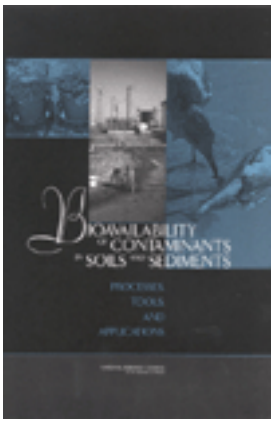

Bioavailability of Contaminants in Soils and Sediments: Processes, Tools, and Applications

Committee on Bioavailability of Contaminants in Soils and Sediments, National Research Council

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Bioavailability of Contaminants in Soils and Sediments

Processes, Tools, and Applications

Committee on Bioavailability of Contaminants in Soils and Sediments
Water Science and Technology Board
Division on Earth and Life Studies
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Preface

Protection of human health and ecosystems is much more challenging today than 33 years ago when the U.S. Environmental Protection Agency (EPA) was founded. Environmental problems in the United States today are diffuse rather than localized, subtle rather than obvious, and involve multiple environmental media (air, water, soil, sediment, and biota) rather than a single medium. Complex environmental questions transcend disciplinary boundaries and involve multiple temporal and spatial scales. Since 1970, advances in analytical measurement techniques have occurred that now allow the detection of more and more chemicals at lower and lower levels. As the new millennium begins, there is a wealth of information about how parts of the environment might function and where chemical contaminants may be found. At the same time it has become all the more difficult to understand what is really important and what should receive highest priority. This is exemplified by our national efforts to assess and manage thousands of acres of contaminated soil and sediment.

It is against this backdrop that the National Research Council (NRC) undertook an examination of the bioavailability of contaminants in soils and sediments. Of primary interest is the risk that contaminated soils and sediments pose to humans and ecological receptors, for which estimating exposure is essential for sound decision-making and devising effective solutions. This report focuses on an assessment of those physical, chemical, and biological factors that may make only a fraction of the total contaminant mass in soil and sediment actually available to humans and ecological receptors. A large amount of empirical data suggests that soils and sediments may sequester chemical contaminants and that chemicals in soils and sediments behave differently than when present in water,

air, or food. The influences that soils and sediments have on contaminant interactions between phases, the transport of contaminants to organisms, the entry of contaminants into living cells, and contaminant accumulation within organisms and possible toxic effects are referred to herein as "bioavailability processes." Understanding these processes is central to improving risk assessment, prioritizing among various problems, and using resources to achieve the greatest benefit.

While the term "bioavailability" is relatively new, bioavailability as a concept has a long history in toxicology, pharmacology, crop science, and nutritional science. Common to all of these contexts is uptake by living organisms. In contrast, the application of bioavailability process understanding in the environmental arena has occurred much more recently, largely within the last decade, and it involves such contextual issues as solubility, mass transfer, mobility, and reaction in addition to uptake by living organisms. Explicitly assessing contaminant bioavailability is viewed by many as a way to help set contaminated site cleanup goals that are more financially or technically feasible, and that involve leaving appreciable amounts of contaminant mass in place, while still being protective of public health and the environment.

Prior to commencing this study, the NRC's Water Science and Technology Board hosted a one-day workshop in November 1998 to assess the need for a NRC study of bioavailability of contaminants in soils and sediments, attended by approximately 25 key experts. A consensus from the attendees was that there is a growing acceptance of incorporating site-specific bioavailability measurements in site management decisions, but that many of the methods being considered for bioavailability assessment have not been critically reviewed or validated. As a result of the workshop, possible study questions were proposed, a prospectus was drafted and circulated, and project sponsors were identified.

The NRC Committee on Bioavailability of Contaminants in Soils and Sediments convened its first meeting in May 2000 and met five additional times over the next two years. The committee's charge included assessing the application of bioavailability concepts for managing hazardous compounds and guiding risk assessment. The committee sought to put the growing interest in bioavailability into perspective by focusing on building a mechanistic-based understanding of bioavailability processes. The primary goal was to define the scientific understanding needed to advance confidence in use of bioavailability concepts, and to assess the tools needed to characterize and measure bioavailability.

