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Guidance for Evaluating the Oral Bioavailability of Metals in Soils for Use in Human Health Risk Assessment

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Guidance for Evaluating the Oral Bioavailability of Metals in Soils for Use in Human Health Risk Assessment

1. Purpose

The purpose of this document is to provide guidance to Regional risk assessors on how to assess site-specific oral bioavailability of metals in soils for use in human health risk assessments. Specifically, this guidance document provides: 1) a recommended process for deciding when to collect site-specific information on the oral bioavailability of metals in soils for use in human health risk assessments; 2) a recommended process for documenting the data collection, analysis, and implementation of a validated method that would support site-specific estimates of oral bioavailability; and 3) general criteria that EPA normally will use to evaluate whether a specific bioavailability method has been validated for regulatory risk assessment purposes. This guidance is focused on media-specific relative bioavailability and does not address adjustments to default absolute bioavailability values. Also, this guidance addresses human health risk assessment and may not be necessarily useful for evaluating ecological receptors. Finally, the guidance document provides information on methodologies for directly assessing bioavailability and does not pertain to indirect methods for predicting bioavailability (*e.g.*, speciation).

This document provides technical and policy guidance to the U.S. Environmental Protection Agency (EPA) staff on making risk management decisions for contaminated sites. It also provides information to the public and to the regulated community on how EPA intends to exercise its discretion in implementing its regulations at contaminated sites. It is important to understand, however, that this document does not substitute for statutes that EPA administers or their implementing regulations, nor is it a regulation itself. Thus, this document does not impose legally-binding requirements on EPA, states, or the regulated community, and may not apply to a particular situation based upon the specific circumstances. Rather, the document suggests approaches that may be used at particular sites, as appropriate, given site-specific circumstances.

2. Introduction

In most cases, the toxicity of an ingested chemical depends, in part, on the degree to which it is absorbed from the gastrointestinal tract into the body. Because oral reference doses (RfDs) and cancer slope factors (CSFs) are generally expressed in terms of ingested dose (rather than absorbed dose), accounting for potential differences in absorption between different exposure media can be important to site risk assessments (U.S. EPA, 1989). This is true for all chemicals, but is of special importance for metals. This is because metals can exist in a variety of chemical and physical forms, and not all forms of a given metal are absorbed to the same extent. For example, a metal in contaminated soil may be absorbed to a greater or lesser extent than when ingested in drinking water or food. Thus, if the oral RfD or CSF for a metal is based on studies using the metal administered in water or food, risks from ingestion of the metal in soil might be underestimated or overestimated. Even a relatively small adjustment in oral bioavailability can have significant impacts on estimated risks and cleanup goals.

3. Scope

This guidance document is limited to evaluating the bioavailability of metals ingested in soil or other soil-like media, for the purpose of assessing human health risks. The basic concepts and principles discussed here are, however, generally applicable, and may also have relevance for other exposure routes (dermal, inhalation), other media (sediment, diet), other receptors (ecological species), and other chemical classes (certain types of organic compounds). This document is not intended to be a state of the science review on bioavailability. Additional information on bioavailability of soil contaminants is available from other sources (*e.g.*, Hrudey *et al.*, 1996; Kelley *et al.*, 2002; NRC, 2003).

This guidance document addresses sites where human health risks from ingestion of chemical contaminants in soil or soil-like media are evaluated under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) process or under the Resource Conservation and Recovery Act (RCRA) Corrective Action process. This document supplements the guidance in Appendix A of the Risk Assessment Guidance for Superfund (RAGS), Part A, regarding adjustments to absorption (U.S. EPA, 1989). In addition, this document provides guidance on how to consider bioavailability in metals risk assessments, which is one of the many key guiding principles outlined in the Framework for Metals Risk Assessment (U.S. EPA, 2007b).

4. Definition of Bioavailability

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

As defined above, bioavailability and oral absorption fraction are equivalent terms. This definition does not consider the case of metals that may act directly upon the gastrointestinal epithelium (*e.g.*, irritants and corrosives), although the same factors that influence bioavailability may also be important in influencing the magnitude of direct effects of a chemical at the portal of entry.

Bioavailability expressed as a fraction (or percentage) of a dose is commonly referred to as *absolute bioavailability*. The term *relative bioavailability* 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, 2007a). 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*.

While absolute bioavailability can never exceed 1.0 (or 100%), relative bioavailability may be either greater than or less than 1. In this guidance document, relative bioavailability is abbreviated as RBA; however, it has been referred to in other reports that are cited in this guidance document as the *relative absorption fraction* (RAF) or the *relative bioavailability adjustment* (RBA).

A related term, pertaining to bioavailability assessment, is *bioaccessibility*. This usually refers to a measure of the *physiological solubility* of the metal at the portal of entry into the body (NRC, 2003). Since solubilization 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).

