EPA 9200.2-86
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Standard Operating Procedure for an *In Vitro* Bioaccessibility Assay for Lead in Soil

1.0 SCOPE AND APPLICATION

1.1 The purpose of this standard operating procedure (SOP) is to define the proper analytical procedure for the validated *in vitro* bioaccessibility assay for lead in soil (U.S. EPA, 2007b), to describe the typical working range and limits of the assay, quality assurance, and to indicate potential interferences. At this time, the method described herein has only been validated for lead in soil (U.S. EPA, 2007b).

1.2 The SOP described herein 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). Users are strongly encouraged to document any deviations as well as any comparisons with other methods and associated Quality Assurance (QA) in any report.

1.3 This document is intended to be used as reference for developing site-specific Quality Assurance Project Plans (QAPPs) and Sampling and Analysis Plans (SAPs), but not intended to be used as a substitute for a site-specific QAPP or a detailed SAP or laboratory Standard Operating Procedure. 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 data quality objectives for the intended application.

1.4 Mention of trade names or commercial products does not constitute endorsement or recommended use by U.S. EPA.

2.0 SUMMARY OF METHOD

Reliable analysis of the potential hazard to children from ingestion of lead in the environment depends on accurate information on a number of key parameters, including (1) lead concentration in environmental media (soil, dust, water, food, air, paint, etc.), (2) childhood intake rates of each medium, and (3) the rate and extent of lead absorption from each medium ("bioavailability"). 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 matrix 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.

3.0 DEFINITIONS

The term *bioavailability* (BA) has many different meanings across various disciplines of toxicology and pharmacology. For the purposes of this SOP, the term bioavailability means:

> *The fraction of an ingested dose that crosses the gastrointestinal epithelium and becomes available for distribution to internal target tissues and organs.*

Bioavailability expressed as a fraction (or percentage) of a dose is commonly referred to as *absolute bioavailability*. The term *relative bioavailability* (RBA) refers to a comparison of absolute bioavailabilities. Relative bioavailability generally is important in risk assessment because we are often most interested in knowing the extent to which the absolute bioavailability of a metal increases or decreases in context with the exposure matrix (*e.g.*, food vs. water vs. soil), or with the physical or chemical form(s) of the metal to which humans are exposed. Often, it is more feasible to assess relative bioavailability than absolute bioavailability (an example of this for lead is demonstrated in U.S. EPA, 2007b). Thus, for the purposes of this guidance document, relative bioavailability means:

> *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*.

A related term, pertaining to bioavailability assessment, is *bioaccessibility*. For the purposes of this SOP, this refers to an *in vitro* measure of the *physiological solubility* of the metal that may be available for absorption into the body. Since solublization is usually required for absorption across membranes, poorly soluble forms of metals, with low bioaccessibility, may also have low bioavailability. In certain circumstances, if solubility is the major determinant of absorption at the portal of entry, bioaccessibility may be a predictor of bioavailability. Lead is an example of this, as is discussed in U.S. EPA (2007a).

$$In\ vitro\ bioaccessiblity = \frac{Pb_{ext} \cdot V_{ext} \cdot 100}{Pb_{soil} \cdot Soil_{mass}}$$

where:

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

The extraction solution volume in this SOP is 0.1 L. For additional definitions for bioavailability-related terms (e.g., Relative Bioavailability) refer to U.S. EPA (2007a). The *in vitro* bioaccessibility assay described in this SOP provides a rapid and relatively inexpensive alternative to *in vivo* assays for predicting RBA 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).

For the purposes of this document, the term batch refers to a group of analytical and control/QC samples that are extracted simultaneously.

4.0 INTERFERENCES AND POTENTIAL PROBLEMS

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). Interferences and potential problems are discussed under Procedures (Section 11.0).

5.0 SAFETY

When working with potentially hazardous materials, follow U.S. EPA, OSHA, or corporate health and safety procedures.

6.0 EQUIPMENT AND SUPPLIES

The equipment that may be used for this procedure is the 1) extraction device shown in Figure 1 OR 2) an end-over-end rotator placed inside of an incubator.

1) The device shown in Figure 1 is an electric motor (the same motor as is used in the Toxicity Characteristic Leaching Procedure or TCLP) that drives a flywheel, which in turn drives a Plexiglass block situated inside a temperature-controlled water bath. The Plexiglass block contains twelve 5-centimeter holes with stainless steel screw clamps, each of which is designed to hold 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.

