United States
Environmental
Protection Agency

OSWER 9200.3-100

Guidance for Sample Collection for *In Vitro* Bioaccessibility Assay for Lead (Pb) in Soil

March 2015

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1.0 Terminology and Application

Definitions:

Absolute Bioavailability (ABA): Fraction of an ingested dose of lead that is absorbed from the gastrointestinal tract and enters the blood and tissues.

Relative Bioavailability (RBA): Ratio of the absolute bioavailability of lead in soil to that of a water soluble reference lead compound (lead acetate).

***In Vitro* Bioaccessibility (IVBA):** Fraction of total amount of lead in a soil sample that is soluble in a gastric-like (i.e., low pH) extraction medium.

The purpose of this document is to provide guidance on the collection of soil samples for measurement of lead IVBA (SW-846, Method 1340) (U.S. EPA, 2013c). The IVBA assay is used as a rapid and inexpensive method for predicting soil lead RBA (U.S. EPA, 2007c). Estimates of lead RBA are used to adjust bioavailability parameters in lead risk assessment models used in site risk assessment (e.g., Integrated Exposure Uptake Biokinetics [IEUBK] model for Lead in Children) (U.S. EPA, 2013a). Soil lead RBA is dependent on physical and chemical properties of the lead in soil and co-occurring elements at any particular site or location within a given site. As a result, site-specific estimates of soil lead RBA that provide representative coverage of the site are recommended for increasing confidence in estimates of risk related to site-specific lead exposures. Sampling plans for estimating soil lead RBA with the IVBA assay should provide a statistically robust estimate of RBA for decision units at the site. Typically, this can be achieved by measuring IVBA in a statistical subsample of soils collected as part of the sampling plan for estimating exposure point concentrations (EPCs) for soil lead. This guidance provides recommendations for data collection requirements, sampling material handling, QA/QC requirements, and health and safety requirements for assessments of site-specific soil lead RBA with the IVBA assay.

2.0 Procedure

Data Collection Requirements: A sampling plan for a site should be developed that considers potential soil exposure pathways for the site and any existing site data; for example, if the site is a residential area, then evaluation of exposure pathways in children's play areas, gardens, and the drip lines of homes should be given special attention (U.S. EPA, 2003a). If existing sampling data are available for a site, the information could assist in targeting the sampling locations where there is likely exposure to these contaminated areas.

Typically soil samples are collected, submitted for metals analysis, and the samples are archived while data are collected and reviewed. Based on the analytical results, a subset of the samples is selected for IVBA assay. At other sites, sample locations could be identified in the sampling plan and IVBA samples collected and analyzed without previous knowledge of lead concentrations at the site, although total metal analysis should be collected and conducted concurrently.

X-ray fluorescence (XRF) could be used to screen samples in the field because there is significant cost saving related to time and financial resources by eliminating the collection of samples that do not meet *a prior* criteria for IVBA analysis. There are many advantages of field screening for lead and other metals

including a reduction of both laboratory and field work. Soils with little to no metals are not collected, shipped, or processed by laboratory staff. Large fluctuations in soil lead concentrations within a site when determined by XRF in the field could be used as justification for collection of additional samples in order to form composites samples in the laboratory. The use of the XRF would allow samplers to immediately collect additional samples which may not be possible following laboratory analyses. Field screening with XRF therefore reduces the turnaround time required to generate IVBA results and reduces the need for additional field deployments as well as generating much less waste (fewer sample reduces shipping cost, processing time, number of analyses, and analytical waste). Field operators of portable XRF instruments should ensure they are following appropriate protocols to obtain reliable results (SW-846, Method 6200, U.S. EPA, 2007b).

When collecting samples for *in vitro* bioaccessibility assay, it is important to note site and sample medium characteristics that may indicate differences in the bioavailability of the lead or indicate that interferences might be present. For example, the lead IVBA assay (SW-846, Method 1340) may not reliably predict RBA of lead in soils that have been amended with phosphate (Scheckel et al., 2013). If phosphate at a site is a concern, it would be worthwhile to analyze the samples for the phosphate concentration. When collecting soils from residential properties it may not be advisable to make composite soil samples from a garden (potentially fertilized with phosphorus) with the surrounding property. Likewise it may not be advisable to composite soil samples from the drip line of a home (possible source of lead contaminated paint) with the remainder of the property (potentially different lead source).

