Guide for Incorporating Bioavailability Adjustments into Human Health and Ecological Risk Assessments at US Department of Defense Facilities

Part 1: Overview of Metals Bioavailability

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Part 1: Overview of Metals Bioavailability

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14. ABSTRACT

This Guide has been developed as a resource on assessment of bioavailability for use by DoD Remedial Project Managers (RPMs) and others involved in remediating DoD sites and designing studies to support remediation. The guide brings together the most current information on bioavailability of metals. Although the guide focuses on bioavailability of metals, many of the basic principles described herein also can be applied to assessing bioavailability of organic compounds. *Part 1: Overview of Metals Bioavailability*, contained in this volume, is a primer on the concept of bioavailability and how it can be used in determining risk levels. The *Overview* provides a definition of bioavailability and general information on the types of situations where it may be beneficial to perform bioavailability studies and outlines the general factors for determining whether bioavailability studies are appropriate and feasible for a particular site. The Guide includes a brief description of test methods; the steps used in conducting a study and important aspects that affect the acceptability of the results. In addition, a brief summary of metal-specific bioavailability information is presented for those metals that are most often found as contaminants at DoD sites (i.e., arsenic, cadmium, chromium, lead, mercury, and nickel for both soil and sediments; and copper, tin and zinc for sediments only.

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EXECUTIVE SUMMARY

The *Guide for Incorporating Bioavailability Adjustments into Human Health and Ecological Risk Assessments at U.S. Department of Defense Facilities, Parts 1 and 2,* has been developed as a resource on assessment of bioavailability for use by DoD Remedial Project Managers (RPMs) and others involved in remediating DoD sites and designing studies to support remediation. The guide brings together the most current information on bioavailability of metals, and synthesizes this information into a practical handbook that explains concepts and identifies types of data that need to be collected to assess bioavailability and incorporate it into risk assessment. Although the guide focuses on bioavailability of metals, many of the basic principles described herein also can be applied to assessing bioavailability of organic compounds. Since the Department of the Navy issued the July 2000 version of this document, bioavailability has achieved much greater prominence as an issue of broad concern to the U.S. Environmental Protection Agency (EPA). Consequently, a number of EPA programs are currently reexamining how bioavailability issues are incorporated into their programs. Several critical draft EPA documents are cited in this Guide, and RPMs are encouraged to check for updates to relevant EPA guidance.

Part 1: Overview of Metals Bioavailability, contained in this volume, is a primer on the concept of bioavailability and how it can be used in determining risk levels. The *Overview* provides a definition of bioavailability and discusses where bioavailability fits in the risk assessment process for both human health and ecological receptors. This volume provides general information on the types of situations where it may be beneficial to perform the additional studies needed to assess bioavailability and outlines the general factors for determining whether bioavailability studies are appropriate and feasible for a particular site. A brief description of test methods used for assessing bioavailability for human health and ecological risk assessment is provided. The steps in conducting a bioavailability study are outlined and important aspects that affect the acceptability of the results are noted. In addition, a brief summary of metal-specific bioavailability information is presented for those metals that are most often found as contaminants at DoD sites (i.e., arsenic, cadmium, chromium, lead, mercury, and nickel for both terrestrial (soil) and aquatic (sediment) settings; and copper, tin and zinc for aquatic settings only).

Part 2: Technical Background Document for Assessing Metals Bioavailability, contained in the following volume, provides more in-depth technical information for those professionals involved in designing and performing bioavailability studies. The *Technical Background Document* includes guidelines on the types of studies that need to be performed and methods for collecting data necessary to assess bioavailability with specific considerations for individual metals. Standard operating procedures (SOPs) and suggested protocols for the recommended studies are provided as appendices so that a user can readily access this information

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ACRONYMS AND ABBREVIATIONS

ABS	absorption fraction
AF	(soil-to-skin) adherence factor
ASTM	American Society for Testing and Materials
AT	averaging time for exposure
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
AVS	acid volatile sulfides
BAF	bioaccumulation factor
BERA	Baseline Ecological Risk Assessment
BRA	Baseline Risk Assessment
BW	body weight
C	concentration
Cal-EPA	California Environmental Protection Agency
CEC	cation exchange capacity
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CF	conversion factor
COPC	chemical of potential concern
CSF	cancer slope factor
DA	absorbed dose
DAD	dermally absorbed dose
DEQ	Department of Environmental Quality
DoD	Department of Defense
DTSC	Department of Toxic Substances Control
ED	exposure duration
EF	exposure frequency
Eh	redox potential
EPC	exposure point concentration
ERL	effects range low
ERM	effects range median
EV	(soil contact) event frequency
f_{oc}	fraction organic carbon
GI	gastrointestinal
GLP	Good Laboratory Practice
HCl	hydrochloric acid
HQ	hazard quotient
IR	ingestion rate
IRIS	Integrated Risk Information System
N	normal
NA	not applicable

NEPI	National Environmental Policy Institute
NJDEP	New Jersey Department of Environmental Protection
NOAA	National Oceanic and Atmospheric Administration
OM	organic matter
PBET	Physiologically Based Extraction Test
ppm	parts per million
PRG	preliminary remediation goal
RAF	relative absorption fraction
RAGS	Risk Assessment Guidance for Superfund
RBC	risk-based concentration
RfD	reference dose
RPM	remedial project manager
SA	(skin) surface area
SEM	simultaneously extracted metals
SMDP	scientific management decision point
SOP	Standard Operating Procedure
SRA	screening risk assessment
SSSL	site-specific screening level
TBD	to be determined
TCLP	Toxicity Characteristic Leaching Procedure
TOC	total organic carbon
TRV	toxicity reference value
U.S. EPA	U.S. Environmental Protection Agency

GLOSSARY

absolute bioavailability: the fraction or percentage of a compound which is ingested, inhaled, or applied on the skin surface that is absorbed and reaches the systemic circulation.

bioaccessibility: a term for the fractional dissolution of a metal from soil in an *in vitro* study.

bioaccumulation: the net accumulation of a chemical by an organism as a result of uptake from all routes of exposure.

bioavailability: the extent to which a substance can be absorbed by a living organism.

bioconcentration: the net accumulation of a chemical directly from aqueous solution by an aquatic organism.

biomagnification: the tendency of some chemicals to accumulate to higher concentrations at higher levels in the food web through dietary accumulation.

cancer slope factor (CSF): a measure of an upper-bound, approximating a 95 percent confidence limit, on the increased cancer risk from lifetime exposure to a chemical, expressed as a proportion affected per mg/kg-day. Current cancer slope factors are available from U.S. EPA's Integrated Risk Information System (IRIS), www.epa.gov/iris.

dissolution: chemical reactions that cause the release of solid phase mineral components of soils to an aqueous phase.

***in vivo*:** within a living organism. In this document, *in vivo* refers to bioavailability studies conducted using live animals.

***in vitro*:** in an artificial environment outside a living organism. In this document, *in vitro* refers to bioavailability studies conducted in a laboratory apparatus that does not use live animals.

ion exchange: a type of sorption reaction occurring at "fixed charge" sites.

oxidation-reduction reactions: the transfer of electrons from one compound to another, resulting in a change in the oxidation state of the compounds involved.

precipitation: chemical reactions that cause aqueous phase inorganic chemicals to become solid phase mineral components of soils.

sorption: chemical processes that retain ions on soils as surface complexes or a surface precipitates or clusters.

reference dose (RfD): an estimate (with uncertainty spanning perhaps an order of magnitude) of daily exposure to a chemical in a human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime. Current reference doses are available from U.S. EPA's Integrated Risk Information System (IRIS)), www.epa.gov/iris.

relative absorption factor (RAF): the fraction obtained by dividing the absolute bioavailability from soil by the absolute bioavailability from the dosing medium used in the toxicity study from which the reference dose for human health risk assessment was determined.

relative bioavailability: a measure of the difference in extent of absorption among two or more forms of the same chemical (e.g., lead carbonate vs. lead acetate), different vehicles (e.g., food, soil, water), or different doses. In the context of environmental risk assessment, relative bioavailability is the ratio of the absorbed fraction from the exposure medium in the risk assessment (e.g., soil) to the absorbed fraction from the dosing medium used in the critical toxicity study.

toxicity reference value (TRV): doses above which ecologically relevant effects might occur to wildlife species following chronic dietary exposure and below which it is reasonably expected that such effects will not occur.

1.0 INTRODUCTION

This guide is intended to provide direction to Department of Defense (DoD) staff and consultants in evaluating the bioavailability of metals in soil and sediment. Bioavailability issues in risk assessment have recently gained national attention and the National Research Council has just released a new book on the subject (NRC, 2002). Since the Department of the Navy issued the July 2000 version of this guide for incorporating bioavailability adjustments into risk assessments, bioavailability has achieved much greater prominence as an issue of broad concern to the U.S. Environmental Protection Agency (EPA). Consequently, a number of EPA programs are currently reexamining how bioavailability issues are incorporated into their programs. A number of critical EPA documents are cited in this guide; several in draft form (e.g., U.S EPA, 2000a, U.S. EPA, 2000b, U.S. EPA, 2000d, U.S. EPA, 2001a, U.S. EPA, 2002d, U.S. EPA, 2002a), and remedial project managers (RPMs) are encouraged to check for updates to relevant EPA guidance. For metals EPA has identified a range of issues related to metal bioavailability, bioaccumulation, and toxicity that are being evaluated in the process of developing a national framework for the assessment of metals (U.S. EPA, 2002a). This guide is intended to provide practical knowledge and tools for more accurately evaluating bioavailability of metals in risk assessments even as EPA continues to develop policy and guidance on this topic.

Site-specific human health risk assessment (HHRA) typically has a conceptual gap between the exposure assessment for chemicals in soil and the toxicity assessment for the chemicals. An exposure assessment usually yields quantitative estimates of dose for each chemical based on bulk concentrations in environmental media such as soil. The toxicity assessment usually generates toxicity values from a dose response assessment using data from studies of the chemical administered to laboratory animals in drinking water or lab chow. Toxicity values based on epidemiology studies of human populations also are not based on exposure to the chemical in soil. Direct application of these toxicity values to doses of a chemical from soil can be inaccurate if the chemical behaves differently in soil, and is less bioavailable.

For ecological risk assessment (ERA) reduced bioavailability of chemicals in soil or sediment may be accounted for when site-specific toxicity studies are conducted. However, some of the same concerns regarding bioavailability in HHRA also arise in ERA when generic cleanup or screening criteria not reflective of site conditions are applied to a site. For example, criteria for contaminated sediments are typically applied based on bulk metal concentrations, while bioavailability and toxicity are more often driven by pore water concentrations that are highly dependent on site-specific conditions. Similarly, ecological screening levels being developed for terrestrial receptors may be based on toxicity reference values derived from laboratory toxicity studies in a manner analogous to the development of toxicity values for human receptors.

1.1 Why Consider Bioavailability in Risk Assessments?

Bioavailability generally refers to how much of a contaminant is “available” to have an effect on humans or other organisms. Bioavailability can be influenced by external physical/chemical factors such as the interactions of metal species with soil or sediment as well as by internal biological factors such as absorption mechanisms within a living organism. Failure to accurately estimate the bioavailability of chemicals in the environment may lead to inaccurate estimates of exposure for both human and ecological receptors. If bioavailability is overestimated, as is often the case, risks from chemicals in the environment may be overestimated and decisions regarding how to address chemical contaminants at sites may be faulty. Conversely, it is possible that in some cases when bioavailability is unusually high, exposure could be underestimated. Figure 1-1 illustrates the relationship between bioavailability and risk-based cleanup levels. As the figure shows, bioavailability has a direct relationship to exposure and risk estimates (i.e., lower bioavailability results in decreased exposure and risk estimates). In contrast,

bioavailability will be inversely related to risk-based cleanup levels (i.e., lower bioavailability will result in an increase in risk-based concentrations with reduced extent of cleanup). Conversely, higher bioavailability results in increased exposure and risk estimates, and will lead to lower risk-based concentrations with a greater extent of cleanup.

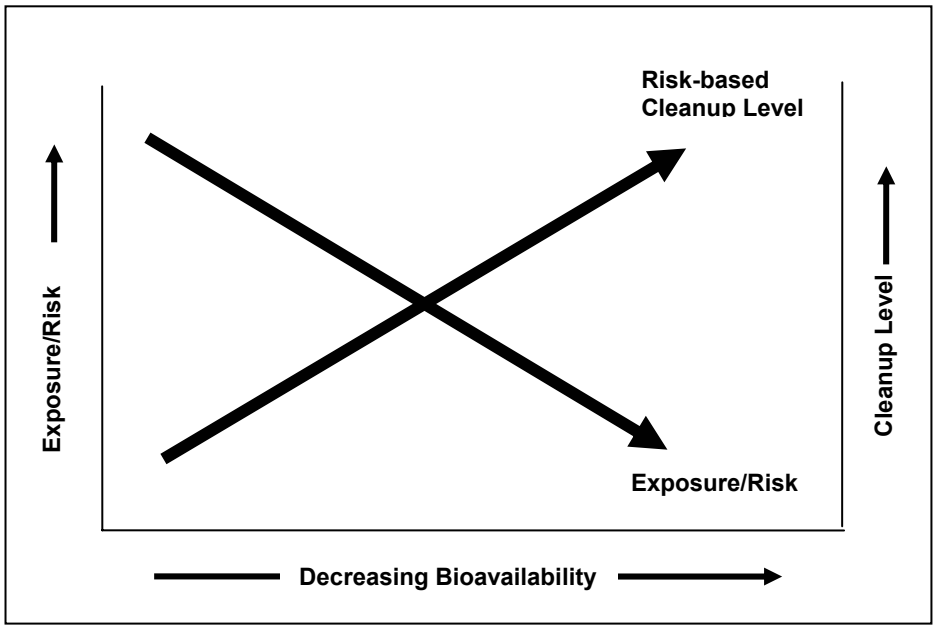


Figure 1-1. Relationship between Bioavailability and Risk Assessment Endpoints

When risk assessments are adjusted to account for lower site-specific bioavailability, the resulting increase in cleanup levels can in some cases substantially reduce the scope and cost of remediation without endangering receptors who come in contact with the site. A good example is the National Zinc Company National Priorities List (NPL) Site in Bartlesville, OK, where soils and house dust in a residential area were contaminated with lead, cadmium, and arsenic from smelting activities. The primary concern at this site was the risk to people living in the area, especially children exposed to lead. Remediation to meet the original cleanup goals would have required extensive soil removal and replacement at an estimated cost of $80 to $100 million. Determining the site-specific bioavailability was identified as an option for revising the exposure estimates to more realistically reflect the conditions at this site. The regulators and other stakeholders were consulted from the beginning of the project, a work plan containing detailed protocols for the bioavailability studies was developed, and independent experts were brought in to review the protocols. The bioavailability tests conducted included a rat feeding study to determine the bioavailability of lead and cadmium, and a laboratory extraction test to determine the bioavailability of arsenic.

The bioavailability studies indicated that the metals in soil at this site were less bioavailable than had been assumed in the initial risk assessment. By incorporating site-specific bioavailability into the risk assessment, the residential soil cleanup level for lead was increased from 500 mg/kg to 925 mg/kg, the cleanup level for cadmium from 30 mg/kg to 100 mg/kg, and the cleanup level for arsenic from 20 mg/kg to 60 mg/kg, resulting in a reduction in remediation costs for this site of more than $40 million. In comparison, the cost of planning, conducting, and reporting the bioavailability studies, which took

approximately seven months, was approximately $200,000. Although this example is not typical of DoD's remediation sites, it does demonstrate how consideration of bioavailability can significantly affect cleanup levels and remediation costs, while still ensuring that the health of residents and workers is protected.

Accurate evaluation of bioavailability is even more critical in cases where no viable option is available for remediation, or where remediation itself may harm the environment. For example, some sites are so vast in size that soil removal from the entire affected area is not feasible. If remediation is not feasible it is critical that bioavailability and exposure estimates be accurate so that the need for alternate risk management strategies can be accurately assessed. Balancing risks of contamination vs. remediation is particularly important for assessing ecological risks such as those associated with contaminated sediments. If the risks associated with contamination are overstated due to overestimates of bioavailability, remediation that causes ecological damage may be implemented unnecessarily. Prediction of changes in bioavailability with time may also be an issue in assessing the permanence of a selected remedy. For metals, many of these issues are being evaluated during the development of a national framework for metals assessment (U.S. EPA, 2002a).

1.2 Purpose of the Document

The *Guide for Incorporating Bioavailability Adjustments into Human Health and Ecological Risk Assessments at U.S. Department of Defense Facilities* consists of two parts. *Part 1: Overview of Metals Bioavailability*, contained in this volume, is designed for use by RPMs and others who want general information on bioavailability. The purpose of the *Overview* is to provide an introduction to the concept of bioavailability (Section 2.0), and to show how it is used in risk assessment and present general guidelines for determining whether bioavailability is worth considering at a particular site (Section 3.0). In addition, the *Overview* provides general information on what a bioavailability study entails and a range of cost, time, and technical requirements needed to conduct such studies (Section 4.0). Profiles of the metals that are most often found to be risk drivers at DoD sites are provided in Sections 5.0 and 6.0 for terrestrial (soil) and aquatic (sediment) settings, respectively. Metals profiled in the terrestrial settings chapter are those most often critical in human health risk assessments, and include arsenic, cadmium, chromium, lead, mercury, nickel. The aquatic settings chapter focuses on ecological risk issues and includes profiles for copper, tin, and zinc, as well. Finally, a brief review of several case studies is provided in Section 7.0. The scope of this document is limited to bioavailability of metals; however, it should be noted that many of the basic principles described herein also apply to organic compounds.

Part 2: Technical Background Document for Assessing Metals Bioavailability, contained in the following volume, provides more in-depth technical information for those professionals involved in designing and performing bioavailability studies. The *Technical Background Document* includes guidelines on the types of studies that need to be performed and methods for collecting data necessary to assess bioavailability with specific considerations for individual metals. Standard operating procedures (SOPs) and suggested protocols for the recommended studies are provided as appendices so that a user can readily access this information.

2.0 WHAT BIOAVAILABILITY IS AND HOW IT IS USED IN RISK ASSESSMENT

This section defines bioavailability and related concepts, discusses the significant factors that affect the form, distribution, and mobility of metals in soil and sediments, and discusses how quantitative measures of bioavailability can be incorporated into human and ecological risk assessments (Section 4.0 provides a more detailed discussion of how bioavailability is measured).

2.1 Definitions and Concepts

For animals, bioavailability is defined as the extent to which a substance can be absorbed and reach the systemic circulation. For environmental risk assessments involving soil and sediment, this definition implicitly includes the extent to which a substance can desorb, dissolve, or otherwise dissociate from the environmental medium in which it occurs to become available for absorption. For incorporation into a risk assessment, bioavailability must be quantified much like any other parameter in a risk calculation. Thus, it is also useful to define bioavailability in the context of how it is measured.

2.1.1 Human Health Risk Assessment

For human health risk assessment, absolute bioavailability and relative bioavailability are two important and separate measures. ***Absolute bioavailability*** is the fraction or percentage of a compound that is ingested, inhaled, or applied on the skin surface that is actually absorbed and reaches the systemic circulation (Hrudey *et al.*, 1996). Absolute bioavailability can be defined as the ratio of an absorbed dose to an administered dose:

$$\text{Absolute Bioavailability} = \frac{\text{absorbed dose}}{\text{administered dose}} \times 100 \qquad (2\text{-}1)$$

For studies of absolute bioavailability, the absorbed dose often is determined by measuring the concentration of the compound in blood over time or by measuring the mass of the compound in such excreta as urine, feces, or exhaled air. Internal (i.e., absorbed) doses are useful for characterizing risk if toxicity factors describing the dose-response relationship (i.e., reference dose [RfD], or cancer slope factor [CSF]) are based on an absorbed dose (Figure 2-1). However, because toxicity parameters are generally based on an administered dose rather than an absorbed dose, it is usually not necessary to determine the absolute bioavailability of a contaminant for use in human health risk assessments.

Relative bioavailability is a measure of the difference in extent of absorption among two or more forms of the same chemical (e.g., lead carbonate vs. lead acetate), or different vehicles (e.g., food, soil, and/or water). Relative bioavailability is important for environmental studies because matrix effects can substantially decrease the bioavailability of a soil- or sediment-bound metal compared to the form of the metal and dosing medium used in the critical toxicity study. In the context of environmental risk assessment, relative bioavailability is the ratio of the absorbed fraction from the exposure medium in the risk assessment (e.g., soil) to the absorbed fraction from the dosing medium used in the critical toxicity study:

$$\text{Relative Bioavailability} = \frac{\text{absorbed fraction from soil}}{\text{absorbed fraction from dosing medium used in toxicity study}} \times 100 \qquad (2\text{-}2)$$

Relative bioavailability expressed in this manner has been termed the relative absorption fraction (RAF). Incorporation of relative bioavailability (i.e., the RAF) into an exposure assessment results in an

improved estimate of the external (i.e., administered) dose (Figure 2-1). It is appropriate to combine the adjusted external dose with toxicity parameters based on an administered dose when characterizing risk.

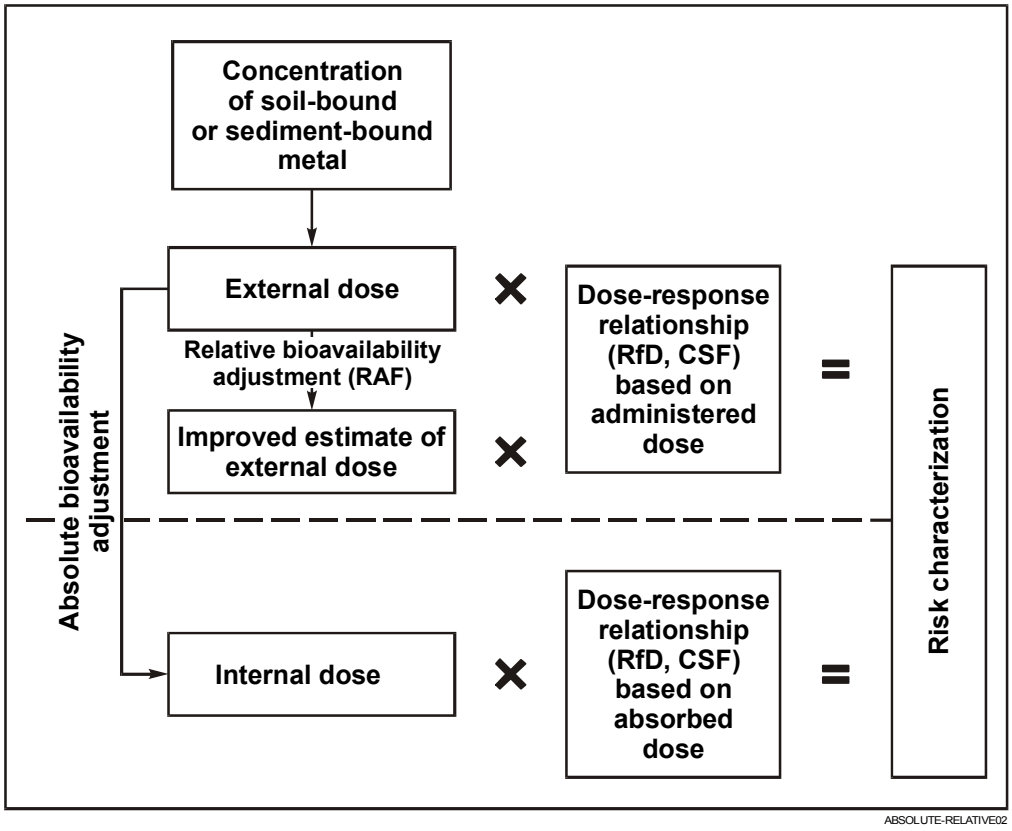


Figure 2-1. Relationship Between Absolute and Relative Bioavailability and Type of Dose for Risk Assessment

The RAF can be calculated using Equation 2-2 when the absolute bioavailability of a chemical is known for both the dosing medium and the exposure medium. However, as this is seldom the case, a more practical approach is to determine the RAF experimentally with animal (*in vivo*) studies or laboratory (*in vitro*) studies without measuring absolute absorption from either the exposure medium or the dosing medium. For example, relative bioavailability can be determined by comparing the accumulation of a compound in a specific target tissue when the compound is administered in soil to the accumulation in the same target tissue when the compound is given in the dosing medium used in the toxicity study.