2) An end-over-end rotator, capable of 30±2 rpm, should be designed to hold at least twelve capped 125-mL wide-mouth HDPE bottles (e.g., Glas-Col® from Terre Haute coupled with an Innova 4230 refrigerated incubator shaker from New Brunswick Scientific, or equivalent). The rotating device should be placed inside of an incubator capable of maintaining 37±2 °C; and the temperature inside of the incubator should be monitored and recorded.

The 125-mL HDPE bottles should have air-tight screw-cap seals, and care should be taken to ensure that the bottles do not leak during the extraction procedure. All equipment should be properly cleaned, acid washed, and rinsed with deionized (DI) water prior to use.

An automated temperature compensation (ATC) pH electrode shall be used for measuring the pH of the extraction fluid prior and post experiment. Additional equipment for this method includes typical laboratory supplies and reagents, as described in Section 7.0.

7.0 REAGENTS AND STANDARDS

All reagents shall be free of lead and the final extraction fluid shall be tested to confirm that lead concentrations are <¼ (<one-fourth) the project-required detection limit (PRDL) of 100 µg/L (i.e., less than 25 µg/L lead in the unprocessed reagent blank). 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. Weigh samples and glycine to the nearest 0.0001 gram using an analytical balance calibrated daily according to the manufacturer's instructions. Pipettes should be calibrated according to manufacturer's instructions.

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°C using trace metal grade concentrated hydrochloric acid (HCl). The extraction fluid is prepared as described below.

Prepare 2 liters (L) of extraction fluid in a volumetric flask (Class A) using ASTM Type II deionized (DI) water. To 1.9 L of DI water, add 60.06 grams glycine (free base, Sigma Ultra or equivalent). Place the flask 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 automated temperature compensation (ATC) pH electrode at 37 °C or pH buffers maintained at 37 °C in the water bath. Add trace metal-grade concentrated hydrochloric acid (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).

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

8.0 SAMPLE PREPARATION, PRESERVATION, AND STORAGE

All test soils should be prepared by drying (<40°C) and sieving to <250 µm. The <250 µm size fraction was used because this particle size is representative of that which adheres to children's hands (U.S. EPA, 2000). Stainless steel sieves are recommended. Samples should be thoroughly mixed prior to use to ensure homogenization. Mixing and aliquoting of samples using a riffle splitter is recommended. Clean HDPE storage bottles are recommended. All samples should be archived after analysis and retained for further analysis for a period of six (6) months. No preservatives or special storage conditions are required.

9.0 QUALITY CONTROL/QUALITY ASSURANCE

For the purposes of this document, the term batch refers to a group of analytical and control/QC samples that are extracted simultaneously.

Recommended quality assurance for the extraction procedure are as follows:

- Reagent Blank — unprocessed (not run through the extraction procedure) extraction fluid analyzed at a frequency of 1 in 20 samples (minimum of 1 per batch).

- Bottle Blank — extraction fluid only (no test soil) run through the complete procedure at a frequency of 1 in 20 samples (minimum of 1 per batch).

- Blank Spike — extraction fluid spiked at 10 mg/L lead, and run through the complete procedure at a frequency of 1 in 20 samples (minimum of 1 per batch).

- Matrix Spikes — subsample of each material used for duplicate analyses used as matrix spike. The matrix spike should be prepared at 10 mg/L lead and run through the extraction procedure at a frequency of 1 in 10 samples (minimum of 1 per batch).

- Duplicate Sample — a duplicate sample extraction performed on 1 in 10 samples (minimum of 1 per batch). The duplicate is treated exactly like a sample and its purpose is to determine laboratory precision.

- Control Soil — National Institute of Standards and Testing (NIST) Standard Reference Material (SRM) 2710 or 2710a or 2711 or 2711a (Montana Soil) used as a control soil. The SRM shall be analyzed at a frequency of 1 in 20 samples (minimum 1 per batch).

Recommended control limits for these quality control samples:

Analysis	Frequency	Control Limits	Corrective Action
Reagent blank	once per batch (minimum 1 in 20 samples)	<25 µg/L lead	Make new extraction fluid and rerun all analyses
Bottle blank	once per batch (minimum 1 in 20 samples)	<50 µg/L lead	Make new extraction fluid and rerun all analyses

Blank spike (10 mg/L)	once per batch (minimum 1 in 20 samples)	85-115% recovery	Ensure dilutions and spike concentrations are correct. If no error is found, re-extract the samples or flag the data.
Matrix spike (10 mg/L)	once per batch (minimum 1 in 10 samples)	75-125% recovery	Ensure dilutions and spike concentrations are correct. If no error is found, re-extract the samples or flag the data.
Duplicate sample	once per batch (minimum 1 in 10 samples)	±20% RPD	Re-extract the samples or flag the data.
Control soil (NIST 2710 or 2710a or 2711 or 2711a)	once per batch (minimum 1 in 20 samples)	NIST 2710a mean 67.5% (acceptable range 60.7-74.2%) NIST 2711a mean 85.7% (acceptable range 75.2-96.2%) (for NIST 2710 and 2711 values see section 13.0)	Re-extract the samples or flag the data.

