Training on Assessment of Relative Bioavailability (RBA) of Soil Arsenic and Lead in Human Health Risk Assessment

Session 4: Laboratory methods to measure RBA/IVBA & soil sampling best practices

OSRTI Technical Review Workgroup

Bioavailability Committee

For More Information

- TRW BAC email (bahelp@epa.gov)
- TRW BAC co-chairs:
  - **Matt Lambert** (Lambert.Mathew@epa.gov)
  - **Sydney Chan** (Chan.Sydney@epa.gov)
  - **Karen Bradham** (Bradham.Karen@epa.gov)
- TRW BAC website (https://www.epa.gov/superfund/soil-bioavailability-superfund-sites-technical-assistance)

Session 1 Recap

- What is soil metal bioavailability (RBA)?
- Brief overview of how RBA is measured:
  - *Directly:* in-vivo animal assays
  - *Estimated:* measure IVBA (via EPA Method 1340) -> use IVBA to predict RBA

Session 2 Recap

- Conceptual site models
- Different ways to apply RBA data in HHRA
- 7 steps of the DQO development process:
  1. State the problem
  2. ID study goal(s)
  3. ID information inputs
  4. Define study boundaries
  5. Develop the analytical approach
  6. Specify performance criteria
  7. Develop plan for obtaining data

Session 3 Recap

- Performance criteria for sample planning/design includes defining & evaluating assessment confidence (i.e., false compliance/exceedance decision error probability objectives).
- Uncertainty/error in sample representativeness & analytical measurements may contribute to false compliance/exceedance error probability.
- A *Bioavailability Sample Planning & Evaluation Tool* has been developed to estimate false compliance/exceedance decision error probabilities (pre-sampling) & evaluate data adequacy (post-sampling).

RBA Assessment Training

Session 4: Laboratory methods to measure RBA/IVBA & soil sampling best practices

In vivo RBA Bioassays

- Various animal models have been used to study oral bioavailability of arsenic & lead in soil (e.g., monkey, swine, mice).
- In vivo bioassays rely on measurements of blood, tissue or urine as metrics of absorbed dose for estimating bioavailability.

$$Relative\ Bioavailability\ (RBA) = \frac{Absolute\ Bioavailability\ (Soil)}{Absolute\ Bioavailability\ (Water)}$$

Mouse RBA Bioassay

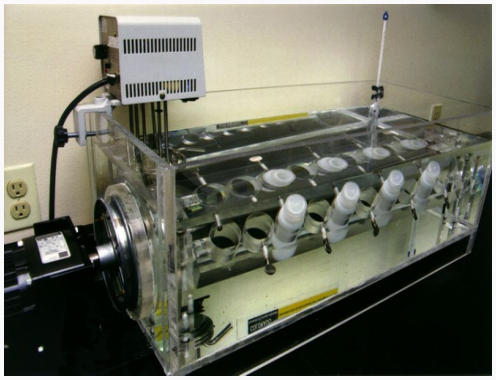
- Developed as a more cost-effective, higher throughput alternative for arsenic and lead bioavailability research.
- Provides highly reproducible estimates of arsenic and lead RBA in soils contaminated from a variety of sources.
- Mouse and swine assays yield similar estimates of RBA.
  - Bradham et al. (2013). Mouse assay for determination of arsenic bioavailability in contaminated soils.
  - Bradham et al. (2018). Comparison of mouse and swine bioassays for determination of soil arsenic relative bioavailability.
  - Li et al. (2016). Arsenic relative bioavailability in contaminated soils: Comparison of animal models, dosing schemes, and biological endpoints.
  - Bradham et al. (2016). Estimating relative bioavailability of soil lead in the mouse.
- Cost is approximately 90% to 95% less than swine or monkey assay.

IVBA Assays

- IVBA (in vitro bioaccessibility) = the fraction of arsenic or lead in soil that can be extracted in a solvent designed to mimic human GI conditions.
- Numerous IVBA assays have been published in the literature. These assays vary with respect to degree of physiological representativeness (e.g., stomach vs. intestinal vs. saliva phases)
- EPA has a process for validating IVBA assays for regulatory use.