The study benefited greatly from contributions of various individuals who made presentations at our meetings, including Fran Kremer, Peter Grevatt, Sarah Levinson, Mark Maddaloni, Elmer Akin, Mark Johnson, Chris Weis, and Mary Reiley, all from the U.S. Environmental Protection Agency; Brad Smith and Cathy Vogel, Strategic Environmental Research and Development Program; Beth Anderson, National Institute of Environmental Health Sciences; Michael Major, U.S. Army; Doris Anders, U.S. Air Force; Sharon Williams-Fleetwood, Agency for Toxic Substances and Disease Registry; Chet Miller, Department of Energy;

Greg Planicka, National Environmental Policy Institute; Teresa Bernhard, Naval Engineering Field Activity Chesapeake; Steve McGrath, IACR-Rothamsted; Monty Elder, Oklahoma Department of Environmental Quality; Todd Bridges and Jeff Steevens, U.S. Army Corps of Engineers; Al Page, University of California at Riverside; Larry Goldstein, Electric Power Research Institute; Hans Stroo, ThermoRetec; Ron Jensen, Southern California Edison; Roman Lanno, Oklahoma State University; and Diane Henshel, Indiana University.

The study would not have been possible without the very capable management and excellent guidance provided by Laura Ehlers of the WSTB. She served as the study director and organized meetings, kept us on track from meeting to meeting, provided important reminders about discussion points, and helped identify places where the committee seemed to be stalled and suggested possible paths forward. She synthesized and edited the final report and was always our tireless cheerleader. Anike Johnson took care of the many mailings and made local meeting arrangements.

More formally, the report has been reviewed by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the authors and the NRC in making the published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The reviews and draft manuscripts remain confidential to protect the integrity of the deliberative process. We thank the following individuals for their participation in the review of this report: Graeme Batley, CSIRO Energy Technology; G. Allen Burton, Wright State University; Kim F. Hayes, University of Michigan; Michael J. McLaughlin, CSIRO Land and Water; Aaron L. Mills, University of Virginia; Joseph J. Pignatello, Connecticut Agricultural Experiment Station; Rosalind A. Schoof, Gradient Corporation; and Eric H. Weyand, Rutgers University.

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of this report was overseen by Bruce E. Rittmann, Northwestern University. Appointed by the NRC, he was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the NRC.

Richard G. Luthy, *Chair*

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Summary

OVERVIEW

The past century of industrial, military, and commercial activity in the United States has resulted in hundreds of thousands of hazardous waste sites where organic compounds and metals contaminate surface and subsurface soils and sediments. In order to reduce risks to human and ecological receptors, considerable time and money have been spent remediating these sites since passage of major environmental legislation (e.g., Superfund). To overcome the difficulties inherent in hazardous waste remediation and resource constraints, as well as to help prioritize cleanup efforts, potentially responsible parties and regulators have recently begun to consider using the concept of bioavailability during hazardous waste site management. This interest stems from observations that some contaminants in soils or sediments appear to be less available to cause harm to humans and ecological receptors than is suggested by their total concentration, such that cleanup levels expressed as bulk concentrations may not correlate with actual risk. This phenomenon, known to involve physicochemical interactions between contaminants and solid particles, can become accentuated with aging of the contaminated soils or sediments.