In addition to total metals analysis and IVBA assay, the samples might also be submitted for lead speciation analysis and animal bioavailability studies. Lead speciation analysis is meant to determine the exact chemical form(s), or species, of lead in a sample, as opposed to the total lead concentration. Speciation analysis may be informative in explaining variability in IVBA across the site, identifying sources of contamination of the soil, and assessing the potential mobility of lead in the soil. While IVBA assay is meant to be a faster and less expensive alternative to *in vivo* animal bioavailability studies, there may be cases (such as potential interference from soil amendment applications [e.g., phosphate], untested lead phases, etc.) when the animal study would be necessary. It is important to ensure that sufficient material is collected for each sample so that additional analyses could be performed. If additional analyses are determined to be necessary, such as lead speciation analysis or *in vivo* animal bioavailability studies, consultation with the Technical Review Workgroup (TRW) Lead Committee is recommended.

Prior to sampling, a determination must be made as to whether the soil is regulated or quarantined by the U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS)/Plant Protection and Quarantine (PPQ) (USDA, 2014). Take special care to segregate regulated or quarantined soil samples from the non-regulated or non-quarantined samples. To determine if the soils collected are regulated or quarantined contact the State Plant Health Director (http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/planthealth?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_plant_health%2Fsa_program_overview%2Fct_sphd).

Number of Samples: The number of samples to collect and analyze for IVBA will depend on the Data Quality Objectives (DQO) for the study. Factors that should be considered in estimating the number of samples include:

- goals of the RBA assessment;
- size and characteristics of the decision units at the site;
- expected variability in RBA within decision units, based on available data or bounding assumptions (U.S. EPA, 2007d); and
- acceptable limits on decision errors.

Project managers should consult with U.S. EPA "Guidance on Systematic Planning Using the Data Quality Objectives Process" or other appropriate guidance when developing DQOs (U.S. EPA, 2006).

In general, sample size estimates for RBA assessments can be based on the same types of power analyses used to evaluate statistical hypotheses in estimating EPCs at decision units (DUs) (see Appendix A). To reduce the cost of analyzing numerous discrete samples, an incremented sampling plan may be a cost effective approach (ITRC, 2012).

Sampling Depth: The appropriate sampling depth for a site will depend on the expected exposure pathway for a site. For most scenarios involving exposure to contaminated surface soil, EPA recommends a sampling depth of the top 0–1 inches of soil below organic litter and sod for lead exposure analysis (http://www.epa.gov/superfund/lead/ieubkfaq.htm). With this rather shallow sample depth it could be challenging to obtain sufficient sample mass for discrete samples especially if the material is particularly course. Incremental composite sampling can provide larger masses for shallow samples. If there are other exposure scenarios for a site, other sampling depth intervals that would represent these scenarios should be collected.

Sample Preparation: To help ensure that sufficient sample material is available for analysis, the field samplers should consider sieving the material in the field to remove larger debris. Sieve screens No. 4 (4.72 mm) or No. 10 (2.0 mm) would be sufficient for removing larger debris in the field.

Sample Mass: For metals analysis, SW-846 recommends that a minimum of 200 g of soil be collected and 2 g of sample be used for the digestions (SW-846, Chapter 3 Inorganic Analytes, Table 3-2, U.S. EPA, 2007a). Method 1340 specifies that 1 g of dried and sieved soil sample be used for IVBA assay for lead for a single replicate (U.S. EPA, 2013c). Additional replicates may be required if the assay does not meet performance specifications for IVBA. The amount of sample required will depend on the particle size distribution of the soil and the moisture content of the soil following course sieving in the field. If the samples will be submitted for animal bioavailability studies or speciation analysis, the laboratories that will be conducting these analyses should be consulted on the amount of sample materials they require to determine the sample mass needed. For further assistance in determining the sample mass for *in vivo* bioavailability and *in vitro* bioaccessibility assays, please contact the TRW Lead Committee.