5. Existing Pertinent EPA Guidance on Use of Site-Specific Bioavailability Factors

Risk Assessment Guidance for Superfund (RAGS) Part A

Under appropriate circumstances, reliable site-specific data may be used in place of default (non-site specific) exposure and toxicity factors. For example, RAGS Part A (U.S. EPA, 1989) states:

> *The specific values* [exposure parameters] *identified should be regarded as general recommendations, and could change based on site-specific information.*

Supplemental guidance for Superfund (U.S. EPA, 1991) reinforces the appropriate use of site-specific parameter values as follows:

> *...exposure parameters presented in this document* [i.e., the defaults] *are generally considered appropriate and should be used in baseline risk assessments unless alternate or site-specific values can be clearly justified by supporting data.*

RAGS Part A (U.S. EPA, 1989) Appendix A specifically addresses the consideration of medium-specific bioavailability information in site-specific risk assessments, which are referred to as *adjustments for absorption efficiency*. In particular, Appendix A of RAGS states:

> *Adjustments also may be necessary for different absorption efficiencies depending on the medium of exposure (e.g., contaminants ingested with food or soil might be less completely absorbed than contaminants ingested with water).*
>
> *If the medium of exposure in the site exposure assessment differs from the medium of exposure assumed by the toxicity value (e.g., RfD values usually are based on*

> *or have been adjusted to reflect exposure via drinking water, while the site medium of concern may be soil), an absorption adjustment may, on occasion, be appropriate. For example, a substance might be more completely absorbed following exposure to contaminated drinking water than following exposure to contaminated food or soil (e.g., if the substance does not desorb from soil in the gastrointestinal tract).*

RAGS (U.S. EPA, 1989) also emphasizes the need for reliable information in support of quantitative relative bioavailability adjustments:

> *In the absence of a strong argument for making this adjustment or reliable information on relative absorption efficiencies, assume that the relative absorption efficiency between food or soil and water is one.*

The absolute bioavailability of the metal in the exposure medium of concern at the site may be greater or less than that in the exposure medium used in the critical toxicity study that formed the basis of the RfD or CSF. Thus, assuming a relative bioavailability of 1.0 for the medium of concern could result in either an underestimate or an overestimate of risk at the site.

When a reliable site-specific RBA value is available, the exposure estimate (i.e., intake) is adjusted when calculating the hazard quotient (HQ):

$$HQ = \frac{(DI \times RBA)}{RfD} \qquad \text{Eq. 1}$$

where DI is the daily oral intake (mg/kg-day), RfD is the reference dose, HQ is the hazard quotient, and RBA is the relative bioavailability. Similarly, the exposure estimate is adjusted when estimating cancer risk (CR):

$$CR = (DI \times RBA) \times CSF \qquad \text{Eq. 2}$$

where CSF is the cancer slope factor and CR is the cancer risk.

6. Medium-Specific Default Values for Bioavailability of Metals

As noted above, Agency guidance (U.S. EPA, 1989) recommends that, in the absence of data to the contrary, the bioavailability of a chemical should be assumed to be equal in soil, diet, and water (i.e., RBA = 1.0). However, the Agency recognizes that some cases may exist where sufficient data are available for a chemical to support development of medium-specific default absorption factors for that chemical. The purpose of these medium-specific and chemical-specific default values is to increase the accuracy of exposure and risk calculations even when site-specific studies are not available. These default absorption factors are determined by EPA based on national data.

Lead is an example of a chemical for which the Agency has established recommended medium-specific default absorption factors for both children and adult populations. The

Integrated Exposure Uptake Biokinetic Model for Lead in Children (IEUBK model) predicts geometric mean blood lead (PbB) concentrations for a hypothetical child or population of children (birth to 84 months of age) resulting from exposure to environmental sources of lead, including soil, dust, air, drinking water, and diet (U.S. EPA, 1994a,b; White *et al*., 1998). An assumption in the model is that the absolute bioavailability of lead in soil and dust for children, at low intake rates, is 0.3 (30%) and the absolute bioavailability of soluble lead in water and food for children is 0.5 (50%). This corresponds to a relative bioavailability of 0.6 (60%) for lead in soil (or dust) compared to soluble lead in water or food for children. The model also allows for the input of site-specific values.

The Agency has developed the Adult Lead Methodology (ALM) for assessing lead risks in adult populations (U.S. EPA, 1996). An assumption in the ALM is that the absolute bioavailability of lead in soil for adults is 0.12 (12%)[1]. This value is based on assumptions that the absolute bioavailability of soluble lead in water for adults is 0.2 (20%) and that the relative bioavailability of lead in soil, compared to soluble lead, for adults is 0.6 (60%).