2.1.2 Ecological Risk Assessment

The uptake by plants and animals of metals from soils, sediments, and water is a complex, dynamic process that involves all levels of the ecological food web. Thus, ecological risk assessment is more complicated than human health risk assessment. Plants and animals absorb metals from soils, sediments, and water by contact with external surfaces; ingestion of contaminated soil, sediment, or water; and inhalation of vapor-phase metals or airborne particles (Brown and Neff, 1993, U.S. EPA, 1998f)). In addition, animals may absorb metals from their food. Metal intake may occur through one of these routes of exposure, or through multiple routes functioning either simultaneously or intermittently. A fish, for

example, can absorb a metal directly from environmental media through its gills and skin, or through incidental ingestion of sediment; however, it also may ingest and ultimately absorb contaminants through consumption of food (Campbell *et al.*, 1988, U.S. EPA, 2002a). Each of these processes involves a different mechanism and, therefore, a different measure of bioavailability.

For ecological evaluations, site-specific bioavailability can be assessed on several levels:

- Evaluation of chemical and physical parameters of soil/sediment, including both general characteristics and specific forms and associations of metals bound to solids

- Measurement of the available fraction of metals present in the environmental media (i.e., sediment or soil) vs. measures of bulk metal concentrations yields an indication of the potential bioavailability

- Site-specific measurements of tissue concentrations in receptor and prey organisms in combination with soil data provide a measure of bioaccumulation and an integrated measure of relative bioavailability by all exposure routes

- Site-specific toxicity tests also provide an integrated measure of relative bioavailability by all exposure routes

- Studies of uptake from ingestion of food may yield relative bioavailability estimates that are particularly useful in modifying TRVs for upper trophic level receptors.

These approaches are described below and illustrated in Figure 2-2. Because of the complexity of the mechanisms associated with bioavailability in the ecological food web, site-specific factors must be considered prior to incorporating bioavailability adjustments into an ecological risk assessment. Specifically, data evaluated during the planning phase (i.e., problem formulation as defined by the U.S. Environmental Protection Agency [U.S. EPA], 1998f) should be reviewed to determine the relevant exposure pathways and ecological receptors of concern at the site.

Soil/Sediment Characterization and Measurement of the Available Fraction in Environmental Media. Metals present in sediments or soils can result in toxicity to organisms directly exposed to them. However, site-specific chemical and physical conditions greatly influence the form in which metals occur in the environment and thus the degree to which they are sorbed to sediments and soils (NRC, 2002). Therefore, evaluating the total metal concentration alone does not accurately reflect the fraction biologically available to aquatic and terrestrial organisms. Use of total concentrations as exposure point concentrations (EPCs) in an ecological risk assessment may overestimate actual exposures. Consideration of qualitative and quantitative evidence related to the physical and chemical conditions of a site can assist in determining what portion of the total measured concentration is actually available to organisms exposed. This information provides a better indication of the actual toxicity associated with metals at a site and may help determine which chemicals and/or sampling locations should be included for evaluation in the assessment.

Measurement of Tissue Concentrations and Bioaccumulation. The relative bioavailability of metals present in soil and sediment may be assessed by measuring metal tissue concentrations in receptor and prey organisms, and by determining the bioaccumulation of the metals (U.S. EPA 2002a).

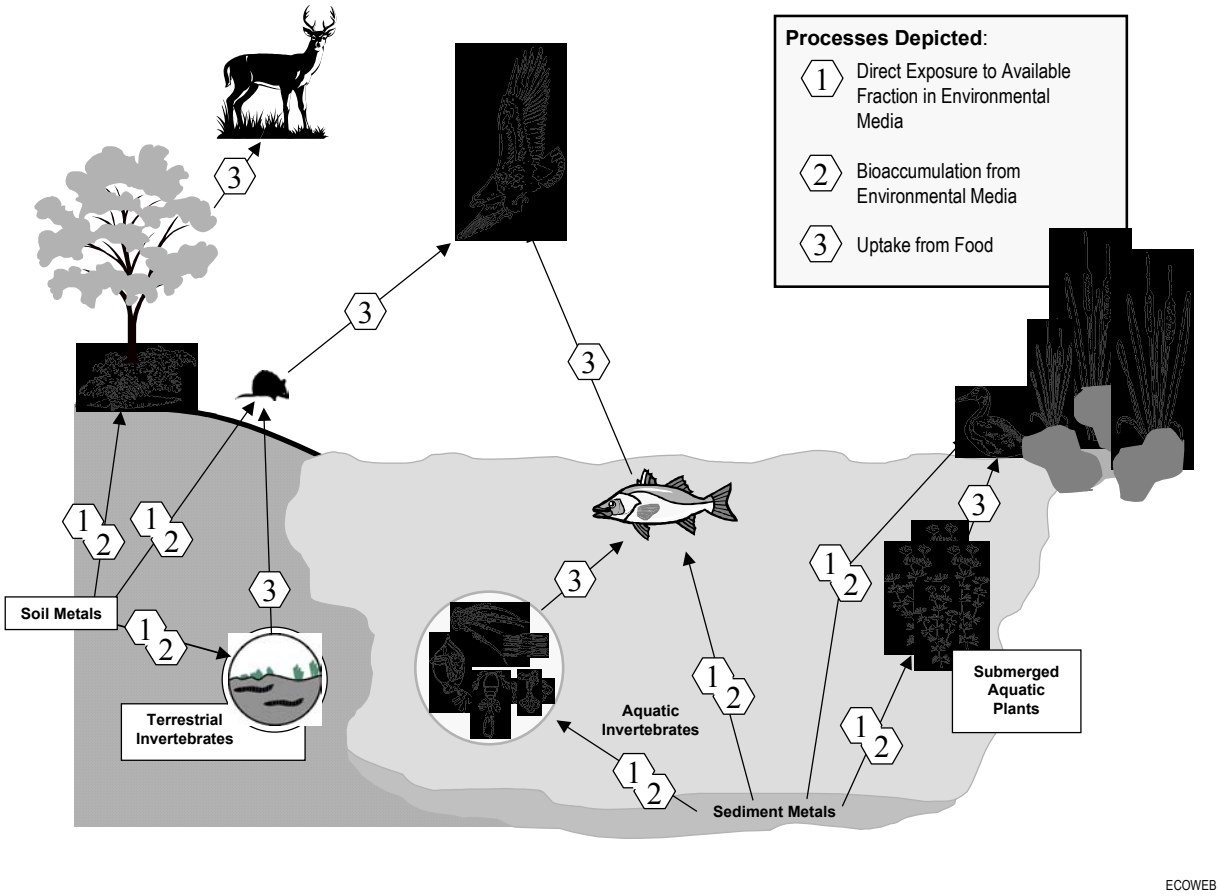


Figure 2-2. Illustration of Bioavailability Processes in the Ecological Food Web

Bioaccumulation is the uptake and retention of a chemical from any one or a combination of possible external sources. Measurement of tissue concentrations provides an estimate of the potential for trophic transfer (i.e., movement of chemicals through the food chain), as well as helping to assess the relative bioavailability of metals in soil or sediment.

Toxicity Tests. The results of site-specific toxicity studies inherently include the effects of variations in bioavailability of the metals being tested. Bioavailability is not quantified from such studies, but properly conducted studies can greatly increase the accuracy of ecological risk assessments by using site-specific data that reflects the bioavailability of metals in site exposure media. These tests are generally limited to lower trophic levels due to practical difficulties in testing larger receptors.

Uptake from Food. Terrestrial, freshwater, and marine animals are able to accumulate most bioavailable forms of metals from their food. When an animal consumes a lower trophic organism, any metals that have accumulated in the tissues of that organism can be transferred to the consumer (i.e., through trophic transfer). This process occurs primarily or exclusively in the unique environment of the gut of the consumer. Metals that are sorbed or bound to the tissues of a food item and are introduced into the gut of the consumer may be desorbed from the food, dissolved in the gut fluids during digestion, and then partitioned from the gut fluids across the gut lining into the tissues of the consumer. As with uptake directly from soils or sediment, the amount of metal desorbed from the food (i.e., the bioavailable fraction) may be dependent on a number of chemical factors (e.g., chemical form, pH). An additional

consideration is that certain metals may become concentrated as the prey is consumed (i.e., biomagnification). Consideration of qualitative and quantitative evidence related to the physical and chemical conditions associated with ingestion and absorption can assist in determining what portion of the total measured concentration is actually available to the organisms exposed. This information may help determine which chemicals and/or sampling locations should be included for evaluation in the ecological risk assessment

2.2 Site-Specific Factors Influencing the Bioavailability of Metals

Changes in the bioavailability of an environmental contaminant are largely a function of environmental processes that act on the contaminant to increase or decrease its mobility, thereby making it more or less accessible to the receptor organism. However, physiological factors within the receptor organism, such as acidic gastric juices in the gastrointestinal tract, may also increase the availability of a soil- or sediment-bound contaminant that would otherwise have limited availability under ambient environmental conditions. Thus, for the oral exposure route, there is not an obvious correlation between environmental mobility and bioavailability, so it is important that oral bioavailability studies mimic the physiological conditions under which absorption occurs. For other exposure routes (i.e., dermal absorption, inhalation, and plant uptake), the factors controlling the mobility of the contaminant in the environment also greatly influence the contaminant's bioavailability. The processes that affect the fate of a metal in soil and sediment systems are briefly described below. More detailed discussions are provided in NRC (2002), U.S. EPA (2000a), and U.S. EPA (2000b).

2.2.1 Factors Influencing the Bioavailability of Metals in Terrestrial (Soil) Environments

Metals can occur in the soil environment in both the solid phase and the aqueous (i.e., soil solution) phase. In solution, metals can exist either as free ions or as various complexes associated with organic (i.e., functional groups such as carboxyl and phenolic) or inorganic (e.g., anions such as OH^-, CO_3^{-2}, SO_4^{-2}, NO_3^-, or Cl^-) ligands. In the solid phase, metal ions either can be retained on organic and inorganic soil components by various sorption mechanisms (e.g., ion exchange or surface complexation), or can exist as minerals or be co-precipitated with other minerals (e.g., carbonates) in the soil. Ions in solution generally are more available for a variety of processes, including plant uptake and transport; however, metal ions in the solid phase may become available if environmental conditions change (NRC, 2002).

Dissolution and ***precipitation*** are the chemical reactions that determine the availability of inorganic *mineral* components of soils. Because most soils are under saturated with respect to their inorganic mineral components, the minerals undergo continuous dissolution; and, dissolution kinetics is the major factor controlling the availability of mineral-derived metal ions. Some of the more common mineral forms occurring in soils for the metals reviewed in this document are listed in Table 2-1.

The extent to which these mineral species occur in a particular soil and their solubility in various biological fluids (e.g., gastrointestinal tract fluid, sweat, or fluid in the alveoli of the lungs) determines the relative bioavailability of the various mineral species. In general, the elemental and sulfide forms of a metal are less soluble in biological fluids and hence less bioavailable than the oxide, hydroxide, carbonate, and sulfate forms of the same metal. However, notable exceptions to this rule of thumb exist, such as the following: the elevated pulmonary and dermal bioavailability of elemental mercury; the low solubility of nickel oxides (in the range of nickel sulfide); and the low solubility of chromium hydroxide, the most prevalent form of natural chromium in soils. At contaminated sites the mineral forms present may reflect the mineral forms used in site operations.

Table 2-1. Possible Mineral Species Controlling Soil Solution for Trace Elements (from Hayes and Traina, 1998)

	Aerobic Soils[(a)]	Anaerobic Soils[(b)]
Arsenic	$Ca_3(AsO_4)_2$, $Mg_3(AsO_4)_2$, As_2O_5	As, As_2S_3
Cadmium	$Cd(OH)_2$, $CdCO_3$	Cd, CdS
Chromium	$Cr(OH)_3$ (low to neutral pH)	$Cr(OH)_3$
Lead	PbO, $PbCO_3$, $Pb_3(CO_3)(OH)_2$	Pb, PbS
Mercury	$HgCl_2$, HgO, $Hg(OH)_2$	Hg, HgS
Nickel	NiO, $NiCO_3$, $Ni(OH)_2$	Ni, NiS

(a) Well-drained soils in upland settings (most soils fall into this category).
(b) Seasonally flooded or wetland soils.

In solution, metals can combine with dissolved organic and inorganic ligands to form complex ions. Examples of such complexes include methylmecury (CH_3Hg^+), cadmium chloride ($CdCl^-$), and lead bicarbonate ($PbHCO_3^+$). In general, metals will complex with the most common anions present in soil solution (i.e., inorganic anious such as SO_4^{-2}, NO_3^-, CO_3^{-2}, HCO_3^-, Cl^-, OH^-; and organic anions such as COO^-). Some metals, such as arsenic and chromium, combine with oxygen to form oxyanions that serve as ligands that can complex with other metals. Arsenite (AsO_3^{-3}), arsenate (AsO_4^{-3}), and chromate (CrO_4^{-2}) are the oxyanions of these metals. The formation of solution complexes can have a significant effect on the mobility of trace metals in soil. For example, trace metals that form chloro-complexes (e.g., $CdCl^-$) are weakly sorbed and thus likely to be more susceptible to leaching and plant uptake. Although it is likely that different dissolved forms of the same metal will have different absorption efficiencies, it is generally assumed that compounds in the dissolved phase can be completely absorbed regardless of the dissolved species. Therefore, it is generally not necessary to distinguish the dissolved forms of a metal in soil solution for a bioavailability study.

Sorption is an important process because it retains ions on the soil and limits their availability in the soil solution. Sorbed compounds can occur as *surface complexed* (i.e., adsorbed); or, if the density of surface complexes is great enough, as a *surface precipitate* or *cluster* (i.e., a three-dimensional growth on the surface of a soil particle). There is a continuum between surface complexation (adsorption) and surface precipitation such that as the amount of metal coverage increases, surface complexation followed by surface precipitation is the predominant sorption mechanism. The formation of surface complexes (i.e., adsorption) of metals occurs on clay minerals, metal oxides (i.e., hydrous oxides, hydroxides, and oxyhydroxides of iron, manganese, and aluminum), amorphous materials, and organic matter. These soil components contain *surface functional groups* (i.e., molecular units such as hydroxyl, carbonyl, carboxyl, and phenol) that can acquire either a positive or a negative charge, depending on the pH of the soil. Surface complexes can be weakly held (referred to as outer sphere complexes) or more tightly held (referred to as inner sphere complexes) to the soil. Outer sphere complexation is usually a reversible process (i.e., sorption and desorption are identical), whereas inner sphere complexation is often not reversible (i.e., the amount of material desorbed from a soil is less than the amount adsorbed). The non-reversible nature of sorption has been observed for contaminants that have been in contact with the soil for some time, thereby indicating that aged contaminants tend to be less bioavailable than fresh contaminants.

Ion exchange is another type of sorption reaction; however, it is distinguished from the other sorption reactions because it occurs mainly at "fixed charge" sites (i.e., the charge is permanent, not pH dependent) of clay minerals that have undergone isomorphic substitution (i.e., replacement of cations in the clay mineral lattice with other cations of lower charge). Soils with significant negative charge have a

high cation exchange capacity (CEC) and low cation mobility. Soils high in clay typically have the highest CEC.

Oxidation-reduction reactions involve the transfer of electrons from one compound to another, resulting in a change in the oxidation state of the compounds involved. The ability of metals to exist in multiple oxidation states is an important property that affects their form and distribution in soils. The most common oxidation states of the soil metals reviewed in this document are as follows: As (III, V), Cd (II), Cr (III, VI), Hg (II), Pb (II), and Ni (II) (copper, tin, and zinc are reviewed in aquatic settings, see Section 2.2.2). Of these metals, only chromium and arsenic are "redox active" (i.e., susceptible to oxidation/reduction reactions) in soil systems. Arsenic exists as As (III) under low redox (i.e., reducing) conditions and as As (V) under high redox (i.e., oxidizing) conditions. Chromium occurs as Cr (III) in most soils under ambient conditions and as Cr (VI) only under highly oxidizing conditions.

In summary, soil conditions that tend to promote precipitation or sorption also tend to reduce the mobility and bioavailability of metals. Thus, the metals that tend to be the most mobile and bioavailable are either those that form weak outer sphere complexes with organic or inorganic (clay, metal oxides) soil components, or those that complex with ligands in solution and are not sorbed. Conversely, metals that form inner-sphere complexes are much less likely to desorb and thus are less mobile and less bioavailable. However, in the presence of dissolved organic carbon, the mobility and bioavailability of metals that form inner-sphere complexes may be higher than expected based on sorption behavior, because these metals tend to also form strong soluble complexes. The relative mobility of the metals reviewed in this document is summarized on Table 2-2.

Table 2-2. Relative Mobility of Selected Metals in Soil (from Hayes and Traina, 1998)

Metal	Most Common Oxidation States in Soil[(a)]	Predominant Forms and Distribution in Soil Systems	Mobility
Arsenic	III	Oxyanion; sorbs more weakly than As(V) to metal oxides and only at higher pH	Moderate
	V	Oxyanion; sorbs strongly to metal oxides; forms relatively insoluble precipitates with iron	Low
Cadmium	II	Cation; sorbs moderately to metal oxides and clays; forms insoluble carbonate and sulfide precipitates	Low to Moderate
Chromium	III	Cation; sorbs strongly to metal oxides and clays; forms insoluble metal oxide precipitates	Low
	VI	Oxyanion; sorbs moderately to metal oxides at low pH, weaker sorption at high pH	Moderate to High
Lead	II (IV)	Cation; sorbs strongly to humus, metal oxides, and clays; forms insoluble metal oxides and sulfides; forms soluble complexes at high pH	Low
Mercury	II (O-I)	Cation; sorbs moderately to metal oxides, and clays at high pH; relatively high hydroxide solubility; forms volatile organic compounds	Low
Nickel	II (III)	Cation; sorbs strongly to humus, metal oxides, and clays; forms insoluble metal oxides and sulfides; forms soluble complexes at high pH	Low

(a) Possible, but less common, oxidation states in soil systems are shown in parentheses; these forms are not discussed.

2.2.2 Factors Influencing the Bioavailability of Metals in Aquatic (Sediment) Environments

Metals are found in all sediments; however, a large amount of the total metals in most sediment is in a residual fraction as part of the natural minerals that make up the sediment particles. The remaining metals in sediments are adsorbed to or complexed with various sediment components. The bioavailability of these metals to benthic organisms and other receptors is influenced by three categories of factors, including physical, chemical and biological factors (Table 2-3), which are summarized by U.S. EPA (2000a).

Table 2-3. Summary of Factors Influencing Bioavailability of Sediment-Associated Chemicals (from U.S. EPA, 2000a)

Physical Factors	Chemical Factors	Biological Factors
- Rate of mixing	- AVS concentrations for Cu, Cd, Pb, Ni, Zn	- Biotransformation
- Rate of sedimentation	- Redox conditions	- Bioturbation
- Diffusion	- pH	- Organism size/age
- Resuspension	- Interstitial water hardness	- Lipid content
	- Sediment organic carbon content	- Gender
	- Dissolved organic carbon content	- Organism behavior
	- Organic matter characteristics	- Diet, including sediment ingestion, feeding mechanism
	- Equilibration time with sediment	- Organism response to physicochemical conditions

Physical factors. Bioavailability of chemicals to benthic organisms and bottom feeders is influenced by the concentration profile of chemicals within sediment, and the concentration profile is, in turn, influenced by physical factors such as rate of sedimentation, turbulence and bioturbation. The concentration profile will control the likelihood of the receptor coming in contact with the chemical, in addition to influencing bioavailability. Resuspension and diffusion are factors that also affect both receptor contact with chemicals and bioavailability of the chemicals.

Chemical factors. As described above, bioavailability of metals in sediments is very closely tied to the amount of sediment-associated metal that is dissolved in interstitial pore water. Methods for determination of simultaneously extracted metals (SEM) and the role of acid volatile sulfide (AVS) in estimating the bioavailable fraction of metals are described in section 2.3.2 below. In oxidized sediments, trace metals may be adsorbed to clay particles, iron, manganese, and aluminum oxide coatings on clay particles, or dissolved and particulate organic matter (Table 2-4). As the concentration of oxygen in sediment decreases, usually due to microbial degradation of organic matter, the metal oxide coatings begin to dissolve, releasing adsorbed metals. In oxygen-deficient sediments, many metals react with sulfide produced by bacteria and fungi to form insoluble metal sulfides. Metals may be released from sorbed or complexed phases into sediment pore water in ionic, bioavailable forms during changes in oxidation/reduction potential and pH. Microbial degradation of organic matter also may release adsorbed

metals to pore water. Certain bacteria are able to methylate some metals, such as mercury, arsenic, and lead, to organic species that are more bioavailable than the inorganic forms. Methylation is a more important factor for the bioavailability of mercury than are AVS conditions (U.S. EPA, 2000a).

Table 2-4. Dominant Adsorbed or Complexed Phases of Metals in Oxic and Anoxic Sediments (from Brown and Neff, 1993)

Metal	Associations in Oxic Sediments	Associations in Anoxic Sediments
Arsenic	AsO_4^{-3}-Fe/MnO	As_2SO_3, AsS, FeAsS
Cadmium	Fe/MnO, OM/S, -CO_3	CdS
Chromium	OM, FeO	OM, $Cr(OH)_3$
Copper	OM, Fe/MnO	Cu_2S, CuS, FeCuS
Lead	Fe/MnO	PbS
Mercury	OM	HgS, OM
Nickel	Fe/MnO	OM/NiS, organic thiols
Tin[(a)]	TBT-Cl-OH-CO_3	TBT-S, OH, -CO_3
Zinc	Fe/MnO, OM	ZnOM/S

(a) Only butyltins are considered.
CO_3 = carbonates.
FeO = iron oxyhydroxides.
Fe/MnO = iron and manganese oxyhydroxides.
OM = organic matter.
S = sulfides (dominant species given).
TBT-Cl, OH, -CO_3, and -S = tributyltin chloride, hydroxide, carbonate, and sulfide.

Biological factors. As described above, bioaccumulation and toxicity of metals to a particular organism is a function of bioavailability, chemical metabolism and distribution, and elimination processes. Chemical conditions in the surrounding medium may alter these functions, for example, changes in temperature may alter food consumption rates while changes in dissolved oxygen concentrations may alter ventilation rates (U.S. EPA, 2000a). Bioturbation has been shown to increase bioavailability (NRC, 2002). Biodegradation and biotransformation will be decreased for chemicals strongly adhering to sediment particles. Organism behaviors, such as burrowing, and variations in diet composition will also influence bioavailability and organism contact with chemicals in sediment.

2.3 How Bioavailability is Incorporated into Risk Assessments

It is important to understand how bioavailability data can be used in human health and ecological risk assessments in order to better understand how this parameter should be quantified. Bioavailability is relevant to many aspects of the risk assessment process (e.g., exposure assessment, toxicity assessment); however, this document focuses on the use of site-specific bioavailability data to refine exposure estimates developed in a risk assessment. It should be recognized, however, that other aspects of bioavailability exist that are beyond the scope of this document (e.g., differences in bioavailability between humans and test animals, and variations in the bioavailability of a compound among human subpopulations).

