ATTACHMENT A: Frequently Asked Questions on Bioavailability Sampling and Assessment

January 4, 2021

1. What is the purpose of this guidance?

The purpose of this guidance is to update the 2015 guidance by providing information to assist risk assessors and risk managers in collecting and effectively utilizing data on *in vitro* bioaccessibility (IVBA) and relative bioavailability (RBA) for use in arsenic and lead human health risk assessments. The guidance provides recommendations on the following major topics:

(1) rationale for collecting RBA data to support human health risk assessment (HHRA);

(2) application of IVBA and RBA data in HHRA;

(3) evaluation and analysis of IVBA and RBA data for use in HHRA;

(4) systematic planning for collection of RBA data; and

(5) collection and processing of samples for measurement of arsenic and lead IVBA at sites.

2. Where can additional information and assistance on RBA sampling and measurement be obtained?

Additional information and assistance with RBA assessments can be found at the U.S. Environmental Protection Agency (U.S. EPA) Technical Review Workgroup (TRW) Bioavailability Committee (BAC) website (https://www.epa.gov/superfund/soil-bioavailability-superfund-sites-technical-assistance) or can be obtained by contacting the BAC through its email or hotline (bahelp@epa.gov; 1-866-282-8622).

3. What are ABA, RBA, and IVBA?

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

Relative bioavailability (RBA): Ratio of the ABA of the contaminant in the medium of interest to that of the same contaminant in the medium used to dose the test organism in the oral toxicity studies.

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

4. What is the purpose of assessing soil arsenic or lead RBA?

RBA is assessed to increase confidence in human health risk estimates and related risk management decisions at sites. The U.S. EPA recommends that site-specific assessments of soil arsenic and lead RBA be performed for improving the characterization of risk at the site (U.S. EPA, 1989, 2007a, 2007b, 2012b, 2017b).

Estimates of RBA are used to adjust soil action levels (ALs) (or other risk-based levels such as screening levels), exposure point concentrations (EPCs), or oral daily intakes (DIs) when bioavailability in site soil differs from bioavailability in the exposure medium that is the basis for the AL or toxicity value.

Site-specific RBA estimates are also used to adjust soil lead bioavailability parameters in risk assessment models used in site risk assessment (e.g., Integrated Exposure Uptake Biokinetic Model for

Lead in Children [IEUBK model], Adult Lead Model [ALM]) when bioavailability of lead in soil at the site differs from the model default value.

Examples of specific types of adjustments made in risk assessments are described in this guidance.

5. What methods are available for measuring soil RBA?

Various animal models (e.g., monkey, mouse, rabbit, rat, swine) have been used to study oral bioavailability of arsenic or lead in soil. Information on these bioassays and pertinent primary literature can be found in U.S. EPA (2019a, 2019b). Bioassays using these models estimate RBA from measurements of tissue levels or urinary levels in relation to the oral dosage of arsenic or lead.

U.S. EPA has validated an IVBA assay for predicting soil arsenic and lead RBA for use in HHRA and recommends using the IVBA assay for characterizing site-specific soil arsenic or lead RBA (U.S. EPA Method 1340; U.S. EPA, 2017b, 2017c). The assay involves a simulated gastric-phase extraction of arsenic or lead from soil in a relatively simple extraction medium. Information on these bioassays and pertinent primary literature can be found in U.S. EPA (2019a, 2019b).

6. How do you convert IVBA data from the laboratory into estimates of RBA?

RBA is predicted from IVBA using a regression model (U.S. EPA, 2017b). The regression model for converting arsenic IVBA to arsenic RBA is as follows:

$$\textit{arsenic RBA percent} = 0.79 \times \textit{IVBA percent} + 3$$

The regression model for converting lead IVBA to lead RBA is as follows:

$$\textit{lead RBA percent} = 0.878 \times \textit{IVBA percent} - 2.8$$

Note that, in both of the above equations, RBA and IVBA and the regression intercept are expressed as percents. If the IVBA data from the laboratory are reported as fractions, rather than percents, then the corresponding equation for arsenic RBA, expressed as a fraction, is as follows:

$$\textit{arsenic RBA fraction} = 0.79 \times \textit{arsenic IVBA fraction} + 0.03$$

and the corresponding equation for the RBA fraction for lead is as follows:

$$\textit{lead RBA fraction} = 0.878 \times \textit{lead IVBA fraction} - 0.028$$