The Agency has also derived RfDs that are specific for an exposure medium based on consideration of bioavailability or other factors that might suggest unique dose-response relationships in that medium. For example, separate RfDs for cadmium in food and drinking water have been derived based on the rationale that the bioavailability of cadmium in water is greater than that of cadmium in food by a factor of 2 (i.e., 5% vs. 2.5%, respectively [U.S. EPA, 2003a]). Similarly, the Agency recommends that a modifying factor of three be applied to the chronic oral RfD for manganese when the RfD is used to assess risks from drinking water or soil to account, in part, for potential differences in bioavailability of manganese in water and soil compared to food (U.S. EPA, 2003b).

However, even in cases where sufficient data exist to support default medium-specific absorption factors for a chemical, site-specific data collection may also be important. Important factors that can affect the bioavailability of metals in soil can be expected to vary from site to site, or within a given site. These include the physical and chemical forms of the metal, as well as the physical and chemical characteristics of the association between the metal and soil particles. Default values for bioavailability may not reflect these factors (*e.g.*, chemistry, particle size, matrix effects) at any given site. Therefore, use of default values should not substitute for site-specific assessments of bioavailability, where such assessments are deemed feasible and valuable for improving the characterization of risk at the site (see *Recommended Decision Framework*, below).

7. Recommended Decision Framework for Data Collection and Utilization of Oral Bioavailability Data for Metals in Human Health Risk Assessment

While existing Agency risk assessment guidance (U.S. EPA, 1989, 1994b, 1996) recommends default oral bioavailability values and also supports the use of site-specific bioavailability information in human health risk assessments, these documents do not provide guidance on how to assess site-specific bioavailability or on how to decide if such assessments

[1] Lead absorption is generally lower in adults than in children, hence the default bioavailability of lead is lower in the ALM than in the IEUBK model.

should be pursued in support of site risk assessments. This document addresses these issues by outlining a recommended decision framework on how to evaluate and incorporate site-specific oral bioavailability information into risk-based decision-making.

The recommended decision framework is intended to improve risk estimates at specific sites where the framework is applied, as well as to encourage the expansion of a knowledge base that can be applied to future assessments of bioavailability of metals in soil at all sites. Thus, the need for site-specific data collection may ultimately be decreased as the knowledge base expands.

The decision framework recommended below uses evaluation criteria and an ordered process for considering these criteria in the assessment of site-specific bioavailability of metals. A flowchart of the recommended framework is presented in Figure 1. This recommended decision framework is intended to help ensure that: (1) decisions about when to collect site-specific data are well thought out and documented; and, (2) that when data are collected, these data will be of appropriate quality to support site-specific risk assessment and risk management decision-making.

As noted above, the recommended decision framework is intended for the collection of data to inform site-specific risk-based decisions. The framework is not intended to address the collection of data for research purposes that may serve to expand scientific knowledge for future assessments (*e.g.*, evaluation or development of methods for assessing bioavailability). In practice, the collection of samples for site characterization may provide opportunities to collect samples for research.

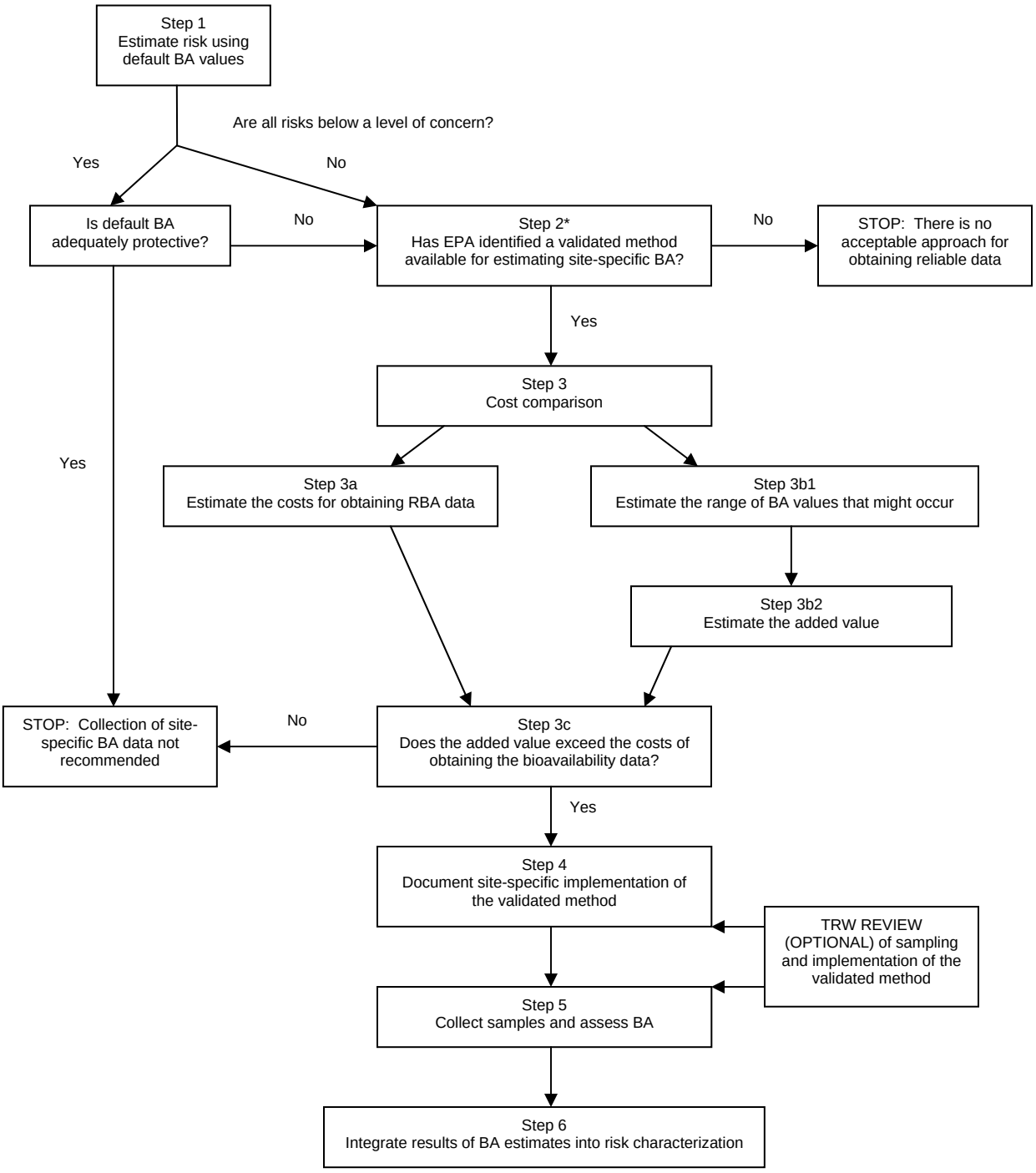
A discussion of the main steps in the recommended decision framework follows.

