bioaccessibility (IVBA) assay for lead in soil, to describe the typical working range and limits of the assay, quality assurance (QA), and to indicate potential interferences. At this time, this method has only been validated for lead-contaminated soil under field conditions and not for other matrices (e.g., water, air, amended soils, dust, food, etc.).

1.2 This method is typically applicable for the characterization of lead bioaccessibility in lead-contaminated soil under field conditions. Users are cautioned that deviations in the method from the assay as described may impact the results and the validity of the method. Users are strongly encouraged to document any deviations, as well as any comparisons with other methods and associated QA in any report.

1.3 It is not recommended to analyze IVBA for soils exceeding a total lead concentration of 50,000 mg/kg in order to avoid saturation of the extraction fluid and because risk management decisions are not likely to be improved by analyzing IVBA for soil with concentrations of lead above this level.

1.4 Knowledge of lead bioavailability is important because the amount of lead that actually enters the blood and body tissues from an ingested medium depends on the physical-chemical properties of the lead and of the medium. For example, lead in soil may exist, at least in part, as poorly water-soluble minerals, and may also exist inside particles of inert matrices such as rock or slag of variable size, shape, and association. These chemical and physical properties may tend to influence (usually decrease) the absorption (bioavailability) of lead when ingested. Thus, equal ingested doses of different forms of lead in different media may not be of equal health concern. For more information, see Reference 13.

1.5 Prior to employing this method, analysts are advised to consult the base method for each type of procedure that may be employed in the overall analysis (e.g., Methods 9040 and 9045 for pH and Methods 6010, 6020, and 6800 for determinative methods for the target analytes) for additional information on QC procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts should also consult the disclaimer statement at the front of the manual and the information in Chapter Two for: 1) guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies; and

2) the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by the Environmental Protection Agency (EPA) as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives (DQOs) for the intended application.

1.6 This method is restricted to use by, or under supervision of, properly experienced and trained personnel. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

After drying and sieving, 1 g of soil sample is rotated with 100 mL of buffered extraction fluid at 37 °C for one hour. The supernatant is separated from the sample by filtration and analyzed for lead by an appropriate analytical method (e.g., Method 6010 and Method 6020).

3.0 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. For this method, RBA is defined as the ratio of bioavailability of lead in soil to lead in water.

3.4 Bioaccessibility – An *in vitro* measure of the physiological solubility of the metal that may be available for absorption into the body.

3.5 Batch – A group of analytical and control/QC samples that are extracted simultaneously and is limited to 20 environmental samples in addition to the batch QC samples.

3.6 Phosphate-amended soil – phosphate rich materials (e.g., fertilizers) applied to lead-contaminated soils

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

3.8 *In vivo* – in the living body of an animal

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

3.10 Refer to Chapter One, Chapter Three, and the manufacturer's instructions for definitions that may be relevant to this procedure.

4.0 INTERFERENCES

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences during sample analysis. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents may be necessary. Refer to each method to be used for specific guidance on QC procedures and to Chapters Three and Four for general guidance on glassware cleaning. Also refer to Methods 9040, 9045, 6010, 6020, 6800, and other determinative methods to be used for information regarding potential interferences.

4.2 At present, it appears that the predictive 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 these types of sites 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 limits on 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).

5.0 SAFETY

This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

6.0 EQUIPMENT AND SUPPLIES

The mention of trade names or commercial products in this manual is for illustrative purposes only, and does not constitute an EPA endorsement or exclusive recommendation for use. The products and instrument settings cited in SW-846 methods represent those products and settings used during the method development or subsequently evaluated by the Agency. Glassware, reagents, supplies, equipment, and settings other than those listed in this manual may be employed provided that method performance appropriate for the intended application has been demonstrated and documented.

This section does not list common laboratory glassware (e.g., beakers and flasks) that might be used.

This method recommends the use of a water bath (Section 6.1) or an incubated air chamber (Section 6.2).

6.1 Water Bath

If the water bath option is used, the specific extraction device is an electric motor (the same motor as is used in the toxicity characteristic leaching procedure (TCLP, Method 1311))

driven flywheel, which drives a rotating block situated inside a temperature-controlled water bath (See Figure 1). The extraction device must be capable of holding a capped 125-mL wide-mouth high density polyethylene (HDPE) bottle. The water bath should be filled such that the extraction bottles are completely immersed. Temperature in the water bath should be maintained at 37 ± 2 °C using an immersion circulator heater, and the water bath temperature should be monitored and recorded. The electric motor must be capable of 30 ± 2 rpm.

6.2 Incubated Air Chamber

If the air incubator option is used, the specific extraction device will rotate the extraction bottles within an incubated air chamber. It must be capable of rotating at 30 ± 2 rotations per minute (rpm) and is designed to hold capped 125-mL wide-mouth HDPE bottles (see Figure 2 for an example of an extraction device in an incubated air chamber). The incubator must be capable of maintaining 37 ± 2 °C. The temperature inside of the incubator should be monitored and recorded. Reference 17 presents results of a study comparing the use of a water bath with the use of an incubated air chamber for performing this method.

6.3 HDPE bottles, 125 mL in size, equipped with airtight screw-cap seals should be used. Care should be taken to ensure that the bottles do not leak and to minimize contamination during the extraction procedure.

6.4 Automated temperature compensation (ATC) pH electrode – used for measuring the pH of the extraction fluid both prior to and after the experiment

7.0 REAGENTS AND STANDARDS

7.1 Reagent grade chemicals, at a minimum, should be used in all tests. Unless otherwise indicated, all reagents should conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society (ACS), where such specifications are available. Other grades may be used, provided the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 Reagent water must be interference free. All references to water in this method refer to reagent water, unless otherwise specified.

7.3 Cleanliness of all materials used to prepare and/or store the extraction fluid and buffer is essential. All glassware and equipment used to prepare standards and reagents shall be properly cleaned, acid washed, and triple-rinsed with deionized water prior to use.

7.4 Extraction fluid – 0.4 M glycine (free base, reagent-grade glycine in deionized water), adjusted to a pH of 1.50 ± 0.05 at 37 °C using trace metal-grade concentrated hydrochloric acid (HCl).