2.3.1 Human Health Risk Assessment

This section illustrates how bioavailability measurements are incorporated into calculations of risk for the oral and dermal exposure pathways, and illustrates how a bioavailability adjustment affects the resulting risk estimates.

For the oral exposure route, relative absorption adjustments can be used to modify the exposure (i.e., intake) estimate (U.S. EPA, 1989). This is illustrated in the following risk equations for carcinogens and for noncarcinogenic effects, respectively, in which the RAF expresses the bioavailability of the soil-bound metal compared to the bioavailability of the metal form and dosing medium in the toxicity study from which the CSF or RfD was derived (i.e., $CSF_{administered}$ or $RfD_{administered}$):

$$\text{Risk} = (\text{Intake} \times \text{RAF}) \times \text{CSF}_{\text{administered}} \tag{2-3}$$

$$\text{Hazard Quotient} = \frac{(\text{Intake} \times \text{RAF})}{\text{RfD}_{\text{administered}}} \tag{2-4}$$

U.S. EPA risk assessment guidance has not routinely included the RAF term in risk calculations as shown in the above equations, although bioavailability adjustments are discussed in an appendix of U.S. EPA (1989) Thus, most risk assessments implicitly assume a default bioavailability of 1 for the oral pathway. The dermal bioavailability of chemicals in soil is expressed as an absorption fraction (ABS_{soil}) that is incorporated directly into the equation for calculating the dermally-absorbed dose (U.S. EPA, 1992, U.S. EPA, 2001a):

$$\text{DAD} = \frac{(C_{soil} \times \text{CF} \times \text{AF} \times \text{ABS}_{soil}) \times \text{EF} \times \text{ED} \times \text{EV} \times \text{SA}}{\text{BW} \times \text{AT}} \tag{2-5}$$

where,

DAD	=	dermally absorbed dose (mg/kg-d)
C_{soil}	=	total concentration in the soil (mg/kg)
CF	=	a conversion factor (10^{-6} kg/mg)
AF	=	soil-to-skin adherence factor (mg/cm^2-event)
ABS_{soil}	=	dermal absorption fraction (dimensionless)
EF	=	exposure frequency (events/year)
ED	=	exposure duration (year)
EV	=	soil contact event frequency (events/day)
SA	=	skin surface area available for contact (cm^2)
BW	=	body weight (kg)
AT	=	averaging time for exposure (days).

The factors in parentheses describe the absorbed dose per event, DA_{event} (mg/cm^2-event). The U.S. EPA (2001a) recommends specific default absorption fractions for a few chemicals, and the use of 10 percent as the default absorption value for semivolatile organic compounds. Among inorganics, default values are provided only for arsenic (3 percent) and cadmium (1 percent).

The dermally-absorbed dose is multiplied by the oral CSF or divided by the oral RfD, adjusted to an absorbed-dose basis, to calculate risks via the dermal pathway:

$$\text{Risk} = \text{DAD} \times (\text{CSF}_{\text{oral}} \text{ x } \text{GI}_{\text{ABS}}) \tag{2-6}$$

and

$$\text{Hazard Quotient} = \frac{\text{DAD}}{(\text{RfD}_{\text{oral}}/\text{GI}_{\text{ABS}})} \tag{2-7}$$

Adjustment of the toxicity factors is required because dermal exposures are expressed as an absorbed (i.e., internal) dose, whereas the toxicity factors are usually derived from orally administered doses. GI_{ABS} is the gastrointestinal absorption factor (dimensionless) that expresses the fraction of the orally administered metal in the toxicity study that was absorbed via the GI tract. The U.S. EPA recommends making adjustments to the toxicity factors only when there is evidence to indicate that the oral absorption in the critical study is significantly less than complete (i.e., <50 percent) (U.S. EPA, 2001a).

2.3.2 Ecological Risk Assessments

In the initial stages of the tiered risk assessment process, estimates of the available fraction of metals in sediment or soil may be limited to a qualitative evaluation of the site-specific chemical and physical parameters that control bioavailability. These data may provide a line-of-evidence argument for inclusion or exclusion of individual chemicals or sampling locations in the risk assessment. The specific parameters considered are discussed further in Section 2.2 and in Sections 5.0 and 6.0 of this document. As the investigation progresses through the tiered evaluation, more complex, quantitative approaches, such as specific analytical techniques or bioassays, may be considered. Section 2.1.2 describes general approaches that can be useful in evaluating site-specific bioavailability of metals to ecological receptors, i.e., assessment of soil chemical and physical characteristics and available fraction of chemicals, measurement of tissue concentrations in receptors and prey, bioaccumulation studies, and toxicity tests. The best approach to use in an ecological assessment may vary with particular receptors and exposure media being evaluated.

For soils, U.S. EPA (2000b) describes approaches for using site-specific information to support bioavailability-based adjustments to ecological soil screening levels (Eco-SSLs) for plants and invertebrates. The Eco-SSLs for these receptors were derived from studies selected to represent soils for which contaminants are more likely to be bioavailable. Therefore, if site conditions indicate that contaminants are likely to have reduced bioavailability in site soils, then literature values for comparable soils may be used to modify the Eco-SSLs. U.S. EPA (2000b) theoretically supports the assessment of available fraction of chemicals, but notes that generally accepted methods of measuring the available fraction is not available for metals in soil. In contrast, site-specific toxicity tests for plants and invertebrates are noted to be readily available and generally acceptable for use in modifying Eco-SSLs (U.S. EPA, 2000b). Ways in which factors that are influenced by bioavailability are incorporated into ecological risk assessments are summarized below.

Assessment of the Available Fraction in Sediments. For sediments analytical techniques, as described in Section 4.1.3, may be applied to quantify the specific concentrations of metals, defined as the simultaneously extracted metals (SEM), that are bioavailable (NRC, 2002, U.S. EPA, 2002a). Concentrations determined from these analytical techniques can be used as adjusted EPCs. For sediments, the estimates of the bioavailable concentration can be further modified based on evaluation of acid volatile sulfides (AVS). In the presence of AVS in sediments, certain metals, including copper, cadmium, lead, nickel, zinc (Ankley, 1996; Ankley *et al.*, 1996), and possibly arsenic and mercury (Luoma, 1989; Allen *et al.*, 1993; Ankley *et al.*, 1996; Neff, 1997a; Berry *et al.*, 1999), precipitate as their respective metal sulfides, which are not bioavailable (DiToro *et al.*, 1990). If the molar concentration of AVS in sediments is higher than the sum of the molar concentrations of these metals in the 1-Normal

hydrochloric acid (1-N HCl) extract (the SEM of the sediment), all of the metals are in non-bioavailable forms in the sediments. This relationship can be summarized in the following manner:

SEM:AVS > 1, metals are present in bioavailable forms

SEM:AVS < 1, metals are not likely to be bioavailable.

If the SEM:AVS>1, then these data can be used to calculate an EPC as discussed below. It is important to note that each of the metals evaluated has a different binding affinity for sulfides (NRC, 2002, U.S. EPA, 2002a). Currently there is considerable debate regarding the relative affinities of each of the metals (U.S. EPA, 2002a); however, typically it is assumed that at equilibrium, copper will preferentially react with AVS, displacing all other metals. If the available AVS is not completely saturated by copper, then the remaining metals will react in the following order: lead, cadmium, zinc, and nickel. In this model, the amount of copper in the sediment that is potentially bioavailable and toxic is defined as follows:

$$Cu_b = (Cu_{SEM} - AVS)*(MW_{cu}) \quad (2\text{-}8)$$

where,

Cu_b = concentration of copper that is bioavailable (mg/kg)
Cu_{SEM} = molar concentration of Cu as defined by simultaneous extraction (moles/kg)
AVS = molar concentration of AVS (moles/kg)
MW_{cu} = molecular weight of copper (mg/moles).

The bioavailable concentration of the other metals in sediment may be determined in the same manner, following the order described above. For each successive metal, the molar concentration of AVS applied should be decreased according to the molar concentration of the preceding chemical; when the concentration of AVS is zero, all remaining metals are assumed to be bioavailable. The metal concentrations derived in this manner can be used as EPCs. Issues related to the consideration of SEM:AVS in contaminated sediments are addressed in a number of recent publications (NRC, 2002, U.S. EPA, 2002a, U.S. EPA, 2000a).

Bioaccumulation and Toxicity Tests. Neither bioaccumulation nor toxicity is a direct measure of bioavailability; however, both will vary as a function of site-specific changes in bioavailability of metals and may be used to provide an indication of the relative bioavailability of metals in site soils or sediment. Generally, both bioaccumulation and toxicity will be lower at sites where metals have reduced bioavailability.

Uptake of sediment-bound or soil-bound metals by organisms (i.e., bioaccumulation) may be measured directly by collecting and analyzing the tissues of representative organisms (U.S. EPA, 2002a). In the initial stages of a risk assessment, estimates are typically derived according to the following equation:

$$C_t = C_s * BAF \quad (2\text{-}9)$$

where,

C_t = concentration in tissue (mg/kg)
C_s = concentration in sediment or soil (mg/kg)
BAF = bioaccumulation factor ([mg/kg_{tissue}] / [$mg/kg_{sed/soil}$]).

In the event that tissue-based toxicity reference values (TRVs) are available, C_t can be used to derive a hazard quotient (HQ) as defined by the equation:

$$HQ = \frac{C_t}{TRV} \tag{2-10}$$

In addition C_t can be used to represent the exposure point concentration for estimating ingested doses for upper trophic level species. For example:

$$Dose_{Ingested} = \frac{C_t * IR}{BW} \tag{2-11}$$

where,

IR = ingestion rate of receptor species (kg/day)
BW = Body weight of receptor species (kg).

BAF values, defined as the ratios of the concentration of the chemical in the tissues of the organism to the concentration of the chemical in sediment or soil, have been derived for various chemicals and species and are available in the literature. In the event that BAF values for relevant chemicals or species are not available in the literature, they may be derived using tissue and soil or sediment data available in the literature or determined experimentally at the site. This relationship may not be valid for those metals that are essential trace nutrients for plants and animals. Additionally, a simple ratio is not applicable over a wide range of concentrations (U.S. EPA, 2002a). BAFs used to evaluate sediments must be based on the evaluation of concentrations in the range of interest either for estimating risk or for setting cleanup goals. For evaluation of risks to terrestrial receptors, U.S. EPA (2000b) acknowledges that regression models may be more appropriate than simple ratio-based BAFs.

Uptake from Food. For upper tropic level species, quantitative data also can be used to modify ingested doses for use in calculating risk estimates. These data would be incorporated as described for the noncarcinogenic human health risk assessment. For example, when evaluating exposures resulting from the ingestion of contaminated prey items, the following simplified equation may be used to determine the risk from food ingested by the ecological receptor:

$$Risk = (Intake \times ABS) / TRV \tag{2-12}$$

where,

Intake = ingested dose (mg/kg/day)
ABS = absorption factor (unitless)
TRV = toxicity reference value (mg/kg/day).

For screening-level evaluations, the ABS is typically assumed to be 1 (i.e., absorption from contaminated prey is assumed to be the same as absorption in the studies used to derive the TRV, i.e., typically a soluble metal form mixed with laboratory chow or drinking water). However, as the investigation progresses through the ecological risk assessment process, it may be possible to refine this value to reflect actual conditions either through a review of the relevant literature, or through bioassays as described for human health exposures.

3.0 WHEN IT IS APPROPRIATE TO CONDUCT A BIOAVAILABILITY STUDY

This section discusses a variety of considerations that RPMs should review when deciding if bioavailability studies would help in characterizing exposures during a site investigation. Approaches for incorporating such studies may into the risk assessment process are also discussed. Section 3.1 discusses where in both the human health and the ecological risk assessment processes it is appropriate to conduct a bioavailability study. Section 3.2 outlines several situations where bioavailability might offer an appropriate solution to a given remediation problem, and Section 3.3 discusses factors that affect whether a bioavailability study is worthwhile for a particular site.

3.1 The Role of Bioavailability in Tiered Risk Assessment Processes

U.S. EPA, many states and DoD have applied tiers to the risk assessment process for assessing human and ecological risks (see Figures 3-1 and 3-2). This section briefly discusses the major steps in tiered risk-assessment processes followed by U.S. EPA and the DoD, and where it is appropriate to conduct a study to support a site-specific bioavailability adjustment. Most tiered processes incorporate a minimum of three tiers, an initial screening level evaluation to identify chemicals of potential concern (COPCs) and areas needing further evaluation, a detailed site-specific assessment, and an assessment of residual risks after remediation or a more complex site-specific assessment. Although site-specific bioavailability is most often considered during a site-specific risk assessment, such data may be considered at the other tiers as well.

3.1.1 Human Health Risk Assessment

U.S. EPA has issued a series of guidance documents (*Risk Assessment Guidance for Superfund)* that constitute a tiered process for human health risk assessment. U.S. EPA (1989) provides baseline risk assessment guidance, U.S. EPA (1991a) describes development of risk-based preliminary remediation goals, and U.S. EPA (1991b) provides guidance for risk evaluation of remedial alternatives.

In addition, in 1996, U.S. EPA released *Soil Screening Guidance* (1996a) that provides a methodology to calculate risk-based, site-specific, soil screening levels (SSLs). These SSLs were described as assisting in the process of identifying and defining areas, contaminants, and conditions at a particular site that do not require further Federal action. The application of these SSLs has been limited; however, due to the fact that they apply only to residential land use, and because only ingestion and inhalation exposures were considered. Additionally the generic SSLs were not regularly updated to reflect changes in underlying toxicity values and other parameters.

A supplement to the SSL guidance addresses many of these issues, adding dermal exposures and industrial/commercial land use (U.S. EPA 2002d). The SSL guidance does allow for modification of the generic SSLs using site-specific data. The supplement does not include oral and dermal absorption in the specified list of site-specific parameters, but these parameters can still be discussed with site managers.

Sources of generic screening levels that are updated at least annually include the U.S. EPA Region III risk-based concentrations (RBCs) (U.S. EPA, 2002b) and the U.S. EPA Region IX preliminary remediation goals (PRGs) (U.S. EPA, 2002c). The Region III RBCs and Region IX PRGs are updated as new toxicity and physico-chemical data become available, but these values are not typically modified using site-specific data. The screening levels that will be applied at a site should be identified early in the site evaluation process.

Whenever a risk assessment process allows for the use of site-specific data, it is possible to incorporate bioavailability adjustments. Figure 3-1 illustrates a three-tiered human health risk assessment process, as applied by the Navy (Department of the Navy, 2001). Other DoD Departments follow similar sequential procedures, although tiers may not be identified (e.g., see USACE, 1999 and AFCEE, 2002). Bioavailability data are most commonly considered during the baseline risk assessment (BRA). The first phase or tier is a risk-based screening step in which site concentrations are compared to generic or site-specific risk-based screening levels. Bioavailability data are not incorporated into the generic screening values because the generic values are based on conservative default exposure assumptions designed to provide screening levels protective of most sites across the country. However, as noted above, the SSL guidance does provide for incorporation of site-specific data into modified SSLs and an argument may be made for including site-specific bioavailability data.

This step is described in the Navy process, where if site concentrations exceed the generic screening values, site-specific screening levels (SSSLs) are calculated in Tier IB and compared to site concentrations (Figure 3-1). SSSLs differ from the generic screening levels in that physical properties of the site are incorporated into the SSSL calculations in place of default values inherent in the generic "look-up" values. In addition, whereas generic screening levels are available for only specific exposure scenarios (typically ingestion, dermal contact, inhalation of vapors and particulates), SSSLs can be developed for other relevant pathways (e.g., food ingestion, vapor intrusion to buildings) or to take into account indirect exposure scenarios (i.e., when receptors are exposed to contaminants that are transported from the source to other exposure media such as groundwater or air). Because the Tier I SSSLs are calculated values rather than "look-up" values, Tier IB provides an opportunity for the incorporation of bioavailability data. Several resources are available for developing SSSLs, including Part B of the U.S. EPA's *Risk Assessment Guidance for Superfund* (*RAGS*) document (U.S. EPA, 1991a), the *Soil Screening Guidance* (U.S. EPA, 1996a, U.S. EPA, 2002d)), and the American Society for Testing and Materials *Standard Guide for Risk-Based Corrective Action Applied at Petroleum Release Sites* (ASTM, 1995) and *Standard Provisional Guide for Risk-Based Corrective Action* (ASTM, 1998).

Under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), the risk-based screening step allows areas of the site with contaminant concentrations below the risk-based screening levels to be eliminated from further action; whereas, areas of the site with contaminant concentrations above the soil screening levels must undergo further assessment (U.S. EPA, 1994a, 1994b, and 1996a). Because the screening step provides a means for eliminating low-risk sites early in the CERCLA process, consideration should be given to conducting a bioavailability study (in the Navy's Tier IB) to support the calculation of realistic risk-based screening levels.

The second step in the human health risk assessment process (or Tier II for the Navy) involves conducting the BRA (Figure 3-1). The U.S. EPA's *RAGS* document (U.S. EPA, 1989) provides guidance on conducting a human health BRA. A BRA involves four basic steps: data collection and evaluation, exposure assessment, toxicity assessment, and risk characterization. As discussed in Section 2.3.1, bioavailability data can be incorporated in the BRA to adjust exposure estimates for key pathways (e.g., soil ingestion), or to extrapolate toxicity data from one route of exposure to another (e.g., GI absorption data are required to adjust oral toxicity factors to an absorbed-dose basis for calculating dermal risks). If bioavailability data are to be incorporated into the BRA, a site-specific bioavailability study is needed early in the BRA to provide the necessary data for making these adjustments. The results of the screening assessment can provide an early indication as to whether or not a bioavailability study might be necessary during the BRA, as this information is useful for identifying contaminants and exposure routes that present the highest risks for the site.

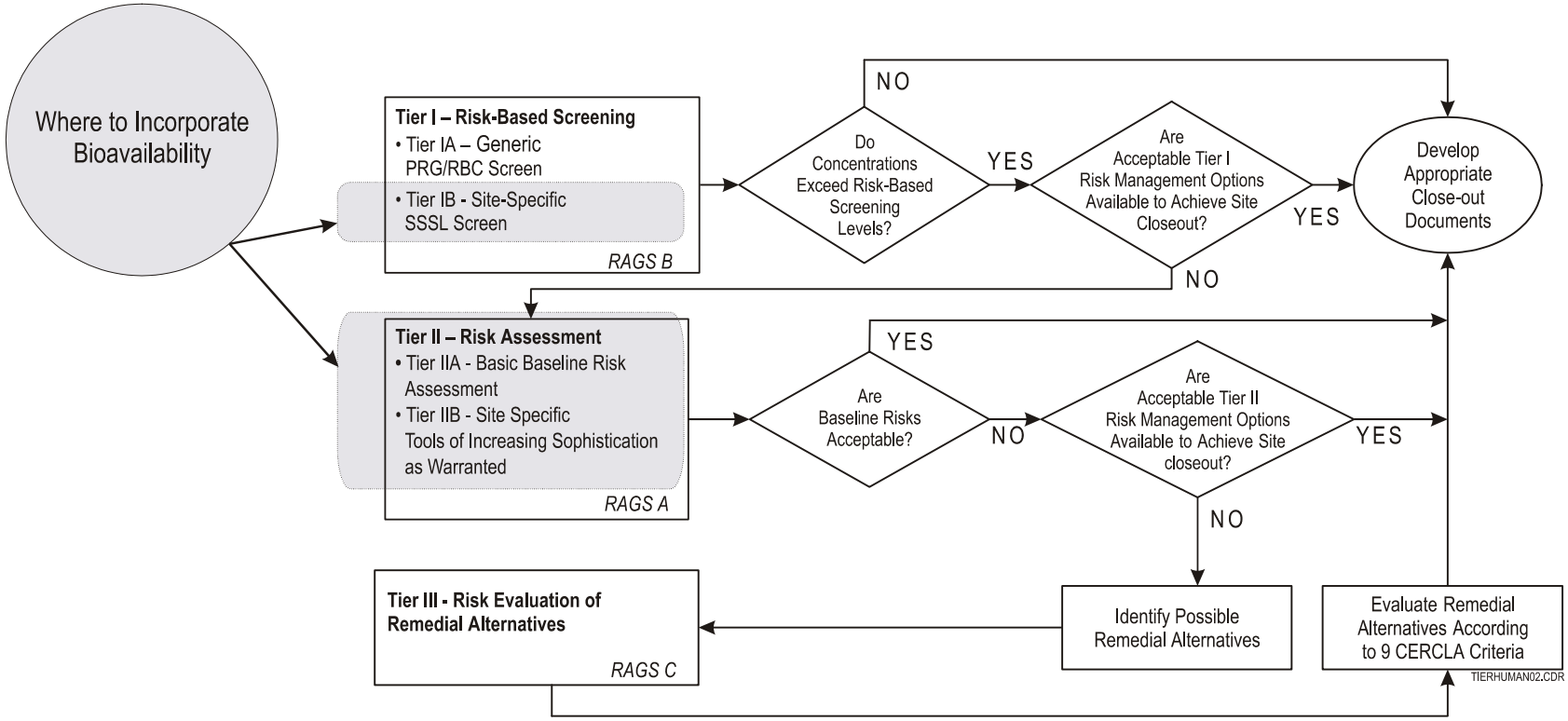


Figure 3-1. Incorporating Bioavailability in the Tiered Human Health Risk Assessment Process

The third step of the human health risk assessment process (the Navy's Tier III) involves an assessment of the risks associated with various remedial alternatives. Guidance for evaluating short-term and long-term risks associated with site remediation activities is provided in Part C of the U.S. EPA's *RAGS* document (U.S. EPA, 1991b). If these risks are assessed in a quantitative manner, incorporation of bioavailability data may also be appropriate in this phase of the risk assessment process.

3.1.2 Ecological Risk Assessment

U.S. EPA's ERA guidance (U.S. EPA, 1997) provides for an eight step process for designing and conducting ERAs. (Figure 3-2). The grouping of EPA's eight steps results in a tiered risk assessment process, with each tier including problem formulation, analysis, and risk characterization. The Tri-services have developed slightly different processes to comply with the U.S. EPA guidance, specifically regarding Tier 3. The Army and Air Force process is shown in Figure 3-2 (Simini, *et al.*, 2000) and the Navy process is shown in Figure 3-3 (Department of the Navy, 1999). The U.S. Army also provides detailed ecological risk assessment guidance (USACE, 1996), and the Air Force has more general guidance referring to U.S. EPA guidance (AFCEE, 2002).

The first tier is a screening risk assessment (SRA), a conservative, screening evaluation of the potential risks at the site based on literature searches and existing site data. Therefore, all chemicals are assumed to be as bioavailable as was the case for studies used to develop toxicity benchmarks used in such screening. All pathways are identified, and EPCs are determined for all relevant environmental media. Toxicity benchmarks are identified based on available water, sediment, and soil criteria. If the EPCs do not exceed the selected toxicity benchmarks, the site passes the SRA and is closed out for ecological concerns. If any of the EPCs exceed the selected toxicity benchmarks, the site proceeds to the second tier, or in the case of the Navy, may proceed to an interim cleanup.

As described in section 2.3.2, U.S. EPA (2000b) has issued draft guidance for the development of ecological soil screening levels (Eco-SSLs). While only a limited number of draft Eco-SSLs are currently available (see section 5.0 for description), as more values are developed they will greatly reduce the scope of literature searching required for evaluation of exposures for terrestrial receptors. In addition, the Eco-SSL guidance provides specific direction for the modification of default Eco-SSLs based on site-specific bioavailability analyses. The modified Eco-SSLs are intended for use in the Tier 2 baseline risk assessment.