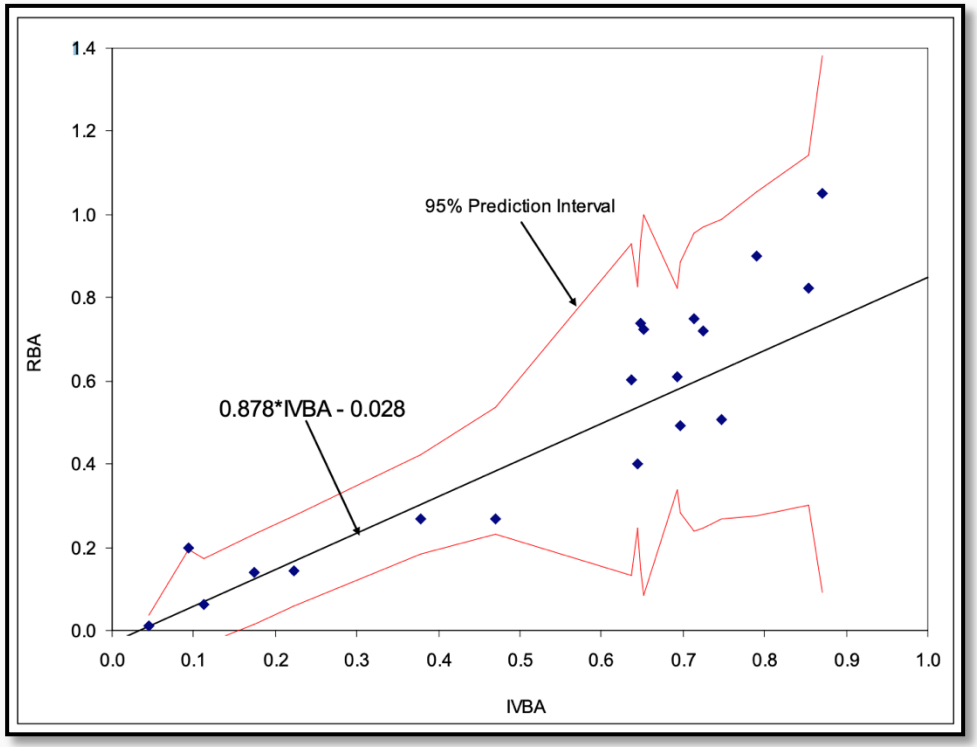
Method 1340 IVBA Assay

- Simulated gastric-phase extraction:
  - 1 g of soil sample + 100 mL buffered extraction fluid heated to 37±2 °C for 1 hr. Filtered solution analyzed for As and/or Pb.
- Validated by EPA for predicting As and Pb RBA in soil, **but not for other contaminants or media (e.g., dust).**
- Other IVBA methods have **NOT** been validated by EPA to predict soil As & Pb RBA for use in HHRA.



Predicting RBA from IVBA | Lead

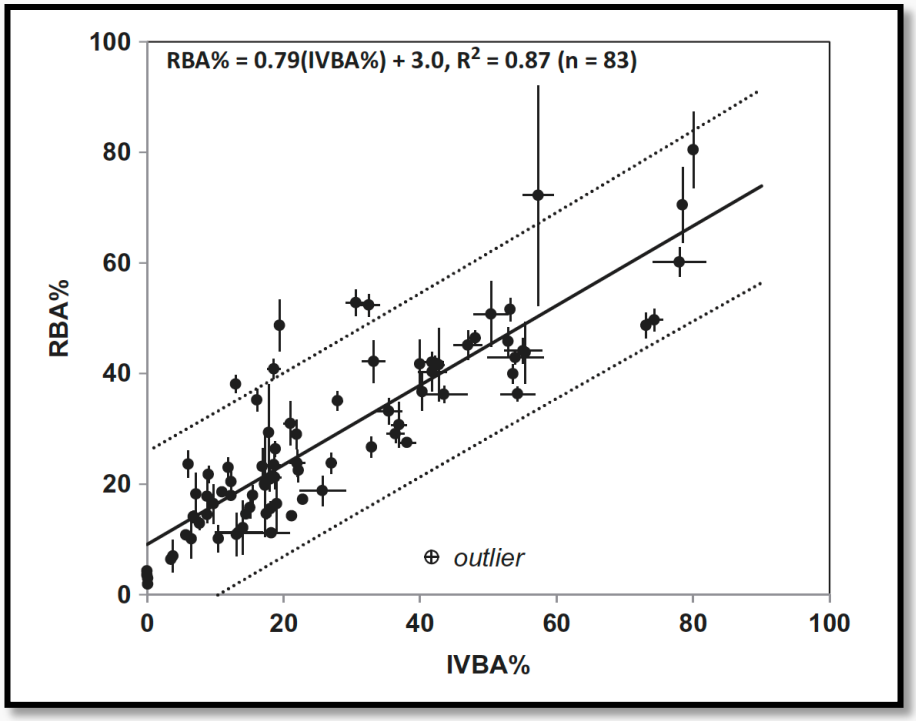
Pb: RBA (%) = 0.878 x IVBA (%) - 2.8 | RBA (fract) = 0.878 x IVBA (fract) – 0.028