Selection of Samples for IVBA: As stated previously, samples for IVBA assay can be designated as part of the sampling plan for estimating EPCs, or they can be selected based on the results from XRF field screening or total metals analysis. The strategy used to select samples for IVBA assay from XRF results or total metals data will depend on the intended use of the IVBA data. If the intended use is for screening, it may be appropriate to select only those samples that have lead concentrations exceeding the risk-based

concentrations used in screening. If the IVBA data are to be used to estimate risk for the site or a DU at the site, a representative statistical subsample should be selected. Samples selected for IVBA assay should have a total lead concentration less than 50,000 mg/kg (SW-846, Method 1340). If the *in vitro* bioaccessibility assay needs to be performed on a sample with a concentration greater than 50,000 mg/kg, the lab performing the assays should be informed of the samples concentrations so that the amount of soil used in the IVBA assay can be adjusted to be within the appropriate lead concentration range.

3.0 Sampling Materials and Handling

Sample Containers: The analytical laboratory/program that will be conducting the metals analysis should be consulted about the appropriate sample container and size required. For the *in vitro* bioaccessibility assay there are no specific sample container requirements. If no sample container is specified by the metals analysis laboratory, then appropriate containers include glass jars, wide-mouth HDPE jars, plastic zippered bags, or any other container that is clean and free of contaminants can be used. A single one-gallon plastic zippered bag should provide sufficient sample material for at least the metals analysis and *in vivo* bioaccessibility assay for most soils. Two-gallon plastic zippered bags may be required for sandy soils and soils with rocks passing through the sieve in the field. If using wide-mouth HDPE jars, a 1000-mL jar should provide sufficient sample, but collect multiple jars per sample if the soil is particularly course. There will be considerable cost reduction using plastic zippered bags compared to HDPE bottle (both cost of sample containers and shipping).

Sampling Equipment: Collection of surface soil samples may be accomplished with a stainless steel cylindrical punch which will capture a constant diameter core for the sampling depth of interest. Sampling using a kick-style cylindrical punch may reduce sample time in the field due to the ease of use. Kick-style punches are not recommended for sandy soils because the soil readily falls out of the probe. Likewise soils with heavy clay content or rocks are not recommended due to the difficulty in removing clay soils from punch and rocky soil will be rejected at the soil surface. For these reasons using plastic or stainless steel spades, trowels, or spoons may be preferable but the sampler should ensure that a sample is collected evenly across the sampling depth. Once the samples are collected, they should be placed in suitable containers for shipment. Any equipment that is not disposable should be thoroughly decontaminated and appropriately stored after sampling. If the exposure pathway being investigated requires deeper sampling depths than 0–1 inches, equipment such as augers, split spoon samplers, and backhoes may be necessary (U.S. EPA, 2000). If sampling at depth, care should be taken during sampling to account for any soil compaction as a result of sampling.

Labeling, Shipping and Storage Temperature, and Hold Times: Sample ID numbering, labeling, documentation, and chain of custody should follow the requirements of the analytical laboratory/program that will be conducting the metals analysis. The samples may be shipped at ambient temperature unless specified otherwise by the analytical laboratory/program.

EPA recommends a hold time of 6 months for metals samples. EPA 9200.2-86 recommends that all samples be archived after metal analysis and retained for further analysis, including *in vivo* bioavailability assay, for 6 months (U.S. EPA, 2012). The samples may be stored at ambient temperature unless specified otherwise by the analytical laboratory/program.

Laboratory Sample Preparation: Once in the lab, the samples should be blended and completely dried at <40°C in an air-drying oven for approximately 5 days to a constant mass. After drying, any clumps in the sample should be gently broken and then fine sieved. However, samples should not be ground by ball

mill or any other grinding method which could result in reduction in the particle sizes of the collected soils.

To ensure composite samples are representative of all of the component locations, the entire composite sample should be processed (i.e. dried and fine sieved). Following sieving, each sample should be thoroughly mixed using ASTM standard D6051-96 (2006) or ITRC Incremental Sampling Methodology (2012) and then transferred to a suitable storage container (U.S. EPA, 2013b).