Step 1. Estimate risk using default values for bioavailability.

The first recommended step is to estimate the current and potential future human health risk based on default values for bioavailability that have been recommended in EPA guidance. As noted earlier, in most cases, the default relative bioavailability factor is 1.0 (U.S. EPA, 1989), although medium-specific default values are available for lead, cadmium, and manganese. If risks predicted using the default bioavailability assumptions are below a level of concern at all parts of the site, and if there is no reason to believe the default bioavailability value has been substantially underestimated, it is generally appropriate to conclude that no further investigation of site-specific bioavailability is needed (although the information may be useful for characterizing uncertainty). This conclusion, however, is predicated on the assumption that the default bioavailability value is an upper bound, health-protective estimate (*e.g.*, default RBA is 1.0). One potential exception is lead, where the default RBA for lead in soil (60%) is intended to be a central tendency value rather than an upper bound value. Hence, it is possible that the actual RBA at the site could be higher or lower than the default. If there is reason to believe that the lead RBA at a site might be substantially higher than the default value (*e.g.*, lead carbonate [EPA, 1994b, 2007a]), it is suggested that the initial assessment (Step 1) be re-run using a higher assumed RBA value (see Step 3b1). If risks predicted using default bioavailability assumptions are above a level of concern at part or all of the site, then collection of site-specific

bioavailability data may be of value for refining risk estimates and/or for determining the extent of remediation. The assessment could proceed to Step 2.

FIGURE 1 Recommended Decision Framework for Assessing Oral Bioavailability (BA) of Metals at Contaminated Sites[2]



*The process of EPA identification of a validated method is independent of the site evaluation process and should occur before a method is used at a contaminated site.

[2] This is not intended to address the collection of samples for research.

Note that if the assessment does proceed beyond Step 1, completing the process and reaching a decision regarding the collection of site-specific bioavailability information should be made early in the site-specific evaluation process. This is important to help avoid substantial delays that might arise from a delayed or late decision to collect additional site-specific information in support of a site-specific bioavailability assessment. It should be apparent very early in the risk assessment process whether any metals may be risk drivers and where additional bioavailability information could have a significant impact.

Step 2. Determine whether or not EPA has identified one or more validated methods for estimating site-specific bioavailability.

Step 2 of the recommended procedure evaluates whether or not EPA has identified one or more validated methodologies for estimating the absolute or relative bioavailability of the metal of concern at the site. Several organizations have addressed the validation of toxicological test methods (ICCVAM, 1997; NRC, 2003). The Agency believes that these regulatory validation approaches are generally applicable to the assessment of bioavailability methods. Across United States federal agencies, the Interagency Coordinating Committee for Validation of Alternative Methods (ICCVAM) typically provides for review and assessment of the validity of toxicological test systems (http://iccvam.niehs.nih.gov). ICCVAM has developed validation criteria and regulatory acceptance criteria for test methods used to generate information to support regulatory decisions. Validation can be achieved by demonstrating that a method is reliable and relevant for its proposed use, while regulatory acceptance can be accomplished when a regulatory (*e.g.*, EPA) or research agency determines that it fills a specific need. Using the ICCVAM criteria as a general guide, the Technical Review Workgroup for Metals and Asbestos (TRW) will evaluate new test methods and determine whether they are suitable for regulatory risk assessment purposes. These criteria (modified from ICCVAM) are presented in greater detail in Section 8.