From OSWER 9200.3-51 June 2009

Predicting RBA from IVBA | Arsenic

As: RBA (%) = 0.79 x IVBA (%) + 3 | RBA(fract) = 0.79 x IVBA (fract) + 0.03



From Diamond et al., Predicting Oral Relative Bioavailability of Arsenic in Soil from in Vitro Bioccessibility, 2016.

Concentration Requirements for IVBA

- Samples for the IVBA assay should have:
  - Total lead concentration < 50,000 mg/kg
  - Total arsenic concentration < 13,000 mg/kg
- If a sample has a lead or arsenic concentration greater than these levels, the lab performing the IVBA assay should be informed of the sample concentrations so that the *soil:extraction solution ratio* can be adjusted to within the range needed to avoid solution saturation.

Phosphate Interference in IVBA

- Method 1340 may not reliably predict RBA of lead in soils that have been amended with phosphate
  - Scheckel et al. (2013) – *Amending soils with phosphate as means to mitigate soil lead hazard: a critical review of the state of the science*
- If phosphate levels are a concern, the phosphate concentration should be measured.
  - Generally, this interference occurs at phosphate concentrations typical for treating a soil to bind lead and reduce its bioavailability (e.g., > 5 g P/kg soil).
  - Naturally occurring levels of soil phosphate are not expected to interfere with Method 1340, and most fertilizers contain little, if any, phosphate.

When to Consider In vivo Assays

- **Goal:** Use IVBA (Method 1340) to more cost-effectively estimate RBA for HHRAs (vs. directly measuring using In vivo assays)
- However, animal models remain important R&D tools to:
  1. Improve IVBA assay predictions
  2. Identify site-specific factors that contribute to variability in RBA (e.g., soil geochemistry & metal speciation)
  3. Conduct R&D on soil metal remediation strategies (e.g., phosphate or jarosite amendment)
  4. Develop new RBA prediction methods for other soil contaminants (e.g., other metals, PAHs, PFAs, etc.)