7. What factors should be considered in choosing between IVBA or *in vivo* RBA assessment methods?

The IVBA assay is a substantially less expensive alternative to an animal bioassay for assessing RBA. The relatively low cost of the IVBA assay compared to an animal bioassay, availability of standard operating procedures (SOPs), and availability of public and commercial laboratories where it can be performed, allows soil samples to be processed more rapidly for the same cost as a single animal bioassay while reducing animal testing. Using the IVBA assay to evaluate multiple soil samples at a site can provide a more thorough assessment of site RBA. However, it is prudent to conduct confirmatory animal RBA bioassays before using an IVBA assay to assess RBA of novel soil types that were not represented in the data used to validate the IVBA assay. These may include soils with chemical and physical characteristics outside the domain of soils used to develop and validate the IVBA assay. It may also include soils that have received treatments with amending agents that alter mobility or solubility of

arsenic or lead. For example, IVBA methods have not been validated for predicting RBA of lead in soils amended with high levels of phosphate to reduce lead bioavailability. Additional information on limitations of the IVBA assays can be found in the technical literature available on the U.S. EPA TRW BAC website or can be obtained by contacting the BAC through its email or hotline (bahelp@epa.gov; 1-866-282-8622).

8. How can RBA be used to adjust the lead bioavailability parameter in the IEUBK model?

The IEUBK model includes a parameter that is used in the calculation of the absorption fraction percent for soil lead (AFP_{soil}) (U.S. EPA, 1994). Users adjust this parameter for RBA when site-specific RBA is to be included in the IEUBK model prediction of the child blood lead distribution. The adjustment is as follows:

$$adjusted\ AFP_{soil} = RBA\ fraction \times 50$$

where RBA is expressed as a fraction, and 50 is the IEUBK model assumption for the absorption fraction percent of lead in drinking water (AFP_{water}). The IEUBK model includes a default value for AFP_{soil} of 0.3, which is equivalent to a default RBA fraction of 0.6 multiplied by the AFP_{water} (50%). A detailed explanation of how to make an RBA adjustment of the IEUBK model is provided in Attachment B (*Calculation of IEUBK Model and Adult Lead Methodology (ALM) Absorption Fraction Parameters from IVBA Results of EPA Method 1340*). An example of an assessment of RBA for the purpose of adjusting the soil absorption parameter in the IEUBK model is provided in Attachment G (*Relative Bioavailability Adjustment of Absorption Fraction Parameters in the Integrated Exposure Biokinetic Model for Lead in Children and Adult Lead Methodology: Cherokee County Railroad Site Case Study*)

9. How can RBA be used to adjust the lead bioavailability parameter in the Adult Lead Methodology (ALM)?

The ALM includes a parameter that represents the absorption fraction of ingested lead in soil and dust lead. Users adjust this parameter for RBA when site-specific RBA is to be included in the ALM prediction of the fetal blood lead distribution. The adjustment is as follows:

$$adjusted\ AF_{S+D} + dust = RBA\ fraction \times 0.2$$

where AF_{S+D} is the ALM parameter for the gastrointestinal absorption fraction of lead in soil and dust, RBA is expressed as a fraction, and 0.2 is the ALM default assumption for the absorption fraction of soluble lead (U.S. EPA, 2003c). A detailed explanation of the adjustment of how to make an RBA adjustment of the ALM is provided in Attachment B (*Calculation of IEUBK Model and Adult Lead Methodology (ALM) Absorption Fraction Parameters from IVBA Results of EPA Method 1340*). An example of an RBA assessment of RBA for the purpose of adjusting the soil absorption parameter in the IEUBK model is provided in Attachment G (*Relative Bioavailability Adjustment of Absorption Fraction Parameters in the Integrated Exposure Biokinetic Model for Lead in Children and Adult Lead Methodology: Cherokee County Railroad Site Case Study*).