The extent to which chemicals are bioavailable has significant implications for the cleanup of contaminated media. If it can be demonstrated that greater levels of contamination can be left in soil or sediment without additional risk, decreased costs and smaller remediation volumes may be realized, and an opportunity for less intrusive remedial approaches exists. Growing interest in this issue led the National Research Council (NRC) in 2000 to undertake a comprehensive

study that would examine the bioavailability of contaminants in soil and sediment, focusing on those factors that influence the percentage of total contaminant levels to which humans and ecological receptors are exposed. Several key questions served to guide the study:

- What scientific understanding is missing that would provide confidence in the use of bioavailability factors for different contaminant classes? That is, what bioavailability mechanisms and processes require better understanding? What are the highest priority research needs? For which contaminant classes, environmental settings, and organism classes are bioavailability assessments most important?
- What tools (biological, chemical, and physical) are available to characterize and measure bioavailability for different contaminant classes, and what new tools are needed? What criteria should be used to validate these tools?
- How do treatment processes affect bioavailability for different contaminant classes? How does bioavailability affect treatment processes that rely on microbial degradation of contaminants?
- How and when should bioavailability information be used? What are its implications for relevant regulations? How can information on bioavailability be reliably communicated, especially to the public?

The NRC committee convened to address these tasks reached several overarching conclusions and recommendations about our current understanding of processes that affect whether contaminants in soils and sediments are bioavailable to humans, animals, microorganisms, and plants. Detailed conclusions and recommendations are found in this summary and throughout the report.

Bioavailability processes are defined as the individual physical, chemical, and biological interactions that determine the exposure of plants and animals to chemicals associated with soils and sediments. In the broadest sense, bioavailability processes describe a chemical's ability to interact with the biological world, and they are quantifiable through the use of multiple tools. Bioavailability processes incorporate a number of steps not all of which are significant for all contaminants or all settings, and there are barriers that change exposure at each step. Thus, bioavailability processes modify the amount of chemical in soil or sediment that is actually absorbed and available to cause a biological response.

Bioavailability processes are embedded within existing human health and ecological risk frameworks. The goal of bioavailability analysis is to reduce uncertainty in exposure estimates and thus improve the accuracy of risk assessment. However, today "bioavailability" is commonly thought of in relation to one process only—absorption efficiency—such that a single "bioavailability

factor" is used as an adjustment to applied dose. Other bioavailability processes are hidden within risk assessment, and assumptions made about these processes are not clear.

Mechanistic understanding of bioavailability processes is ultimately needed to improve the scientific basis of risk assessment. Thus, tools for measuring bioavailability processes that further mechanistic understanding and promote predictive model development are preferred over conventional empirical approaches. In the short term, empirical approaches are useful in generating site-specific information—provided that their results are analyzed using a weight-of-evidence approach and with an understanding that they will be replaced with more mechanistic tools as they are developed. At any given site, a suite of tools will be necessary to describe bioavailability processes in soils or sediments.

The potential for the consideration of bioavailability processes to influence risk-based decision-making is greatest where certain chemical, environmental, and regulatory factors align, that is:

- where the contaminant is (and is likely to remain) the risk driver at a site;
- where the default assumptions made for a particular site are inappropriate;
- where significant change to remedial goals is likely (e.g., because large amounts of contaminated soil or sediment are involved);
- where conditions present at the site are unlikely to change substantially over time; and
- where regulatory and public acceptance is high.

These factors should be evaluated before committing the resources needed for a detailed consideration of bioavailability processes.

DEFINING BIOAVAILABILITY PROCESSES

The individual physical, chemical, and biological interactions that determine the exposure of organisms to chemicals associated with soils and sediments are defined herein as "bioavailability processes" (Figure ES-1). The report adopts the term "bioavailability processes" because "bioavailability" has been defined in different ways that are often discipline-specific—creating a semantic stumbling block that can confound use of the term. Presently, our mechanistic understanding of the bioavailability processes described below is highly variable, and quantitative descriptive models of bioavailability processes in most cases are lacking.