Confirmatory In vivo Bioassays

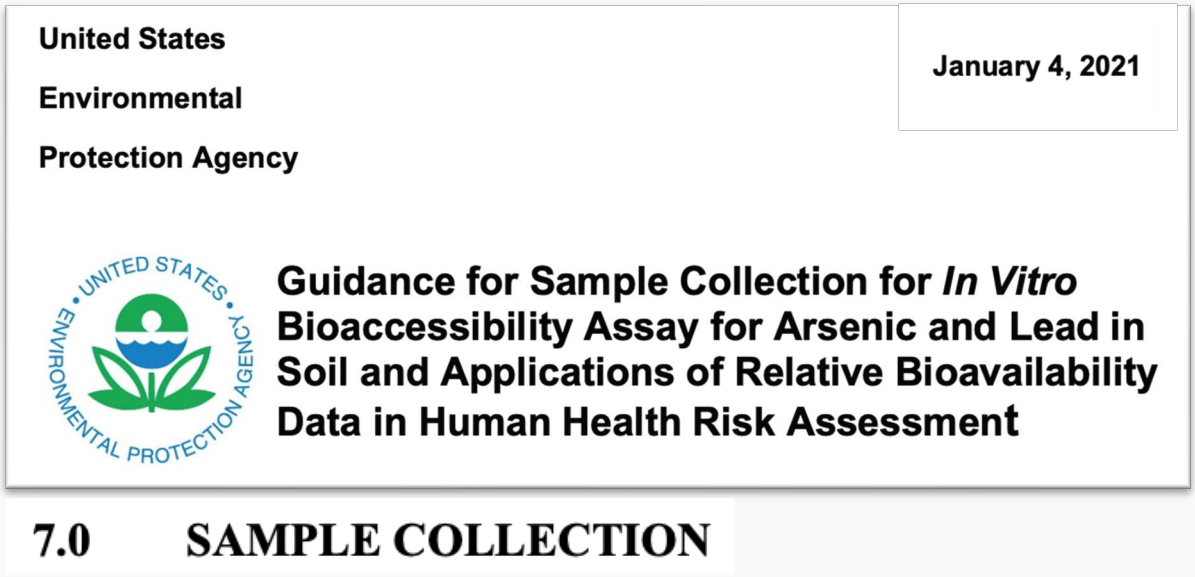
- May be conducted to assess RBA of soil types not represented in data used to validate the IVBA assay. For example…
  - Soils treated with amending agents that alter mobility or bioavailability of arsenic or lead. IVBA methods have been used for R&D purposes, but have NOT been validated for use in HHRAs.
  - Soils or contaminant sources that are “unusual”. Consultation with EPA BAC is recommended in these cases.
- If In vivo bioavailability studies are determined to be necessary, consultation with the TRW BAC is recommended.

Q&A Break

Selection of Samples for IVBA Testing

Useful resources

1. Guidance on Choosing a Sampling Design for Environmental Data Collection (EPA 2002)
2. Guidance for Sample Collection for IVBA | Section 7 (EPA 2021)



United States

Environmental

Protection Agency

January 4, 2021

Guidance for Sample Collection for *In Vitro* Bioaccessibility Assay for Arsenic and Lead in Soil and Applications of Relative Bioavailability Data in Human Health Risk Assessment

7.0 SAMPLE COLLECTION

Selection of Samples for IVBA Testing, cont.

Two general approaches:

1. Collect (& process) samples -> submit for totals analysis -> select subset for IVBA analysis based on totals data.
   - Enables team to target specific total concentrations relevant to decision making.
2. Collect (& process) samples -> submit for concurrent totals & IVBA analysis (i.e., measure IVBA without prior knowledge of total concentrations).

- Regardless of the approach used, incorporating RBA needs into the DQO process prior to sampling makes the field effort more efficient, simplifies data analysis, and clarifies use of data.

Field Screening with Portable XRF

- Benefits:
  - May reduce unnecessary collection of samples that do not meet the established criteria for IVBA analysis (such as concentrations below the decision range), need for additional field deployments, and waste generation.
  - Can inform variability in soil metal concentrations within or between exposure or decision units.
- Useful resources
  - SW-846, Method 6200, U.S. EPA, 2007b
  - U.S. EPA Region 4 Superfund X Ray Fluorescence Field Operations Guide
- Pb:As ratios > 10:1 may interfere with XRF measurements of As, leading to erroneous non-detect or “less-than” values.

Sampling Depth

- Will depend on the expected exposure pathway(s) of concern.
- For most scenarios involving exposure to contaminated surface soil (e.g., incidental soil ingestion), EPA generally recommends a sampling depth of the top 0 –1 inches of soil below organic litter and sod.
  - At this shallow sample depth, obtaining sufficient sample mass may require collecting a larger soil mass than is typical, especially if the material is particularly coarse or for discrete sampling. Composite sampling can provide larger masses for shallow samples.
- If there are other exposure scenarios for a site, other sampling depth intervals representative of these scenarios should be collected.