7.4.1 Prepare 2 liters (L) of extraction fluid in a volumetric flask (Class A) using American Society for Testing and Materials (ASTM) Type II deionized (DI) water. Add 60.06 grams of glycine (free base) to a flask containing 1.9 L of deionized water. Solution can be transferred to a wide-mouth HDPE bottle for ease of handling. Place the HDPE bottle containing the extraction fluid in a water bath at 37 °C and heat until the extraction fluid reaches 37 °C. Standardize the pH meter using an ATC pH electrode at 37 °C or pH buffers maintained at 37 °C in the water bath. Add trace metal-grade concentrated HCl (12.1 N) until the solution pH reaches 1.50 ± 0.05 . Bring the solution to a final volume of 2 L (0.4 M glycine).

7.4.2 If the extraction fluid is prepared in advance of the extraction, the extraction fluid must be heated to 37 °C and the pH shall be adjusted to 1.5 using trace metal grade concentrated HCl prior to conducting the extraction batch.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Sample collection, preservation and storage requirements may vary by EPA program and may be specified in a regulation or project planning document that requires compliance monitoring for a given contaminant. Where such requirements are specified in the regulation, those requirements must be followed. In the absence of specific regulatory requirements, see Chapters Three and Four in SW-846 as guidance in determining the sample collection, preservation and storage requirements.

Once the samples are prepared as described in Section 11.1, no preservatives or special storage conditions are required.

9.0 QUALITY CONTROL

9.1 Refer to Chapter One for guidance on QA and QC protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific criteria and Chapter One criteria; technique-specific QC criteria take precedence over Chapter One criteria. Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a quality assurance project plan (QAPP) or a sampling and analysis plan (SAP), which translates project objectives and specifications into directions for those who will implement the project and assess the results.

Each laboratory should maintain a formal QA program. The laboratory should also maintain records to document the quality of the data generated. Development of in-house QC limits for each method is encouraged. Use of instrument-specific QC limits is encouraged, provided such limits will generate data appropriate for use in the intended application. All data sheets and QC data should be maintained for reference or inspection. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulatory community in making judgments necessary to generate results that meet the DQOs for the intended application.

9.2 Initial demonstration of proficiency (IDP)

Each laboratory must demonstrate initial proficiency by generating data of acceptable precision and bias for target analytes in a clean matrix. It is recommended that the laboratory repeat the demonstration of proficiency whenever new staff members are trained or significant changes in instrumentation and/or procedures are made.

9.3 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.

9.4 Method blank – Extraction fluid only (i.e., no test soil) is carried through all steps of the method at a frequency of 1 in 20 samples (minimum 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.

9.5 Laboratory Control Sample (LCS) – A LCS consisting of a spiked blank may be run once per batch (minimum 1 in 20 samples). 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.

9.6 Matrix Spike (MS) – A MS should be run once per batch (minimum 1 in 20 samples). 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.

9.7 Duplicate sample – A duplicate sample should be run once per batch (minimum 1 in 20 samples) 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.

9.8 Control soil – Any one of the following National Institute of Standards and Testing (NIST) standard reference materials (SRMs) may be used as a control soil: 2710a or 2711a (Montana soil). The reference material shall be carried through all steps of the method and analyzed at a frequency of 1 in 20 samples (minimum of 1 per batch). The IVBA is calculated using the equation in Sec. 12.3.1.

9.8.1 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.

9.8.2 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.

9.8.3 NIST SRMs 2710a and 2711a are primary standard reference materials and are an integral part of the method quality control protocol. If NIST SRMs 2710a and 2711a are not available for purchase through the NIST website, check the following EPA website at: <http://www.epa.gov/superfund/bioavailability/trw.htm> or send an email to the EPA at bahelp@epa.gov to inquire about alternative SRMs.

9.9 Lower limit of quantitation check standard

9.9.1 The laboratory should establish the LLOQ as the lowest point of quantitation which, in most cases, is the lowest concentration in the calibration curve. 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 $\pm 35\%$ of the true value and the RSD should be $\leq 20\%$. In-house

limits may be calculated when sufficient data points exist. The monitoring of recovery data for the LLOQ check standard over time is useful for assessing precision and bias. Refer to a scientifically valid and published method (such as Chapter 9 of *Quality Assurance of Chemical Measurements* (Taylor 1987)) or the Report of the Federal Advisory Committee on Detection and Quantitation Approaches and Uses in Clean Water Act Programs (<http://water.epa.gov/scitech/methods/cwa/det/index.cfm>) for calculating precision and bias for LLOQ.

9.9.2 Ongoing LLOQ verification, at a minimum, is carried out on a quarterly basis to validate quantitation capability at low analyte concentration levels. This verification may be accomplished either with clean control material (e.g., reagent water, method blanks, Ottawa sand, diatomaceous earth, etc.) or a representative sample matrix (free of target compounds). Optimally, the LLOQ should be less than or equal to the desired regulatory action levels based on the stated project-specific requirements.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Prior to measurement of extraction fluid pH, the pH meter should be calibrated using a minimum of two points that bracket the expected pH (1.5) of the samples and are approximately two pH units or more apart. Repeat adjustments on successive portions of the two buffer solutions until readings are within 0.05 pH units of the buffer solution value as indicated in SW-846 method 9045D. The pH meter should be calibrated and checked with a standard that is of a different source from the buffers used to calibrate and within the calibration range (e.g., pH = 2) in accordance with the manufacturer's instructions.

10.2 Thermometers capable of measuring 37 ± 2 °C are needed.

10.3 The analytical balance should be calibrated daily in accordance with the manufacturer's instructions.

10.4 Pipettes should be calibrated in accordance with the manufacturer's instructions and the laboratory QA plan.

11.0 PROCEDURE

11.1 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. Samples should be thoroughly mixed, prior to use, to ensure homogenization. The mixing and aliquoting of samples using a riffle splitter is recommended. The use of clean HDPE storage bottles is recommended.

11.2 The extraction fluid for this procedure is 0.4 M glycine (free base, reagent-grade glycine in deionized water), adjusted to a pH of 1.50 ± 0.05 at 37 ± 2 °C using trace-metal grade concentrated hydrochloric acid (HCl). The extraction fluid should be pre-heated to 37 ± 2 °C. See Sec. 7.5 for extraction fluid preparation details.

11.3 Pre-heat the TCLP extractor water bath or incubator (See Sec. 6.0) to 37 °C. Record the temperature at the beginning and end of each extraction batch.