10. How can RBA be used to adjust a soil exposure point concentration (EPC)?

The EPC should represent the average exposure experienced by the receptor within the exposure unit or decision unit (U.S. EPA, 2002b). For contaminants other than lead, removal and remedial decisions are often made at sites based, in part, on a calculation of the risk from the EPC using a toxicity value (e.g., oral reference dose [RfD], oral cancer slope factor), which represents an upper limit of the DI of the contaminant in soil that poses negligible risk. The EPC can be adjusted to account for differences between the bioavailability of the contaminant in soil and the bioavailability assumed in the derivation of the toxicity value or screening level. This adjustment facilitates comparisons of EPCs to screening levels

that are based on specific RBA assumptions. In lead risk assessments, RBA-adjusted EPCs can be used in batch file processing of input data for the IEUBK model. The adjustment is as follows:

$$adjusted\ EPC = EPC \times RBA\ fraction$$

where RBA is expressed as a fraction. An example of an assessment of RBA for the purpose of adjusting an EPC for arsenic and lead is provided in Attachment C (*Relative Bioavailability Adjustment of Decision Unit Exposure Point Concentrations for Arsenic and Lead: Upper Columbia River Case Study*).

11. How can RBA be used to adjust a soil contaminant daily oral intake?

For contaminants other than lead, removal and remedial decisions are made at sites based, in part, on comparison of the oral DI of a contaminant to a toxicity value such as a chronic oral RfD, which represents an upper limit of the contaminant intake soil that poses negligible risk. The DI for arsenic can be adjusted to account for differences between the bioavailability of the contaminant in soil and the bioavailability assumed in the derivation of the RfD. The adjustment is as follows:

$$adjusted\ DI = DI \times RBA\ fraction$$

where RBA is expressed as a fraction. An example of an assessment of RBA for the purpose of adjusting an oral DI for soil arsenic is provided in Attachment D (*Bioavailability Adjustment of Daily Oral Intake of Arsenic in a Baseline Human Health Risk Assessment: A Case Study*). An example of how to adjust a time-weighted soil lead concentration is provided in Attachment H (*Relative Bioavailability Adjustment of Soil Lead Exposure Point Concentrations for a Time-Weighted Exposure to Soil*).

12. How can RBA be used to adjust a soil arsenic or lead risk-based screening level or action level (AL)?

At sites where removal and remedial decisions are made based, in part, on comparison of the EPC to an AL or risk-based concentration or screening level, the AL can be adjusted to account for differences between the bioavailability of the contaminant in soil and the bioavailability assumed in the derivation of the AL. The adjustment should be made to the AL or to the EPC (see Section 5.3), but not to both. The exact adjustment to be made will depend on what assumptions about RBA are incorporated into the AL. For example, if a soil AL for arsenic has been derived assuming an RBA for arsenic of 1.0, then a site-specific RBA adjustment of the AL must be a value relative to 1. For example:

$$adjusted\ AL = AL \times 1.0/RBA\ fraction$$

where RBA expressed as a fraction. An example of adjustment of a soil AL for arsenic is presented in Attachment E (*Retrospective Relative Bioavailability Assessment in Support of a Removal Decision: A Case Study*). Lead ALs derived from the IEUBK model that assume that the default model RBA value of 0.6 (absorption fraction for lead in soil = 0.3, absorption fraction for lead in drinking water = 0.5), would be adjusted as follows:

$$adjusted\ AL = AL \times 0.6/RBA\ fraction$$

An example for the adjustment of a risk-based concentration for lead is provided in Attachment F (*Relative Bioavailability Adjustment of a Risk-Based Concentration for Lead: A Case Study – Adjusting RBA in the IEUBK Model and ALM*).