Sample Mass

- For totals, SW-846 (Chapter 3 Inorganic Analytes, Table 3-2) recommends a minimum of 200 g of bulk soil be collected and 2 g of sample (sieved to < 150 um) be used for analysis.
  - Field samplers should consider coarse-sieving samples in the field to remove larger debris. Sieve screens No. 4 (4.72 mm) or No. 10 (2.0 mm) are sufficient for removing larger debris in the field.
- For IVBA, Method 1340 specifies 1 g of dried and sieved (< 150 um) soil sample be used for analysis of a single replicate.
  - Standard Operating Procedure for an In Vitro Bioaccessibility Assay for Lead and Arsenic in Soil. EPA Office of Land & Emergency Response. April 20, 2017.
- Discuss sample mass needs with the analytical lab(s) performing totals or IVBA analyses, including QC sample requirements.

Sample Mass (cont.)

- The actual amount of bulk or coarse-sieved sample required will depend on the particle size distribution of the soil and the moisture content of the soil.
- If 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 required.
- For further assistance in determining sample mass needs for In vivo bioavailability and IVBA assays, please contact the TRW BAC.

Compositing of Soils

- Soil samples from garden areas generally should not be composited with samples from surrounding land use areas if garden exposure pathways of concern differ from exposure to soils in other areas, and there is some possibility that a garden may have elevated phosphate levels.
- For lead, soil samples from home drip lines generally should not be composited with samples from the remainder of the property, as lead within the drip line may include lead paint and warrant special consideration

Sample Containers

- The analytical laboratory/program that will be conducting analyses should be consulted about appropriate sample container and size requirements.
- Appropriate containers include glass jars, wide-mouth high density polyethylene (HDPE) jars, plastic zippered bags, or similar container that is clean and free of contaminants of concern.
  - For IVBA analyses, there are no “specific” sample container requirements beyond general recommendations discussed.

Sample Containers (cont.)

- A single one-gallon plastic zippered bag (e.g., plastic freezer bag) should provide sufficient sample material for at least the metals analysis and IVBA 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 coarse.
- There may be cost reduction using plastic zippered bags compared to HDPE bottles (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.
  - Kick-style cylindrical punches may reduce sample time in the field due to the ease of use, but are (usually) not recommended for sandy soils, soils with heavy clay content or rocky soils.
- Alternatively, use of plastic or stainless-steel spades, trowels, or spoons may be preferred, but the sampler should ensure that a sample is collected evenly across the sampling depth.

Sampling Equipment (cont.)

- If the exposure pathway being investigated requires deeper sampling depths than 0–1 inches, equipment such as augers, split spoon samplers, or backhoes may be necessary.
  - If sampling at depth, care should be taken during sampling to account for any soil compaction from sampling.
- Any equipment that is not disposable should be thoroughly decontaminated between samples to maintain sample representativeness and prevent cross-contamination.

Field Notes

- Samplers should take thorough field notes and 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 note any potential interferences (e.g., phosphate-amended soils) present.

Sample Processing in Lab | Drying

- Prior to analysis, analytical labs should…
  - Homogenize and dry samples in an air-drying oven at <40ºC for up to 5 days or until a constant mass is achieved.
  - Gently break up any clumps in the sample using a clean, gloved hand in preparation for passing through a No. 10 (2 mm) standard test sieve.
- Bulk samples should be sieved, **NOT** ground by ball mill, mortar and pestle, or any other grinding method that could result in reduction in the particle sizes of the collected soils.

Sample Processing in Lab | Coarse-Sieving

- For samples that were not coarse-sieved in the field…
  - First sieve using a No. 3.5 (5.66 mm) sieve placed on top of a No. 10 sieve to remove pebbles or conglomerated soils.
- Personal protection equipment (e.g., face mask, lab coat, gloves) should be worn when sieving soils in the field or lab.
- If possible, a dust containment system such as a vent hood should be utilized in labs to reduce exposure when sieving contaminated soils.
- **Note:** Brass sieves or sieves with lead solder should **NOT** be used as they can contaminate samples with trace amounts of heavy metals.

Sample Processing in Lab | Fine Sieving

- Samples should be fine-sieved to a particle size limit appropriate to the exposure scenario (e.g., <150 µm for dermal contact with surface soil).

- Affix No. 100 (150 µm) standard test sieve, with a receiver pan at the bottom. Fill sieve (up to) halfway with soil. Sieve until visual inspection indicates soil has been sufficiently sieved (~ 5–10 min. for sandy soils & 20–30 min. for clay soils). Repeat as needed.
- For coarse-sieved samples where clogging of the No. 100 sieve is suspected, place intermediate sized sieves (No. 30 or No. 40) between the No. 10 and No. 100 sieves as needed.