If EPA has not identified a validated methodology, we recommend that further pursuit of site-specific values generally not proceed without the development and validation of a suitable method. The latter efforts usually would not be undertaken as part of site-specific risk assessment efforts, but original research on the development of alternative bioavailability methods is encouraged, where resources are available.

Step 3. Evaluate the costs and potential value added by obtaining the data.

Step 3a. Estimate the costs.

In this recommended step, information is collected on the cost (including both time and money) that would be required to obtain reliable site-specific bioavailability data. This should include the level of effort that would be needed to plan for and collect appropriate site samples for analysis, the time and cost of performing the bioavailability measurements using the validated method(s), and the effort needed to summarize, evaluate, and apply the results to the risk assessment process.

Step 3b. Estimate the potential value added by obtaining the data.

Step 3b1. Estimate the range of bioavailability values that are plausible.

In this recommended step, information should be assembled from the site under consideration, or from other similar sites, that may be useful in judging whether the bioavailability of the metal in soil at the site could be substantially different from the default value used in the screening-level calculations performed in Step 1. Examples of the types of information that may be relevant include:

a) Bioavailability values obtained using validated methods at other sites that are thought to be similar to the site under consideration.

b) Data on the chemical forms of the metal in the soil. These data might have been derived from measurements made at the site or from knowledge about the sources of soil contamination.

c) Data on the soil types at the site may also be assembled, with specific attention to those characteristics that are known or suspected to affect reactivity and solubility with the metal. In general, these would include the organic content and the nature of the organic fraction (*e.g.*, fulvic acid, humic acid), mineral composition, and the physical characteristics of the metal-soil particles (*e.g.*, particle size, chemical phase, extractability of the metal).

The specific types of information that would be relevant for a particular metal of concern should be assessed from the available scientific literature. A recent review of these topics can be found in NRC (2003).

Based on the available information, the range of bioavailability values that might be plausible at the site should be estimated. These estimates may be based, in large part, on observations at other sites and on professional judgment applied to extrapolations to the site of interest. The objective is to provide plausible bounds on the absolute or relative bioavailability of the metal at the site, which may then be used in estimating costs and potential value added by collecting site-specific bioavailability data.

Step 3b2. Estimate the added value.

Step 3b2 of the recommended decision framework estimates the added value that might be realized if reliable site-specific bioavailability data were obtained. For example, cost savings could be realized if the site-specific bioavailability values were in the lower part of the plausible range. This estimation could be accomplished by first using the plausible range of bioavailability values to estimate the current and potential future human health risk. Then one could determine the extent of the site soils that would fall above a level of concern using the default bioavailability assumption, and compare that to the area that would be above a level of concern based on the potential alternative (lower) assumed value. The difference in areas of concern is then multiplied by the estimated cost of remediation per unit area, and the result is a crude estimate of the potential cost savings from reduced remediation. Collection of site-specific bioavailability data could also provide additional value through improved confidence in the estimate and enhanced information for risk communication.

Step 3c. Cost comparison.

In the final part of recommended Step 3, the estimated costs (time and money) of obtaining the data are compared to the added value that may be realized, and a decision is reached based on the cost comparison. For example, at a site where the area of concern based on default bioavailability assumptions is relatively small, the cost of cleanup might be the same or less than the cost of obtaining the data. Conversely, at a large site, the potential cost savings might outweigh the cost of data collection if collection of site-specific bioavailability data resulted in even a small decrease in the extent of the site determined to be above a level of concern. In the absence of cost savings, the value of continuing with the bioavailability study may still be worth the added expense (*e.g.*, in order to improve confidence in the estimate and provide additional information for risk communication). It is also important to consider whether additional data collection activities can be completed within an adequate time frame. Depending upon the type of information needed, data collection could take a few weeks to several months. If additional collection of site-specific bioavailability data is not feasible, either due to resource or schedule constraints, then the plausible range of bioavailability values and their potential impacts on risk estimates should be discussed in the uncertainty section of the human health risk assessment. However, if the cost comparison and feasibility evaluation support collection and analysis of additional data, then the assessment could proceed.

Step 4. Document site-specific implementation of validated method.

In this recommended step, a site-specific risk assessment should document the 1) rationale for use of the selected validated method at the site; 2) the basis for the selection of soil samples assayed for the purpose of predicting bioavailability at each area of concern; and 3) the approaches (conceptual and quantitative) used to integrate the site bioavailability information into the risk characterization. We also recommend that the risk assessment document the basis for selecting the appropriate sample size needed to ensure that the bioavailability assay yields a reliable estimate of bioavailability (or relative bioavailability). Additionally, the risk assessment should describe how the estimates of bioavailability are used in the risk characterization, such as whether the bioavailability estimates were used to adjust an exposure or risk term in the risk

estimation algorithm; or, if a biokinetic model is being used in the risk assessment (*e.g.*, IEUBK Model), what specific parameters in the model were adjusted based on the bioavailability information collected.