Total metals analysis and other analyses should be conducted on the same dried, sieved, and homogenized sample material that will also be used for the *in vitro* bioaccessibility assay. To split a sample into equivalent aliquots for the different analyses, the processed soil should be passed through a riffle splitter and the aliquots collected in clean, 250 ml high-density polyethylene bottles (U.S. EPA, 2003b). Samples that have been dried and sieved can be submitted for total metals analysis, metals speciation, IVBA assay, and *in vivo* animal bioavailability studies but should not be used for analysis of other contaminants of concern.

4.0 Quality Assurance/Quality Control

The field samplers should consult with the metals analysis laboratory/EPA program to determine in advance the requirements for blanks, duplicates, and matrix spikes for the metals analysis samples. For the IVBA assay, Method 1340 does not require field blanks, field duplicates, or matrix spikes to be prepared or collected by field samplers. Material for the matrix spike and duplicates for Method 1340 can be taken from the samples at the laboratory's discretion and will not require that samplers collect and designate separate matrix spike and duplicates in the field.

Samplers should take thorough field notes and should retain any photographs taken, logbooks, and notes following the sampling event. The field group should make note of any differences in the media between the sample locations and indicate if there is any potential interferents (i.e., phosphate amended soils) present.

5.0 Health and Safety

When working with potentially hazardous materials, follow U.S. EPA, Occupational Safety and Health Administration (OSHA), and any contractor's corporate health and safety procedures, in addition to the procedures specified in the site-specific Health and Safety Plan.

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Appendix A

Guidance for Sample Collection for *In Vitro* Bioaccessibility Assay for Lead (Pb) in Soil

Objectives:

Predicting the minimum sample number needed to estimate the RBA-adjusted mean soil Pb concentration involves evaluating and setting limits on the probability of two types of errors. We define the null hypothesis as:

H_0: RBA-adjusted mean soil Pb concentration ≥ Risk based concentration (RBC)

And the alternate as:

H_1: RBA-adjusted mean soil Pb concentration < Risk based concentration (RBC)

A Type 1 error occurs if we reject H_0 when it is true; we conclude that the RBA-adjusted mean soil Pb concentration is less than the RBC, when it is actually greater than the RBC. A Type I error could result in underestimating risk at the site.

A Type 2 error occurs if we accept H_0 when it is false; we conclude that the RBA-adjusted mean soil Pb concentration exceeds the RBC, when it is actually less than the RBC. A Type 2 error could result in overestimating risk at the site.

The objective of a sample number assessment is to identify sample numbers that are expected to satisfy specified requirements for Type 1 and Type 2 error rates. These error rates depend on several factors:

- the difference between the mean soil Pb concentration and the RBC;
- the variability in the soil Pb concentration; and
- the mean and variability of the soil RBA at the site.

Larger sample numbers will be required to achieve a given error rate when the actual RBA-adjusted mean soil Pb is closer to the RBA, or when variability (i.e., standard deviation) of the soil Pb concentrations or RBA at the site are higher.

Assumptions for calculating sample number:

An example of sample number calculation is presented here. Assumptions in the analysis are as follows:

1. The underlying distribution of measured Pb concentrations in discrete soil samples at the decision unit (DU) is lognormal (the incremental-composite sampling [ICS] design should collect adequate samples to ensure a normal distribution of the concentrations of multiple composites).
2. Distribution of measured RBA within a DU is normal (e.g., single source of Pb contamination and uniform soil characteristics). An analysis of IVBA measurements of soil RBA at 10 different sites, at which multiple IVBA measurements were made (range: 12–86 discrete samples per site), showed that the average coefficient of variation (standard deviation/mean) was 13% (range: 5–22) (update to EPA TRW, 2003 that included data from Bunker Hill).
3. The RBA-adjusted mean soil Pb concentration for the DU is:

$$Adjusted\ Mean\ PbSoil = Mean\ PbSoil \cdot \frac{Mean\ RBA}{0.8}$$

where PbS is the soil Pb concentration and 0.8 is the default values for RBA in the IEUBK Model.