Splitting Samples for Analyses

- Ideally, analytical analyses should be conducted on the same dried, sieved, and homogenized sample (totals, IVBA, speciation, etc.).
- To split samples into equivalent aliquots for different analyses, processed soil should be passed through a riffle splitter and aliquots collected in clean, 250-mL high-density polyethylene bottles (or equivalent).

Note: *Homogenized samples for Pb/As analyses prepared as described here may not be suitable for analysis of other contaminants of concern.*

Packaging Processed Soils

- Transfer sieved soils into clean, pre-weighed polyethylene bags, or similar toxic element-free storage containers (wide-mouth HDPE jars, aluminum pan, etc.).
- Label all storage bags/containers with date, soil ID, soil particle size, and personnel initials plus any other relevant information.
- Weigh (± 0.01 g per container) all sieved soils.
- Record weights of all processed samples (± 0.01 g per container) in laboratory notebook or electronic database.

Labeling, Shipping and Storage

- Sample ID numbering, labeling, documentation, & chain of custody should follow requirements of the analytical laboratory/program that will be conducting the analyses.
- Samples may be shipped & stored at ambient temperature unless otherwise specified by the analytical laboratory/program.
- EPA recommends a holding time of < 6 months for metals samples.
- Method 1340 recommends that all samples be archived after metal analysis and retained should further analyses be needed.

QA/QC Samples

- Method 1340 does not “require” field blanks, field replicates, or matrix spikes be collected/prepared in the field; however, field samplers should consult with the laboratory or the EPA program to determine:
  - Requirements for blanks, duplicates, and matrix spikes for analytical analyses to ensure sufficient sample mass is collected.
  - Field sample processing requirements (don’t just assume analytical lab will, for example, coarse-sieve bulk samples).

Health and Safety

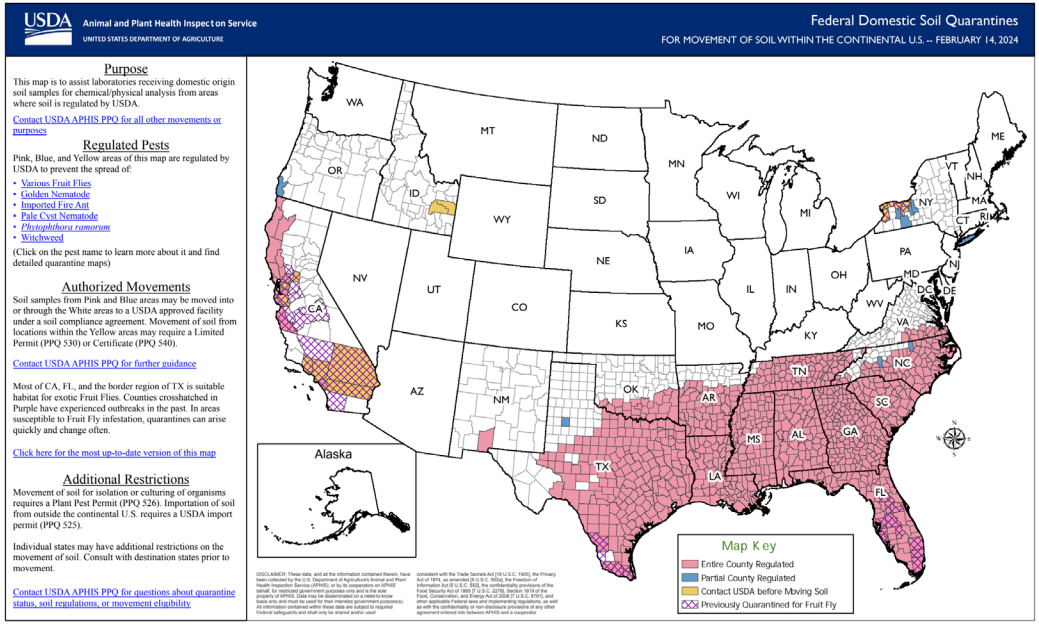
- When working with potentially hazardous materials, health and safety procedures should be adhered to, including but not limited to those of:
  - U.S. EPA
  - OSHA
  - Health & safety procedures required by contractor(s)
  - Site-specific health and safety plans