The first part of the site-specific documentation (i.e., rationale) should address the basis for relying on data from the selected method for predicting bioavailability (relative or absolute) for receptors of concern at the site (note, this documentation should address site-specific issues, not already addressed in existing generic guidance for use of the selected method). This site-specific documentation should satisfy the data quality objectives and methodology validation for acceptance. The site-specific documentation should also summarize the pertinent results of these evaluations and why they support the use of the method for the assessment of site-specific bioavailability. Limitations of the selected method for the intended application, in comparison to alternatives, should be documented as well.

The second part of the site-specific documentation should address the approach used to translate the results from bioavailability assays into estimates of absolute or relative bioavailability of the metal in the receptors of concern at the site. For example, if statistical transformations of the data, such as regression models, were used in translating the data output from the methodology into bioavailability estimates, these statistical models should be documented (see U.S. EPA, 2007a, for an example of a regression model applied to the output of an *in vitro* solubility assay for lead).

The third part of the documentation should address selection and procuring of samples that allow prediction of bioavailability at each area of concern. The ultimate goal of the bioavailability assessment is to arrive at a bioavailability adjustment(s) that can be applied to risk estimations for all or part of the site. In some cases, the bioavailability of the metal of concern may be similar across the entire site, and a single sample (usually a composite sample) may be adequate for derivation of a site-specific RBA estimate. In other cases, the bioavailability of the metal of concern may vary within or between sub-areas of the site due to differences in soil characteristics, metal concentrations, form of metal, aging, land use, or other factors. In these cases, bioavailability should be assessed in representative samples collected from each sub-area of potential concern. In all cases, the documentation for the selection of samples to be assessed should address the adequacy of the sample size and sample locations for assessing both within-area and between-area variability, and explain how the estimates of variability will be integrated into the bioavailability assessment at each area. The TRW is available for consultation and review of site-specific implementation plans as needed. For additional information on sampling, see U.S. EPA Site Assessment Guidance (U.S. EPA, 2001b).

Step 5. Collect soil samples and assess bioavailability.

Step 5 of the recommended process is the collection of the soil samples and measurement of bioavailability in those samples using the selected methodology. Sample collection, laboratory procedures, data handling, and archiving should be consistent with Agency guidance for data quality objectives and assurance (U.S. EPA, 2000a,b,c; 2002a,b,c). A report on the methodology used and results of the assessment, including a thorough discussion and, where

possible, a quantitative assessment of confidence in the bioavailability estimates should be developed for inclusion in documents supporting the risk assessment.

In the case that a validated *in vitro* method is used to estimate bioavailability, it is recommended that the protocol specified in the methodology be followed for making the extrapolation from *in vitro* data to *in vivo* values. That is, there is no *a priori* assumption that all validated *in vitro* methods must yield results that are identical to *in vivo* values. Rather, it is assumed that a mathematical equation will exist such that the *in vitro* result (entered as input) will yield an estimate of the *in vivo* value (as output).

In general, the mathematical equation that links *in vitro* results to *in vivo* results will yield an estimate of the expected (average) value of the *in vivo* bioavailability value. However, some range of uncertainty or variability in actual RBA values around this average value can be expected, due either to authentic inter-sample variability in RBA and/or to measurement error in *in vitro* bioaccessibility or RBA. Thus, the true *in vivo* bioavailability value may be either lower or higher than the best estimate predicted from the *in vitro* value. Risk assessors and risk managers should exercise their judgment in deciding whether to use the average value, a range of values, or a conservative point estimate when applying the results to the risk assessment.

Step 6. Integrate results of bioavailability estimates into risk characterization.

In Step 6 of the recommended procedure, the results of the site-specific bioavailability assessment should be incorporated into the characterization of the site risks. This approach is consistent with other EPA risk assessment guidance (U.S. EPA, 1989, 1991) which recommends that, in general, reliable site-specific parameter values are preferred over default values that may not represent site-specific conditions. The uncertainty assessment section of the risk characterization should discuss the basis for confidence in the site-specific estimates of bioavailability, the limitations in the estimates, and any issues related to extrapolating these values over time (i.e., whether the value measured at present is likely to change over time due to potential physical or chemical changes in the test material over time). The uncertainty assessment should also provide at least a qualitative, but preferably a quantitative, assessment of uncertainty in the site-specific bioavailability estimates, as well as the potential impacts of this uncertainty on the risk characterization. Guidance regarding approaches to quantitative uncertainty assessment of site risk estimates can be found in RAGS Volume 3 (U.S. EPA, 2001a).