4. For evaluating Type 1 error, we assume that the RBA-adjusted mean soil Pb concentration at the DU exceeds the RBC. For evaluating Type 2 error, we assume that the RBA-adjusted mean soil Pb concentration at the DU is below the RBC.
5. An acceptable Type 1 error rate is 5% (i.e., the probability of concluding that the RBA-adjusted mean soil Pb concentration is less than the RBC, when it is actually greater than the RBC, is equal to or less than 5%).
6. An acceptable Type 2 error rate is 20% (i.e., the probability of concluding that the RBA-adjusted mean soil Pb concentration is greater than the RBC, when it is actually less than the RBC, is equal to or less than 20%). We are typically less concerned about a Type 2 error than a Type 1 error (overestimating risk) than a Type 1 (underestimating risk).
7. The ICS design consists of *n*=C composites are collected at the DU, each composite consisting of *n*=I increments, and *n*=R composites are randomly selected for IVBA analysis.
8. The estimated mean soil Pb concentration for the DU is the mean of measured Pb concentrations of *n*=C composites.
9. The estimated mean RBA for the DU is based on the mean of measured IVBA of *n*=R composites.
10. Values assumed for soil Pb concentration, RBC, and RBA for evaluating Type 1 and Type 2 error rates are presented in Table A-1.

A Monte Carlo Simulation (MCS) was used to estimate Type 1 and Type 2 error rates. The MCS consisted of 10,000 random draws from soil Pb concentration and RBA distributions (see Table A-1) and calculation of 10,000 corresponding values for the mean RBA-adjusted soil Pb concentration. The Type 1 error rate is the number of means that are less than the RBC (divided by 10,000) when the assumed (true) concentration equals or exceeds the RBC (see Figure A-1). The Type 2 error rate is the number of means that are greater than or equal to the RBC (divided by 10,000), when the true mean is less than the RBC.

Predictions:

Type 1 and Type 2 error rates for various ICS designs are presented in Table A-2. The single composite design is equivalent to a discrete sampling design with *I*=n discrete samples per DU. The estimated probability distribution of the RBA-adjusted mean soil Pb concentration for the sampling design *C*=3, *I*=20, and *R*=1 is shown in Figure A-1. A plot of the Type 1 error rates corresponding to various combinations of *C*, *I*, and *R* is shown in Figure A-2.

As noted previously, error rates depend on the values selected for the various parameters listed in Table A-1. This is illustrated in Figure A-3 which shows the probability of rejecting H_0 as a function of increasing mean RBA-adjusted soil Pb concentration for a design in which 3 composites of 30 increments each are collected. When the mean soil Pb concentration is well below 400 ppm (<200 ppm), the probability of rejecting H_0 is 100% (Type 1 error = 0). Similarly, when it is well above 400 ppm (>600 ppm) the probability of rejecting H_0 is 0% (Type 2 error = 0). However, at a soil Pb concentration of 500 ppm, the probability of rejecting H_0 is 5%, even though the mean exceeds the 400 ppm RBA (Type 1 error = 5%).

Figure A-3 also shows the effect of variability in RBA on the Type 1 error rate. Three coefficients of variation are shown (0.15, 0.30, 0.50). If the coefficient of variation is 0.50 (RBA=0.6±0.30), rather than 0.15 (RBA=0.6±0.09), the Type 1 error rate at a 500 ppm mean soil concentration increases from 5% to 18%. In order to decrease the Type 1 error rate to an acceptable 5%, the number of increments in each of the 3 composites would have to increase from 20 to 60. If the coefficient of variation is 0.30 (RBA=0.6±0.18), a 5% Type 1 error rate can be achieved with 25 increments in each of 3 composites.