"A" in Figure ES-1—contaminant binding and release—refers to the physical and [bio]chemical phenomena that bind, unbind, expose, or solubilize a contaminant associated with soil or sediment. Binding may occur by adsorption on solid surfaces or within a phase like natural organic matter, or by change in form

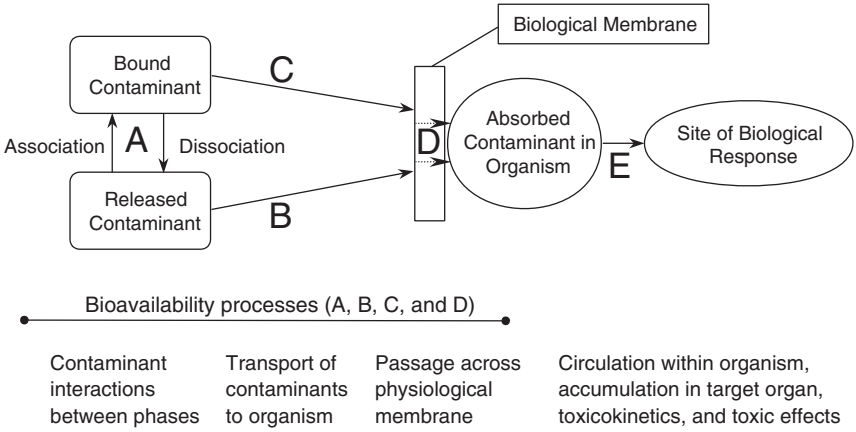

FIGURE ES-1 Bioavailability processes in soil or sediment, including release of a solid-bound contaminant and subsequent transport, direct contact of a bound contaminant, uptake by passage through a membrane, and incorporation into a living system. Note that A, B, and C can occur internal to an organism such as in the lumen of the gut.

as by covalent bonding or precipitation. Contaminants become bound to solids as a result of chemical, electrostatic, and hydrophobic interactions, the strength of which vary considerably. An important aspect governing contaminant–solid interactions is time; with aging, a contaminant generally is subject to transformation or incorporation into a more stable solid phase that can lead to a decrease in contaminant bioavailability. Contaminants can be released to fluid in contact with soil or sediment in response to changes in water saturation, in water and gas chemistry, and in solid surface properties. Biologically induced release is common in natural systems, including release mediated by digestive processes, microorganisms, plants, and bioturbating invertebrates.

"B" in Figure ES-1 involves the movement of a released contaminant to the membrane of an organism, while C involves the movement of contaminants still bound to the solid phase. Contaminants dissolved in the aqueous or gas phases are subject to transport processes such as diffusion, dispersion, and advection that may carry the contaminant to the surface of a living organism. These same processes can also transport contaminants still bound to small solid particles (colloids) to within close proximity of potential receptors. As contaminants are being transported, they can undergo transformation reactions (including oxidation–reduction reactions, hydrolysis, acid–base reactions, and photolysis) that can affect greatly the bioavailability and toxicity of the contaminant. It should be noted that if association–dissociation processes have occurred internally (as in the gut lumen), fate and transport processes prior to uptake across a biological membrane may be limited.

The bioavailability process depicted as D entails movement from the external environment through a physiological barrier and into a living system. Because of the enormous diversity of organisms and their physiologies, the actual process of contaminant uptake into a cell—or factors that may impede or facilitate uptake—varies depending on receptor type. One common factor among all organisms is the presence of a cellular membrane that separates the cytoplasm (cell interior) from the external environment. Most contaminants must pass through this membrane (by passive diffusion, facilitated diffusion, or active transport) before deleterious effects on the cell or organism occur. For bacteria and plants, contaminants must be dissolved in the aqueous phase before they can be taken up. However, elsewhere in the natural world there are exceptions to the notion that bioavailability is directly dependent on solubility. For example, contaminant-laden particles that undergo phagocytosis can be delivered directly into some cells (although within the cell the contaminant may eventually need to be solubilized to reach its site of biological action). Uptake mechanisms relevant to humans include absorption across the gut wall, the skin, and the lining of the lungs.