8. TRW Review of Bioavailability Assessments

Review by the Technical Review Workgroup for Metals and Asbestos (TRW) may be sought at any point in the process described above, when new methods or novel applications of existing methods are implemented. In particular, the TRW normally will use the "method validation" and "regulatory acceptance" criteria modified from ICCVAM (1997) to evaluate new bioavailability test methods in determining whether they are suitable for regulatory risk assessment purposes. Table 1 provides the general criteria that EPA typically considers to determine if a specific bioavailability method has been validated for regulatory risk assessment purposes (adapted from ICCVAM, 1997). Generally, a test methodology may be accepted for

regulatory use (*i.e.*, is a *regulatory methodology*) only after it has been adequately evaluated, documented, and undergone independent review. Some examples of the general criteria that EPA will typically use for evaluating the regulatory acceptance of a test methodology are provided in Table 2 (adapted from ICCVAM, 1997). The Agency does not expect that all of the recommended criteria in Tables 1 and 2 will need to be addressed to the same extent for each alternative bioavailability methodology. Rather, EPA intends to evaluate the recommended criteria on a case-by-case basis.

TABLE 1 Recommended Criteria for Validation of Test Methods (adapted from ICCVAM, 1997)

U.S. EPA typically will consider the following criteria in evaluating whether a new or revised test method may be considered validated for regulatory risk assessment purposes. The extent to which these criteria are relevant typically will vary with the method and its proposed use.

- The scientific and regulatory rationale for the test method, including a clear statement of its proposed use.
- The relationship of the test method endpoint(s) to the biologic effect of interest.
- A detailed protocol for the test method, including a description of the materials needed, a description of what is measured and how it is measured, acceptable test performance criteria (e.g., positive and negative control responses), a description of how data will be analyzed, a list of the species for which the test results are applicable, and a description of the known limitations of the test including a description of the classes of materials that the test can and cannot accurately assess.
- The extent of within-test variability and the reproducibility of the test within and among laboratories, including the degree to which biological variability affects this test reproducibility.
- The test method performance using reference chemicals or test agents representative of the types of substances to which the test method will be applied, including both known positive and known negative agents.
- Sufficient data to permit a comparison of the performance of a proposed substitute test with that of the test it is designed to replace.
- Data supporting the validity of a test method and reported in accordance with Good Laboratory Practices (GLPs).
- Data supporting the assessment of the validity of the test method.
- The methodology and results have been subjected to independent scientific review.

TABLE 2 Recommended Criteria for Regulatory Acceptance of Toxicological Test Methods (adapted from ICCVAM, 1997)

A test method proposed for regulatory acceptance generally should be supported by the following attributes:

Has undergone independent scientific peer review by disinterested persons who are experts in the field, knowledgeable in the method, and financially unencumbered by the outcome of the evaluation.

Has a detailed protocol with standard operating procedures (SOPs), a description of operating characteristics, and criteria for judging test performance and results.

Data generated adequately measure or predict the toxic endpoint of interest and demonstrate a linkage between either the new test and an existing test or the new test and effects in the target species.

Adequate test data for chemicals and products representative of those administered by the regulatory program or agency and for which the test is proposed.

Generate data useful for risk assessment purposes, i.e., for hazard identification, dose-response assessment, and/or exposure assessment. Methods may be useful alone or as part of a battery or leveled approach.

The specific strengths and limitations are clearly identified and described.

Be robust (relatively insensitive to minor changes in protocol) and transferable among properly equipped and staffed laboratories.

Be time and cost effective.

Can be harmonized with similar testing requirements of other agencies and international groups.

Suitable for international acceptance.

Provide adequate consideration for the reduction, refinement, and replacement of animal use.

It is strongly recommended that both "method validation" and "regulatory acceptance" criteria be met before the quantitative use of a method in site-specific risk assessments. Such methods would generally be identified by EPA as *regulatory methodologies*, in contrast to *research methodologies* that may have been explored for assessing bioavailability, but may not be appropriate for applications to site risk assessments. In support of this guidance document, the Agency intends to identify *regulatory methodologies* for assessing bioavailability of metals in soils as it has already done for lead (*e.g.*, see U.S. EPA, 2007a).

The TRW is available to review the plans for incorporation of the site-specific bioavailability data (generated by a validated method) into the site assessment. Interested parties also have the option of submitting for TRW review the proposed sampling plan for input on whether the plan adequately captures spatial variability in metal species or soil types across the site.