RPD = Relative percent difference

10.0 pH METER CALIBRATION AND STANDARDIZATION

An automated temperature compensation (ATC) pH electrode shall be used for measuring the pH of the extraction fluid prior and post experiment. Each instrument/electrode system must be calibrated at 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 for Soil and Waste pH (http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/9045d.pdf). The pH meter should be calibrated and checked with another standard solution within the calibration range (e.g., pH = 2) according to the manufacturer's instructions. Thermometers capable of measuring 37 °C ± 2 are needed. After calibration, the meter is ready to analyze samples.

11.0 PROCEDURE

11.1 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). See Section 7.0 for extraction fluid preparation details.

11.2 Pre-heat the TCLP extractor water bath OR incubator (See Section 6.0) to 37°C. Record the temperature at the beginning and end of each extraction batch (an example of an extraction data recording form is provided in Attachment A).

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

11.4 The extraction procedure is begun by placing 1.00±0.05 g of sieved test material (<250 μm) into a 125mL wide-mouth HDPE bottle. Record weight of soil to 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.

11.5 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 extraction fluid to the 125-mL wide-mouth HDPE bottle.

11.6 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.

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

11.8 Samples are extracted by rotating the samples at 30±2 rpm for 1 hour.

11.9 After 1 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.

11.10 A 15-mL sample of supernatant fluid is removed directly from the extraction bottle into a disposable 20-cc syringe. After withdrawal of the sample into the syringe, a Luer-Lok attachment fitted with a 0.45-μm cellulose acetate disk filter (25 mm diameter) is attached, and the 15 mL aliquot of fluid is filtered through the attachment to remove any particulate matter into a pre-acid washed 15-mL polypropylene centrifuge tube or other appropriate sample vial for analysis.

11.11 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).

11.12 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.

> 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-analyzed. If the second test also resulted 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 HCl.

11.13 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. The samples should be analyzed for lead by ICP-AES or ICP-MS (U.S. EPA Method 6010C or Method 6020A). The method detection limit (MDL) in extraction fluid should be approximately 20 µg/L for Method 6010 and 0.1-0.3 µg/L for Method 6020 (U.S. EPA 2012a,b).

11.14. A check list of minimum data recording requirements is provided at the end of Appendix A.

11.15. Once received by the laboratory, all samples and extracts should be checked-in, verified, and maintained under standard chain-of-custody (e.g., U.S. EPA, 2012c).

12.0 DATA ANALYSIS AND CALCULATIONS

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 the U.S. EPA SW-846 (U.S. EPA 2012d) 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 SW-846 Method 3050B (December 1996 revision) or 3051A (microwave-assisted digestion, February 2007 revision), and the digestate analyzed for lead concentration determined by ICP-AES analysis (method 6010C, February 2007 revision) or ICP-MS (method 6020A, February 2007 revision).

12.1 *In vitro* bioaccessibility (IVBA) is calculated and expressed on a percentage basis using the following equation:

$$In\ vitro\ bioaccessiblity = \frac{Pb_{ext}\ \cdot V_{ext} \cdot 100}{Pb_{soil}\ \cdot Soil_{mass}}$$

where:

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

12.2 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. Because there is measurement error not only in RBA but also in IVBA, linear fitting was also performed taking the error in both RBA and IVBA into account. There was nearly no difference in fit, so the results of the weighted linear regression were selected for simplicity (U.S. EPA, 2007b). This decision may be revisited as more data become available. Based on this decision, the currently preferred model is:

RBA = 0.878•IVBA – 0.028

where RBA and IVBA are expressed as fractions (not percent). It is important to recognize that 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 NIST SRM (NIST SRM 2710, 2710a, 2711, or 2711a) should be used as a control soil. The SRM will be analyzed at a frequency of 1 in 20 samples (minimum 1 per batch). These SRMs are available from the National Institute of Standards and Technology, Standard Reference Materials Program (http://www.nist.gov/srm/). Information on the recent round study used to develop the following new lead IVBA means (calculation for percent IVBA located in section 12.1) for 2710a and 2711a is provided in Appendix A.

13.2 NIST SRM 2710: Analysis of The NIST SRM 2710 standard should yield an IVBA result of 75.5% (see Figure 3.3 of U.S. EPA, 2007b).

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%).