13. What is a soil RBA data quality objective?

A data quality objective (DQO) process is used to establish performance or acceptance criteria, which serve as the basis for designing a plan for collecting data of sufficient quality and quantity to support site assessment and remedial decision making. As with planning any environmental sampling, DQOs should be developed for RBA data collection. See the *Guidance on Systematic Planning Using the Data Quality Objectives Process* (U.S. EPA, 2006) for further discussion. The development of DQOs is a 7-step process:

(1) state the problem;

(2) identify the goal of the study;

(3) identify information inputs;

(4) define the boundaries (in space and time) of the study;

(5) develop the analytical approach;

(6) specify the performance criteria; and

(7) develop a detailed plan for obtaining the data.

The final step of the DQO process is to develop a sampling and analysis plan. This plan should consider potential soil exposure pathways for the site and any existing site data. If existing sampling data are available for a site, the information could assist in understanding the variability of data at the site and in planning a representative sampling design. Samples collected to assess RBA and total metal concentrations should be representative of the bioavailability throughout the area of exposure (i.e., the exposure unit). The *Guidance on Choosing a Sampling Design for Environmental Data Collection for Use in Developing a Quality Assurance Project Plan* is a useful resource for selecting a design to meet the project DQOs and provide representative data (U.S. EPA, 2002a). An example of application of DQOs to RBA assessment is presented in Attachment C (*Relative Bioavailability Adjustment of Decision Unit Exposure Point Concentrations for Arsenic and Lead: Upper Columbia River Case Study*). Consultation with a qualified statistician who has experience with sampling design is recommended.

14. What factors should be considered in designing a retrospective RBA assessment based on archived soils samples?

Retrospective RBA assessments are sometimes undertaken at sites based on RBA measurements made on archived soils collected for some other purpose (e.g., discovery, preliminary site characterizations, assessments to support removal decisions). In these instances, the original sampling design may not have considered DQOs for characterizing RBA. Therefore, development of a DQO for RBA assessment based on the archived soils is advised so that an appropriate approach to selecting soils for RBA measurement may be developed. For example, if the DQO is to estimate a site-wide RBA value, then consideration should be given to whether or not the archived soils actually provide a representative sample of RBA at the site. If not, sources of sampling bias should be identified and incorporated into the approach to selecting soils for RBA measurements. If these biases cannot be controlled with the method used to select samples, then they should be considered in the interpretation of the results and in any decisions that are made based on the results. In the absence of a DQO and appropriate sampling design, RBA assessments would be based on a "convenience sample" (e.g., random sample of the archive), rather than on a statistical sample of the site. Use of convenience samples to estimate a site-wide or area-wide RBA introduces larger uncertainty into the RBA estimate. For this reason, the selection of the statistic to represent the site or area RBA may need to recognize greater uncertainty in the mean. For example,

rather than using a mean or 95% upper confidence limit (95UCL) of the mean, an upper percentile or maximum might be considered to represent RBA at the site. An example of a retrospective RBA assessment at a site based on measurement of the RBA using archived samples is provided in Attachment E (*Retrospective Relative Bioavailability Assessment in Support of a Removal Decision: A Case Study*).

15. How do you evaluate data adequacy in RBA assessments?

Evaluation of adequacy of RBA data begins with a thorough evaluation of the data against the quality control limits for the methods used to collect the data. Quality control criteria of arsenic and lead IVBA assays can be found in the SOPs for the assay (U.S. EPA, 2017b). Quality evaluation of RBA data also includes evaluation of the implementation of sample collection methods to determine whether or not the sample design was followed and, if not, the causes, effects, and implications of deviations from the plan. Provided that quality control requirements for sampling and analysis have been achieved, adequacy of the RBA data should be evaluated against the DQO for RBA at the site. The DQO should specify performance and acceptance criteria of the data. More information on DQOs and performance criteria can be found in the *Guidance on Systematic Planning Using the Data Quality Objectives Process* (U.S. EPA, 2006). For DQOs that test hypotheses such as, "is the EPC greater than an AL," the collected data should result in acceptable false compliance decision error (Type 1) and false exceedance decision error (Type 2) probabilities. A false compliance decision error occurs if it is concluded that the EPC is less than the AL, when it is actually greater than the AL. This outcome is also referred to as a false rejection error (U.S. EPA, 2006). A false compliance decision error could result in underestimating risk at the site and/or not taking an action when action is needed to reduce risk. A false exceedance decision error occurs if it is concluded that the EPC exceeds the AL, when it is actually less than the AL. This outcome is also referred to as a false acceptance error (U.S. EPA, 2006). A false exceedance decision error could result in overestimating risk at the site and/or taking action at the site to reduce risk when no action is needed. An example of how to estimate decision error probabilities that rely on estimates of RBA-adjusted EPCs is provided in Appendix A (*Guidance for Sample Collection for Estimating an RBA-adjusted Exposure Point Concentration for Soil*). The example is presented from the perspective of systematic planning for data collection; however, the data collected can be analyzed using the same methods to evaluate whether data collected were within acceptable limits of decision error.