Conclusions:

1. If the mean RBA-adjusted soil Pb concentration is 500±500 ppm and the mean soil Pb RBA is 0.60±0.09, an acceptable Type 1 error (5%) is predicted with:
    a. 1 composite made up of 60 increments;
    b. 2 composites made up of 30 increments; or
    c. 3 composites made up of 20 increments.
2. If 3 composites of 20 increments are collected, RBA assessment of a single randomly selected composite would yield an acceptable Type 1 error rate. A minimum of 30 increments has been recommended (ITRC, 2012).
3. Higher variability in RBA will require a larger number of increments per composite to achieve an acceptable Type 1 error rate.
    a. If the RBA coefficient of variation is 0.30 (RBA=0.60±0.18), 25 increments would be needed per composite.
    b. If the RBA coefficient of variation is 0.50 (RBA=0.60±0.0.30), 60 increments would be needed per composite.
4. A larger number of increments will be needed if the actual mean soil Pb concentration is closer to the RBC, and fewer will be needed if the actual mean Pb concentration is further from the RBC.
5. In general, for most risk assessment applications, acceptable Type I error rate can be expected if ITRC (2012) recommendations are followed (30 increments per composite).

TABLE A-1. Parameter Values for Sample Number Calculation

Parameter	False Negative Assessment	False Positive Assessment	Basis
Soil Pb RBC (ppm)	400	400	OSWER screening level corresponding to P_{10}=5% (approximately)
Mean RBA-adjusted soil Pb concentration (ppm)[a]	500	300	Assumption Type 1 error = RBC x 1.25 Assumption Type 2 error = RBC x 0.75
Mean RBC-adjusted soil Pb standard deviation (ppm)	500[b]	300[b]	CV=1 for Bunker Hill soil (or dust)
Mean soil RBA	0.60	0.60	Site-wide median (U.S. EPA OSRTI TRW)
Soil RBA standard deviation	0.09[c]	0.09[c]	CV=0.15, based on median CV for 11 sites (TRW: Estimation of Lead Bioavailability in Soil and Dust: Update to the Default Values for the Integrated Exposure Uptake Biokinetic Model for Lead in Children (11/02/11) plus Bunker Hill (CV=0.11)
IEUBK model default RBA	0.80	0.80	IEUBK Model

[a]RBA-adjusted mean soil Pb=Mean soil Pb x mean soil RBA/0.8, where 0.8 is the IEUBK Model default soil RBA
[b]Soil Pb distribution: lognormal (mean, SD).
[c]RBA distribution Normal (mean, SD, min, max), with min=0, max=1.

TABLE A-2. Error Rates for ICS Designs

Number of Composites for Pb Analysis (C)[a]	Number of Composites for IVBA Analysis (R)	Number of Increments per Composite (I)	Type 1 Error Rate (%)	Type 2 Error Rate (%)
1	1	10	29	14
1	1	20	18	8.2
1	1	40	9.0	3.1
1	1	50	6.6	2.0
1	1	60	4.1	1.3
1	1	80	3.2	0.5
2	2	10	18	8.1
2	2	20	8.5	3.3
2	2	30	4.5	1.3
2	2	40	2.6	0.5
3	3	5	22	10
3	3	10	13	4.8
3	3	20	4.6	1.3
3	3	30	1.8	0.5
3	1	5	23	10
3	1	10	13	5.4
3	1	20	5.3	1.5
3	1	30	2.3	0.6

[a]If we are interested only in estimating the mean soil Pb concentration (i.e., not the upper confidence limit of the mean), a single composite of *I*=n increments is equivalent to *I*=n discrete samples.