Tier 2, the baseline ecological risk assessment (BERA), entails a more detailed approach incorporating site-specific exposure factors. In both the EPA and the Tri-service processes, bioavailability is considered during the exposure assessment (i.e., what the Navy refers to as Step 3a, the refinement of conservative exposure assumptions). Bioavailability considerations may be incorporated into the initial stages of Tier 2 in a number of ways, depending on the data, funding, and time available. For example, as a first effort, chemical and physical parameters, such as sediment and soil pH, total organic carbon (TOC), redox potential (Eh), specific form of the metal, SEM/AVS, can be evaluated. Evaluation of each of these factors provides qualitative information for use in a line-of-evidence approach to eliminating individual metals or the site from future consideration. Similarly, application of literature-based bioaccumulation factors or absorption fractions, if appropriate, can provide evidence demonstrating a lack of bioavailability. If, based on these refinements, evidence indicates that the site poses acceptable risks, then the site exits the ecological risk assessment process. Otherwise, the evaluation proceeds further into the baseline, which involves a more extensive evaluation of site-specific information.

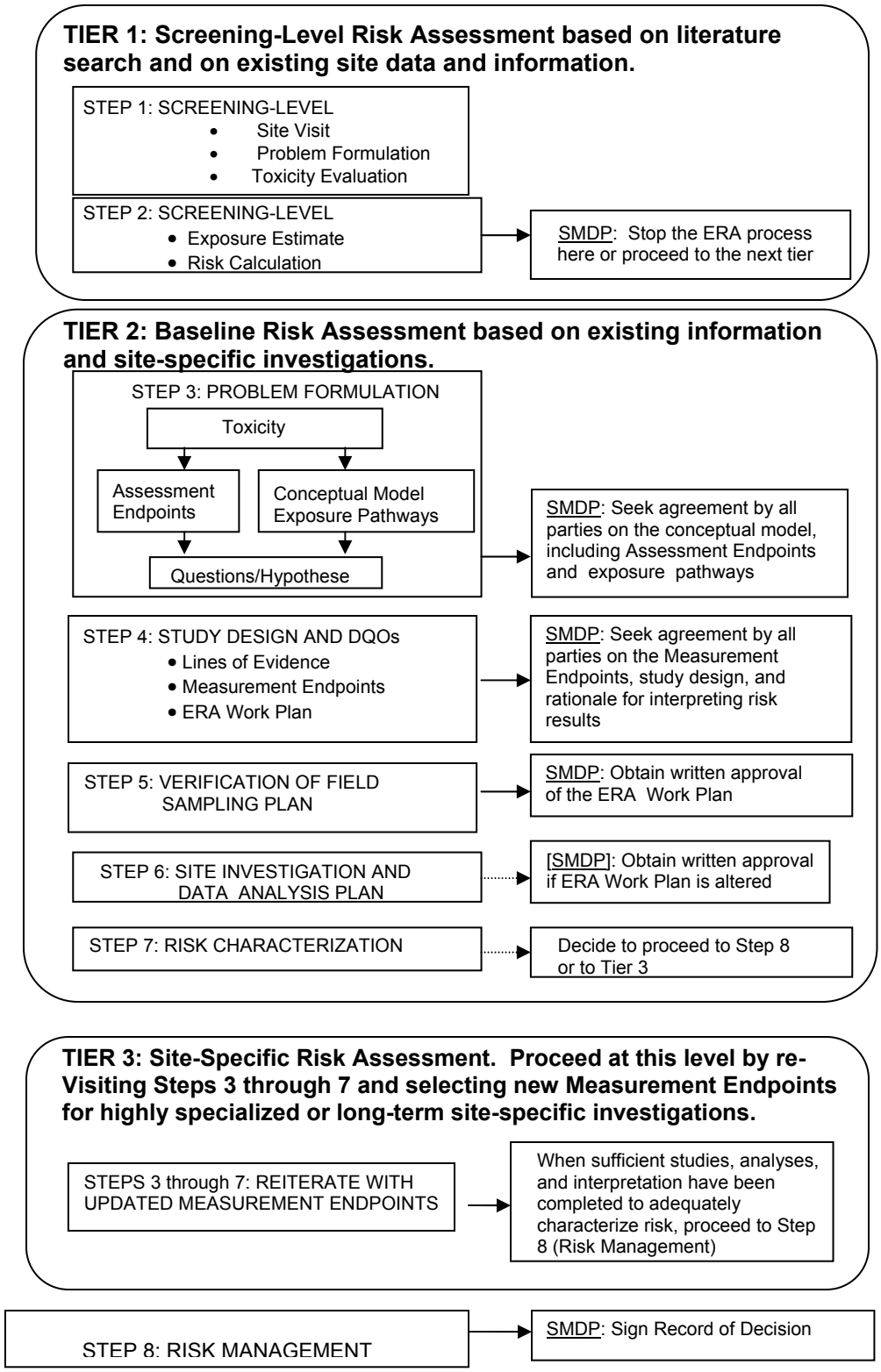
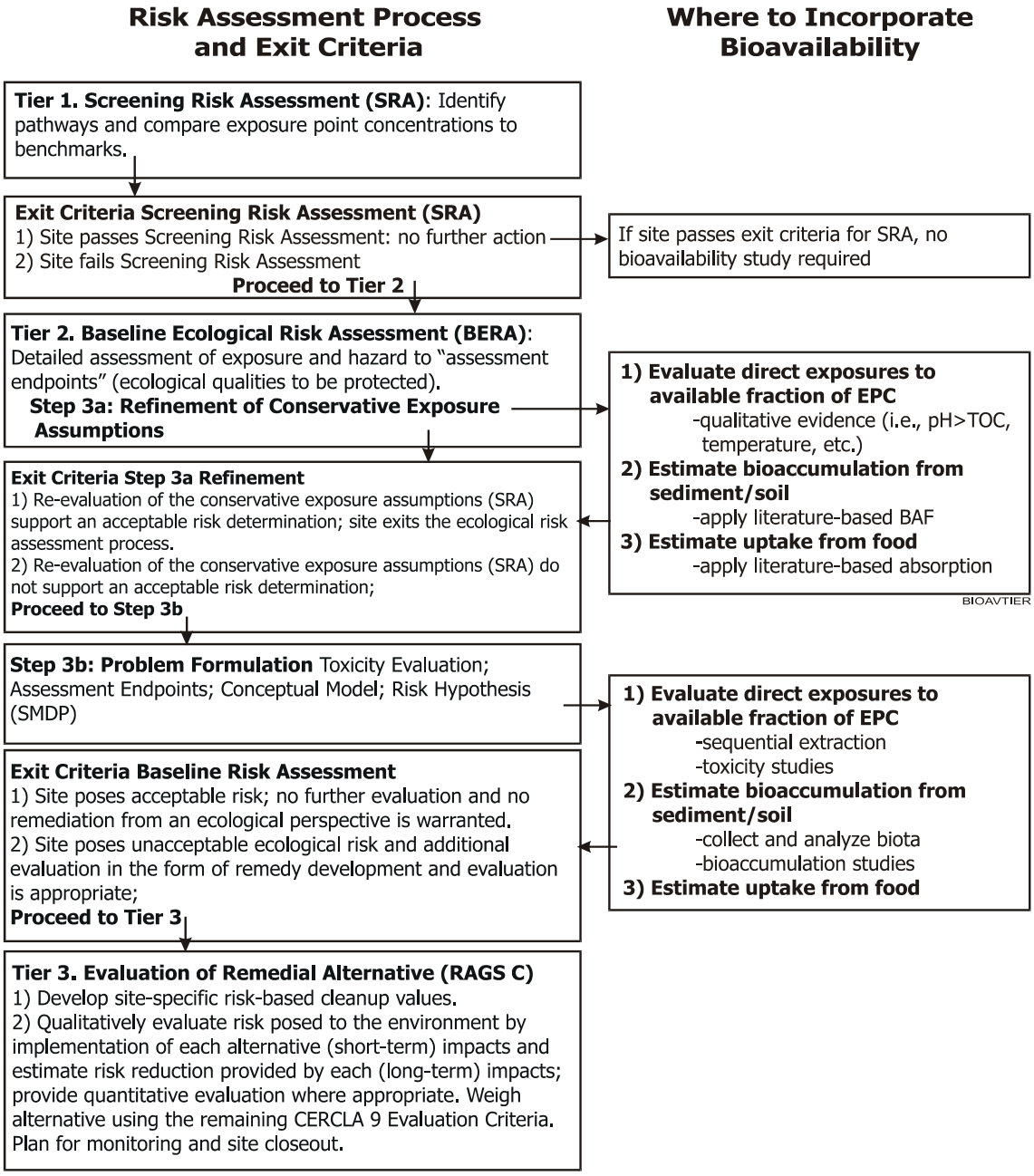


Figure 3-2. The Tri-Service Tiered ERA Process in Relation to EPA's Steps.



Note: Modified from the Navy Ecological Risk Assessment Tiered Approach (http://web.ead.anl.gov/ecorisk), which is based on the U.S. EPA's 8-Step Ecological Risk Assessment Process.

Figure 3-3. Incorporating Bioavailability in the Navy's Tiered Ecological Risk Assessment Process

In evaluating bioavailability, additional site-specific data may be collected, such as concentrations of metals in tissues of organisms from the site, or measurement of the bioavailable fraction in sediment or soil through sequential extraction techniques. In addition, site-specific bioassays such as bioaccumulation tests or relative bioavailability are considered. It is important to note that site-specific information collected previously should be carefully evaluated to determine the potential effectiveness of proceeding with these more complex and time-consuming bioassays. If determined to be appropriate, the results of these tests, combined with the data previously collected, can be evaluated to determine if the site poses acceptable risks. If the risks are determined to be acceptable, no further evaluation or remediation from an ecological perspective is required. If the risks are determined to be unacceptable, and additional evaluation is appropriate, the process proceeds to the third tier.

For the Army and Air Force, Tier 3 is a site-specific risk assessment that may involve revisiting Steps 3 through 7, and selecting new measurement endpoints for highly specialized or long-term site-specific investigations. Many of the site-specific investigations at this level will inherently assess bioavailability during long-term field studies. The focus of the Navy's Tier 3 is quite different, and involves an evaluation of remedial alternatives to develop site-specific, risk-based cleanup goals and to determine the appropriate remedial strategy. It should be noted that USEPA considers this evaluation to be part of the feasibility study. All site information collected during the assessment, including that pertaining to the potential for bioavailability, should be evaluated when considering the various remedial alternatives.

3.2 Site Factors Affecting the Usefulness of Bioavailability Studies

On a scientific basis, site-specific bioavailability studies will always be useful in characterizing exposures of human and ecological receptors at a site more accurately than is done using generic default assumptions. As a practical matter, such studies require time and resources, and the benefits will vary from site to site. A number of site factors determine whether bioavailability studies might help clarify if action is needed to reduce risks at a site. In some cases it may be clear that site-specific bioavailability studies are needed prior to conducting the baseline risk assessment, whereas in other cases the need for such studies may only be apparent after completion of a baseline risk assessment.

For example, when risk-based cleanup goals require extensive remediation it is likely to be particularly important to accurately characterize exposure and risks. Sites with large areas of elevated contaminant concentrations over much of the site that may not be technically feasible to remediate or that will cause great disruption of a community are likely to require the investment of greater resources in more detailed site investigations. In these cases, it may be appropriate to conduct site-specific bioavailability studies and revise the risk-based cleanup goals if the contaminant is less available than was assumed in the original risk assessment. Using a revised risk assessment, it may be possible to focus the remediation on those areas posing the greatest risk to receptors. At the Butte, MT Superfund site where mining activities had resulted in widespread lead contamination, bioavailability studies found that availability of lead from soil at the site was only 12 percent compared to the default assumption of 30 percent (Weis, *et al*., 1993). As a result, the cleanup goal for lead was increased from the default of 500 ppm to 1,200 ppm, and the scope of the cleanup was reduced. The reduction in the cleanup area also reduced disruption in the residential neighborhoods affected.

In some cases, the remediation activities required to achieve the cleanup goals for a site would have adverse impacts on the environment. Such impacts include habitat destruction, increased potential for erosion, or re-release of contaminants into other environmental media. At such sites, bioavailability studies may allow for habitat preservation without excessive residual risks. At the East Fork Poplar Creek site in Tennessee, mercury contamination was spread over 650 acres of the creek's forested watershed. Further study revealed that most of the mercury was in a form that has low bioavailability. This was confirmed by animal uptake and simulated human digestion studies. Cleanup goals based on

human health risks from contact with inorganic mercury in soil and sediment were adjusted from the original goal of 10 ppm mercury in soil to 400 ppm. Cleanup costs were cut from an estimated $1.2 billion to approximately $8 million, while leaving a large tract of wildlife habitat undisturbed (NEPI, 1998).

In other cases remediation may not be technically feasible due to either site conditions or the lack of an effective remediation technology to achieve the required cleanup goals. If the contaminants at the site are shown to be less bioavailable than was assumed in the initial risk assessment, the risk estimate might be decreased to an acceptable level requiring no cleanup or calculation of risk-based cleanup goals might yield higher goals that are feasible to achieve.

An RPM should consider the following factors when deciding whether a site-specific bioavailability study is likely to be beneficial for a site.

- **Nature of exposures and chemicals driving risk.** Critical exposure pathways and exposure routes vary among sites and by kinds of chemicals. For human health risk assessment, most bioavailability studies of metals in soil have focused on oral exposures because this is generally the most important human exposure route for metals in soil. In contrast, volatile organic chemicals typically have inhalation as the primary exposure route. Consequently, if the primary COPCs at a site are volatile organic chemicals, bioavailability studies may not be warranted.

- **Form of the chemical or the exposure medium for the site compared to the reference dose.** If the form of the chemical found at a site is different than the form used in the toxicity study on which the reference dose is based, then the bioavailability of that compound may be different and conducting a site-specific bioavailability study potentially could result in a significant reduction in risk. An example of this situation is when the form of metal used in a toxicity study is a very soluble form (as is often the case), and the form of metal found in soil has a low solubility. Also, if the exposure medium is different between the reference dose toxicity study and the site (e.g., reference dose was given in water while site exposure is to soil), the bioavailability at the site may be sufficiently different from that reported in the toxicity study to justify a bioavailability study. If the forms or exposure media are similar, then bioavailability is more likely to be similar and a bioavailability adjustment may not be worthwhile.

- **Potential for regulatory acceptance.** Although most regulatory policies allow for bioavailability adjustments, there is no requirement that these adjustments be considered or accepted by the regulators. Therefore, it is important to consider the regulatory climate for the site before undertaking a bioavailability study. The regulators for the site should be contacted to determine if they are receptive to the concept of a bioavailability adjustment. Also, it may be helpful to determine whether there are any precedents for approval of bioavailability adjustments by that agency.

- **Whether bioavailability studies can be completed within the required time frame for the site.** The time required for a bioavailability study can vary depending on the type of study required to collect the necessary data. Generally, simple *in vitro* (laboratory) tests require less time than *in vivo* (live animal) feeding studies. More detailed information on time required for various types of studies is provided in Section 4.3.

- **The cost of bioavailability testing compared to the cost of cleanup.** The cost of performing bioavailability studies and incorporating the results into risk assessment must be weighed against the cost of cleanup and the potential cost savings that could result from the bioavailability study.

Costs of bioavailability studies can vary substantially depending on what tests are done and who is selected to do them. Section 4.3 provides some rough guidelines on the costs of various types of studies.

- **Existing site data support a bioavailability study.** Information commonly collected during a site investigation should be reviewed when evaluating whether to proceed with a site-specific bioavailability study. Both historical site information and soil parameter data bear on the likely results of such a study. Under certain circumstances, it may be possible to use existing site data to indicate the likely outcome of a bioavailability study, and thereby help determine whether to proceed with the study itself. In general, however, site data cannot be used in place of site-specific bioavailability studies. The following information on using site data to "estimate" bioavailability is intended as a general guideline; soils at specific sites may not conform to all of the general trends discussed here. Furthermore, the generalizations apply mainly to the oral (ingestion) exposure route, which has been the most extensively studied to date. The impact of site history and soil chemistry parameters on the oral bioavailability of metals from soil is indicated in Table 3-1.

  - ☛ Historical site information to consider includes both the types of metals contamination present and the length of time that the contamination has been resident in soils or sediments (i.e., the weathering or aging time). The source of contamination can indicate the likely forms in which the metals were deposited in the soils. In general, soils that contain sulfide or elemental metal forms yield lower bioavailability values than soils that contain oxide or carbonate metal forms. Nickel is a notable exception to this trend, and forms several insoluble oxide species. In addition, small mineral particles yield higher bioavailability than large mineral particles. Soil weathering reactions change the bioavailability of metals over time. In general, metal forms with high bioavailability (oxides and carbonates) alter to less bioavailable forms, while metals with low bioavailability (sulfides and elemental forms) alter to more bioavailable forms. The length of time that the metals have been present in the soil will determine the extent of these weathering reactions, and the current bioavailability of the metals in soil.

  - ☛ Site-specific soil chemistry determines the products of the soil weathering reactions discussed above. Measurements of soil parameters such as pH, TOC, total carbonate (alkalinity), and iron and manganese concentrations may therefore indicate the likely outcome of a site-specific bioavailability study. In general, soil conditions that tend to promote precipitation or sorption also tend to reduce mobility and bioavailability of metals.

  - ☛ Most of the metals reviewed in this document (cadmium, lead, mercury, and nickel) can alter to carbonate forms in alkaline soils, and these carbonate metal forms are highly bioavailable via the oral exposure route. Soils containing elevated TOC (greater than 5 to 10 percent) tend to contain metals that are complexed to organic matter; these organically complexed metals appear to have elevated oral bioavailability (this is particularly true for lead and mercury). These same soils/sediments will often contain relatively insoluble sulfides as a result of the action of sulfate-reducing bacteria. This mechanism is limited to cadmium, mercury, lead, and nickel in seasonally flooded soils. Finally, soils with elevated iron and manganese concentrations (greater than 3 to 5 percent combined) tend to have reduced bioavailability for other metals, particularly for arsenic due to increased sorption on these soil components.

  - ☛ The research to date indicates that regulatory leaching tests, such as the Toxicity Characteristic Leaching Procedure (TCLP), do not predict the oral bioavailability of metals

from soil. Therefore, results from TCLP testing should not be used in estimating the extent of metals bioavailability from soil.

Table 3-1. Impact of Soil Characteristics on the Oral Bioavailability of Metals for Mammals

Site History	Bioavailability		
	Low	Medium	High
Metal Forms:			
Sulfides	X		
Elemental (metallic)	X		
Sulfates		X	
Carbonates			X
Oxides			X (except Ni)
Particle Size (of metal-bearing grains):			
Small			X
Large	X		
Weathering/Aging Time:			
Sulfides	X →		
Elemental	X →	→	
Carbonates		←	X
Oxides		←	X
Soil Chemistry			
pH:			
Acidic		X	
Basic			X (Cd, Hg, Pb, Ni)
Alkaline soils			X (Cd, Hg, Pb, Ni)
High TOC			X (Hg, Pb)
High Fe and Mn		X (As)	
Sulfide-producing soil		X (Cd, Hg, Pb, Ni)	

4.0 DESIGNING/CONDUCTING A BIOAVAILABILITY STUDY

For assessing potential human health risks, it should be assumed that bioavailability adjustments must be supported by a site-specific study because it generally is not possible to predict the bioavailability of a compound based on other, more fundamental physical or chemical properties of the site or the contaminant. For ecological risk assessments, there are a variety of ways to incorporate bioavailability, and adjustments can be determined either experimentally or with estimation techniques (e.g., bioaccumulation is often modeled using literature-derived bioaccumulation factors). This section provides background information on the types of tests that can used to assess the bioavailability of a metal to human and ecological receptors and the resources (i.e., cost, time, and technical expertise) required to conduct such tests. The discussion is presented from the perspective that a site-specific bioavailability study will be designed and conducted during risk assessment activities. Thus, recommendations are offered regarding the appropriate steps to include in a bioavailability study to ensure that the study is acceptable to involved regulatory agencies.

4.1 Test Methods for Assessing Bioavailability

A wide variety of methods have been used to study the bioavailability of metals in soils and sediments. A comprehensive review and evaluation of these methods is provided in NRC, 2002). For soils, the focus has been on studies in laboratory animals and simple *in vitro* extraction tests to assess the oral bioavailability of metals in soils relative to the bioavailability of more soluble metal compounds. Most studies of soils have been conducted for use in human health risk assessment (Kelley, *et al.*, 2002, NEPI, 2000). For sediments, the bioavailability of metals to ecological receptors has been the focus of most research to date (NRC, 2002, U.S. EPA, 2000a).

Site-specific studies are generally required to support changes from default bioavailability assumptions. Studies conducted using soluble metal compounds freshly mixed with soil or sediment generally do not show significant reductions in bioavailability, and will not provide a representative indication of the relative bioavailability of metals in soil or sediment at a specific site. Consequently, studies should be conducted using weathered soils or sediments. In addition, it is important that the samples being tested be characterized for parameters such as pH, TOC, CEC, particle size (sand, silt, clay), total metals (Fe, Mn, Al), and available anions (PO_4, SO_4, CO_3) (NRC, 2002, U.S. EPA, 2000a, U.S. EPA, 2000b). Also, it is also important that, for studies predicting human oral absorption of metals in soils, the soils be sieved to include particle sizes of less than 250 microns, because it is these finer particles that are thought to adhere to hands and be ingested during hand-to-mouth activities. For dermal absorption studies, particle sizes of less than 150 microns are the most likely to adhere to skin. Soil samples should never be ground prior to testing.

4.1.1 *IN VITRO* METHODS FOR HUMAN HEALTH

This section describes the application of simple laboratory extraction tests (*in vitro* tests) that are predictive of the bioavailability of metals from soil to humans. These methods are both rapid and inexpensive, requiring only a day to conduct and costing only a small fraction of what an *in vivo* study (discussed below) would cost. Although *in vitro* work has focused primarily on determining the oral bioavailability of arsenic and lead, results from these two elements can be extrapolated to other metals based on universal solubility-limiting factors and similarities in the aqueous geochemistry of certain elements. In addition, the dermal absorption of chromium from soil and waste materials has been

evaluated by extraction tests using both real and synthetic human sweat (Horowitz and Finley, 1993; Wainman *et al.*, 1994).

Simple extraction tests have been used for several years to assess the degree of metals dissolution in a simulated GI-tract environment, i.e., bioaccessibility (Kelley, *et al.*, 2002, NRC, 2002, Ruby *et al.*, 1993, 1996, and 1999). The predecessor of these systems was developed originally for nutrition studies to assess the bioavailability of iron from food (Miller *et al.*, 1981; Miller and Schricker, 1982). In these systems, various metal salts, or soils containing metals, are incubated in a low-pH solution for a period intended to mimic residence time in the stomach. The pH then is increased to near neutral, and incubation continues for a period intended to mimic residence time in the small intestine. Enzymes and organic acids are added to simulate gastric and small-intestinal fluids. The fraction of a metal that dissolves during the stomach and small-intestinal incubations represents the fraction that is bioaccessible (i.e., is soluble and available for absorption).

The currently available *in vitro* tests (Medlin, 1997; Rodriguez *et al.*, 1999; Ruby *et al.*, 1996) are designed around human pediatric gastrointestinal conditions, and are intended to mimic fasting conditions. Critical design factors that have been evaluated include extraction fluid chemistry and temperature, extraction time, mixing rate, and the particle size of the test material. Because the goal is to develop the simplest test possible, which will yield the highest repeatability and reproducibility, these tests have been streamlined to include only those factors that control the dissolution of a particular metal.