16. What RBA statistic should be used to represent an RBA for a decision unit?

Selection of a statistic to represent the RBA for a decision unit will depend on the DQO established for the decision. If the RBA is to be used to adjust the EPC for the decision unit (i.e., adjusted EPC = EPC × RBA), the statistic selected to represent the RBA should be consistent with the definition of the EPC [see Attachment C (*Relative Bioavailability Adjustment of Decision Unit Exposure Point Concentrations for Arsenic and Lead: Upper Columbia River Case Study*)]. Often, in HHRA, the decision unit represents an exposure unit, within which the receptor has an equal probability of being exposed to soil contaminants anywhere within the decision unit. In this context, the EPC should be the average concentration in the decision unit, estimated as the arithmetic mean or the 95UCL of the mean, from a representative set of soil samples collected from the decision unit (U.S. EPA, 1989, 2002b, 2019c). If the EPC is intended to represent the average exposure concentration at the decision unit, then, consistent with the EPC representing the average exposure, the RBA-adjusted exposure should also represent the average and the statistic to be used to represent the RBA should be the mean or 95UCL of the mean.

The RBA may also be used to adjust the AL applied to evaluating the decisions such as whether or not to remediate at the decision unit [e.g., adjusted AL = AL/RBA; Attachment E (*Retrospective Relative Bioavailability Assessment in Support of a Removal Decision: A Case Study*)]. This adjustment, conceptually, also represents an adjustment of the EPC, in that, an upward adjustment of the AL implies that the EPC can be higher without exceeding the AL. Therefore, the adjustment of the AL should also be

consistent with the definition of the EPC. If the EPC is intended to represent the average exposure concentration at the decision unit, then the mean or 95UCL should be selected to represent the RBA.

In some circumstances, it may be prudent to consider statistics other than the mean (or 95UCL) to represent the RBA [see Attachment E (*Retrospective Relative Bioavailability Assessment in Support of a Removal Decision: A Case Study*)]. For example, heterogeneity in RBA within the decision unit, if detected from sampling or inferred from other information about sources of contamination, may prompt consideration of a percentile to represent the RBA. The selection of the percentile will depend on the observed distribution of RBA within the decision unit. The RBA distribution can be estimated from a properly designed discrete sampling plan. In selecting a percentile rather than a mean to represent the RBA, the resulting adjusted EPC or AL will no longer represent the average adjusted exposure. This bias may be warranted on the basis of ensuring that risk is not underestimated at a decision unit in which there is high variability in RBA. Selection of an upper percentile to represent the RBA at the decision unit will decrease false compliance decision error and increase false exceedance decision error [see Appendix A (*Guidance for Sample Collection for Estimating an RBA-adjusted Exposure Point Concentration for Soil*), for further explanation of decision errors].

17. How would you estimate a site-wide RBA from RBA data on multiple decision units?

A site-wide RBA may be estimated to simplify risk assessment calculations at sites where RBA is found be (or is assumed to be) homogenous across decision units. The method used to estimate a site-wide RBA will depend on the DQO and the conceptual site model (i.e., how well decision units represent the site), as well as the distribution of observed RBAs in the decision unit.