13.3 NIST SRM 2711: The NIST SRM 2711 standard should yield an IVBA result of 84.4% (see Figure 3.3 of U.S. EPA, 2007b).

NIST 2711a: The NIST SRM 2711a standard should yield a mean IVBA result of 85.7% (acceptable IVBA range 75.2-96.2%).

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity 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 Agency 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* available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street, NW, Washington, DC 20036, http://www.acs.org.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices are consistent with all applicable rules and regulations. The Agency urges laboratories to protect the 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's Department of Government Relations and Science Policy, 1155 16th Street, NW, Washington, DC 20036, (202) 872-4477.

16.0 REFERENCES

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Appendix A. Additional information on methods development for EPA Method 9200.2-86:

The dissolution of lead from a test material into the extraction fluid depends on a number of variables including extraction fluid composition, temperature, time, agitation, solid/fluid ratio, and pH. Any alterations in these parameters should be evaluated to determine the optimum values for maximizing sensitivity, stability, and the correlation between *in vitro* and *in vivo* values. Additional discussion of these procedures is available in U.S. EPA (2007b) and Drexler and Brattin (2007).

Most previous *in vitro* test systems have employed a more complex fluid intended to simulate gastric fluid. For example, Medlin (1997) used a fluid that contained pepsin and a mixture of citric, malic, lactic, acetic, and hydrochloric acids. When the bioaccessibility of a series of test substances were compared using 0.4 M glycine buffer (pH 1.5) with and without the inclusion of

these enzymes and metabolic acids, no significant difference was observed (p=0.196). This indicates that the simplified buffer employed in the procedure is appropriate, even though it lacks some constituents known to be present in gastric fluid.

Water vs. air extraction comparison

A statistical comparison (t-test) was made between the SRM data derived from IVBA extractions that were performed by laboratories employing air (incubator type) as the temperature controlling (37± 2ºC) medium, versus water (aquarium type). The comparison showed that, for this set of results, there was no statistical difference between the two (2) techniques of controlling the temperature of sample bottles during the extraction.

Additional testing to confirm these results was conducted by EPA's NERL and included four *in vitro* scenarios using SRM NIST 2710a (n = 27 for each scenario):

1. Water bath + preheated gastric solution
2. Water bath + room temperature gastric solution
3. Air incubator + preheated gastric solution
4. Air incubator + room temperature gastric solution

Results of the t-tests indicate that there was no statistically significant difference in observed mean Pb IVBA values for NIST 2710a SRM between scenarios 1 and 2; 1 and 3; and 2 and 3. The mean Pb IVBA value from scenario 4 (air temperature controlled, gastric solution not-preheated) was slightly lower. Therefore, the mean Pb IVBA value for scenario 4 was statistically different from the other three scenarios.

Extraction fluid

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°C using trace metal-grade concentrated hydrochloric acid (HCl)

Temperature

A temperature of 37°C is used because this is approximately the temperature of gastric fluid *in vivo*.

Extraction time

The time that ingested material is present in the stomach (i.e., stomach-emptying time) is about 1 hour for a child, particularly when a fasted state is assumed (see U.S. EPA 2007a, Appendix A). Thus, an extraction time of 1 hour should be used. It was found that allowing the bottles to stand at room temperature for up to 4 hours after rotation at 37°C caused no significant variation (<10%) in lead concentration.

pH

Human gastric pH values tend to range from about 1 to 4 during fasting (see U.S. EPA 2007b, Appendix A). Excess phosphate in the sample may result in interference with the IVBA assay and IVBA results for phosphate-treated soils have not been shown to correlate with extraction results from the juvenile swine in vivo assays.

Agitation

If the test material is allowed to accumulate at the bottom of the extraction apparatus, the effective surface area of contact between the extraction fluid and the test material may be reduced, and this may influence the extent of lead solubilization. Depending on which theory of dissolution is relevant (Nernst and Brunner, 1904, or Dankwerts, 1951), agitation will greatly affect either the diffusion layer thickness or the rate of production of fresh surface. Previous workers have noted problems associated with both stirring and argon bubbling methods (Medlin, 1987). Although no systematic comparison of agitation methods was performed, an end-over-end method of agitation is recommended.

Soil/Fluid Ratio and Mass of Test Material

A solid-to-fluid ratio of 1/100 (mass per unit volume) should be used to reduce the effects of metal dissolution when lower ratios (1/5 and 1/25) were used. Tests using Standard Reference Materials (SRM 2710) showed no significant variation (within ±1% of control means) in the fraction of lead extracted with soil masses as low as 0.2 gram (g) per 100 mL. However, use of low masses of test material could introduce variability due to small scale heterogeneity in the sample and/or to weighing errors. Therefore, the final method employs 1.0 g of test material in 100 mL of extraction fluid.