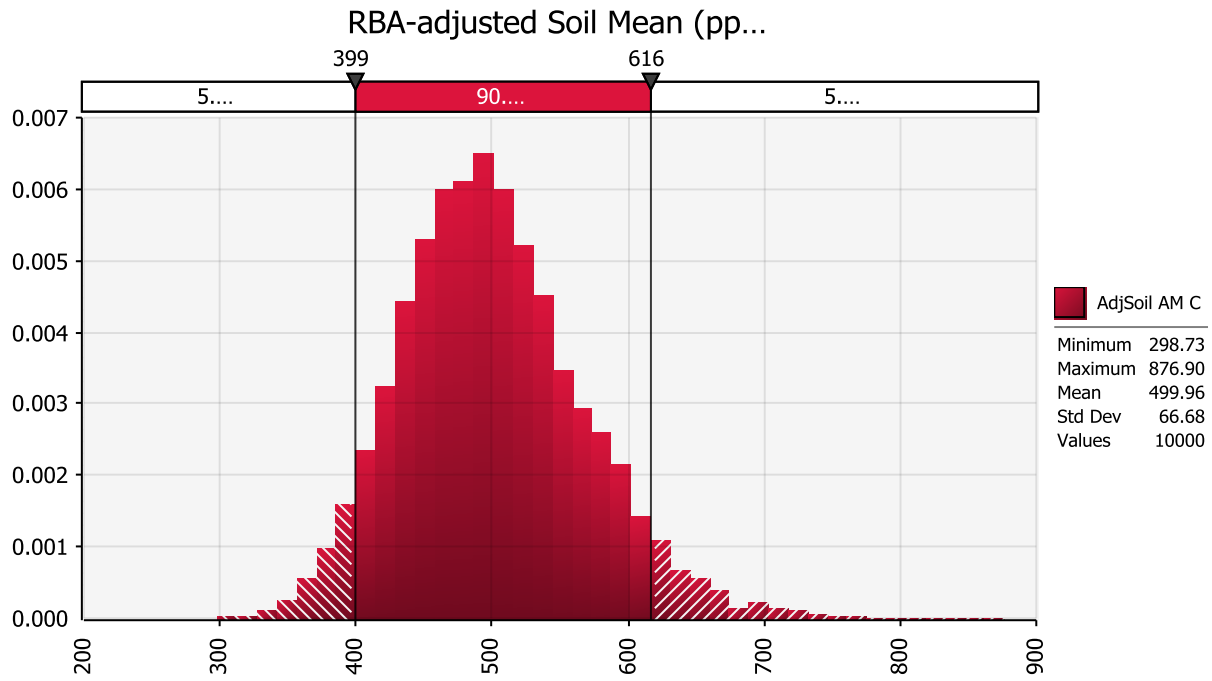

FIGURE A-1. Probability distribution (vertical axis) of estimated mean RBA-adjusted soil Pb concentration (horizontal axis) based on a 3 composite samples consisting of 20 increments with RBA measured on 1 randomly selected composite (C3xI20xR1). Cumulative distribution (percentile) is shown at the top of the graph. Actual soil Pb RBA is 0.60, actual mean soil Pb concentration is 500 ppm; RBC is 400 ppm. The probability of obtaining estimates that are less than 400 ppm (which would lead to Type 1 errors) is approximately 5%. In this case, a Type 2 error is not possible because the true mean exceeds the RBC.