The research to date indicates that the fractional extraction of arsenic or lead during a one-hour incubation in acidic fluid (pH 1.5 in hydrochloric acid) is a good surrogate for relative arsenic or lead bioavailability values derived from *in vivo* studies (Medlin, 1997; Rodriguez *et al.*, 1999; Ruby *et al.*, 1996). Figure 4-1 shows the correlation of *in vivo* and *in vitro* tests for lead bioavailability. Many laboratories currently are using a specialized test system such as that shown in Figure 4-2 for these studies; however, Rodriguez *et*

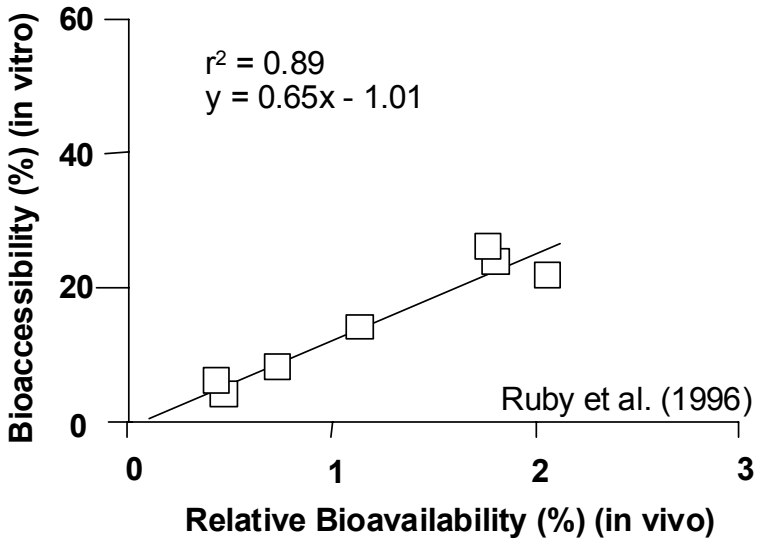


Figure 4-1. *In vitro* to *In vivo* Correlation for Lead in Soil

al. (1999) replaced this cell with mason jars and achieved equally good results. It is important to maintain a constant pH during the test (i.e., 1.5 ± 0.3), because the solubility of most metals is highly pH dependent, and allowing the pH to fluctuate may influence the test results. Note that incorporating the food material used during the Rodriguez *et al.* (1999) studies of arsenic bioaccessibility is not recommended, because the food material contained elevated phosphate concentrations (nearly 3 percent available phosphate), which enhanced the solubilization of soil arsenic.

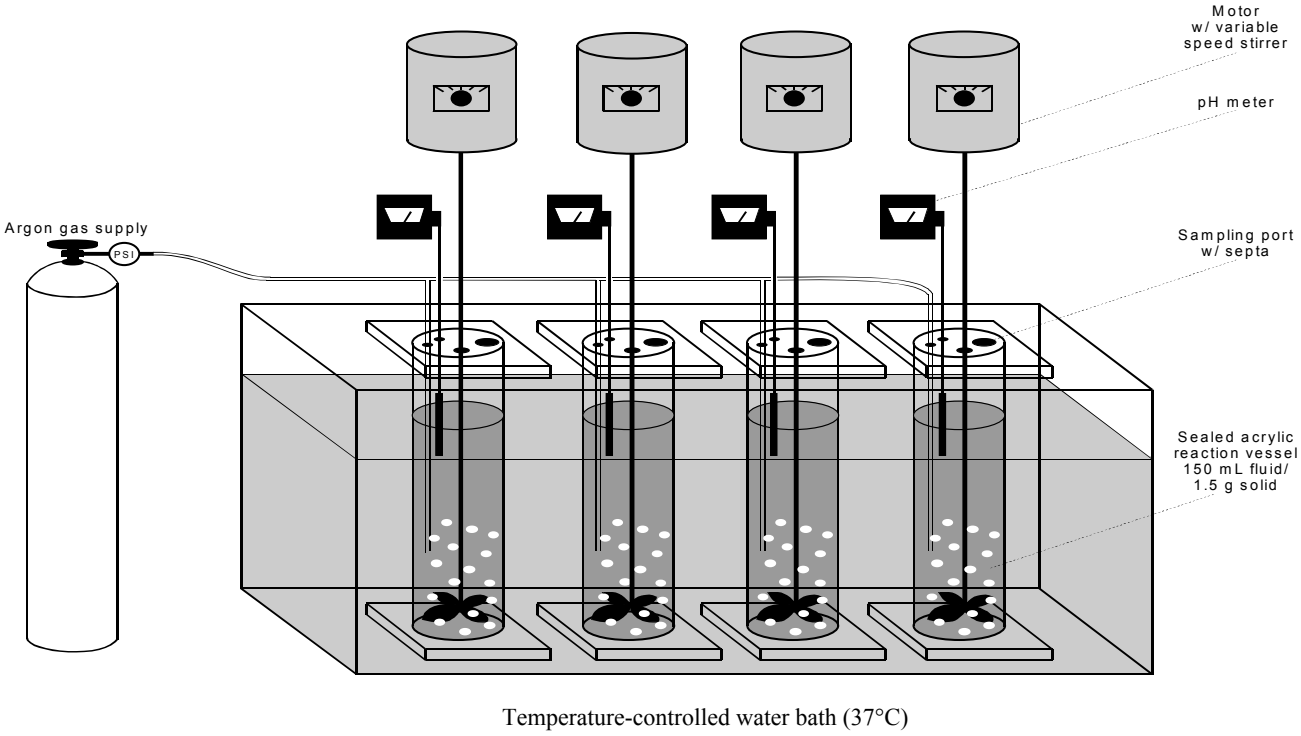


Figure 4-2. *In vitro* Test System

No published in-vitro-to-in-vivo correlations exist for cadmium, chromium, mercury, or nickel. Because all of these metals may occur in soil as discrete mineral forms with varying oral bioavailability, it appears that the same controls on bioavailability will be in effect for these metals as those for arsenic and lead. At this time, it is recommended that the *in vitro* test, which consists of a stomach-phase (i.e., acidic) incubation, be applied to determining the bioaccessibility of arsenic, cadmium, lead, and nickel from soil. Chromium and mercury are best evaluated using sequential stomach-phase and intestinal-phase incubations.

Before undertaking an *in vitro* study, it is important to consider the desired use for the data. Will the data be used primarily as a range-finding tool, and for guiding further study of site soils using an *in vivo* model, or are the data intended for use in making a quantitative adjustment to a human health risk assessment? If it is the latter, it is critical to establish a dialogue with the relevant regulatory agency as early as possible, because the use of *in vitro* data for making adjustments to human health risk assessments is not widely accepted by regulatory toxicologists. Submittal of a study protocol to the regulatory agency is generally a good place to start the dialogue over study design issues and the

acceptable uses for these types of data. Appropriate protocols (i.e., Standard Operating Procedures [SOPs]) for *in vitro* methods may be found in Part 2 of this Guide.

4.1.2 *In Vivo* Methods for Human Health

Most of the *in vivo* research to date has focused on the oral bioavailability of metals in soils (NEPI, 2000, Kelley, *et al.*, 2002). This focus reflects the observation that human health risk-based soil cleanup levels for metals are typically driven by ingestion exposures. New dermal risk assessment guidance from U.S. EPA (2001a) that includes default assumptions of 1 percent dermal bioavailability for cadmium and 3 percent for arsenic may result in estimates of dermal exposures that influence cleanup levels at some sites. Consequently, this section focuses on methods for assessing oral bioavailability using laboratory animals. Dermal absorption studies are described briefly. Inhalation studies are not discussed because site-specific studies will seldom be relevant, as inhalation is not a pathway that typically contributes significantly to risk from metals in soil. When evaluating whether to conduct a bioavailability study, and what form it should take, the Data Quality Objectives (U.S. EPA, 1994b) process should be used to develop the study.

Although the oral bioavailability study methods described are generally used for studies in laboratory animals, it is useful to note that many of these same methods may be used for studies in humans. Recently, lead bioavailability studies in humans have been conducted. The protocols for these studies must undergo scrutiny by institutional review boards to ensure that no unacceptable risks will be imposed, and that informed consent will be obtained.

Oral bioavailability studies generally involve measuring chemical concentrations in body tissues or excreta at various time points after dosing. The specific study design needs to be selected after considering how the metal being studied is handled by the body. Some metals are well absorbed and rapidly excreted in the urine (arsenic is a good example), while other chemicals may have more limited absorption and may accumulate in body tissues. For example, lead is accumulated in bone, while cadmium is accumulated in the kidneys and liver. Different study designs are needed to reflect these different characteristics. Thus, there is no one oral bioavailability study protocol that can be applied uniformly to all metals.

The four primary methods used to study the oral bioavailability of metals are:

- **Measurement of blood concentrations over time for oral and intravenous doses.** The area under the curve (AUC) is calculated, and oral absorption is determined by comparing the AUC_{oral} to the $AUC_{intravenous}$ (see Figure 4-3). This method works best for metals that are well absorbed, and rapidly and completely excreted (e.g., arsenic).
- **Measurement of the fraction of the dose that is excreted in the feces.** This measurement generally reflects unabsorbed metal, so absorbed dose is calculated by subtracting the excreted dose from the administered dose. This method may underestimate absorption if a metal is absorbed, then excreted via bile back to the gastrointestinal tract.

- **Measurement of the fraction of the dose that is excreted in urine.** This fraction provides an estimate of absorbed dose for metals that are rapidly excreted primarily in the urine (e.g., arsenic).

- **Comparison of tissue concentrations after administration of different forms of a metal.** This method provides an estimate of relative bioavailability, and is most useful for metals that are preferentially accumulated in specific tissues.

For all of these methods, if metals in soil are compared to a soluble form of the metal, the resulting relative bioavailability estimate may be used to derive exposure estimates. The specific animal model selected for use in the studies should be based on an understanding of the behavior of the metal being studied in that animal, and on any significant differences between the animal selected and humans. Other factors to consider include the age of the animals (for example, lead is absorbed more completely in young animals), and the nutritional status and diet for the animals (for example, lead is better absorbed in fasted animals).

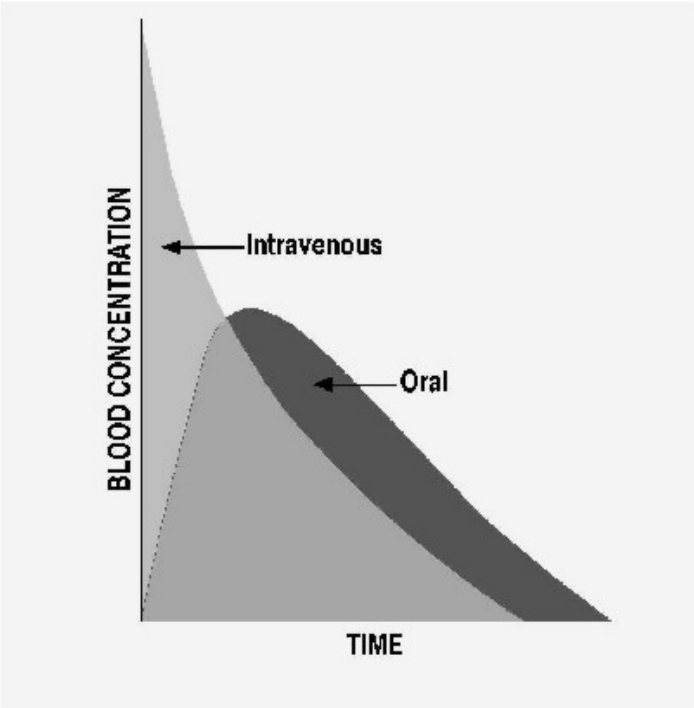


Figure 4-3. Comparison of AUCs for Blood Concentrations

A study protocol or work plan must be prepared that specifies dose levels, frequency of dosing, number of animals per group, samples to be collected and the timing and frequency of sample collection, and quality assurance procedures to be followed. The U.S. EPA has issued specific regulations for quality assurance for laboratory studies called Good Laboratory Practices (GLPs) (40 CFR Part 792). These regulations specify the elements to be included in a study protocol, and quality assurance procedures to follow. It is advisable to require a contractor to conduct studies in accordance with the GLPs.

The preferred methods for studying dermal absorption of metals include *in vivo* studies and *in vitro* studies. Rhesus monkey and swine are useful animal models for *in vivo* dermal studies. *In vitro* dermal studies are performed using human cadaver skin. No simple *in vitro* extraction methods have been developed for routine use in screening a series of site soils for relative dermal bioavailability. In designing dermal absorption studies for use in risk assessment, it is critical that the nature of potential exposures be mimicked as closely as possible. Critical factors include the use of a fine fraction of the soil (particles less than 150 microns are thought to be most likely to adhere to skin), the use of a soil load that will not exceed a monolayer on the skin surface (generally less than 5 mg soil/cm^2 of skin), and an exposure period representative of expected exposures at the site. An extensive review of methods for

studying dermal absorption can be found in the U.S. EPA's *Dermal Exposure Assessment* document (1992).

4.1.3 Test Methods for Ecological Receptors

As discussed in Sections 2.1.2 and 2.3.2, a variety of approaches may be used to incorporate bioavailability into ecological risk assessments. For each of these approaches, several specific test methods may be used to provide a quantitative or qualitative measure of metal bioavailability. The methods selected depend on the complexity of the site and the site-specific factors discussed in section 3. Table 4-1 summarizes the categories of test methods, their purpose and limitations.
A more detailed review and evaluation of tools for assessing bioavailability in provided in NRC (2002).

Evaluation of Chemical and Physical Parameters of Soil/Sediment. It is possible to qualitatively determine the potential for bioavailability based on general chemical and physical parameters (e.g., pH, fraction organic carbon [f_{oc}], TOC, Eh). For example, adsorption of inorganic cations (e.g., Pb^{2+}) to soil increases with pH, with a resulting decrease in bioavailability, while the reverse is true for inorganic anions (e.g., $H_2AsO_4^{1-}$). Similarly, metals in sediments tend to be more bioavailable in acidic freshwater bodies than in neutral or basic waters. Seawater is naturally buffered at a pH of about 8.0 (alkaline), so most metals in marine sediments are less bioavailable than those in most freshwater systems. Based on this information, evaluation of soil pH can provide a quick, qualitative indication of whether measured metals are likely to be bioavailable.

Bioavailability and toxicity may vary depending on the form or species of the metal (see Section 6.0 of this document and U.S. EPA, 1992). Therefore, use of techniques such as X-ray diffraction (XRD) and scanning electron microscopy (SEM) to identify the specific forms of the metal present in soil and sediment can assist in determinations of relative bioavailability. Standard protocols are currently available for these methods, and many other methods are being used in research (NRC, 2002).

Biological Approaches to Measuring Uptake.

Uptake from Food or Solid Media. As discussed in Section 2.3.2, estimates of the uptake of metals from food or solid media by ecological receptors may be made by conducting laboratory bioassays. However, the multiple exposure routes for many receptors can complicate designing studies to support an ecological assessment. Section 2.1.1 describes the concept of relative bioavailability for human health assessments. Increasingly, relative bioavailability is being considered in ecological assessments as well particularly to estimate the fraction of metal in food available to ecological receptors (Menzie-Cura and TN Associates, 2000, U.S. EPA, 2000b). To apply this approach to ecological assessments, tests should be designed to incorporate species representative of the key receptors identified at the site.

Bioaccumulation from Environmental Media. Uptake and retention of metals by organisms (i.e., bioaccumulation) may be measured directly by collecting and analyzing the tissues of representative organisms from a site (U.S. EPA, 2000a, U.S. EPA, 2000b, U.S. EPA, 2000d, and U.S. EPA, 2002a). BAF values can be determined experimentally from tissue and soil or sediment data from the site. Determination of site-specific BAF values requires correlated concentrations in sediment or soil and tissues to provide an accurate representation.

Table 4-1. Test Methods for Assessing Bioavailability in Ecological Risk Assessments

Approach	Methodology	Purpose	Limitation
Evaluation of chemical and physical parameters of soil/sediment	General characteristics, specific forms of contaminants bound to solids	Provides qualitative evidence for line-of-evidence argument	Evidence is only qualitative, but may allow application of alternative quantitative literature values
Measurement of the available metal fraction in soil or sediment	Extraction techniques that change the solid phase (e.g., 1-N HCl for soil)	Provides estimate of bioavailable fraction for specific receptors	Used mostly to assess plant uptake and nutrient deficiency. May not be applicable to high concentrations.
	Passive extracts and pore water measurements (e.g., comparison of AVS/SEM for sediment)	Provides additional modification to bioavailable fraction estimate	Recent data indicate that the AVS/SEM model is not always a good predictor
Biological approaches to measuring uptake	Measure uptake from food, soil or sediment (laboratory bioassay)	Provides measure of actual absorption of site-specific dose	Bioassays may be costly and time consuming
	Bioaccumulation: field survey of site specific tissue data	Provides an integrated measure of amount of chemical that is taken up by resident species by all exposure pathways and routes	Measured concentrations may be impacted by sources other than those at the site
	Bioaccumulation: bioassays	Can be used to modify default BAFs	Soil/sediment often must be removed from field for studies, which may alter character, studies may be costly and more time consuming
	Toxicity studies, either in laboratory or *in situ*	Can be used directly to modify TRVs, absence of toxicity provides line-of-evidence support for lack of bioavailability	Results of toxicity tests can be difficult to interpret and may be costly and time consuming

Bioaccumulation of metals also may be evaluated through the use of bioaccumulation assays. These studies involve laboratory exposure of relevant species to metals to sediments or soils collected from the site. At the end of the test, the concentrations of metals in the tissues of the organism are determined. For the purpose of the bioassay, lower accumulation of metals from site soils or sediments relative to a reference material would indicate limited bioavailability at the site. Similar to toxicity studies, these bioassays may be used in the latter stages of an ecological risk assessment to provide an additional line of evidence regarding assumptions based on more qualitative approaches earlier in the process.

Toxicity Studies, Either in Laboratory or In Situ. Toxicity tests using environmental media such as sediment and soil in a laboratory setting or in the field can be used to directly modify TRVs for some receptors, and to evaluate the relative bioavailability. Although such tests do not provide a numerical estimate of the bioavailable fraction, the relative toxicity in organisms exposed to site materials versus reference materials provides an estimate of relative bioavailability. The combination of qualitative evidence indicating limited bioavailability and bioassays exhibiting low toxicity has also been used successfully to demonstrate that metals at a site have reduced bioavailability.

4.2 Steps in Conducting a Bioavailability Study

The key steps in conducting a bioavailability study are outlined in Figure 4-4. These steps apply mainly to human health bioavailability studies; however, they also can be used to guide bioavailability studies for ecological risk assessments, particularly if animal feeding studies are involved. As discussed in Section 3.1, bioavailability studies are typically done during the second tier of the risk assessment process. There are several factors in the figure that should be emphasized.

First, it is important to thoroughly evaluate whether a bioavailability study is appropriate and feasible for the site before the study is undertaken (see Section 3.3). The key question that must be answered is whether a bioavailability study is likely to reduce the uncertainty in exposure estimates for chemicals that are risk drivers at a site, and whether more accurate exposure estimates will facilitate the process of identifying appropriate future actions at a site.

Second, in development of the work plan, it is important to consider factors that will support the credibility of the study results, such as involving a qualified peer reviewer in development of the work plan, collecting representative samples, using accepted GLPs or the equivalent, and selecting a reputable testing laboratory.

Finally, one of the most important factors is involving the regulators, and possibly other stakeholders, at the outset and giving them the opportunity to provide input throughout the process. By involving them early and giving them the opportunity for input along the way, they are more likely to accept the results. On the other hand, if they are not receptive to the concept of bioavailability adjustments, it is best to find this out early, before time and money are spent on bioavailability studies.

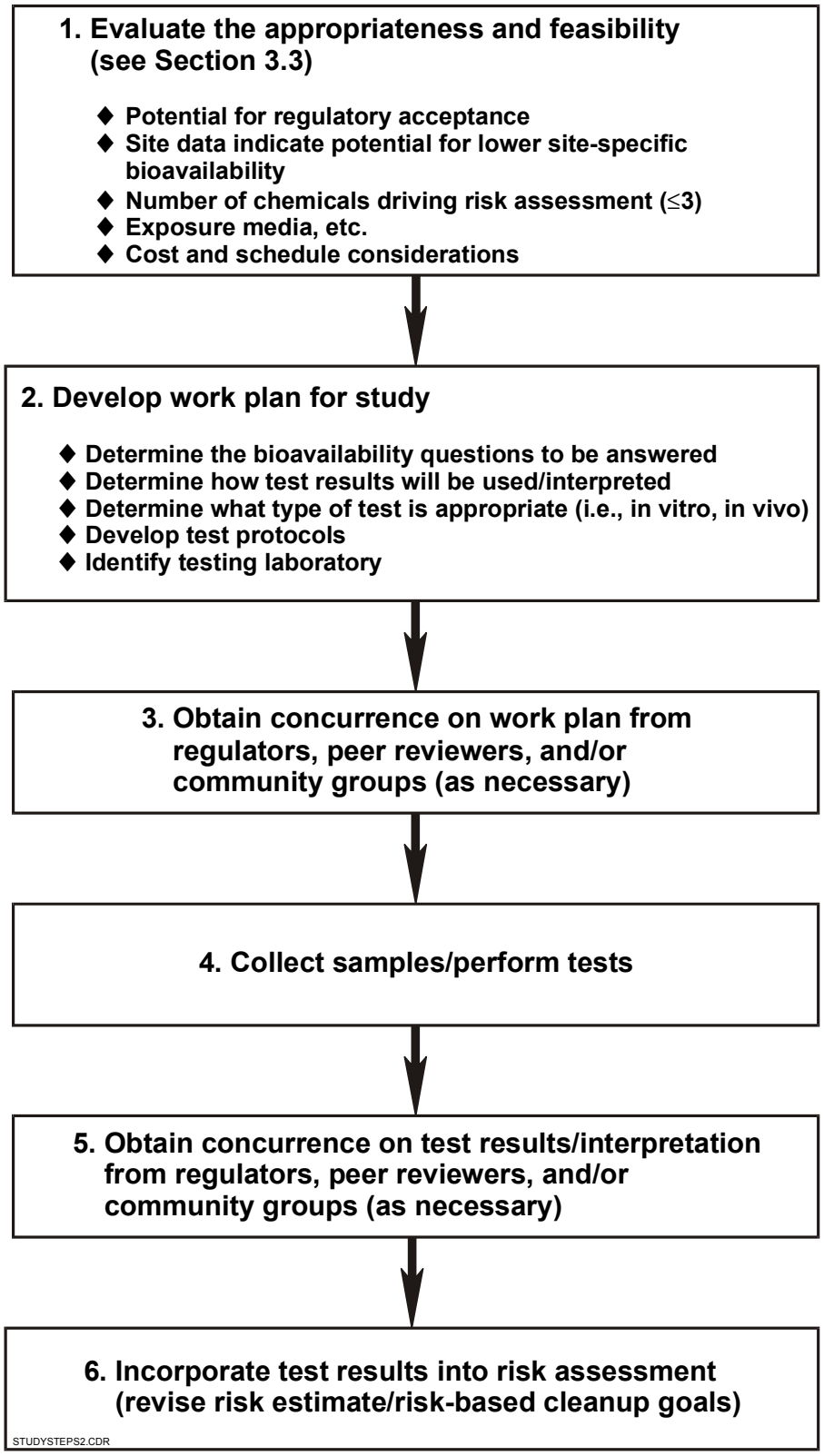


Figure 4-4. Steps in Conducting a Bioavailability Study

4.2.1 Human Health Risk Assessments

Table 4-2 presents a summary of technical resources for conducting both *in vitro* and *in vivo* studies to estimate the relative bioavailability of metals from soil. Because *in vitro* methods are relatively well established for arsenic and lead, it is appropriate to perform these studies in commercial laboratories. *In vitro* studies for cadmium, chromium, and nickel are no more complicated than those for arsenic and lead, and the same laboratory references are therefore applicable. Mercury, on the other hand, is more complicated to work with due to the potential for elemental mercury to volatilize, and it is recommended that a consulting firm that has qualified specialists in mammalian toxicology, soil chemistry, and aqueous geochemistry be contacted to perform these types of studies.