Use of a site-wide RBA to adjust decision EPCs or decision unit ALs: Often, in HHRA, the decision unit represents an exposure unit, within which the receptor has an equal probability of being exposed to soil contaminants anywhere within the decision unit. In this context, the EPC representing exposure within the decision unit should be the average concentration in the decision unit, estimated as the arithmetic mean or the 95UCL of the mean. The assumption of equal probability of exposure may not apply across decision units. If it did, the entire site could be considered a single decision unit. If exposure cannot be assumed to be random across the site, then use of a site-wide RBA to adjust decision unit EPCs or ALs is not advised, and these adjustments should be made at the decision unit level. If a site-wide RBA is to be used to assess risk at the decision unit level, and exposure is not random across the site, then some form of spatial or activity weighting of the decision units should be considered in the calculation of a site-wide RBA. However, it must be kept in mind that a weighted or unweighted estimate of a site-wide RBA (e.g., weighted mean) may over- or underestimate RBA at any given decision unit and, as a result, there will be lower confidence in the resulting adjusted EPC or adjusted AL for the decision unit if adjusted by a site-wide RBA. For this reason, consideration should be given in decision unit-level assessments for measuring RBA at each decision unit being assessed. If only a subset of decision units is assessed for RBA, then the DQO should address the following: (1) plan for selecting decision units for RBA measurement that ensures that resulting data can be used to predict RBAs at these decision units that are not selected for RBA measurement and (2) statistic to be used to represent the RBA at decision units not selected for measurement of RBA [see Attachment C (*Relative Bioavailability Adjustment of Decision Unit Exposure Point Concentrations for Arsenic and Lead: Upper Columbia River Case Study]*).

Use of a site-wide RBA to characterize RBA variability at the site: Assessment of site-wide variability in RBA can support decisions to assess RBA at the decision unit level. It may also reveal heterogeneity in RBA across the site that may be related to multiple sources of contamination with materials that have different RBA. If the objective is to understand variability in RBA at the site, then decision unit RBAs can be analyzed in a variety of ways, including probability plots and spatial

distribution plots. The outcome of these analyses will determine how the site-wide RBA is to be estimated (e.g., unweighted or spatially weighted statistics).

18. How many samples should be collected to estimate a soil RBA for a decision unit?

The minimum sample number needed to estimate the RBA-adjusted mean soil concentration of a contaminant will depend on the DQO. Data can be collected for the purpose of estimating soil concentrations and/or RBA at a site (estimation study) or for the purpose of supporting decision making (hypothesis testing; U.S. EPA, 2006). The number of samples needed will depend on numerous factors, which may need to be assumed before the study is undertaken. These factors include concentration and RBA variability at the site, the difference between the average soil concentration and the AL (or risk-based concentration, screening level, removal management level, etc.) that is to inform the decision, and the sampling design (e.g., discrete, incremental composite sampling [ICS]). An example of how to estimate sample numbers needed for decision making that relies on estimates of RBA-adjusted EPCs is provided in Appendix A (*Guidance for Sample Collection for Estimating an RBA-adjusted Exposure Point Concentration for Soil*).

19. How can the conceptual site model be used to inform RBA sampling?

Selection of an appropriate sampling design and sample numbers used to assess RBA at a site will depend, in part, on the RBA variability at the site. Often, in developing sampling design to support a DQO, accurate information of RBA variability may not be available (e.g., if site was not previously sampled) and would have to be assumed. These assumptions can be informed by the conceptual site model, which may identify factors that could contribute variability of RBA across the site. Examples of these factor include:

- Would the source(s) of contamination be expected to result in low or high variability in RBA? For example, multiple sources may release different forms of arsenic or lead, which could have different RBAs, depending on the initial source of contamination, timing of release, and environmental conditions that affect leaching and redistribution of the contamination and mixing with background sources.

- Does the soil or sediment geochemistry vary across the site? For example, local and regional variability in soil characteristics could contribute to RBA variability across the site.

- What are the expected soil concentrations? For example, decisions about contaminant concentrations that are more than 100 times the AL may not be appreciably affected by RBA assessments.

20. How can information on soil concentrations be used to select samples for RBA measurement?

RBA of soil arsenic and lead can be expected to range from 0 to 100%. Over the RBA range of 1 to 100%, adjustments of the EPC or AL to account for RBA will be less than a factor of 100, and decisions about contaminant concentrations (removal, remediation, control) that are more than 100 times the AL may not be appreciably affected by RBA assessments.