"E" in Figure ES-1 refers to paths taken by the chemical following uptake across a membrane, for example, metabolic processing or exerting a toxic effect within a particular tissue. In general, the magnitude and the nature of the effect will be determined by the form and concentration of the chemical at its active site(s). If concentrations of the chemical achieved at the biological targets are too low, or if the chemical has been converted to a form that no longer interacts with the target, no effect will be observed. On the other hand, exposure may lead to concentrations that are sufficiently high so as to be lethal. Between these extremes is the potential for non-lethal, yet deleterious effects such as reduced metabolic activity, impaired reproduction, and increased sensitivity to physical or chemical stresses. Of particular importance is the bioaccumulation of contaminants (e.g., polychlorinated biphenyls or PCBs) to storage sites within tissues that are often inaccessible to normal elimination mechanisms such as metabolism and excretion. Slow release of the chemical from these storage sites can result in protracted "exposure" within the body even when exposure outside the body has been reduced.

Bioaccumulated contaminants may become available at some point to higher-order organisms that eat the plant or animal in which the contaminants are stored. In fact, food chain transfer is probably a more important exposure pathway to contaminants in soils and sediment for higher-order animals than is direct ingestion of soil or sediment. Depending on the extent of bioaccumulation in each organism, animals can be exposed to contaminants at concentrations higher than those found in the solids from which the compound originated (biomagnification).

The committee's definition of "bioavailability processes" incorporates all the steps that take a chemical from being bound or isolated in soil or sediment to being absorbed into an organism (Processes A through D). Although of great

importance in determining the overall effect of a contaminant on an organism, E processes are not considered bioavailability processes per se because soil and sediment no longer play a role. While it is instructive to consider bioavailability processes in isolation, it is imperative to realize that they occur in concert and often are interdependent. Nonetheless, typically a few steps will be most restrictive and thus impart the greatest impact on total bioavailability (i.e., for a given situation, a select few processes are expected to dominate). In planning a bioavailability assessment, which typically will involve measurement of various physical-chemical properties and some kind of biological response, the objective should be to characterize only the most critical features of the system using appropriate tools.

CURRENT USE OF BIOAVAILABILITY IN THE MANAGEMENT OF CONTAMINATED SOIL AND SEDIMENT

Bioavailability processes overlap with many of the exposure pathways commonly considered during risk assessment and thus are an integral part of exposure assessment. However, their consideration is not always obvious or explicit. For both human health and ecological risk assessment, bioavailability processes may be dealt with by using either default values in exposure equations or site-specific data and information.

Human Health Risk Assessment

In human health risk assessment, "bioavailability" is specifically used in reference to absorption into systemic circulation—consistent with the toxicological use of the term. This encompasses bioavailability process D in Figure ES-1 as well as some process E steps, such as liver processing. Bioavailability processes leading up to absorption (A–C) are also included in human health risk assessments, but are instead described as "fate and transport" processes.

When considering bioavailability as the fraction of the chemical that is absorbed into systemic circulation, two operational definitions are important—*absolute* and *relative* bioavailability. Absolute bioavailability is the fraction of the applied dose that is absorbed and reaches the systemic circulation (and can never be greater than 100 percent). Relative bioavailability represents a comparison of absorption under two different sets of conditions—for example from a soil sample vs. food—and can be greater than or less than 100 percent. These values are used in exposure assessments, particularly for exposure by direct ingestion of soil or sediment and by dermal contact. For example, the exposure intake equation for incidental ingestion of soil invokes a relative bioavailability adjustment factor if the absolute bioavailability for the case of concern is known to differ from the absolute bioavailability implicit in the toxicity value used. Dermal exposure equations have additional relative correction factors because there are very

few toxicity values available specifically for the dermal route. The inhalation pathway presents even more complexity, and there are few examples of situations where a bioavailability adjustment factor has been used to refine an inhalation risk assessment.