9. Implementation of Bioavailability Guidance

The Agency recognizes that conducting a bioavailability assessment is complex and crosses several scientific disciplines (geochemistry, toxicology, etc.). Using this guidance often will involve considerable scientific judgment and expertise. As a result, EPA believes it is important to provide technical support to those engaged in human health risk assessment at contaminated sites and has established a "Bioavailability Committee" which will operate under EPA's Technical Review Workgroup for Metals and Asbestos (TRW). This committee will be composed of EPA staff with expertise in bioavailability assessment and its application to site-specific risk assessments. The Bioavailability Committee of the TRW will act as the primary point of contact, information archive, and repository of outreach materials for the methods recommended in the guidance document. It will meet on an as-needed basis to review site-specific applications, provide assistance to the Regions, and issue additional guidance as necessary. Moreover, the Committee will review new methods for assessing bioavailability of inorganic soil contaminants (new method validation). Additional information, technical assistance, and future bioavailability guidance will be provided on the following website: http://www.epa.gov/superfund/bioavailability.

10. Cited References

Hrudey, S.E., W. Chen, and C.G. Rousseaux. 1996. Bioavailability in Environmental Risk Assessment. CRC Press, Boca Raton, FL, USA.

ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods). 1997. Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the Ad Hoc Coordinating Committee on the Validation of Alternative Methods. NIH Publication 97-3981. National Institute of Environmental Health Sciences, Research Triangle Park, N.C.

Kelley, M.E., S.E. Brauning, and R.A. Schoof. 2002. Assessing Oral Bioavailability of Metals in Soil. Battelle Press, Columbus, OH, USA.

National Research Council. 2003. Bioavailability of Contaminants in Soils and Sediments : Processes, Tools, and Applications. National Academies Press: Washington, DC. http://www.nap.edu/openbook/0309086256/html/

U.S. EPA. 1986. Air Quality Criteria for Lead Volume I-IV. Environmental Criteria and Assessment Office, Office of Research and Development, RTP, NC. EPA 600/8-83-028 a-d.

U.S. EPA. 1989. Risk Assessment Guidance for Superfund. Volume I. Human Health Evaluation Manual (Part A). EPA/540/1-89/002.

U.S. EPA. 1991. Human Health Evaluation Manual, Supplemental Guidance: Standard Default Exposure Factors. OSWER Directive 9285.6-03.

U.S. EPA. 1993. Data Quality Objectives Process for Superfund. EPA540-R-93-071.

U.S. EPA. 1994a. Technical Support Document: Parameters and Equations Used in the Integrated Exposure Uptake Biokinetic Model for Lead in Children (v. 0.99d). EPA/540/R-94/040, PB94-963505.

U.S. EPA. 1994b. Guidance Manual for the Integrated Exposure Uptake Biokinetic Model for Lead in Children. EPA/540/R-93/081, PB93-963510.

U.S. EPA. 1996. Recommendations of the Technical Review Workgroup for Lead for an Interim Approach to Assessing Risks Associated with Adult Exposures to Lead in Soil. U.S. Environmental Protection Agency Technical Review Workgroup for Lead.

U.S. EPA. 1999. US Submission to Meeting of OECD Working Party on Existing Chemicals. February, 1999 HPV Chemical Human Health Testing: Animal Welfare Issues and Approaches.

U.S. EPA. 2000a. Guidance for the Data Quality Objectives Process (G-4). EPA600/R-96/055

U.S. EPA. 2000b. Guidance for the Data Quality Objectives Process for Hazardous Waste Sites (G-4HW). EPA/600/R-00/007.

U.S. EPA. 2000c. Guidance for Data Quality Assessment: Practical Methods for Data Analysis (G-9). EPA/600/R-96/084.

U.S. EPA. 2001a. Risk Assessment Guidelines for Superfund: Volume III - Part A. Process for Conducting Probabilistic Risk Assessment. EPA 540-R-02-002.

U.S. EPA. 2001b. Site Assessment Guidance CD. Available online: http://www.epa.gov/oerrpage/superfund/sites/phonefax/descript/siteassess.htm.

U.S. EPA. 2002a. Guidance on Quality Assurance Project Plans (G-5). EPA/600/R-02/009.

U.S. EPA. 2002b. Guidance on Choosing a Sampling Design for Environmental Data Collection (G-5S). EPA/600/R-02/005.

U.S. EPA. 2002c. Guidance on Environmental Data Verification and Validation (G-8). EPA/240/R-02/004.

U.S. EPA. 2003a. Reference Dose for Chronic Oral Exposure: Cadmium. IRIS. http://www.epa.gov/iris/.

U.S. EPA. 2003b. Reference Dose for Chronic Oral Exposure: Manganese. IRIS. http://www.epa.gov/iris/.

U.S. EPA. 2007a. Estimation of Relative Bioavailability of Lead in Soil and Soil-like Materials Using in Vivo and in Vitro Methods. OSWER 9285.7-77.

U.S. EPA. 2007b. Framework for Metals Risk Assessment. EPA 120/R-07/001.

White P.D., P. Van Leeuwen P., B.D. Davis, M. Maddaloni, K.A. Hogan, A.H. Marcus, and R.W. Elias. 1998. The conceptual structure of the Integrated Exposure Uptake Biokinetic Model for Lead in Children. *Environ. Health Perspect.* 106(Suppl 6):1513-1530.