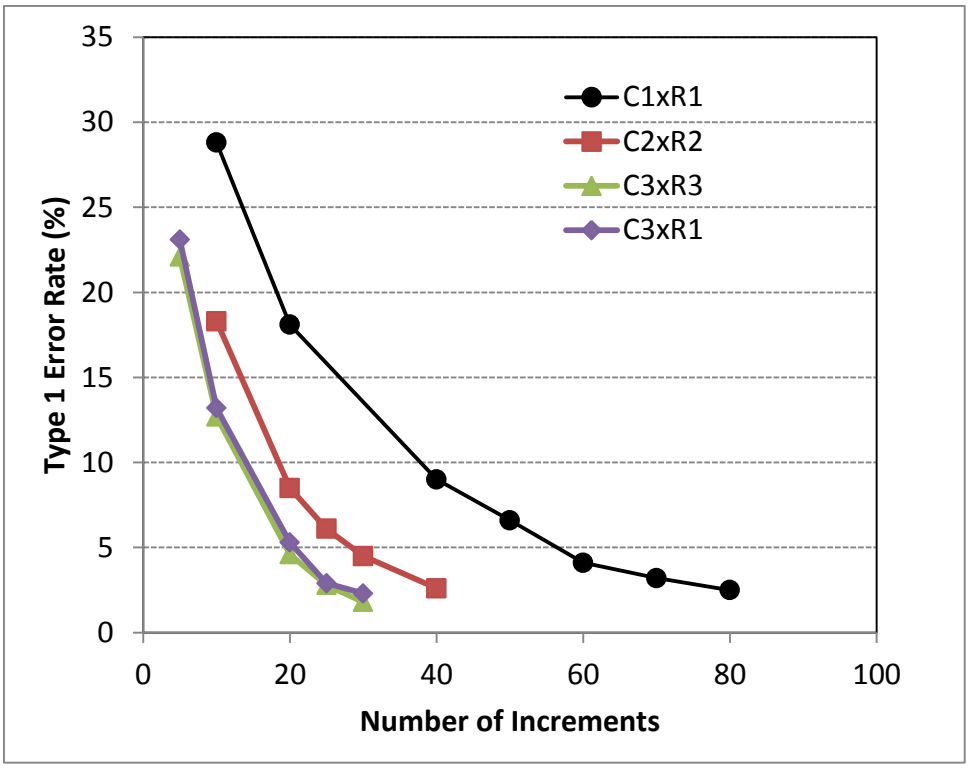

FIGURE A-2. Type 1 error rate (%) predicted for increasing number of increments for 1, 2, or 3 composite samples (20 increments per composite). Mean soil Pb RBA is 0.60±0.09, mean soil Pb concentration is 500±500 ppm; RBC is 400 ppm. The single composite design (C1xR1) is equivalent to a discrete sampling design with *I*=n discrete samples per DU.

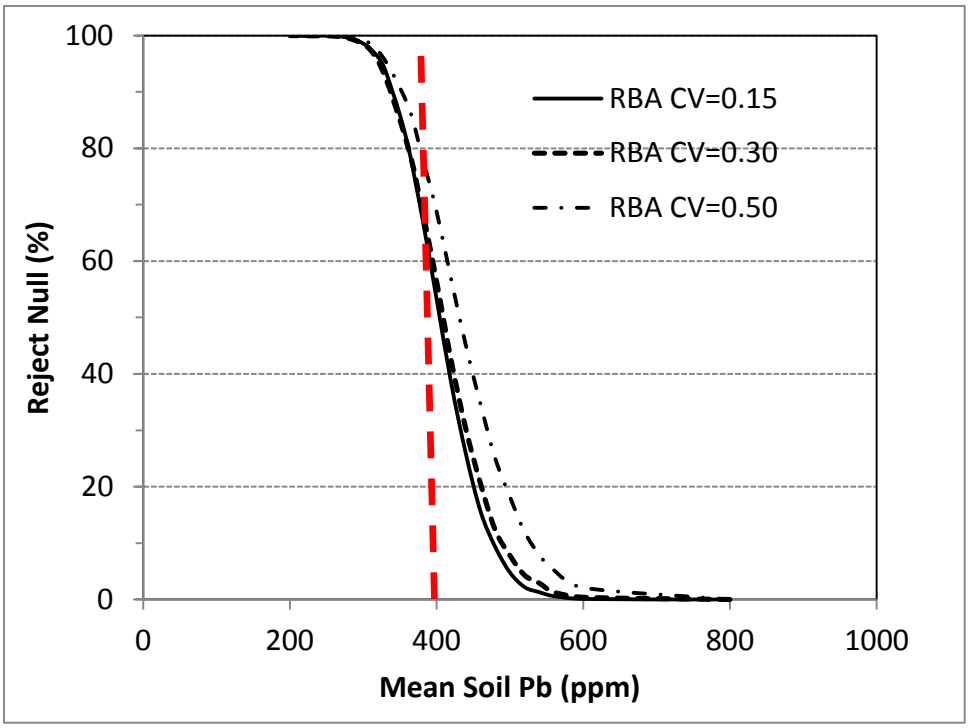

FIGURE A-3. Probability of rejecting H_0 as the mean RBA-adjusted soil Pb concentration increases when the coefficient of variation of RBA is 0.15 (RBA=0.60±0.09), 0.30 (RBA=0.60±0.18), or 0.50 (RBA=0.60±0.30). Soil Pb coefficient of variation is 1.0; RBC is 400 ppm. The area under the probability curve, to the right of the vertical line representing the RBC is the Type 1 error. Sample design is 3 composites of 20 increments per composite, with a single composite for RBA (C3xI20xR1).