Studies using animals as surrogates for humans have been conducted at a small number of sites to determine relative bioavailability (and to a lesser extent absolute bioavailability) for different chemical–solid combinations. These studies have shown that there is considerable variability in the relative bioavailability values measured for a certain contaminant in different soil types. Nonetheless, there is a general paucity of absorption data that has led to the extensive use of simplifying or default adjustment factors regarding chemical absorption in human health risk assessments. Federal and state regulatory agencies, as a practical matter, often specify the defaults they regard as acceptable, mainly for dermal contact and oral ingestion of soil. Default values are sometimes given for single chemicals or, where less information is available, for classes of chemicals. The use of national default values for relative and absolute bioavailability has been most thoroughly developed for lead-contaminated sites.

The most prominent default is that relative bioavailability is assumed to be 100 percent unless there is compelling contrary evidence and a scientifically defensible adjustment factor can be derived. In most instances, an assumption of 100 percent relative bioavailability is conservative, because most toxicity tests utilize forms of a chemical that tend to be readily absorbed. However, this is not always the case, and treatment with the chemical in diet, for example, may represent sub-optimal conditions for absorption. Under these circumstances, it is possible that exposure to the chemical in an environmental medium like soil may entail greater absorption than during the critical toxicity study. In this situation, an assumption of 100 percent relative bioavailability will underpredict the potential for exposure.

Ecological Risk Assessment

Bioavailability processes are also considered in exposure intake equations for ecological risk assessment. However, when compared with human health risk assessment there is greater complexity in ecological risk assessment because of the many species, physiologies, and physicochemical processes that must be considered. Some organisms feed directly on soils and sediments and thereby access contaminants, other species absorb dissolved chemicals across their external membranes, and still other species access contaminants that originated in soils and sediments by eating organisms exposed via the first two routes.

Two pathways frequently drive ecological risk assessments—direct contact of invertebrates with soils or sediments and exposure to wildlife feeding on soil invertebrates and plants. For the direct contact pathway, relatively simple techniques have been developed that predict the partitioning of metals and organics

between different phases—solid, aqueous, or within an organism—with the latter two representing the bioavailable fraction. These estimates of the bioavailable fraction of a contaminant pool are directly compared to threshold concentrations known to cause negative effects, if thresholds are known. Or, estimates of the bioavailable fractions can be used to model contaminant transfer to higher trophic levels.

Two partitioning techniques have become commonplace. For metals, normalizing their concentrations in sediment to acid volatile sulfides (AVS) has been suggested as a universal explanation of metal availability from sediments. The theory assumes that low pore water concentrations of metal translate into limited bioavailability. However, there are numerous environmental settings and organisms for which AVS is not applicable, thus limiting its potential. For organics, much attention has been given to the biota-soil/sediment-accumulation-factor (BSAF), an empirical ratio defined as the chemical concentration in tissue over the chemical concentration in soil or sediment. Because BSAF values are dependent on the physical–chemical properties of both the organic compound and solid as well as on the lipid content of the organism, they are site- and species-specific, although there have been attempts to apply BSAF values measured in one location to other locations. Thus, the commonly used normalization paradigms for the direct contact pathway have substantial uncertainties, and, at best, may capture only the crudest influences.

The wildlife exposure pathway includes not only direct ingestion of soils and sediments but also exposure to chemicals accumulated in the tissues of prey. As such, approaches to determining the bioavailability of contaminants in lower-order animals like invertebrates (discussed above) are important in wildlife exposure modeling. Although wildlife also may be exposed via incidentally ingested soils or sediments, little effort has been spent determining relative bioavailability adjustment factors because of difficulties in making such measurements; they typically are assumed to be 100 percent. Other than this assumption, there are few if any default relative bioavailability values commonly used in ecological risk assessment—unlike with human health risk assessment.