For the *in vitro* evaluation of all these elements, the cost of conducting the extraction and analyzing the extract is only a fraction of total study cost, if the study includes protocol development, external review, reporting, and negotiations with the appropriate regulatory agency. Although individual samples may cost only a few hundred dollars to process through the simplified lead protocol, at least five samples per site are typically evaluated, and whenever any more complicated protocols are developed the total cost of developing protocols, running the study, and preparing a report will likely cost $5,000 to $15,000. At the upper end, these studies also would include mineralogical analyses to support interpretation of the *in vitro* extraction test results. Typically, *in vitro* studies can be planned, run, and reported in 6 to 8 weeks.

As described in Section 5.0, *in vivo* studies have been conducted to determine the relative bioavailability of arsenic, cadmium, and lead in soil.. The costs for *in vivo* studies, including protocol development and report preparation, will range from $30,000 to $100,000 depending on study design and number of samples tested. A minimum of 3 months is needed to order animals, allow for a quarantine period once the animals are ordered, run the study, get samples analyzed (with quality assurance review), and prepare a preliminary report. In planning a site investigation, it would be more realistic to allow for a total of 6 months from protocol development and review to final study report.

Most contract toxicology laboratories should be capable of performing these types of studies. Contract laboratories are also likely to routinely conduct studies in accordance with GLPs (see Section 4.1.2), but generally will be unfamiliar with handling soil samples. University laboratories may provide a lower cost alternative for conducting these studies, but generally do not follow GLPs to conduct studies. Because successful relative bioavailability studies have not been conducted for chromium, mercury, and nickel, the initiation of such a study will require development of a detailed study protocol, external peer-review of the protocol, and possibly one or more pilot studies to ensure that an appropriate animal model has been selected.

Because no dermal absorption studies have been conducted for soils that contain the forms of metals commonly found in the environment, undertaking such a study will require careful planning and execution. Dr. Ronald Wester, who is a research dermatologist at the University of California at San Francisco, performed the existing studies on the dermal absorption of soluble forms of arsenic, cadmium, and mercury in the presence of soil using a monkey model. Swine have also been used for dermal absorption studies.

Table 4-2. Technical Resources for Conducting Bioavailability Studies for Use in Human Health Risk Assessments

Studies	Animal Model	Time Required	Cost[a]
***In vitro* (oral)**			
Arsenic, cadmium, chromium, lead, and nickel (data only)	NA	3 weeks[b]	$150/sample[c]
Arsenic, lead, cadmium, chromium, nickel, and mercury (full study)[d]	NA	6-8 weeks	$5,000-15,000/study[e]
***In vivo* (oral)**			
Arsenic	Monkeys	3-6 months[f]	$50-80,000/substrate[g]
Lead	Rats	3-6 months[f]	$60-85,000/substrate[g]
	Swine	3-6 months[f]	$45,000/substrate[g]
Cadmium	Rats	3-6 months[f]	$60-85,000/substrate[g]
Mercury	TBD	5-8 months[h]	$75-100,000/substrate[i]
Chromium	TBD	5-8 months[h]	$60-85,000/substrate[i]
Nickel	TBD	5-8 months[h]	$60-85,000/substrate[i]
Dermal Absorption			
Arsenic, cadmium, chromium, lead, mercury, and zinc	Monkeys	3 months[f]	$45-55,000/substrate

(a) With the exception of the first *in vitro* listing, costs include drafting study protocols, conducting study and preparing a report for submission to regulators.
(b) Assumes sample extraction, and two-week analytical turnaround on analysis of a single metal in the extract and the test soil.
(c) Average per sample cost for data production only at a commercial analytical laboratory.
(d) Includes protocol development, sample handling and testing, report, production, and limited negotiations with a regulatory agency (phone calls only).
(e) Actual cost depends on number of samples, and project specific requirements.
(f) Includes external review of existing protocol, study, and reporting, but no agency negotiations.
(g) Actual cost depends on laboratory that is conducting the study and study design.
(h) Includes protocol development and external review, study, and reporting. No agency negotiations included.
(i) Represents an approximate cost estimate. No such study has been conducted to date.
TBD = To be determined.
NA = Not applicable.

4.2.2 Ecological Risk Assessments

Table 4-3 provides a summary of the estimated cost and time for each of the different tests and analyses proposed for measuring bioavailability in ecological risk assessments. These costs are intended to provide an indication of the analytical level of effort necessary to address these issues and may not reflect actual total costs associated with each task. In general, all of the tests proposed are standard laboratory protocols for which specific methods have been developed. For example, ASTM and EPA have published guidance on the appropriate methodologies for evaluating the toxicity of metals to aquatic and terrestrial invertebrates (e.g., for freshwater invertebrates, see U.S. EPA, 2000e). Similarly, the analytical

methods discussed rely on standard analytical techniques. EPA has also provided guidance for collection, storage, and manipulation of contaminated sediments for chemical and toxicological analyses (U.S. EPA, 2001b). As a result, these tests can be performed by any qualified laboratory. The cost estimates provided are averages for contract laboratories; other laboratory facilities (e.g., universities) may offer lower costs for some of these analyses.

It is important to note that the exact cost of a bioavailability study will vary from site to site, depending on the existing data and the complexity of the site. For example, if all chemical and physical parameters are available from existing data, it may not be necessary to collect additional samples. In addition, costs could not be estimated for qualitative evaluations, or for interpretation of results or negotiations with agencies. It is impossible to accurately predict the costs associated with these tasks because their scope is entirely dependent on site-specific factors including the size of the site, tests selected for inclusion, and the technical expertise available to the RPM. In some instances, the RPM may require additional technical expertise for assistance in data interpretation, while at other sites; such assistance may not be required. Therefore, the costs in Table 4-3 are offered to provide a general background on the relative costs of the various tests proposed.

Table 4-3. Time and Cost Associated with Test Methods for Assessing Bioavailability in Ecological Risk Assessments

Test Type	Description	Estimated Cost per Sample[a]	Time per Test
Evaluation of Chemical and Physical Parameters			
Measurement of general chemical and physical parameters	Chemical form, pH, TOC, Eh, f_{oc}, etc.	$200	Allow 3-4 weeks for sample analysis
Measurement of specific metal forms and associations	X-ray diffraction and scanning electron microscopy (EM)	$500/sample for EM	Allow 3-4 weeks for sample analysis
Direct Exposures to the Available Fraction			
Extraction techniques		$120	Allow 3-4 weeks for sample analysis
Comparison of AVS/SEM	Compare ratio of measured SEM to AVS	$250	Allow 3-4 weeks for sample analysis
Biological Approaches to Measuring Uptake			
Uptake from food	Relative bioavailability study	*In vivo*: $30,000-$100,000/substrate (see Section 4.3.1)	*In vivo*: 3-6 months (see Section 4.3.1)
Collect and analyze site-specific tissue data	Metals in fish, invertebrates, birds, mammals, etc.	$300-400[b]	Allow 3-4 weeks for sample analysis
Conduct bioaccumulation studies	Standard test methods for aquatic or terrestrial invertebrates, and plants	$1,900 per species (includes cost of 5 replicates and chemical analyses)	Test lengths can vary from 10 to 56 days
Toxicity tests	Standard test methods for aquatic or terrestrial invertebrates, and plants	$500-1,200 per sample[c]	Test lengths can vary from 10 to 56 days

(a) Costs provided are estimated based on standard procedures. Total may vary depending on such factors as the specifics of project protocol and the number of chemicals analyzed.
(b) Costs provided assume analysis of whole body concentrations.
(c) Total cost for standard test methods requiring multiple concentrations and analytical support will be at least 10-fold higher.

5.0 CHEMICAL-SPECIFIC CONSIDERATIONS FOR ASSESSING BIOAVAILABILITY IN TERRESTRIAL (SOIL) SETTINGS

This section provides a review of chemical-specific issues to consider when determining whether to proceed with site-specific bioavailability studies. The six metals included are those that are commonly important in human health risk assessments at DoD sites, specifically arsenic, cadmium, chromium, lead, mercury, and nickel. For each metal, the predominant forms in soil are briefly described. Differences in toxic endpoints in humans for the different forms of the same metal are noted because evaluation of relative bioavailability is relevant only for forms of a metal that have the same toxic endpoints. The focus of the toxicity discussion is on oral toxicity. Generally, little or no toxicity data are available related to systemic effects of dermally applied metals. As described in section 4.1.2, it is unlikely that site-specific bioavailability studies of inhaled metals from resuspended soil particles will be useful. Consequently, inhalation toxicity and bioavailability of metals is excluded from this discussion.

For each metal, studies of oral bioavailability from different media are described. Oral absorption of arsenic and lead from soil has been studied quite extensively and studies of cadmium and mercury, although limited, have been conducted. The oral bioavailability of chromium and nickel in soil is not well characterized. The database for dermal bioavailability is much more limited. Dermal absorption studies have been conducted for arsenic, cadmium, and mercury in soil, but in all three cases, soluble forms of the metals were mixed with soils and tested without time for weathering reactions to occur. Thus, there are no data currently available to predict the dermal bioavailability of these metals in weathered soils at contaminated sites.

Many of the metal-specific considerations for assessing bioavailability to human receptors are applicable to terrestrial ecological receptors that are exposed to metals in soils through direct contact. However, direct comparisons are limited to monogastric mammalian receptors (e.g., small mammals and other wildlife), and do not necessarily apply to ruminants (e.g., deer or cows), reptiles, amphibians, and avian species. Small mammals that burrow in soils and exhibit preening behavior, or that ingest earthworms for a large portion of their diet, have elevated soil ingestion rates. For example, short-tailed shrew and eastern cottontail rabbits are estimated to consume 13 and 6.3 percent soil in their diet, respectively (Talmage and Walton, 1993; Sample and Suter, 1994). As a result, these receptors often drive ecological risk assessments for metals in upland soils.

As described in section 2.3.2, bioavailability is a critical factor considered in the derivation of TRVs and Eco-SSLs. U.S. EPA selected 24 chemicals to be addressed initially by the Eco-SSL guidance, including 17 metals and 7 semivolatile organic chemicals (U.S. EPA, 2000b). Only 8 of the 17 metals are included in the 2000 draft guidance document (Table 5-1). U.S. EPA (2000b) includes summaries of the

Table 5-1. Metals for Which Eco-SSLs are Being Developed

Metal Type	Eco-SSL metals[a]
Metal cations	**aluminum**, **antimony**, barium, beryllium, **cadmium**, **cobalt**, **copper**, iron, lead, manganese, nickel, silver, and **zinc**
Metal anions	**arsenic**, **chromium**, selenium, and vanadium

[a]Metals in bold are those for which Eco-SSL derivation is included in U.S. EPA (2000b).

derivation of Eco-SSLs for these 8 metals that include limited descriptions of the forms found in soils, and factors affecting the bioavailability of various forms to ecological receptors. These summaries do not include a discussion of the forms of the metal used in the studies that were considered in deriving the TRVs. Instead it is necessary to review the toxicity summary tables in the appendices to determine the metal forms tested. In many cases, the metal forms tested are not those found in the environment. For example, antimony potassium tartrate, the form tested in 9 of 25 studies assessing antimony toxicity to mammals, is a pharmaceutical preparation.

Because the TRVs used in ecological risk assessment are often based on laboratory studies where soluble metal salts were added to the diet of these animals, bioavailability of the metals in soil should be compared to the bioavailability of the soluble forms of the metal. Although the following sections discuss relative bioavailability estimates for humans exposed to the metals in soil, much of the information provided is directly applicable to assessing relative bioavailability for mammalian ecological receptors.

5.1 Arsenic

Default risk-based soil cleanup levels for arsenic are frequently below local background soil concentrations of this element. If cleanup levels in soil are based on background concentrations, site-specific bioavailability data may have a limited impact on cleanup levels when the adjusted risk-based cleanup levels are still below background concentrations. Nevertheless, in situations where there is some flexibility in target risks, bioavailability data may be a powerful tool for adjusting cleanup goals.

5.1.1 Predominant Forms in Soil

Trivalent and pentavalent inorganic arsenic compounds are the predominant forms in soils. Inorganic arsenic compounds vary widely in their water solubility, with sodium arsenate and arsenic trioxide representing highly water-soluble forms. Discrete arsenic mineral phases present in soils commonly include less soluble forms such as sulfide minerals, complex oxides, and arsenic present in iron, manganese, and phosphate mineral species. All but the sulfide minerals may be formed over time in surficial (oxygen-rich) soils, as weathering reactions occur that favor the most thermodynamically stable metal forms. Arsenic may also be present in soil in ionic forms that may be adsorbed to soil constituents. Reduced bioavailability of arsenic in soil is thought to be primarily a function of the presence of less soluble mineral phases and ionic forms that are strongly adsorbed to soil particles or coprecipitated with other elements in soil.

5.1.2 Toxicity Assessment

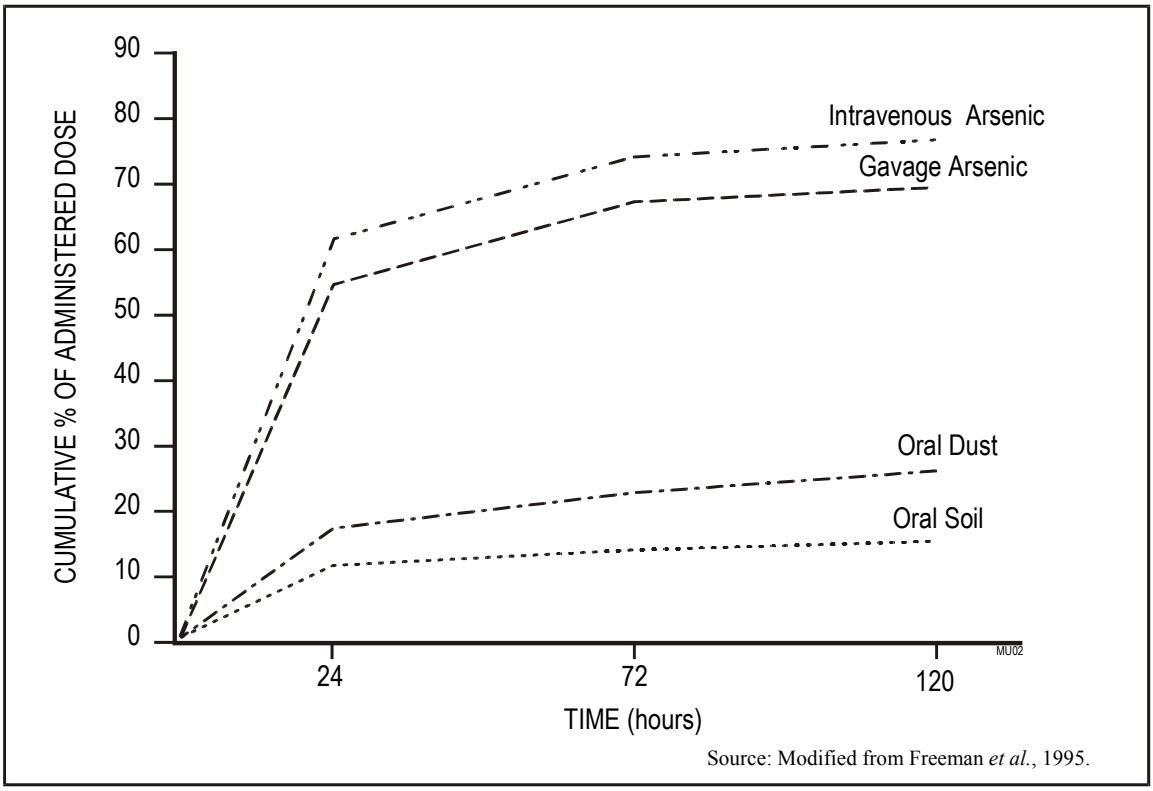
All inorganic arsenic compounds induce chronic toxic effects by the same mechanism, regardless of valence state. Ingested inorganic arsenic compounds cause cancer at high doses, so all inorganic arsenic compounds may be considered together when assessing bioavailability. The toxicity of arsenic to humans is described by ATSDR (2000a). The oral toxicity values used in risk assessments are based on epidemiology studies of human populations exposed to soluble inorganic arsenic dissolved in drinking water, so these soluble forms should be the point of comparison in studies of relative bioavailability.

5.1.3 Relative Bioavailability Via Oral Exposure

After ingestion, water-soluble forms of inorganic arsenic are almost completely absorbed from the gastrointestinal tract of humans and many laboratory animals. Ingestion of less soluble forms of arsenic leads to reduced absorption. Studies have been conducted in laboratory animals that demonstrate reduced absorption of arsenic from soil taken from many different sites (Freeman *et al.*, 1993; Freeman *et al.*,

1995; Groen *et al.*, 1994; Casteel *et al.*, 1997b; Rodriguez *et al.*, 1999). These studies indicate that arsenic in soil is typically only one-half to one-tenth as bioavailable as soluble arsenic forms. In other words, these studies support relative bioavailability adjustments ranging from 0.5 to 0.1 in exposure assessments for these sites.

Monkeys, dogs, rabbits, and swine have been used to study arsenic in soil, mainly from mining and smelting sites. Bioavailability estimates have been based on the fraction of the dose excreted in the urine, and on the AUC values for arsenic concentrations in the blood. Figure 5-1 illustrates differences in excretion of soluble arsenic and arsenic from soil and indoor dust from Anaconda, MT in the urine of monkeys. The animal studies are supported by mineralogical analyses demonstrating the presence of less soluble arsenic forms in the soils tested, and by *in vitro* studies (i.e., PBETs) that indicate reduced bioaccessibility of arsenic in the samples studied.



Source: Modified from Freeman *et al.*, 1995.

Figure 5-1. Monkey Bioavailability Study: Arsenic Excretion in Urine

5.1.4 Bioavailability Via Dermal Exposure

The dermal bioavailability of a water-soluble arsenic form (sodium arsenate) mixed with a soil matrix has been evaluated *in vivo* in monkeys, yielding estimates of arsenic absorption from soil ranging from 3.2 to 4.5 percent (Wester *et al.*, 1993a). The same soil mixture was tested with human skin *in vitro*, yielding an estimate of approximately 1 percent absorption. As a result of this study, a value of 3 percent dermal absorption of arsenic from soil is being used in some risk assessments.

5.1.5 Summary of Pertinent Data

Inorganic forms of arsenic vary in water solubility and bioavailability. Most of the oral bioavailability studies of soil arsenic conducted to date used soil from mining or smelting sites, and support relative bioavailability adjustments ranging from 0.5 to 0.1. A simple *in vitro* test system is available that has shown good agreement with the results of studies in laboratory animals using the same soils (Rodriguez *et al.*, 1999; see Section 4.1.1).

5.2 Cadmium

Risk-based soil cleanup levels for cadmium may be influenced by dermal exposures and by uptake into homegrown produce, as well as by direct ingestion of soil. Therefore, the relative importance of these pathways should be evaluated prior to planning site-specific bioavailability studies.

5.2.1 Predominant Forms in Soil

Cadmium in soil may be found in forms that range in solubility from sparingly (sulfides) to moderately (cadmium sulfate) to highly soluble (cadmium carbonate).

5.2.2 Toxicity Assessment

The reference dose for cadmium is based on effects of a soluble form of cadmium (cadmium chloride) on the kidney. All inorganic cadmium forms commonly present in soils induce chronic toxic effects after ingestion by the same mechanism. Consequently, all inorganic cadmium compounds may be considered together when assessing bioavailability.

5.2.3 Relative Bioavailability Via Oral Exposure

Oral absorption of cadmium in humans generally is reported to be very low (1 to 7 percent) (ATSDR, 1999a). Evidence that the bioavailability of cadmium in soil may be reduced compared to the bioavailability of soluble cadmium forms is available from a limited number of studies. Several studies have reported reduced oral bioavailability of a soluble cadmium form, cadmium chloride, mixed with soil (Griffin *et al.*, 1990; Schilderman *et al.*, 1997). For cadmium in weathered soil, data are available for soil from a single site (the site of a former zinc smelter) that has been evaluated *in vivo* in rats (Schoof and Freeman, 1995; PTI, 1994). A relative cadmium bioavailability estimate of 33 percent was obtained based on comparison of liver and kidney tissue concentrations in animals fed rodent chow mixed with soil, versus those fed rodent chow mixed with cadmium chloride. An *in vitro* study of this same soil yielded a higher value, which suggests that the *in vitro* method might overestimate the relative bioavailability of soil cadmium.

5.2.4 Bioavailability Via Dermal Exposure

An *in vitro* study of dermal absorption in human cadaver skin of cadmium chloride mixed with soil yielded an estimate of 0.02 to 0.07 percent absorption based on cadmium in receptor fluid (Wester *et al.*, 1992). An additional 0.06 to 0.13 percent of the dose was retained in the skin. The U.S. EPA default value of 1.0 percent for dermal absorption of cadmium compounds from soil is more than 10 times higher than the maximum percent of the cadmium chloride dose reaching the receptor fluid and 5 times higher than the maximum combined percent dose in receptor fluid and skin. Dermal absorption of cadmium from weathered soils may be even lower.

5.2.5 Summary of Pertinent Data

Limited evidence is available that oral absorption of cadmium in soil is reduced compared to absorption of soluble cadmium. For any site in which dermal exposures are quantified, the highest priority for site-specific studies may be studies of dermal exposure from soil. This priority reflects the likelihood that default assumptions overestimate dermal absorption of cadmium from soil by a factor of 10 or more, but may only overestimate oral absorption by a factor of 3.

5.3 Chromium

The two primary oxidation states of chromium are trivalent and hexavalent, with hexavalent chromium generally being more bioavailable and more toxic than trivalent chromium. Sometimes soil cleanup levels for total chromium are based on the toxicity value for hexavalent chromium. In such cases, it clearly would be prudent to characterize the form of chromium present before trying to decide if bioavailability studies would be useful.

5.3.1 Predominant Forms in Soil

Unlike many of the other metals discussed in this document (e.g., arsenic, cadmium, and lead), anthropogenic sources of chromium for soils are generally in a soluble form (with the exception of sites that contain chromite ore processing residue). As a result, the soil alteration processes that control chromium bioavailability generally have these soluble chromium species as a starting point. The solubility and mobility of trivalent chromium is minimal, whereas hexavalent chromium is both highly soluble and mobile. The relative concentrations of trivalent chromium and hexavalent chromium in a particular soil sample will depend on the form of the chromium contaminant and the soil redox conditions and geochemistry, particularly the pH and presence of oxidizing or reducing agents.

5.3.2 Toxicity Assessment

Trivalent chromium is a required nutrient. The oral reference dose for trivalent chromium applies to insoluble salts, and is based on a study in which no adverse effects were observed at any dose tested when Cr_2O_3 was baked into bread and fed to rats. The oral reference dose for hexavalent chromium applies to soluble salts, and is based on doses that caused no adverse effects in a rat drinking water study. Based on their respective reference doses, soluble salts of hexavalent chromium are considered to be almost 1,000 times more toxic than insoluble salts of trivalent chromium.

5.3.3 Relative Bioavailability Via Oral Exposure

The oral bioavailability of chromium depends on its valence state, with hexavalent chromium being more readily absorbed than trivalent chromium (ATSDR,2000b). Oral absorption of nondietary trivalent chromium compounds is extremely low (approximately 1 percent). Absorption of hexavalent chromium compounds is somewhat higher (approximately 10 percent). There is evidence that hexavalent chromium is converted to trivalent chromium in the acid environment of the stomach, which would limit the oral bioavailability of hexavalent chromium. Two oral *in vivo* studies using environmental soil chromium samples are reported in the literature, one performed in humans and one in laboratory animals (Gargas *et al.*, 1994; Witmer *et al.*, 1989, 1991). Both studies used soils containing chromite ore processing residues, and therefore contained a mixture of trivalent and hexavalent chromium. Although these studies suggested limited oral absorption of the soil chromium, no reliable estimates of relative bioavailability were obtained.