11.4 Soil samples should be thoroughly mixed immediately prior to subsampling for extraction to ensure homogenization (i.e., rotate sample bottles using X, Y, Z motion).

11.5 The extraction procedure begins by placing 1.00 ± 0.05 g of sieved test material (<250 μm) into a 125-mL wide-mouth HDPE bottle. Record the weight of the soil to the nearest 0.0001 g. Care should be taken to ensure that static electricity does not cause soil particles to adhere to the lip or outside threads of the bottle. If necessary, an antistatic brush should be used to eliminate static electricity prior to adding the test substrate.

11.6 Measure 100 ± 0.5 mL of the 37 ± 2 °C buffered extraction fluid (0.4 M glycine, pH 1.5), using a graduated cylinder or automated dispenser and transfer the extraction fluid to the 125-mL wide-mouth HDPE bottle.

11.7 The bottle should be tightly sealed and then shaken or inverted to ensure that there is no leakage and that no soil is caked on the bottom of the bottle.

NOTE: Care should be taken to prevent contamination of the samples during rotation (e.g., getting bath water in the threads around the cap and possibly into the sample when the cap is removed). Precautions that laboratories may consider include but are not limited to: the type of bottle that is used, sealing the samples in plastic freezer bags with air expelled before installing in the water bath extractor, and/or sealing the bottles with tape or Parafilm[®].

11.8 Fill the extractor (TCLP extractor or rotating extractor inside of a pre-heated incubator, see Sec. 6.0 for details) with 125-mL bottles containing test materials or QC samples (see Sec. 7.0). Record start time of rotation.

11.9 Samples are extracted by rotating the samples at 30 ± 2 rpm for one hour.

11.10 After one hour, the bottles should be removed from the rotator, dried, and placed upright on the bench top to allow the soil to settle to the bottom.

11.11 A 40-mL sample of supernatant fluid is then removed directly from the extraction bottle into a disposable syringe. After withdrawal of the sample into the syringe, a Luer lock attachment (equipped with a 0.45- μm 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 clean (e.g., acid-washed or pre-cleaned) polypropylene centrifuge tube or other appropriate sample vial for analysis.

11.12 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 90 minutes, the test must be repeated (i.e., Steps 11.1-11.11).

11.13 Measure and record the temperature and 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 slag soils), 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 0.5 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 >0.5 units, it is reasonable to conclude that the test material is buffering the solution. In these cases, the test should be repeated using

manual pH adjustment during the extraction process, stopping the extraction at 5, 10, 15, and 30 minutes and manually adjusting the pH down to pH 1.5 at each interval by drop-wise addition of trace-metal grade HCl.

11.14 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 lead by an appropriate method (see Sec. 2.0 for examples of appropriate methods).

NOTE: In some cases high dissolved solids in the extracts may cause nebulizer performance issues by inductively coupled plasma-optical emission spectrometry (ICP-OES) or inductively coupled plasma-mass spectrometry (ICP-MS). If this is encountered, dilution of the extracts tenfold is recommended before analysis. Correct for any dilutions in the calculations. Alternately, a high solids nebulizer may be useful. Graphite furnace atomic absorption spectrophotometry (GFAA) should be avoided due to the high levels of HCl in the extracts.

Note: In some cases, the amount of lead present in the sample will begin to saturate the extraction fluid, and the extraction response will cease to be linear. If the concentration of lead in the extract exceeds approximately 500 mg/L (depending on the sample matrix and mineralogy), this upper limit may have been reached. It is not recommended to analyze IVBA for soils exceeding a total lead concentration of 50,000 mg/kg in order to avoid saturation of the extraction fluid and because risk management decisions are not likely to be improved by analyzing IVBA for soil with concentrations of lead above this level. Reference 19 can be consulted for more information on how different liquid to solid ratios impact the bioaccessibility of metals in soils.

11.15 A check list of minimum data recording requirements is provided in Sec. 17.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 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 lead concentration using analytical procedures taken from SW-846 or a non-destructive method such as Instrumental Neutron Activation Analysis. If 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 lead concentration by an appropriate analytical method.

NOTE: Since this method may be applied to samples containing high amounts of lead, the analyst should read section 8.4 of Method 3050 in case linear range or digestion capacity are exceeded for high level samples.

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

12.3 *In vitro* bioaccessibility (IVBA)

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

Where: Pb_{ext} = *in vitro* extractable lead in the *in vitro* extract (mg/L)
 V_{ext} = extraction solution volume (L)
 Pb_{soil} = lead concentration in the soil sample being assayed (mg/kg)
 $Soil_{mass}$ = mass of soil sample being assayed (kg)

12.3.2 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). Due to the measurement error in RBA, as well as in IVBA, a linear regression calibration fit was used to minimize the error in both the RBA and IVBA approach. There was no significant difference in fit observed, so the results of the weighted linear regression were selected for simplicity. This decision may be revisited as more data become available. Based on the available data, the currently preferred calibration model is:

where RBA and IVBA are expressed as fractions, not as percentages. It is important to recognize that the use of this equation to calculate RBA from a given IVBA measurement will yield the "typical" RBA value expected for a test material with that IVBA, and the true RBA may be somewhat different (either higher or lower).

13.0 METHOD PERFORMANCE

13.1 Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance criteria for users of the methods. Instead, performance criteria should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method. Performance data must not be used as absolute QC acceptance criteria for purposes of laboratory QC or accreditation.

13.2 Refer to the appropriate determinative method for performance data examples and guidance.

13.3 Information on the recent round-robin study used to develop the new lead IVBA means (calculation for percent IVBA is located in Sec. 12.3) for NIST 2710a and 2711a are provided in Reference 9. This data is provided for guidance purposes only.

13.4 Reference 17 presents results of a study comparing the use of a water bath with the use of an incubated air chamber for performing this method. This data is provided for guidance purposes only.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the EPA recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, a free publication available from the ACS, Committee on Chemical Safety, http://portal.acs.org/portal/fileFetch/C/WPCP_012290/pdf/WPCP_012290.pdf.

15.0 WASTE MANAGEMENT

The EPA requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Laboratories are urged to protect air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* available from the American Chemical Society at the web address listed in Sec. 14.2.

16.0 REFERENCES

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17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The following pages contain the tables and figures referenced by this method.