Large variations in concentrations across the site may also be indicative of multiple sources of contamination and, possibly, associated variation in RBA. This information may be useful for developing sampling designs in the DQO process. However, selection of soils for RBA assessment based on contaminant concentrations should be done in a manner that avoids biasing the data. The DQO planning process should be used to ensure that the resulting data can satisfy the DQO. For example, if the DQO is to estimate a site-wide RBA, selection of soils based on concentration may bias the site-wide estimate if some areas are sampled much less densely than others. This consideration is particularly important if the

RBA results are to be used to predict RBA based on concentrations at locations where RBA was not measured.

An alternative to selection of soils for RBA assessment based on concentration is to select a random sample of soils and then analyze the data for RBA variance attributable to concentration (e.g., analysis of variance, regression modeling). Often, this approach may be preferable, given the relatively low additional expense of IVBA assays, the importance of understanding variability, and the need for samples to be representative (i.e., in addition to the expense of contaminant concentration measurements).

21. How can information on mineralogy and speciation be used to select samples and methods for RBA measurement?

Information on mineralogy and speciation can be useful to explain RBA variability at the site. This information may be useful for developing sampling designs in the DQO process. Speciation of soil metals is a technically complex and is often applied to a small subset of samples for the purpose of explaining observed RBA rather than for predicting RBA in advance of measurements. For example, unusual or unexpected RBA values may be followed up with speciation measurements to better understand why the RBA values were observed or to improve predictions of RBA from IVBA.

22. What depth should be sampled for RBA?

The appropriate sampling depth for a site will depend on the expected exposure pathways for a site. For most scenarios involving exposure to contaminated surface soil, U.S. EPA recommends a sampling depth of the top 0–1 inches of soil below organic litter and sod for lead exposure analysis (U.S. EPA, 2020). With this shallow sample depth, obtaining sufficient sample mass for discrete samples may require collecting a larger mass of soil than is typical, especially if the material is particularly coarse. ICS can provide larger masses for shallow samples. If there are other exposure scenarios for a site, alternative sampling depth intervals that would represent these scenarios should be collected

23. How should the samples be prepared for delivery to the laboratory?

A detailed description of recommendations on preparation of field samples is provided in Section 5 of the *Guidance for Sample Collection for In Vitro Bioaccessibility Assay for Arsenic and Lead in Soil*. The guidance includes recommendations on sample containers and field sieving.

References:

U.S. EPA (U.S. Environmental Protection Agency). (1989) Risk Assessment Guidance for Superfund Volume 1 Human Health Evaluation Manual (Part A). U.S. Environmental Protection Agency, Office of Emergency and Remedial Response: Washington, DC. EPA/540/1-80/0023. December. Available online at: https://www.epa.gov/risk/risk-assessment-guidance-superfund-rags-part.

U.S. EPA (U.S. Environmental Protection Agency). (1994) Technical Support Document for the Integrated Exposure Uptake Biokinetic Model for Lead in Children (v0.99d). U.S. Environmental Protection Agency, Office of Emergency and Remedial Response: Washington, DC. EPA 9285.7-22. December. Available online at: https://www.epa.gov/superfund/lead-superfund-sites-software-and-users-manuals.

U.S. EPA (U.S. Environmental Protection Agency). (2002a) Guidance on Choosing a Sampling Design for Environmental Data Collection for Use in Developing a Quality Assurance Project Plan. U.S. Environmental Protection Agency, Office of Environmental Information. Washington, DC. EPA QA/G-5S. EPA/240/R-02/005. Available online at: https://www.epa.gov/sites/production/files/2015-06/documents/g5s-final.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2002b) Calculating Upper confidence Limit for Exposure Point Concentrations at Hazardous Waste Sites. U.S. Environmental Protection Agency, Office of Emergency and Remedial Response: Washington, DC. OSWER 9285.6-10. July. Available online at: https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P100CYCE.txt.