5.3.4 Bioavailability Via Dermal Exposure

Hexavalent chromium and trivalent chromium exhibit very limited ability to penetrate the skin, with somewhat greater penetration observed for hexavalent chromium. Less than 1 percent absorption of hexavalent chromium from water was observed for dosing periods of 5 hours (Wahlberg and Skog, 1963). No studies of dermal absorption of chromium from soil were identified.

5.3.5 Summary of Pertinent Data

The complexity of the factors affecting chromium geochemistry combined with differences in toxicity make it necessary to characterize the valence states of chromium in soils at a site prior to beginning any site-specific bioavailability studies.

5.4 Lead

Direct ingestion of lead in soil and dust generally drives soil lead cleanup levels. Lead is the only chemical for which the U.S. EPA's default assumption is that oral bioavailability from soil is less than the oral bioavailability of soluble forms (U.S. EPA, 1994a). Methods for assessing the oral bioavailability of lead in soil are well developed, and are relatively easy to conduct on a site-specific basis (U.S. EPA, 1999).

5.4.1 Predominant Forms in Soil

Inorganic lead is present in geologic materials and soils in more than 200 minerals that vary greatly in solubility. The majority of lead in geologic materials is in the form of galena (lead sulfide), anglesite (lead sulfate), and cerussite (lead carbonate). Organic forms of lead are rare in soils and are not evaluated in this document.

5.4.2 Toxicity Assessment

The toxicity assessment for lead used by the U.S. EPA is unique, incorporating specific assumptions for lead absorption from ingested water, food, and soil in a pharmacokinetic model that predicts lead levels in blood. Inorganic forms of lead in soil all have the same toxic endpoints and may be considered together when assessing bioavailability.

5.4.3 Relative Bioavailability Via Oral Exposure

Gastrointestinal absorption of lead varies with the age, diet, and nutritional status of the subject, as well as with the chemical species and the particle size of lead that is administered (ATSDR, 1999b). Age is a well-established determinant of lead absorption; adults typically absorb 7 to 15 percent of lead ingested from dietary sources, while estimates of lead absorption from dietary sources in infants and children range from 40 to 53 percent. In U.S. EPA's childhood lead model, it is assumed that 50 percent of an oral lead dose is absorbed from food and water, while 30 percent of a soil lead dose is assumed to be absorbed. Thus, the default assumption for lead is that the relative bioavailability of soil lead compared to soluble lead forms is 0.6 (i.e., 30 percent divided by 50 percent) (U.S. EPA, 1994a).

The oral bioavailability of lead in soil has been more extensively studied than that of any other metal. Soil lead absorption has been studied in rats, swine, and humans. The swine model has been used to test

soils from numerous sites. A physiologically based extraction method is also well developed (Ruby *et al.*, 1993, 1996; Medlin, 1997) and is undergoing detailed validation studies.

The studies in rats and swine have indicated that absorption of lead from soil will vary with the source of the lead, ranging from near zero to greater than 50 percent absolute bioavailability (i.e., relative bioavailability of 1.0, or more compared to soluble lead forms) (Casteel *et al.*, 1997a; Dieter *et al.*, 1993; Freeman *et al.*, 1992, 1996a; Schoof *et al.*, 1995; U.S. EPA, 1996b-e; 1998a-e). On average, the results of these studies support the use of a default assumption that 30 percent of an oral lead dose is absorbed from soil (i.e., relative bioavailability of 0.6). A study in adult humans indicates that absolute lead bioavailability from a mining-area soil varies from approximately 3 to 26 percent, depending on how recently the test subject had eaten (Maddaloni *et al.*, 1998).

5.4.4 Bioavailability Via Dermal Exposure

It is generally assumed that absorption of inorganic lead compounds through the skin is negligible in comparison to the oral or inhalation routes, and dermal exposure to soil lead is generally excluded from risk assessments. No studies of the dermal absorption of lead from soil or dust were identified.

5.4.5 Summary of Pertinent Data

A substantial body of research has demonstrated that the relative oral bioavailability of soil lead varies from site to site. On average, the current default assumption that the relative oral bioavailability of soil lead is 0.6 has been found to be appropriate. A simple *in vitro* extraction method, currently being validated for lead, may offer a rapid, cost-effective method for generating site-specific data.

5.5 Mercury

Mercury is the only metal for which inhalation of vapors released from soil may be an exposure pathway of concern. If elemental mercury is present in soils at a site, the relative importance of the inhalation exposures compared to oral exposures should be assessed prior to determining whether oral or dermal bioavailability studies would be useful.

5.5.1 Predominant Forms in Soil

Mercury in contaminated soils generally is present as either elemental mercury or inorganic mercury compounds. Organic mercury compounds are rarely present in soil in significant quantities. Consequently, only the inorganic forms of mercury are considered here. Inorganic mercury species in weathered soils range from forms with extremely limited solubility (i.e., elemental mercury and mercuric sulfide) to much more soluble forms (i.e., mercury adsorbed into organic matter or clays, and mercury oxides, hydroxides, and carbonates).

5.5.2 Toxicity Assessment

Because of significant differences in pharmacokinetic characteristics and toxicity, elemental mercury and all other inorganic mercury compounds must be addressed separately. The oral reference dose typically applied to inorganic mercury compounds is specifically described as a reference dose for mercuric chloride, a water soluble form of mercury. This reference dose is based on autoimmune effects observed in rats. There is no oral reference dose for elemental mercury due to its extremely limited oral absorption.

However, if elemental mercury is present in surface soils, risk-based cleanup levels will be driven by predicted inhalation exposures from mercury vapor released from soil.

5.5.3 Relative Bioavailability Via Oral Exposures

Soluble forms of inorganic mercury, such as mercuric chloride or mercuric nitrate, appear to be 15 to 25 percent absorbed across the gastrointestinal tract (Rahola *et al.*, 1973; Nielsen and Anderson, 1990). Several studies suggest that mercuric sulfide, a relatively insoluble inorganic mercury compound, has a much lower bioavailability than mercuric chloride (i.e., approximately 30 to 60 times lower) (Schoof and Nielsen, 1997). The oral absorption of elemental mercury is quite low, perhaps on the order of 0.01 to 0.1 percent (ATSDR, 1999c).

One study has been identified that attempted to estimate the bioavailability of mercury in environmental soil samples using an animal model (Revis *et al.*, 1989, 1990), but the study did not yield reliable bioavailability estimates because of study design limitations. Another study suggests that the presence of soil alone decreases the oral bioavailability of inorganic mercury compounds (Sheppard *et al.*, 1995). Several *in vitro* studies performed to measure the dissolution of mercury from soil found that relative bioavailability was generally estimated to be less than 10 percent (SAIC, 1994; CDM, 1992).

5.5.4 Bioavailability Via Dermal Exposure

A study of dermal absorption of mercuric chloride from water and soil used an *in vitro* model with human cadaver skin (Wester *et al.*, 1995). In this study, very little mercury passed through the skin and appeared in the receptor fluid (0.7 percent for water, 0.06 percent for soil), but a substantial amount of mercury was retained in the skin (28.5 percent for water, 7.9 percent for soil). It is not clear what proportion of the mercury retained in the skin would subsequently be absorbed.

5.5.5 Summary of Pertinent Data

Due to differences in toxicity and predominant routes of exposure, it is necessary to identify the mercury species present in soil whenever bioavailability studies are performed. Speciation studies of mercury are technically challenging, and peer review of proposed methods is recommended. Studies of oral absorption of mercury from weathered soils are very limited, and no dermal absorption studies have used weathered soils.

5.6 Nickel

Little is known about the bioavailability of nickel compounds in soil, largely due to the relatively low toxicity of nickel in soil. Oral absorption of nickel compounds is very limited, so dermal exposures may also need to be quantified to accurately characterize exposures.

5.6.1 Predominant Forms in Soil

Nickel may be present in soils in a variety of mineral forms, from forms with very limited solubility (sulfide and sulfate forms) to the much more soluble carbonate form. Given that nickel may be present as discrete mineral phases of varying solubility in soils, or adsorbed onto organic matter or clay particles, the solubility of nickel in soils will vary with different nickel sources and soil geochemistry.

5.6.2 Toxicity Assessment

The nature of the oral toxicity of nickel does not vary among the different forms expected to be present in soil. The oral reference dose is based on a study in which a soluble nickel salt (nickel sulfate hexahydrate) administered to rats after being mixed with their diet caused reduced body and organ weights. Roughly 10 to 15 percent of the population will show an immunological contact dermatitis reaction in response to nickel applied to the skin (Peltonen, 1979). This localized effect will not be dependent on systemic absorption, but may be affected by the solubility of nickel forms contacting the skin.

5.6.3 Relative Bioavailability Via Oral Exposures

Nickel generally is not well absorbed from the gastrointestinal tract in either laboratory animals or humans (ATSDR, 1997). Less than 5 percent of the most soluble nickel salts are absorbed orally in humans and animals. The gastrointestinal absorption of nickel correlates directly with the solubility of the metal, with less than 1 percent of the least soluble forms (oxides and sulfides) being absorbed.

When a soluble nickel form, nickel chloride, was mixed with soil and administered to rats as an aqueous slurry, the bioavailability was reduced relative to nickel chloride administered to the rats in water (Griffin *et al.*, 1990). The sandy-loam slurry produced a relative bioavailability of 63.1 percent, and the clay-loam slurry a 33.5 percent relative bioavailability, as measured by nickel in blood. No studies of the relative oral bioavailability of nickel in weathered soils were identified.

5.6.4 Bioavailability Via Dermal Exposures

No studies of dermal absorption of nickel from soil were identified.

5.6.5 Summary of Pertinent Data

Because of the great variation in solubility of nickel compounds, site-specific studies of the relative oral bioavailability of nickel in soil could have a significant effect on risk-based cleanup levels.

6.0 CHEMICAL-SPECIFIC CONSIDERATIONS FOR ASSESSING BIOAVAILABILITY IN AQUATIC (SEDIMENT) SETTINGS

All sediments contain metals. The metals in freshwater and marine sediments originate from several natural and human sources and are present in the sediments in several different physical and chemical forms (Goldberg, 1954). Ecological receptors are the focus of sediment quality assessments for most metals. Direct contact with sediments is usually limited for humans, and the primary exposure route to metals from sediment, i.e., dermal uptake, is expected to be low. The primary exposure pathway of concern from sediments to humans is consumption of aquatic organisms that have bioaccumulated metals from sediment, with methyl mercury being the primary metal of concern. Thus, bioavailability issues for human exposures will be addressed in the context of bioavailability of metals in sediments to aquatic organisms. Consequently this section addresses only ecological receptors.

The chemical species and forms of complexed, adsorbed, and solid metals in sediments have a profound effect on the bioavailability and toxicity of the metals to aquatic/marine plants and animals (Nelson and Donkin, 1985). Each metal has unique physical and chemical properties that determine the forms of the metals in sediments and pore water and their relative bioavailability to aquatic receptors. Metals in highly insoluble solid forms are not bioavailable to sediment-dwelling organisms. Metals in solution or colloidal suspension in sediment pore water or in adsorbed forms that are readily desorbed (leached) into the dissolved phase by small changes in oxygen concentration, pH, and Eh are bioavailable. Therefore, it is important to understand the chemical forms of metals in sediments if bioavailability is going to be used in ecological risk assessment.

Chemicals of potential concern due to possible bioaccumulation from sediments were recently identified by the U.S. EPA Bioaccumulation Analysis Workgroup (U.S. EPA, 2000a). Eleven metals were included in the resulting list of chemicals: arsenic, cadmium, chromium VI, copper, lead, methylmercury, nickel, selenium, silver, tributyltin (oxide) and zinc. The sections that follow are a brief summary of the forms, bioavailability, and toxicity of the metals thought to be of primary concern at DoD sediment sites, including arsenic, cadmium, chromium, copper, lead, mercury, nickel, tin and zinc

Table 6-1 summarizes information on background concentrations and effects levels for the metals discussed in this section with the exception of tin. In addition, “high” concentrations developed by Daskalakis and O’Connor (1995) based on data from the National Oceanic and Atmospheric Administration’s (NOAA’s) National Status and Trends Program are included. Daskalakis and O’Connor (1995) examined chemical residue data for large numbers of marine sediment samples collected as part of the NOAA National Status and Trends Program and several other monitoring programs in coastal marine environments in the United States. They defined a “high” concentration of chemicals in sediments as the geometric mean concentration plus one standard deviation of the National Status and Trends site means.

Table 6-1. Typical Background Concentrations and "High" Concentrations of Metals in Coastal Sediments

Metal	Background Conc. (µg/g dry wt)	High Conc. (µg/g)	ERL[(a)] (µg/g)	ERM[(a)] (µg/g)	Acute/Chronic Water Quality Criteria (µg/L)
Arsenic (As)	5-15	13	8.2	70	69/36
Cadmium (Cd)	0.1-0.6	0.54	1.2	9.6	43/9.3
Chromium (Cr)	50-100	125	81	370	1,100/50
Copper (Cu)	10-50	42	34	270	4.8/3.1
Lead (Pb)	5-30	45	46.7	218	220/8.5
Mercury (Hg)	≤ 0.2	0.22	0.15	0.71	2.1/1.11[(b)]
Nickel (Ni)	≤ 50	42	20.9	51.6	75/8.3
Zinc (Zn)	1.2->100	135	150	410	95/86

(a) Effects Range Low (ERL) and Effects Range Median (ERM) Screening Levels for Marine Sediments and Acute/Chronic Marine Water Quality Criteria are Included

(b) Marine water quality values are for inorganic mercury. The chronic value of methylmercury is 0.025 µg/L.

6.1 Arsenic

6.1.1 Predominant Forms in Sediment

Concentrations of total arsenic in uncontaminated nearshore estuarine and marine sediments usually fall in the range of 5 to 15 µg/g dry wt (Neff, 1997a) (Table 6-1). Daskalakis and O'Connor (1995) defined a "high" concentration of chemicals in sediments as the geometric mean concentration plus one standard deviation of the National Status and Trends site means. The "high" concentration of arsenic in coastal sediments is 13 µg/g. This concentration is exceeded frequently in sediments near natural (e.g., phosphate deposits) and anthropogenic sources of this chemical.

Arsenate (+V) is the most abundant form of arsenic in oxidized marine sediments, whereas arsenite (+III) is the dominant dissolved and solid species in reduced sediment layers (Neff, 1997a) (Table 2-3). Arsenite in oxidized sediments is oxidized rapidly to arsenate (De Vitre *et al.*, 1991). Much of the arsenic in the oxidized layers of sediment is associated (coprecipitated or adsorbed) with the hydrous iron and manganese oxide fraction or is present as $Fe_3(AsO_4)$. Under these conditions, the amount of arsenic in solution in potentially bioavailable forms in oxidized sediment pore water is low and 65 to 98 percent is present as the less bioavailable arsenate (Masscheleyn *et al.*, 1991).

Under moderately reducing conditions, iron and manganese oxide phases begin to dissolve, releasing adsorbed arsenate into pore water (Masscheleyn *et al.*, 1991). Arsenate is reduced to arsenite in reducing sediments and, if sulfur is abundant (as is the case in most marine sediments), most of the arsenic reacts with sulfides to form realgar (AsS), impurities in copper and zinc sulfides, arsenopyrite (FeAsS), and orpiment (As_2S_3) (Morse, 1994). These sulfides have low solubility, mobility, and bioavailability.

However, in estuarine and freshwater sediments containing low concentrations of sulfur, arsenic solubility is less limited by formation of insoluble sulfide minerals. Arsenite, often as arsenolite (As_2O_3), may

remain quite mobile and tends to diffuse upward to be released into the overlying water column as either arsenate or arsenite (Soma *et al.*, 1994). Because of this behavior, the bioavailability of arsenic usually is highest in freshwater sediments, is intermediate in estuarine sediments, and is lowest in marine sediments.

6.1.2 Bioavailability and Toxicity in Sediments

Sediments are a major source of arsenic in bottom-living freshwater and marine animals (Bryan and Langston, 1992). There is a direct relationship between the concentration of arsenic in tissues of sediment invertebrates and the arsenic/iron (As/Fe) ratio in the easily extractable (1-N HCl) fraction of sediments in which the invertebrates reside. In uncontaminated or slightly contaminated oxidized sediments, most of the non-residual arsenic is adsorbed to iron oxyhydroxides and is relatively unavailable.

Concentrations of total arsenic in the tissues of marine invertebrates and fish are very high. Most of the arsenic is present as various organo-arsenic compounds, particularly arsenobetaine, which are not toxic to the marine animals or their consumers, including humans (Neff, 1997a).

Inorganic arsenic is more toxic to aquatic plants than aquatic animals. Arsenite and arsenate have similar toxicities to aquatic organisms, but different species differ markedly in sensitivity to arsenic (Neff, 1997a). Methyl-arsenic compounds, frequently present at trace concentrations in sediments, are bioavailable, but have a low toxicity. The U.S. EPA acute and chronic water quality criteria for arsenic (as arsenite) for protection of marine life are 69 µg/L and 36 µg/L, respectively (Table 6-1). ERL and ERM concentrations of arsenic in marine sediments are 8.2 µg/g and 70 µg/g, respectively (Long *et al.*, 1995). Concentrations below the ERL values are considered to be rarely, if ever, toxic to bottom-dwelling marine animals. Concentrations between the ERL and ERM may be toxic to some species. Concentrations above the ERM are nearly always toxic to most species.

6.2 Cadmium

6.2.1 Predominant Forms in Sediment

Cadmium concentrations in uncontaminated marine sediments usually are in the range of 0.1 to 0.6 µg/g dry wt (Warren, 1981) (Table 6-1). The "high" concentration of cadmium in coastal sediments is 0.54 µg/g (Daskalakis and O'Connor, 1995). There is a direct correlation in relatively uncontaminated sediments between concentrations of cadmium and aluminum (an indicator of clay minerals) (Schropp *et al.*, 1990).

Cadmium in oxidized sediments is associated primarily (50 to 70 percent) with the carbonate plus iron/manganese oxide fractions of the sediment (Rosental *et al.*, 1986) (Table 2-3). Most of the remainder is associated with the organic/sulfide fraction. Only about 1 percent is in the completely non-bioavailable residual fraction, indicating that cadmium associated with oxidized sediments is likely to be moderately mobile and bioavailable (Samant *et al.*, 1990).

Cadmium in anoxic sediments appears to be associated almost exclusively with the sulfide phase (Salomons *et al.*, 1987). Cadmium forms solid sulfides and strong complexes with sulfides. However, soluble cadmium sulfide complexes are formed (e.g., $Cd(HS)_x^{x-2}$ where x = 1 or 4) only at high concentrations of sulfide ($>10^{-3}$ M). Cadmium sulfide complexes are moderately soluble; therefore, the mobility of cadmium in reducing environments may be quite high (Boulègue, 1983). Various insoluble hydroxide complexes may be present in freshwater sediments containing low sulfide concentrations. Nearly 90 percent of the cadmium in anoxic marine sediments is present as cadmium sulfide (Lee and Kittrick, 1984).

6.2.2 Bioavailability and Toxicity in Sediments

Marine invertebrates and fish bioaccumulate cadmium primarily from food and sediments (Canli and Furness, 1995; Wen-Xiong and Fisher, 1996). Oysters are able to filter 85 to 95 percent of cadmium-contaminated particles (sediment and diatoms) from water and retain about 60 percent of the cadmium supplied (Hardy *et al.*, 1984). More than half the cadmium in the oyster tissues is from ingested particles; the rest is from bioconcentration from the water. When mice are fed cadmium-contaminated oysters, they retain about 0.83 percent of the administered dose in their tissues (Sullivan *et al.*, 1984). Thus, the trophic transfer of cadmium from sediment particles and primary producers to a primary consumer is moderately efficient, but transfer to a secondary consumer, the mouse, is inefficient. Cadmium is not biomagnified in aquatic food webs.

Cadmium in ionic, bioavailable forms is one of the more toxic metals to freshwater and marine animals (Eisler, 1985). Toxicity tends to decrease with increasing salinity, because of complexation of the toxic ionic species with chloride. The U.S. EPA acute and chronic marine water quality criteria for cadmium are 43 μg/L and 9.3 μg/L, respectively (Table 6-1). ERM and ERL values for cadmium in sediments are 1.2 μg/g and 9.6 μg/g, respectively (Long *et al.*, 1995).

6.3 Chromium

6.3.1 Predominant Forms in Sediment

Concentrations of total chromium in uncontaminated estuarine and marine sediments usually are in the range of 50 to 100 μg/g dry wt (Mayer, 1988) (Table 6-1). The "high" concentration of chromium in U.S. coastal sediments is 125 μg/g (Daskalakis and O'Connor, 1995). Much of the chromium in sediments is associated with the clay fraction, as indicated by a close correlation between aluminum and chromium concentrations (Schropp *et al.*, 1990).

The distribution of chromium in sediment seems to depend in part on the source of the chromium. Generally, chromic chromium (+III) is more abundant than chromate chromium (+VI) in sediments. Chromate is a strong oxidizing agent and is reduced rapidly by organic matter and some metals in sediments. The small amounts of chromate in sediments usually is tightly bound to soil organic matter and iron oxide coatings on clay particles, or is coprecipitated with iron sulfides (Olazabal *et al.*, 1997). In estuaries receiving chromium from tanneries and electroplating operations, more than 80 percent of the total chromium in the sediment is associated with the organic/sulfide fraction (Loutit *et al.*, 1988). Because chromium is not known to form sulfides, carbonates, or phosphates (Mayer, 1988), and because of the stability of solid $Cr(OH)_3$, it is probable that most of the chromium in these sediments is bound to organic matter or is present as the hydroxide (Table 2-3).

Chromium in less contaminated oxidized sediments often is adsorbed primarily to amorphous iron oxide (50 to 70 percent) and organic/sulfide (25 to 40 percent) fractions of the sediment (Kersten and Förstner, 1986). Coarse-grained sediments contain a greater proportion of the total chromium in the non-bioavailable, residual fraction; clayey, organic-rich sediments contain a greater proportion of the total chromium in the more bioavailable organic fraction. More than 70 percent of the chromium in uncontaminated sediments may be associated with the non-bioavailable, residual fraction (Prohic and Kniewald, 1987). The residual chromium is associated primarily with the heavy minerals chromite, chromiferous magnetite, and spinels, as well as with the aluminosilicate lattice of clays (Mayer and Fink, 1980).

6.3.2 Bioavailability and Toxicity in Sediments

Marine and freshwater organisms have evolved efficient mechanisms for bioaccumulating and regulating chromium and other essential trace metals (Simkiss and Taylor, 1989). Concentrations of essential metals (including arsenic, chromium, copper, nickel, and zinc) in tissues of aquatic organisms are regulated at relatively constant values over a wide range of concentrations in the ambient media or food (Chapman *et al.*, 1996). Chromium (III) compounds, because of their low aqueous solubilities, have a low bioavailability to freshwater and marine organisms. Chromium bioaccumulated by marine animals tends to be sequestered in insoluble granules and is not bioavailable to predators of the marine animals (Nott and Nicolaidou, 1996).

Hexavalent chromium is moderately toxic, and trivalent chromium, because of its low aqueous solubility, is practically non-toxic to aquatic organisms. The U.S. EPA acute and chronic marine water quality criteria for chromate are 1,100 µg/L and 50 µg/L, respectively (Table 6-1). Marine sediment ERL and ERM values for chromium are 81 µg/g and 370 µg/g, respectively.