TABLE 1
LABORATORY RESULTS AND THE PREDICTION AND
CONFIDENCE INTERVALS FOR NIST 2710A

NIST 2710a Analyte: Lead Units: mg/Kg							
Laboratory	A	B	C	D	E	F	G
Extraction Type	Water	Water	Water	Water	Water	Air	Air
Rep 1	3290	3520	3320	3567.5	3652.5	3372	3430
Rep 2	3270	3470	3300	3592.6	3623.4	3314	3370
Rep 3	3290	3483	3360	3495.6	3663.2	3321	3420
Rep 4	3300	3479	3330	3536.2	3632.6	3347	3430
Rep 5	3290	3538	3370	3617.0	3605.6	3348	3460
Average	3288.0	3498.0	3336.0	3561.8	3635.5	3340.4	3422.0
Std Dev	10.95	29.39	28.81	47.61	22.94	23.31	32.71
RSD	0.33	0.84	0.86	1.34	0.63	0.70	0.96

Pooled n=35	
Average	3440.23
Std Dev	124.58
RSD	3.62

Extracted Pb 99 - Percentile Prediction Interval (mg/Kg)		
99 low	Average	99 high
3095.56	3440.23	3784.91
10.02% = ± 99 prediction interval in percent		

Lead IVBA 99 - Percentile Prediction Interval		
99 low	Average	99 high
60.70	67.46	74.21
NIST 2710a Digestion EPA Method 3050 median result from the NIST certificate is 5100 mg/Kg		
IVBA = 67.46 or 67.5%	SD = 2.44	RSD = 3.62

Confidence Interval of the Mean		
3440.23 = Mean	21.05798 = SD of the Mean	0.61 = RSD of the Mean
99 low	Average	99 high
3382.79	3440.23	3497.68
1.67 % = ± 99 percentile of the confidence interval of the mean		

Std Dev = Standard Deviation
RSD = Relative Standard Deviation
CI = Confidence Interval

Source: Shaw 2011

TABLE 2
SRM 2710A BATCH QC SAMPLE RESULTS, LEAD

Laboratory	A	B	C	D	E	F	G	Mean
Extraction Type	Water	Water	Water	Water	Water	Air	Air	
Reagent Blank <25 ug/L	<30	<5	<40	<0.95	1.98	2.67	9.6	na
Bottle Blank ug/L <50 ug/L	<30	<5	<40	<0.95	1.86	NA	5.1	na
Blank Spike Percent Recovery (85-115%)	96.1	98.6	96.3	99.0	100.0	97.0	98.0	97.9
Control Soil SRM 2711 mg/Kg (nominal =928.4 mg/Kg)	865	953	910	977.8	1007.2	906.6	953	938.9
IVBA Control Soil SRM 2711 mg/Kg IVBA = 84.4 (%)	78.6%	86.6%	82.7%	88.9%	91.6%	82.4%	86.6%	85.4%
IVBA Control Soil SRM 2711 Percent Recovery (%)	93.2%	102.6%	98.0%	105.3%	108.5%	97.7%	102.6%	101.1%

na = not applicable

Source: Shaw 2011

TABLE 3
NIST 2710A ROUND ROBIN RESULTS ANALYSIS OF VARIANCE

ANOVA: Single Factor (Lead)
Note: alpha at 0.05 (95 percentile)

Groups	Count	Sum	Average	Variance
Laboratory A	5	16440	3288	120
Laboratory B	5	17490	3498	864
Laboratory C	5	16680	3336	830
Laboratory D	5	17809	3562	2266
Laboratory E	5	18177	3635	526
Laboratory F	5	16702	3340	543
Laboratory G	5	17110	3422	1070

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Inter-laboratory	502813.7789	6	83802.29648	94.31778649	2.93938E-17	2.445259395
Intra-laboratory	24878.28	2	888.51			
Total	527692.0589	34				

SS = Sum of Squares
df = Degrees of Freedom
MS = Mean Square
F = F Value Calculated
F Crit = Critical Value of F
P-value = Probability Value

Source: Shaw 2011

TABLE 4
NIST SRM 2710A RESULTS, AIR VERSUS WATER TEMPERATURE CONTROL MEDIUM, T-TEST

NIST 2710a Analyte: Lead Units: mg/Kg									
Extraction Type	WATER						AIR		
Laboratory	A	B	C	D	E		F	G	
Rep 1	3290	3520	3320	3567.5	3652.5		Rep 1	3372	3430
Rep 2	3270	3470	3300	3592.6	3623.4		Rep 2	3314	3370
Rep 3	3290	3483	3360	3495.6	3663.2		Rep 3	3321	3420
Rep 4	3300	3479	3330	3536.2	3632.6		Rep 4	3347	3430
Rep 5	3290	3538	3370	3617	3605.6		Rep 5	3348	3460
Average	3288.0	3498.0	3336.0	3561.8	3635.5		Average	3340.4	3422.0
Std Dev	10.95	29.39	28.81	47.61	22.94		Std Dev	23.31	32.71
RSD	0.33	0.84	0.86	1.34	0.63		RSD	0.70	0.96

	WATER	PERCENT DIFFERENCE	AIR
	n=25	2.41%	n=10
AVG	3463.9	AVG	3381.2
Std Dev	137.8	Std Dev	50.7
RSD	4.0	RSD	1.5

Excel t-Test: Two-Sample Assuming Equal Variances
alpha = 0.05

	WATER	AIR
Mean	3463.85	3381.2
Variance	18991.74177	2566.622222
Observations	25	10
Pooled Variance	14512.16371	
Hypothesized Mean Difference	0	
df	33	
t Stat	1.833590061	
P(T ≤ t) two-tail	0.075747815	
t Critical two-tail	2.034515287	

t-Stat = t-statistic

t crit = t critical value

P(T ≤ t) two tail = if the value is less than 0.05 indicates a 95% probability that the means of the two groups do not come from the same population