U.S. EPA (U.S. Environmental Protection Agency). (2003c) Recommendations of the Technical Review Workgroup for Lead for an Approach to Assessing Risks Associated with Adult Exposures to Lead in Soil. U.S. Environmental Protection Agency: Washington, DC. EPA-540-R-03-001, OSWER Directive #9385.7-54. December. Available online at: https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P100A16H.txt.

U.S. EPA (U.S. Environmental Protection Agency). (2006) Guidance on Systematic Planning Using the Data Quality Objectives Process. EPA QA/G-4. U.S. Environmental Protection Agency, Office of Environmental Information: Washington, DC. EPA/240/B-06/001. February. Available online at: https://www.epa.gov/sites/production/files/2015-06/documents/g4-final.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2007a) SW-846. Chapter Three – Inorganic Analytes. Revision 4. U.S. Environmental Protection Agency, Office of Resource Conservation and Recovery: Washington, DC. February. Available online at: https://www.epa.gov/sites/production/files/2015-10/documents/chap3_1.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2007b) Method 6200 – Field Portable X-ray Fluorescence Spectrometry for the Determination of Elemental Concentrations in Soil and Sediment. SW- 846, Update VI. Revision 0. U.S. Environmental Protection Agency, Office of Resource Conservation and Recovery: Washington, DC. February. Available online at: https://www.epa.gov/sites/production/files/2015-12/documents/6200.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2012b) Compilation and Review of Data for Relative Bioavailability of Arsenic in Soil and Recommendations for Default Value for Relative Bioavailability. U.S. Environmental Protection Agency, Office of Superfund Remediation and Technology Innovation: Washington, DC. EPA 9200.1-113. December. Available online at: https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P100FKWO.txt.

U.S. EPA (U.S. Environmental Protection Agency). (2017b) Standard Operating Procedure for an *In Vitro* Bioaccessibility Assay for Lead and Arsenic in Soil. U.S. Environmental Protection Agency, Office of Superfund Remediation and Technology Innovation: Washington, DC. OLEM 9200.2-164. July. Available online at: https://semspub.epa.gov/src/document/HQ/100000153.

U.S. EPA (U.S. Environmental Protection Agency). (2017c) Validation Assessment of the *In Vitro* Bioaccessibility Assay for Predicting Relative Bioavailability of Arsenic in Soil and Soil-like Materials at Superfund Sites. U.S. Environmental Protection Agency, Office of Superfund Remediation and Technology Innovation: Washington, DC. OLEM 9355.4-29. April. Available online at: http://semspub.epa.gov/src/document/HQ/196751.

U.S. EPA (U.S. Environmental Protection Agency). (2019a) Fact Sheet: Arsenic Relative Bioavailability (RBA) and *In Vitro* Bioaccessibility (IVBA). U.S. Environmental Protection Agency, Office of Superfund Remediation and Technology Innovation, Technical Review Workgroup Bioavailability Committee (TRW BAC): Washington DC. December.

U.S. EPA (U.S. Environmental Protection Agency). (2019b) Fact Sheet: Lead Relative Bioavailability (RBA) and *In Vitro* Bioaccessibility (IVBA). U.S. Environmental Protection Agency, Office of Superfund Remediation and Technology Innovation, Technical Review Workgroup Bioavailability Committee (TRW BAC): Washington DC. December.

U.S. EPA (U.S. Environmental Protection Agency). (2019c) Guidelines for Human Exposure Assessment. U.S. Environmental Protection Agency, Risk Assessment Forum: Washington, DC. EPA/100/B-19/001. October. Available online at: https://www.epa.gov/sites/production/files/2020-01/documents/guidelines_for_human_exposure_assessment_final2019.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2020) Lead at Superfund Sites: Frequent Questions from Risk Assessors on the Integrated Exposure Uptake Biokinetic (IEUBK) Model. U.S. Environmental Protection Agency, Office of Superfund Remediation and Technology Innovation, Technical Review Workgroup Lead Committee: Washington DC. January. Available online at: https://www.epa.gov/superfund/lead-superfund-sites-frequent-questions-risk-assessors-integrated-exposure-uptake#Sampling.