Metals Speciation

- Speciation analysis is meant to determine the exact chemical/mineralogical form(s), or species, of lead or arsenic in a soil sample.
- While speciation analysis is not necessary, it may help:
  - Explain variability in IVBA/RBA across geographical scales (e.g., site, decision or exposure unit)
  - Identify contamination sources
  - Assess mobility of arsenic or lead in soil.
- If speciation analyses are determined to be necessary, consultation with the TRW BAC is recommended.

USDA Regulated Soils

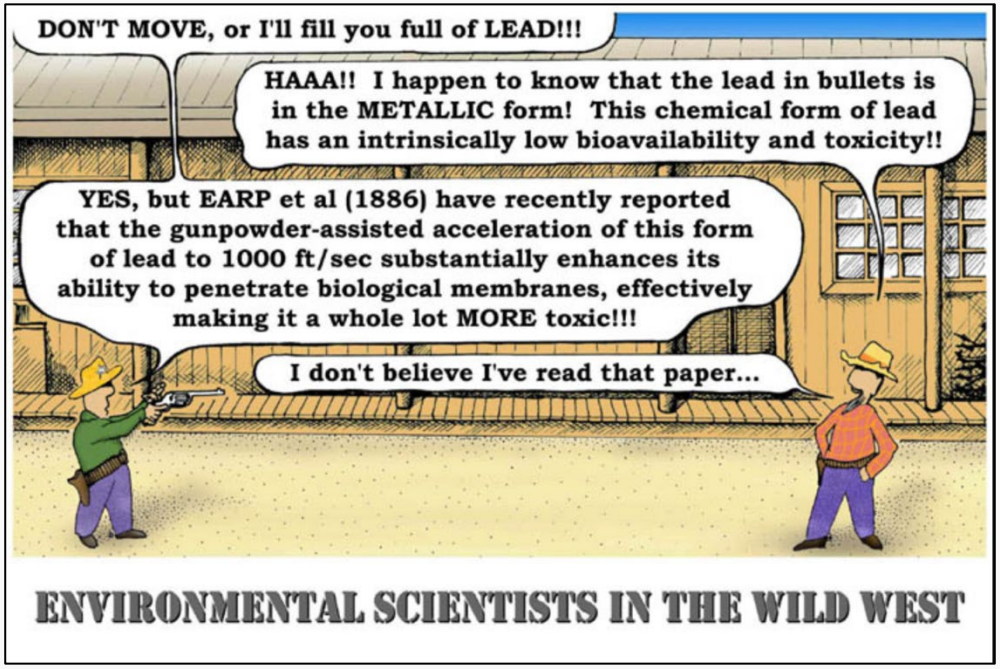
- Prior to sampling, a determination must be made if soils are regulated or quarantined by USDA (APHIS PPQ), as special shipping and handling requirements may be needed.



www.aphis.usda.gov/plant_health/permits/organism/soil/downloads/Fed-SoilRegs.pdf

Closing Reminder: Why is bioavailability important?

The total concentration of arsenic or lead in soil may not provide an accurate measure of risk. Risk will be more accurately assessed after adjusting total soil concentrations for the fraction that is bioavailable.



For More Information

- TRW BAC co-chairs:
  - **Matt Lambert** (Lambert.Mathew@epa.gov)
  - **Sydney Chan** (Chan.Sydney@epa.gov)
  - **Karen Bradham** (Bradham.Karen@epa.gov)
- TRW BAC email (bahelp@epa.gov)
- TRW BAC website (https://www.epa.gov/superfund/soil-bioavailability-superfund-sites-technical-assistance)

Previous Training Sessions

#	Topic	Date*
1	Intro to RBA assessment	2/12/24
2	Applying RBA data to human health risk assessment	3/1/24
3	Sample planning to meet site assessment decision confidence objectives	3/18/24
4	**Laboratory methods to measure IVBA & RBA & soil sampling best practices**	**4/1/24**

Previous trainings are archived and can be viewed at https://clu-in.org/live/archive/