TABLE 5
LABORATORY RESULTS AND THE PREDICTION AND
CONFIDENCE INTERVALS FOR NIST 2711A

NIST SRM 2711a Analyte: Lead Units: mg/Kg							
Laboratory	A	B	C	D	E	F	G
Extraction Type	Water	Water	Water	Water	Water	Air	Air
Rep 1	1040	1145	1080	1138.3	1181.7	1099	1130
Rep 2	1030	1147	1100	1121.3	1194.2	1057	1130
Rep 3	1040	1122	1080	1155.1	1177.6	1089	1130
Rep 4	1030	1157	1080	1150.8	1182.2	1086	1120
Rep 5	1030	1165	1060	1151.1	1190.8	1082	1130
Average	1034.0	1147.2	1080.0	1143.3	1185.3	1082.6	1128.0
Std Dev	5.48	16.22	14.14	13.83	6.92	15.63	4.47
RSD	0.53	1.41	1.31	1.21	0.58	1.44	0.40

Pooled	n=35
Average	1114.4
Std Dev	49.4
RSD	4.4

Extracted Pb 99 – Percentile Prediction Interval (mg/Kg)		
99 low	Average	99 high
979.64	1114.35	1249.05
12.09 = ± 99 percentile prediction interval in percent		

IVBA 99-Percentile Prediction Interval		
99 low	Average	99 high
75.21	85.72	96.23
NIST 2711a Digestion EPA Method 3050 the median result from the NIST certificate of analysis is 1300 mg/Kg		
so IVBA =85.72 or 85.7% SD= 3.80 RSD = 4.43		

Confidence Interval of the Mean at 99 percentile		
1114.35 = Mean	8.346 = SD of the Mean	0.749 = RSD of the Mean
99 low	Average	99 high
1091.58	1114.35	1137.11
2.04% = ± 99 percentile confidence interval of the mean		

Std Dev = Standard Deviation
RSD = Relative Standard Deviation
CI = Confidence Interval

Source: Shaw 2011

TABLE 6
SRM 2711A BATCH QC SAMPLE RESULTS, LEAD

Laboratory	A	B	C	D	E	F	G	Mean
Extraction Type	Water	Water	Water	Water	Water	Air	Air	
Reagent Blank <25 ug/L	<30	<5	<40	<0.95	1.7	0.55	11.4	na
Bottle Blank ug/L <50 ug/L	<30	<5	<40	<0.95	1.42	nr	4.6	na
Blank Spike Percent Recovery (85-115%)	95.7%	96.6%	95.7%	95%	98.6%	98%	98%	96.8%
Control Soil SRM 2711 mg/Kg (nominal =928.4 mg/Kg)	861.1	967	900	958.8	1014	921.7	958	940.1
IVBA Control Soil SRM 2711 mg/Kg IVBA = 84.4 (%)	78.3%	87.9%	81.8%	87.2%	92.2%	83.8%	87.1%	85.5%
IVBA Control Soil SRM 2711 Percent Recovery (%)	92.8%	104.2%	96.9%	103.3%	109.2%	99.3%	103.2%	101.3%

nr = not reported na = not applicable

Source: Shaw 2011

TABLE 7
NIST 2711A ROUND ROBIN RESULTS ANALYSIS OF VARIANCE

Anova: Single Factor (Lead)
note alpha at 0.05 (95 percentile)

Groups	Count	Sum	Average	Variance
Laboratory A	5	5170	1034	30
Laboratory B	5	5736	1147	263
Laboratory C	5	5400	1080	200
Laboratory D	5	5716.6	1143	191
Laboratory E	5	5926.5	1185	47.8
Laboratory F	5	5413	1083	244
Laboratory G	5	5640	1128	20

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Inter-laboratory	78913.57886	6	13152.26314	92.37418704	3.87283E-17	2.445259395
Intra-laboratory	3986.648	28	142.3802857			
Total	82900.22686	34				

SS = Sum of Squares
df = Degrees of Freedom
MS = Mean Square
F = F Value Calculated
F Crit = Critical Value of F
P-value = Probability Value

Source: Shaw 2011

TABLE 8
NIST SRM 2711A RESULTS, AIR VERSUS WATER TEMPERATURE
CONTROL MEDIUM, T-TEST

NIST 2711a Analyte: Lead Units: mg/Kg								
Extraction Type>	WATER					AIR		
Laboratory>	A	B	C	D	E		F	G
Rep 1	1040	1145	1080	1138.3	1181.7		1099	1130
Rep 2	1030	1147	1100	1121.3	1194.2		1057	1130
Rep 3	1040	1122	1080	1155.1	1177.6		1089	1130
Rep 4	1030	1157	1080	1150.8	1182.2		1086	1120
Rep 5	1030	1165	1060	1151.1	1190.8		1082	1130
AVG	1034.0	1147.2	1080.0	1143.3	1185.3		1082.6	1128.0
Std Dev	5.48	16.22	14.14	13.83	6.92		15.63	4.47
RSD	0.53	1.41	1.31	1.21	0.58		1.44	0.40

	WATER	PERCENT DIFFERENCE		AIR
	n=25	1.14		n=10
AVG	1117.96		AVG	1105.30
Std Dev	56.10		Std Dev	26.27
RSD	5.02		RSD	2.38

Excel t-Test: Two-Sample Assuming Equal Variances
alpha = 0.05

	Water	Air
Mean	1117.964	1105.3
Variance	3147.690733	690.0111111
Observations	25	10
Pooled Variance	2477.414473	
Hypothesized Mean Difference	0	
df	33	
t Stat	0.679997865	
P(T ≤ t) two-tail	0.501248691	
t Critical two-tail	2.034515287	

t-Stat = t-statistic

t crit = t critical value

P(T ≤ t) two tail = if the value is less than 0.05 indicates a 95% probability that the means of the two groups do not come from the same population

Source: Shaw 2011

TABLE 9

ROUND ROBIN STUDY SRM IVBA RESULTS COMPARED TO PREVIOUS IVBA RESULTS

SRM	Mean IVBA	Standard Deviation	RSD	CV	N
2710 Previous Lot	75.5%	4.7	6.2	0.062	68
2711 Previous Lot	84.4%	4.7	5.5	0.055	66
2711 This Study	85.4%	4.3	5.0	0.050	14
2710a	67.5%	2.4	3.6	0.036	35
2711a	85.7%	3.8	4.4	0.044	35

Source: Shaw 2011

TABLE 10

NIST SRMS 2710A AND 2711A 99 PERCENTILE ROUNDED VALUES

SRM	Low 99	Average	High 99
SRM 2710a (mg/Kg)	3100	3440	3780
SRM 2710a IVBA	60.7	67.5	74.2
SRM 2711a (mg/Kg)	980	1110	1250
SRM 2711a IVBA	75.2	85.7	96.2