6.4 Copper

6.4.1 Predominant Forms in Sediment

Concentrations of copper in uncontaminated estuarine and marine sediments are in the range of 10 to 50 µg/g dry wt (Salomons and Förstner, 1984) (Table 6-1). The "high" concentration of copper in marine sediments is 42 µg/g (Daskalakis and O'Connor, 1995). Approximately 25 percent of coastal sediments monitored as part of U.S. monitoring programs contain concentrations of copper equal to or higher than the high value.

Much of the copper in sediments containing low concentrations of organic matter is in the residual fraction associated with the silicate lattice of clays (Chester *et al.*, 1988). In sediments containing high concentrations of organic matter, copper is associated primarily with the organic/sulfide fraction or with extractable organic matter (Luoma, 1985) (Table 2-3). Much of the remainder of the copper in oxidized sediments in associated with the reducible iron and manganese oxides (Prohic and Kniewald, 1987). In anoxic sediments, copper may undergo a variety of reactions with different inorganic and organic sulfur species to form a variety of soluble and insoluble complexes (Shea and Helz, 1988). Polysulfide complexes with cuprous copper (I) are soluble. Thus, the dominant form of copper in solution in the pore water of anoxic sediment layers is $CuS(S_5)^{-2}$. The dominant forms of copper in the solid phase of sediment include chalcocite (Cu_2S), covellite (CuS), and possibly chalcopyrite ($CuFeS_2$) (Shea and Helz, 1988). These sulfides have a low mobility and bioavailability.

6.4.2 Bioavailability and Toxicity in Sediments

Copper is an essential trace nutrient and is bioaccumulated by aquatic organisms primarily from the water. The most bioavailable forms of copper to aquatic organisms are the inorganic hydroxide complexes [$CuOH^+$, $Cu(OH)_2$, $Cu(OH)_3$, and $Cu_2(OH)_2$] (Simkiss and Taylor, 1989). The free ion (Cu^{+2}) also is bioavailable (Phinney and Bruland, 1994). Most organic complexes of copper are bioaccumulated inefficiently. Aquatic organisms regulate concentrations of copper in their tissues within a narrow, species-specific range and net accumulation to higher than natural concentrations occurs only when concentrations of bioavailable forms of copper in water or sediments greatly exceed natural levels. Water is the main source of copper in tissues of aquatic organisms (Ettanjani *et al.*, 1992). Copper does not biomagnify in aquatic food webs (Schafer *et al.*, 1982).

Dissolved, reactive copper is toxic to aquatic plants and animals. Free ionic copper at concentrations as low as 0.3 μg/L decreases primary production in several species of oceanic phytoplankton (Brand *et al.*, 1986). However, most of the copper in seawater is complexed with organic matter or in less toxic, bioavailable forms. The U.S. EPA acute and chronic marine water quality criteria for copper are 4.8 μg/L and 3.1 μg/L, respectively (Table 6-1). The ERL and ERM for copper in marine sediments are 34 and 270 μg/g, respectively (Long *et al.*, 1995).

6.5 Lead

6.5.1 Predominant Forms in Sediment

Concentrations of lead in uncontaminated estuarine and nearshore marine sediments generally fall in the range of 5 to 30 μg/kg dry wt (Salomons and Förstner, 1984) (Table 6-1). Freshwater sediments may contain lower concentrations. The "high" concentration of lead in marine sediments is 45 μg/kg (Daskalakis and O'Connor, 1995). Most of the lead in sediments is associated with fine-grain sediment particles (Krumgalz *et al.*, 1992). Residual lead (part of the mineral matrix of sediment particles) in uncontaminated sediments, which may represent up to 80 percent of the total lead, is associated primarily with aluminosilicates, sulfide minerals, and barite (Loring, 1982). This residual lead is immobile and not bioavailable. The non-residual lead in oxidized surficial sediments appears to be associated primarily with reducible iron and manganese oxide coatings on clay particles (Luoma and Bryan, 1981) (Table 2-3), as indicated by the strong positive correlation between concentrations of aluminum (from aluminosilicate clay particles) and lead in sediments (Schropp *et al.*, 1990).

In anoxic (oxygen-depleted) sediments, the most stable valence state of lead is the +2 state (Harada and Tsunogai, 1988). Divalent lead (Pb^{+2}) reacts with inorganic sulfide in sediment to form highly insoluble lead sulfide (PbS) (Kersten and Förstner, 1986). However, in highly reducing sediments with an Eh of less than about –0.4 volts, lead may form bisulfide complexes with sulfur. These bisulfide complexes are slightly soluble and the dissolved lead may be mixed up into the water column by sediment disturbance (Shea and MacCrehan, 1988). Most of the lead in oxidized and anoxic sediments is in insoluble and non-bioavailable forms.

6.5.2 Bioavailability and Toxicity in Sediments

Marine deposit-feeding clams and polychaete worms are able to bioaccumulate lead from oxidized sediments (Luoma, 1985). The bioavailability of lead to sediment-associated animals is proportional to the lead/iron concentration ratio in weak acid extracts of the sediment, indicating that the lead absorbed to iron oxide coatings on sediment particles is not bioavailable. In moderately hypoxic or anoxic sediments, most of the lead is precipitated as lead sulfide and is not bioavailable (Bourgoin *et al.*, 1991). Lead is biodepleted in marine food chains relative to calcium, which behaves similarly to lead in the environment (Smith *et al.*, 1990), meaning that it does not biomagnify.

Inorganic lead is moderately toxic to freshwater and marine organisms. U.S. EPA acute and chronic water criteria for inorganic lead for protection of marine life are 220 μg/L and 8.5 μg/L, respectively (Table 6-1). The ERL and ERM concentrations in marine sediments are 46.7 μg/g and 218 μg/g, respectively.

6.6 Mercury

6.6.1 Predominant Forms in Sediment

Concentrations of total mercury in uncontaminated estuarine and marine sediments generally are 0.2 μg/g dry wt or lower (Salomons and Förstner, 1984) (Table 6-1), except in areas of natural mercury-containing deposits, such as the East Pacific Rise and the Mid-Atlantic Ridge (Jonasson and Boyle, 1972). The "high" concentration of mercury in coastal sediments is 0.22 μg/g (Daskalakis and O'Connor, 1995).

Mercury may occur in three valence states in water and sediments: zero (elemental mercury), +1 (mercurous compounds), and +2 (mercuric compounds) (Moore and Ramamoorthy, 1984). The +2 valence state is the most common in well-oxygenated and hypoxic aquatic environments. Mercury (II) is reduced to elemental mercury, mercuric sulfide, and methylmercury in anoxic sediments (Weber *et al.*, 1998).

Most of the labile (non-residual) mercury in sediments is complexed with particulate and dissolved organic matter in the sediments and not with clay particles or iron oxide coatings on clay particles (Table 2-3). Inorganic and organic mercury salts form very strong and stable complexes with organic ligands in water and sediment (Moore and Ramamoorthy, 1984). These organic complexes have a low bioavailability to aquatic organisms.

Most mercury methylation takes place in hypoxic or anoxic sediment layers (Gagnon *et al.*, 1996). Mercury methylation is performed primarily by sediment-dwelling, sulfate-reducing bacteria. Under certain conditions, volatile dimethylmercury also is formed (Weber *et al.*, 1998). It may diffuse through the sediment layers into the overlying water column from which it evaporates into the atmosphere. Elemental mercury, also produced by sulfate-reducing bacteria, is slightly volatile and may be lost rapidly from sediments to the atmosphere (Nakamura *et al.*, 1990). In oxidized sediment layers, methylmercury is demethylated to produce inorganic divalent mercury. Because of rapid interconversions of inorganic and organic mercury in oxidized and reduced layers of freshwater and marine sediments, methylmercury rarely represents more than 1 percent of the total mercury in sediments (Berman and Bartha, 1986). Dissolved methylmercury may represent up to about 30 percent of the total dissolved mercury in sediment pore water, but less than 1 percent of the methylmercury adsorbed to sediment particles in the anoxic layers of sediments (Gagnon *et al.*, 1996). Although much of the dissolved methylmercury in sediment pore water is actually complexed to dissolved organic matter, particularly fulvic acids, it should be considered potentially bioavailable to sediment-dwelling organisms. The main pathway for movement of methylmercury from anoxic pore water into the overlying water column is through bioaccumulation by sediment-dwelling animals that are part of the aquatic food web.

High concentrations of sulfide in sediments may inhibit methylmercury formation (Berman and Bartha, 1986). This is thought to be due to formation of extremely insoluble mercuric sulfide (solubility product $10^{-52.4}$). Mercuric sulfide tends to be quite stable and non-bioavailable in hypoxic and anoxic sediments. However, if sulfide concentrations are very high, more soluble disulfide (HgS_2^{-2}) or polysulfide complexes may be formed. These sulfides are more soluble than HgS (Lu and Chen, 1977).

6.6.2 Bioavailability and Toxicity in Sediments

Because of their high affinity for dissolved and particulate organic matter, both inorganic and organic mercury readily complex with organic matter in water and sediments. Mercury bound to organic particles has a low bioavailability to freshwater and marine organisms (Jenne and Luoma, 1977). Methylmercury is more readily bioaccumulated than inorganic mercury (Phillips and Buhler, 1978). This probably is a

result of the much slower release of bioaccumulated organic than inorganic mercury by aquatic animals (Thompson, 1990).

Quantitatively, the most important sources of mercury, particularly methylmercury, in the tissues of aquatic animals are probably from ingestion of mercury-contaminated sediments and food. Methylmercury in the tissues of aquatic animals is derived from microbial methylation of inorganic mercury in hypoxic and anoxic layers in the water column and sediments (Rolfhus and Fitzgerald, 1995; Gagnon *et al.*, 1996). The dominant form of mercury in the tissues of most freshwater and marine animals is methylmercury. The concentration of organo-mercury tends to increase with increasing trophic level in aquatic food webs, indicating that organic mercury compounds can be biomagnified in aquatic food webs (Schafer *et al.*, 1982). Very high concentrations of total mercury may be present in the livers of fish-eating marine birds and mammals (Neff, 1997b).

Mercury as the reactive, free inorganic ion and as various organo-mercury compounds in solution is one of the most toxic metals to marine organisms. Acutely toxic concentrations of inorganic mercury in solution are in the range of 3 to 1,000 μg/L. However, mercury that is complexed with dissolved or particulate organic matter in the water is not readily bioavailable and has a low aquatic toxicity. The U.S. EPA chronic marine water quality criterion for mercury (II) is 1.106 μg/L; the chronic value for methylmercury is 0.025 μg/L (Table 6-1). However, methylmercury rarely represents more than 10 percent of total mercury in oxygenated surface waters (Mason and Fitzgerald, 1993). Therefore, the chronic value for this form of mercury rarely is exceeded in surface waters. The sediment screening levels for total mercury are 0.15 μg/g ERL and 0.71 μg/g ERM.

6.7 Nickel

6.7.1 Predominant Forms in Sediment

Nickel often is relatively abundant in soils and sediments. Uncontaminated estuarine and marine sediments usually contain 50 μg/g dry wt or less of nickel, the concentration often being positively correlated with the clay content of the sediments (Bowen, 1979) (Table 6-1). The "high" concentration of nickel in sediments from coastal areas of the United States is 42 μg/g (Daskalakis and O'Connor, 1995). However, much higher concentrations of nickel are reported frequently in apparently uncontaminated sediments (Breckenridge and Crockett, 1995). Some soils and sediments, particularly of deep-sea origin, may contain up to 1,000 μg/g nickel (Loring and Asmund, 1996). Similarly, igneous rocks contain 2 to 3,600 ppm nickel (Adriano, 1986), and volcanic minerals may contain high nickel concentrations.

In oxidized sediments, much of the potentially bioavailable nickel is complexed to iron and manganese oxides (Luther *et al.*, 1986) (Table 2-3). Nickel forms weak coordination complexes with oxygen donors such as carboxylate, hydroxyl, and other oxy-ligands (e.g., humic and fulvic acids, clays, and metal oxides) (Wood, 1987). It also becomes tightly bound to anionic groups of bacterial polysaccharides (Wood, 1987). Nickel forms stable, insoluble complexes with surfides and organic thiols in anoxic sediment layers (Wood, 1987). However, most of the nickel (often more than 90 percent) in relatively uncontaminated sediments is in the residual fraction, associated primarily with oxide minerals, such as magnetite, spinels, and silicates (Loring, 1982). Thus, the bioavailability of nickel in sediments usually is low.

6.7.2 Bioavailability and Toxicity in Sediments

Like other essential metals, nickel concentrations in the tissues of aquatic organisms do not covary with nickel concentrations in the ambient water, sediments, and prey items. Of the dominant forms of nickel in

sediments and sediment pore water [Ni^{+2}, $Ni(OH)_2$, and NiS], only nickel ion is readily bioavailable (Förstner and Wittmann, 1981). However, nickel sulfide is the most soluble of the common metal sulfides and readily dissolves when the oxygen concentration in sediment increases. Similarly, nickel weakly complexed to organic matter in surface sediments readily exchanges with divalent cations in the water, releasing bioavailable nickel ion to the overlying water column (Morse, 1995). The hydroxide and sulfide are insoluble. Nickel in soils generally is not bioavailable to earthworms (Sample *et al.*, 1998).

Inorganic nickel has a relatively low toxicity to aquatic organisms. The U.S. EPA marine acute and chronic water quality criteria for nickel are 75 µg/L and 8.3 µg/L, respectively (Table 6-1). ERL and ERM values for nickel in marine sediments are 20.9 µg/g and 51.6 µg/g, respectively (Long *et al.*, 1995). These screening values often are exceeded (usually without adverse effects in benthic organisms) as a result of the high abundance of residual nickel in several crustal rocks and minerals.

6.8 Tin

6.8.1 Predominant Forms in Sediment

The concentration of inorganic tin in uncontaminated sediments is about 2 µg/g dry wt. Although inorganic tin compounds may be moderately toxic to aquatic organisms, contamination of aquatic ecosystems with inorganic tin is rarely perceived as a problem, except possibly near some metal smelting and mining operations (Skei *et al.*, 1972). However, various organotin compounds, some of which are extremely toxic to aquatic organisms, are used for a variety of commercial purposes that favor their entry into the marine environment. Most organotins contain tetravalent tin covalently bonded to one to four organic substituents (Müller *et al.*, 1989). Tripropyl-, tributyl-, and triphenyl-tins are extremely effective biocides that are used as wood preservatives, antifoulants for boat hulls and other submerged structures, and disinfectants and slimicides for cooling and paper mill waters (Snoeij *et al.*, 1987). Although organotins do not adsorb strongly to particles, they do tend to accumulate in sediments in the vicinity of major sources in the water column (e.g., marinas and ship yards), though their concentrations rarely are as high as those of inorganic tin.

Tributyltin (the most common organotin in antifouling coatings) is present in aerobic sediment primarily as tributyltin chloride, tributyltin hydroxide, and tributyltin carbamate (Eng *et al.*, 1986). In anaerobic sediment, the dominant chemicals forms appear to be the sulfide, hydroxide, and carbonate. Tributyltins undergo sequential de-alkylation in sediments to yield dibutyltin, monobutyltin, and finally inorganic tin (Maguire and Tkacz, 1985). The degradation half-life of tributyltin in oxidized marine sediments is approximately 162 days (Stang and Seligman, 1986). Biodegradation of tributyltin in hypoxic or anoxic sediments is negligible.

6.8.2 Bioavailability and Toxicity in Sediments

Organotins in water, sediments, and tissues of aquatic organisms are relatively bioavailable (Laughlin and French, 1988). They also are highly toxic to aquatic organisms (Langston *et al.*, 1990).

Concentrations as low as 1-2 ng/L (parts per billion) of dissolved tributyltin causes severe reproductive and developmental effects in freshwater and marine invertebrates. These concentrations are observed in the water of marinas and ports where vessels are protected with tributyltin-based paints from biofouling organisms. Because of their high toxicity, tributyltin antifouling paints recently were banned for most marine and freshwater uses in the United States and Europe.

6.9 Zinc

6.9.1 Predominant Forms in Sediment

Concentrations of zinc in uncontaminated sediments vary widely. Coarse-grained sandy sediments may contain as little as 1.2 µg/g dry wt zinc; clay sediments may contain more than 100 µg/g total zinc (Larsen and Gaudette, 1995) (Table 6-1). The "high" concentration of zinc in U.S. coastal sediments is 135 µg/g (Daskalakis and O'Connor, 1995).

Most of the zinc in sediments is residual, rendering it non-bioavailable. The residual zinc is associated with the mineral lattice of clays and with a variety of heavy minerals, including chromite, ilmenite, and magnetite (Loring, 1982). Sphalerite (ZnS) and zincite (ZnO) are important carriers of residual zinc in some sediment. The nonresidual zinc in many oxidized sediments is associated primarily with the reducible iron and manganese oxide fractions. In reducing sediments, much of the zinc is associated with the organic/sulfide fraction (Rosental *et al.*, 1986) (Table 2-3). During transitions of oxidation/reduction potential in sediments, zinc may be released in soluble form into sediment pore water, from which it diffuses into the overlying water column. The total flux of zinc from sediments into the waters of the whole of southern San Francisco Bay is approximately 298 kg/day (Wood *et al.*, 1995).

6.9.2 Bioavailability and Toxicity in Sediments

Zinc is an essential micronutrient in all aquatic organisms, being a cofactor in several enzymes. Most aquatic species have efficient mechanisms for bioaccumulating zinc, and some species store zinc in non-toxic forms in their tissues. Freshwater and marine organisms accumulate zinc from water, food, and sediments. Sediment-dwelling aquatic invertebrates can accumulate zinc adsorbed to iron oxides in oxidized sediments (Harvey and Louma, 1985). Much of the zinc in tissues of aquatic organisms is sequestered in phosphate granules and is not bioavailable to predators (Nott and Nicolaidou, 1993). Zinc is not biomagnified in aquatic food webs.

The toxic species of zinc is the free ion, which represents only a small fraction of the total zinc in natural water and sediment pore water. Acutely lethal concentrations of total zinc in solution usually are in the range of 100 to 50,000 µg/L. Sublethal responses are observed, particularly in aquatic plants, at much lower concentrations. Invertebrates and plants seem to be more sensitive than fish and higher animals to zinc poisoning. The U.S. EPA acute and chronic water quality criteria for zinc are 95 µg/L and 86 µg/L, respectively (Table 6-1). The ERL and ERM for zinc in marine sediments are 150 µg/g and 410 µg/g, respectively, reflecting the relatively low toxicity of sediment-bound zinc (Long *et al.*, 1995).

7.0 SUMMARY OF SELECTED CASE STUDIES

Bioavailability adjustments have been incorporated into human health risk assessments for several sites having metals contamination. The number of such sites continues to grow as the concept of bioavailability is better understood and gains acceptance among the regulatory community. Bioavailability studies have been used both at sites where U.S. EPA is the lead regulatory agency (Regions III, VII, VIII, IX, and X) and at sites where the state agency has the lead (Oklahoma, Michigan, California, Illinois, Wisconsin, and New Jersey). Bioavailability adjustments have been supported by *in vivo* animal studies, *in vitro* testing, environmental health studies, mineral speciation, or some combination of these methods. To date, most bioavailability adjustments have been made for the oral route of exposure. Only one case study was identified for dermal bioavailability, and none were identified for the inhalation pathway. Bioavailability adjustments have been made for arsenic, lead, mercury, and cadmium; however, the majority of adjustments have been for lead and arsenic associated with mining and smelting activities.

Results of several case studies are presented in Table 7-1. Most of the case studies presented here illustrate decreased bioavailability compared to the default assumptions and thus increased cleanup levels; however, it should be noted that in some cases (particularly for lead, where the default assumption is 30 percent absolute bioavailability from soil) bioavailability studies can support the default assumption or even demonstrate higher bioavailability than the default. One such example in Table 7-1 is the Palmerton, PA site, where swine studies supported the default bioavailability value of 30 percent.

Among the case studies presented in Table 7-1, the National Zinc Company NPL Site in Bartlesville, OK illustrates several factors that are important in getting a bioavailability study accepted. In this case study, the regulators and other stakeholders were involved from the beginning. A detailed work plan including protocols for the bioavailability studies was prepared. Protocols were developed with input from toxicologists with training in pharmacokinetics to select appropriate animal models and testing endpoints. These protocols followed GLP Standards and were peer reviewed by an outside toxicologist brought in by the stakeholders. Also, the regulators and stakeholders were given the opportunity to review the results prior to making final interpretation. The bioavailability studies for this site supported RAFs of 0.25 for arsenic, 0.33 for cadmium, and 0.20 (vs. default of 0.30) for lead (PTI, 1994). Using these adjustments for bioavailability, the Oklahoma Department of Environmental Quality (DEQ) accepted a threefold increase in cleanup levels for arsenic and cadmium (from 20 to 60 ppm for arsenic and from 30 to 100 ppm for cadmium) and almost a twofold increase in the cleanup level for lead (from 500 to 925 ppm)(ODEQ, 1994). In this case, the process from drafting the work plan to draft remedial investigation report for public comment required only seven months. The costs related to the bioavailability studies (work plan development and laboratory testing) were approximately $200,000; however, the increased cleanup goals reduced remediation costs by approximately $40 million.

Table 7-1. Selected Case Studies for Bioavailability Adjustments

Site	Contaminant	Test	Bioavailability Test Results	Cleanup Level	Regulatory Agency
National Zinc Co. NPL Site, Bartlesville, OK	Lead	*In vivo* – rat; and speciation	40% (20% absolute)	925 mg/kg	Oklahoma DEQ
	Cadmium	*In vivo* – rat; and speciation	33%	100 mg/kg	
	Arsenic	*In vitro* (PBET); and speciation	25%	60 mg/kg	
Butte, MT	Lead	*In vivo* – rat	24% (12% absolute)	1,200 mg/kg	U.S. EPA Region VIII
Palmerton, PA	Lead	*In vivo* – swine, Monte Carlo analysis	30% absolute (same as default)	650 mg/kg	U.S. EPA Region III
Anaconda, MT	Arsenic (soil)	*In vivo* – monkey	18.3%	250 ppm	U.S. EPA Region VIII
	Arsenic (dust)	*In vivo* – monkey	25.8%		
Rushton/North Tacoma, WA Off-Site	Arsenic (soil)	None – Regulators accepted adjustment	80%	230 ppm	U.S. EPA Region X
Oak Ridge National Laboratory, TN	Mercury	*In vivo*, *in vitro*, speciation	10%	400 ppm	U.S. EPA Region IV
Carson River, NV	Mercury (insoluble 90%, soluble 10%)	Speciation	(20% for insoluble; 100% for soluble) 30% overall	80 ppm	U.S. EPA Region IX
Crego Park, MI	Arsenic	*In vitro* (PBET) and speciation	10%	68 ppm (from 6.8 ppm)	Michigan DEQ
Almaden Quicksilver County Park, Los Gatos, CA	Mercury	*In vitro* and speciation	30%	300 to 500 ppm for various areas in park	Cal-EPA DTSC
Union Pacific Railroad Yard, Sacramento, CA	Arsenic	*In vivo* – swine	0-1% absorption from slag vs. 59% absorption of soluble control	No cleanup required (slag up to 1,800 ppm As)	Cal-EPA DTSC
Hudson Co., NJ	Chromium	*In vitro* extraction (ASTM method 3987)	Endpoint allergic contact dermatitis	State has recommended test but no results yet	NJDEP

Cal-EPA = California Environmental Protection Agency.
DEQ = Department of Environmental Quality.
DTSC = Department of Toxic Substances Control.
NJDEP = New Jersey Department of Environmental Protection.
PBET = Physiologically Based Extraction Test.

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