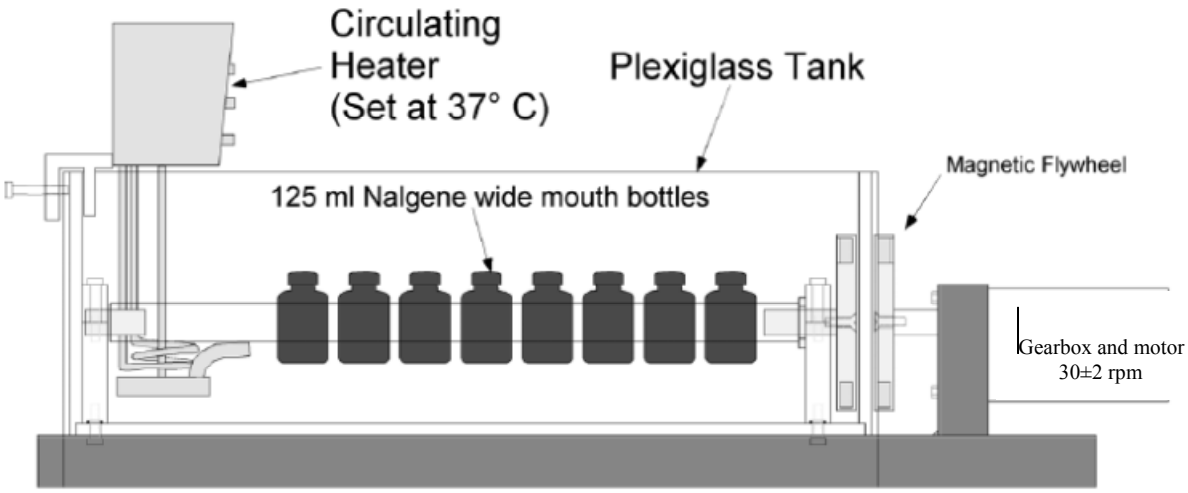
In special cases, the mass of test material may need to be <1.0 g to avoid the potential for saturation of the extraction solution. Tests performed using lead acetate, lead oxide, and lead carbonate indicate that if the bulk concentration of a test material containing these relatively soluble forms of lead exceed approximately 50,000 ppm, the extraction fluid becomes saturated at 37°C and, upon cooling to room temperature and below, lead chloride crystals will precipitate. To prevent this from occurring, the concentration of lead in the test material should not exceed 50,000 ppm, or the mass of the test material should be reduced to 0.50±0.01 g.

NIST 2710a and 2711a consensus values

The previous lots of these materials, which have the same SRM number without an "a" suffix, became unavailable for purchase from NIST in late 2008. Therefore, it was necessary to develop new lead IVBA means and acceptance ranges for the recently released replacement SRMs NIST SRMs 2710a and 2711a. A Round Robin study was conducted in late 2010 using seven (7) participating laboratories. Each laboratory analyzed each of the SRMs in five (5) replicate analyses, along with the EPA IVBA SOP-required Quality Control (QC) samples. Statistical analysis of the Round Robin sample results provided a mean and acceptable ranges (based on 99-percentile prediction interval) for the each of the two (2) NIST SRMs that are consistent with previous studies. The extracted lead prediction interval was converted to the IVBA prediction interval by dividing by the strong leach digestion value presented in the respective SRM certificates of analysis. The lead values for the EPA Method 3050 strong leach digestion of the SRMs 2710a and 2711a, are 5100 mg/Kg and 1300 mg/Kg, respectively. No outlying sample

results were indentified within each laboratory (n=5), or collectively for the n=35 data set for the individual SRMs, based on statistical analysis. The associated Quality Control (QC) sample results provided by the laboratories for the reagent blank, bottle blank, spiked blank, matrix spike, and Control Soil were all within the acceptance criteria presented in the EPA IVBA SOP 9200.1-86.

Figure 1. Example of *In vitro* Bioaccessibility Extraction Apparatus.



Checklist of minimum reporting requirements for EPA 9200.2-86

- Each batch must include the following:

  Bottle blank

  Blank spike

  Control soil (NIST SRM 2710 or 2710a or 2711 or 2711a)

  Reagent blank

  Duplicate

- The sample mass of control soil and soil samples
- ICP concentrations of QCs and sample extracts
- Minimum detection limit for ICP
- QCs run as part of ICP analysis
- "Total" Pb concentration of soil samples used to calculate % IVBA