Source: Shaw 2011

FIGURE 1

EXAMPLE OF AN *IN VITRO* BIOACCESSIBILITY EXTRACTION APPARATUS WITH WATER BATH

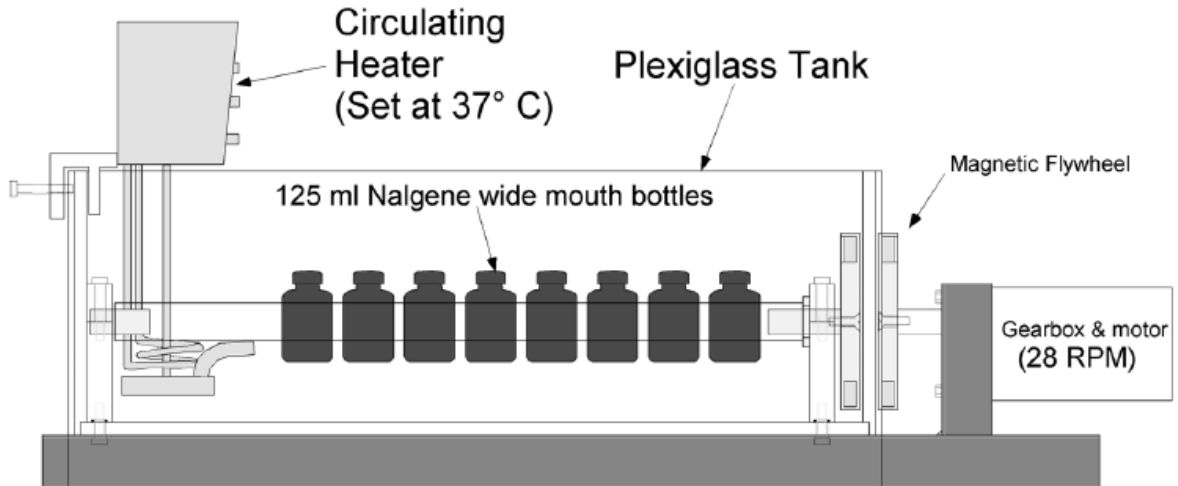


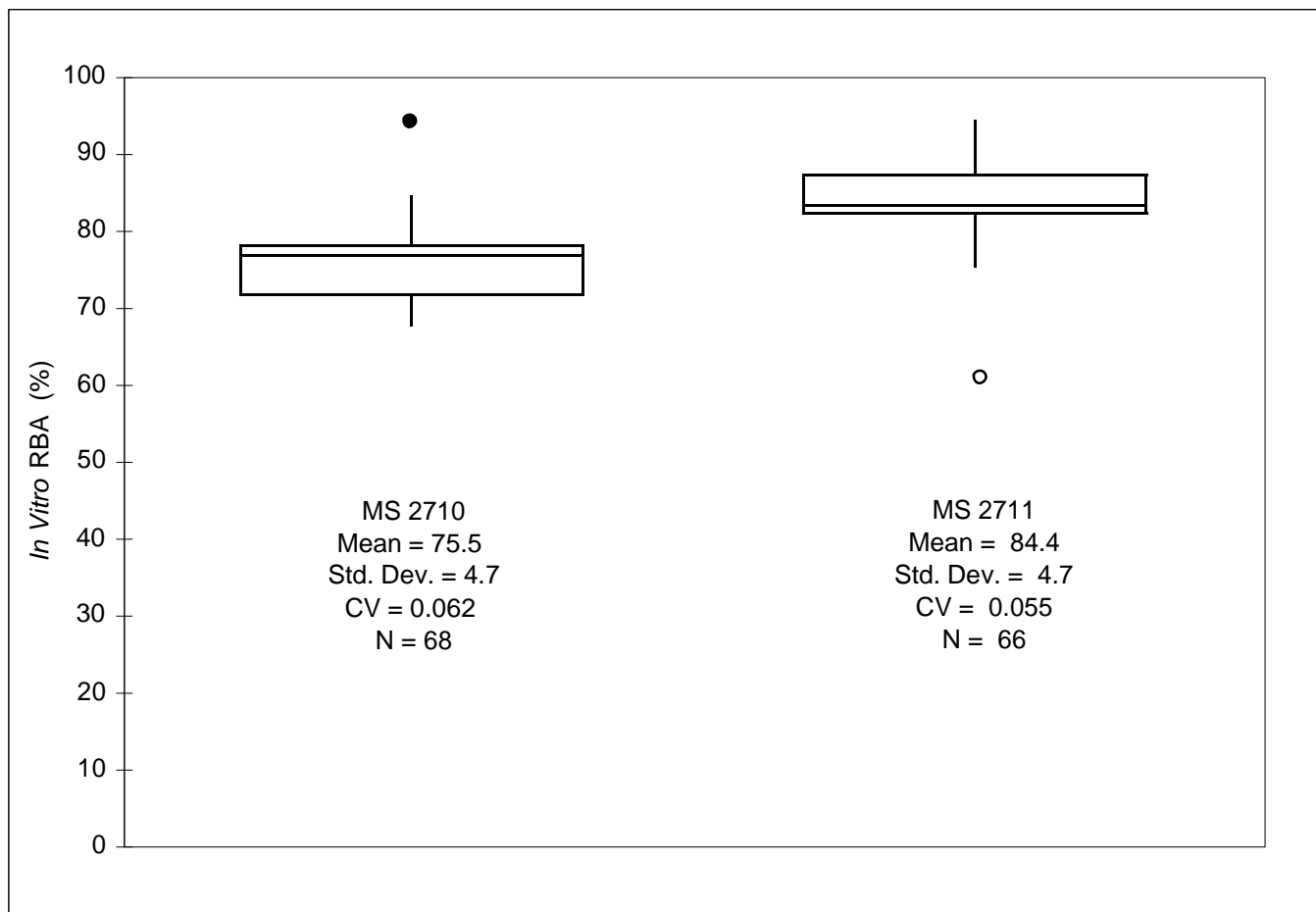
FIGURE 2

EXAMPLE OF AN *IN VITRO* BIOACCESSIBILITY EXTRACTION APPARATUS IN AN AIR INCUBATOR



FIGURE 3

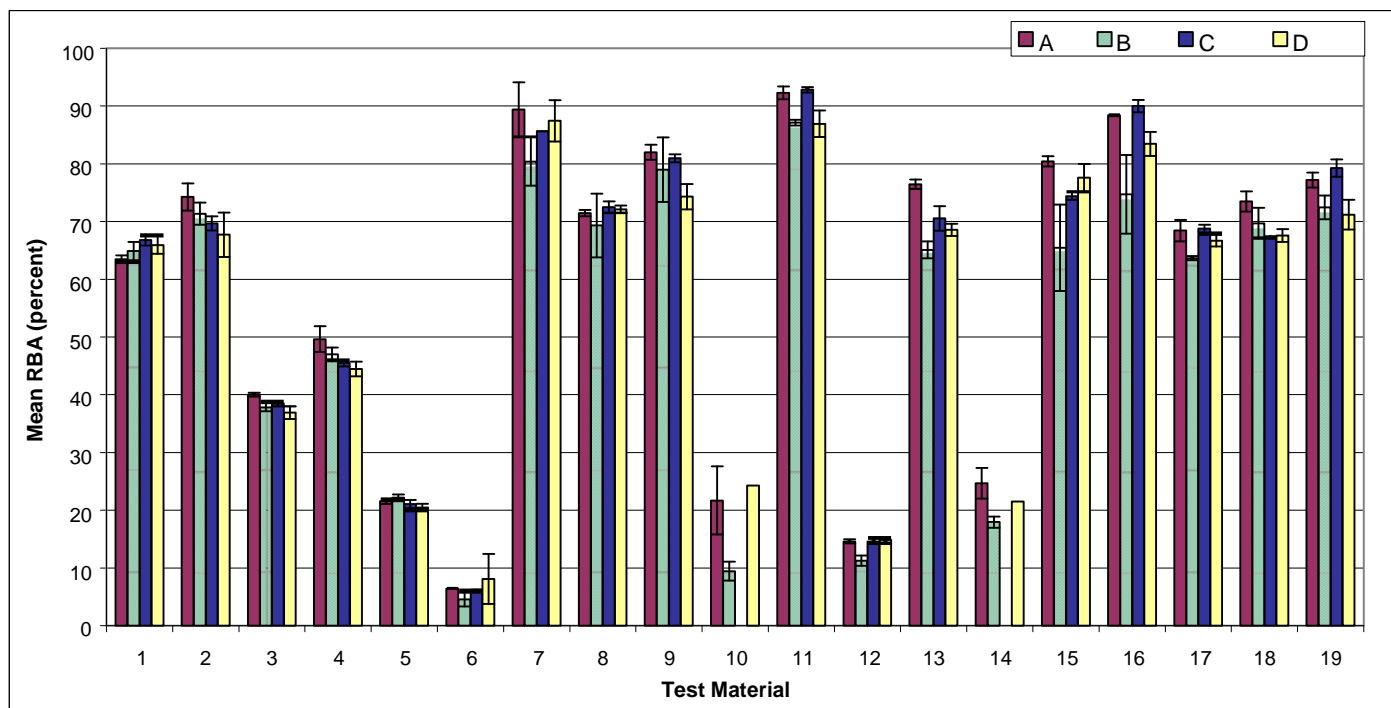
PRECISION OF *IN VITRO* BIOACCESSIBILITY MEASUREMENTS



Source: OSWER 2007b

FIGURE 4

REPRODUCIBILITY OF *IN VITRO* BIOACCESSIBILITY MEASUREMENTS

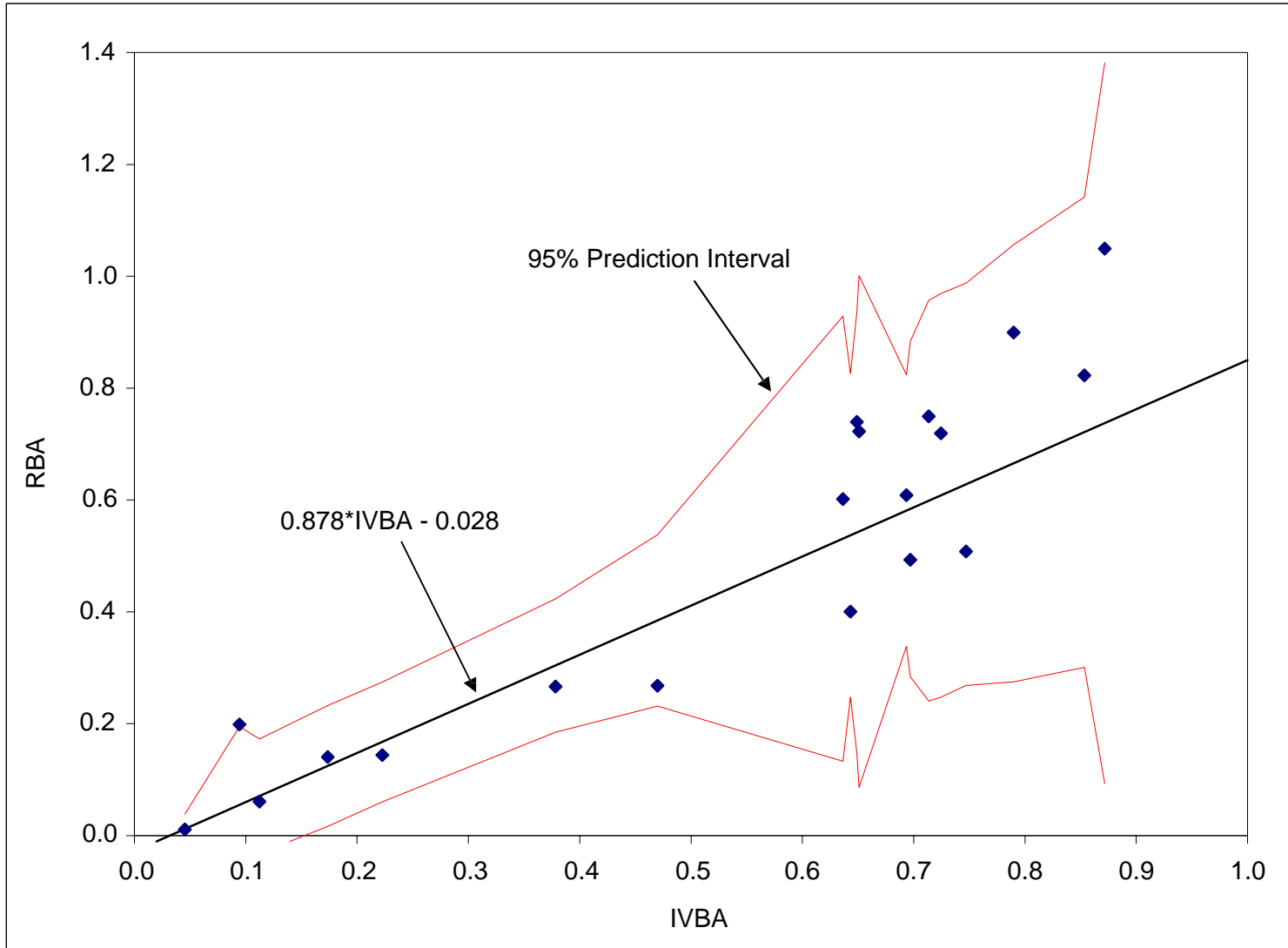


Test Materials

- | | | |
|----------------------------------|--|---------------------------|
| 1 = Aspen Berm | 8 = Jasper County High Lead Smelter | 14 = Midvale Slag |
| 2 = Aspen Residential | 9 = Jasper County Low Lead Yard | 15 = Murray Smelter Slag |
| 3 = Bingham Creek Channel Soil | 10 = California Gulch AV Slag | 16 = Murray Smelter Soil |
| 4 = Bingham Creek Residential | 11 = California Gulch Fe/Mn PbO | 17 = Palmerton Location 2 |
| 5 = Butte Soil | 12 = California Gulch Oregon Gulch Tailings | 18 = Palmerton Location 4 |
| 6 = Galena-enriched Soil | 13 = California Gulch Phase I Residential Soil | 19 = NIST Paint |
| 7 = Jasper County High Lead Mill | | |

Source: OSWER 2007b

FIGURE 5
PREDICTION INTERVAL FOR RBA BASED ON MEASURED IVBA



Source: OSWER 2007b

FIGURE 6 EXAMPLE EXTRACTION RECORD

Date: Sample ID:

BATCH No:

Extraction Fluid ID: Glycine & HCl, pH 1.5; SRM ID:

Spike solution concentration: 10mg/L Pb

Lead Spiking Solution Vendor, Lot No. (X mL of standard added to X mL extraction solutions (100mL total volume) labeled as "spikes")

Sample ID	Sample Preparation			Extraction					
	Bottle No.	Volume (mL)	Sample Mass (g)	Time (min)	Initial pH	Final pH	Start Temp (C)	End Temp (C)	Total Time (min)
Acceptance Range		100 ± 0.5	1.00 ± 0.01	60 ± 5	1.50 ± 0.05	1.50 ≥ 0.50	37 ± 2	37 ± 2	≤ 90
Bottle Blank	1		NA						
Blank Spike	2		NA						
NIST SRM ID	3								
Sample ID	4								
Sample ID	5								
Sample ID	6								
Sample ID	7								
Sample ID	8								
Sample ID	9								
Sample ID	10								
Sample ID	11								
Sample ID	12								
reagent blank	13		NA	NA	NA	NA	NA	NA	NA

reagent blank is not extracted through in vitro process

FIGURE 7

GASTRIC EXTRACTION FLUID PREPARATION

Sample Batch No: Date Prepared:					
Component	Lot ID	Fluid Preparation		Actual Quantity	Comments
		1 L	2 L		
Deionized Water	ASTM Type II	0.95 L (approx.)	1.90 L (approx.)		
Glycine	Sigma Lot No.	30.04±0.05g	60.08±0.05g		
HCL (12.1 N; Tr.metal)	Fisher Optima	(approx.)	(approx.)		
Final Volume	_____	1.0 L (class A)	2.0 L (class A)		
pH at 37C	_____	1.50±0.05	1.50±0.05		

FIGURE 8 EXAMPLE BATCH FORMAT AND IVBA CALCULATION

Date: Sample ID:

BATCH No:

Extraction Fluid ID: Glycine & HCl, pH 1.5; SRM ID:

Spike solution concentration: 10mg/L Pb

Lead Spiking Solution Vendor, Lot No. (X mL of standard added to X mL extraction solutions (100mL total volume) labeled as “spikes”)

Batch #	Bottle No.	Type	Sample ID	Soil weight grams	Soil weight kg	Volume (ml)	Volume (L)	ICP (Pb) mg/L	Soil [Pb] (mg/kg)	% IVBA	Avg % IVBA	S.D. of % IVBA
Insert No.	1	QC	Bottle blank	n/a	n/a	100	0.1		n/a	n/a		
	2	Blank spike	Blank + spike	n/a	n/a	100	0.1		n/a	n/a		
	3	Control soil	SRM 2710a	1.0019	0.00100	100	0.1	34.24	5100	67		
	4	Sample	Sample1 a	1.0016	0.00100	100	0.1	32.24	5100	63	64.1	1.4
	5	Sample	Sample1 b	1.0006	0.00100	100	0.1	33.24	5100	65		
	6	Matrix spike	Sample + spike	0.9985	0.00100	100	0.1					
	7	Sample	Sample2 a	1.0029	0.00100	100	0.1				Avg of dups	SD
	8	Sample	Sample2 b	1.0022	0.00100	100	0.1					
	9	Matrix spike	Sample + spike	1.0028	0.00100	100	0.1					
	10	Sample	Sample3 a	1.0004	0.00100	100	0.1				Avg of dups	SD
	11	Sample	Sample3 b	1.0029	0.00100	100	0.1					
	12	Matrix spike	Sample + spike	0.9972	0.00100	101	0.1		n/a	n/a		
	13	Reagent blank	unprocessed sample		n/a	n/a	100	0.1		n/a	n/a	

Example calculation:

$$\% \text{ IVBA} = \frac{(\text{Concentration in IVBA extract mg/L})(0.1 \text{ L})}{(\text{Concentration in solid mg/kg})(\text{weight of sample kg})} * 100$$

METHOD 1340 FLOWCHART

IN VITRO BIOACCESSIBILITY ASSAY FOR LEAD IN SOIL