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Site-specific assessments that have been labeled specifically as "incorporating bioavailability" have occurred for a small subset of risk assessments across the country. Typical measurements of relative bioavailability reflect the difference between uptake of soil-bound contaminant vs. contaminant in the dosing medium used for the toxicity study. For human health risk assessment, such studies are most prevalent for the oral route of exposure and for inorganic contaminants (arsenic, cadmium, lead, and mercury). Bioavailability processes are commonly included in ecological risk assessments, although they have not been labeled as "bioavailability assessments or adjustments" per se. Nonetheless, there

are certain pathways (e.g., sediment to invertebrates) and chemicals (persistent, bioaccumulative compounds) for which bioavailability information has been frequently sought and has gained regulatory acceptance.

Legal and Regulatory Framework

One of the most prominent and explicit uses of bioavailability is its incorporation into the regulatory standards for biosolids (sludge) disposal. Biosolids are the residual material from municipal water treatment, and they are sometimes used to restore or remediate soils. Since the late 1970s, the U.S. Environmental Protection Agency (EPA) has developed standards to assure that no adverse effects would occur as a result of land application of biosolids. Over time these Part 503 regulations have incorporated a great deal of research data, such that for all exposure pathways other than human ingestion of biosolids, the bioavailable fraction, rather than the total concentration of the compounds of concern, forms the basis of the regulations.

Other examples of using bioavailability concepts in managing hazardous waste are less obvious. Within the contaminated soil field, the legal and regulatory view of "bioavailability" is narrower than the processes illustrated in Figure ES-1, in that the primary focus has been on absorption (particularly absorption into systemic circulation for humans) and thus on direct contact with soils via the oral and dermal pathways. As mentioned above, the most common default assumption about absorption has been that contaminants are equally bioavailable from soil as from the medium used in the critical toxicity study, although some states have set default values other than 100 percent relative bioavailability for broad use. The replacement of default values with site-specific measurements has not been acknowledged in laws or regulations for hazardous waste cleanup at the federal or state level, although there is also no formal prohibition against doing so.

EPA's only quasi-official recognition of bioavailability is in the Risk Assessment Guidance for Superfund, which refers to "adjustments for absorption efficiency." There is no agency-wide guidance on the data necessary to substantiate such an adjustment, however, leaving that critical determination to EPA regional offices, state agencies, or the judgment of risk assessors and others. An informal survey conducted by the committee to determine how EPA regional offices were considering bioavailability in hazardous waste programs revealed that recognition, acceptance, and utilization of bioavailability factors in state and federal cleanup projects are limited at best, with wide variations among the regions. These differences may be explained only partially by regional differences in the nature, types, and costs of cleanups. Hesitancy to replace default values with site-specific measurements of bioavailability, especially for human health risk assessment, may reflect agency concern with increased analytical costs, anxiety about public acceptance of the concept and methods, concerns

about legal challenges, and the absence of more formal national guidance. Thus, despite the lack of legal impediments, bioavailability studies are not a regular feature of site-specific risk assessment.

With regard to contaminated sediments, several federal agencies routinely conduct surveys of sediment quality and biological effects, and in doing so try to account for certain bioavailability processes. Similar to the lack of guidance apparent in the soil remediation arena, the approaches used by the different agencies are highly variable. The National Oceanic and Atmospheric Administration uses an empirical, statistical approach for screening sediment quality that does not explicitly address bioavailability processes. EPA's more chemical approach has been to develop criteria for protecting ecosystems from sediment toxicity using equilibrium partitioning theory (e.g., AVS). The U.S. Army Corps of Engineers' experimental approach tests the toxicity of every sediment (for disposal of dredge spoils), and thereby implicitly considers bioavailability on a sediment-by-sediment basis. These differences serve as a point of confusion for practitioners hoping to better quantify the risks involved in various sediment management scenarios, and they reflect the lack of consensus among environmental managers about how to deal with bioavailability processes.

Although consideration of bioavailability processes is inherent to risk assessment, usually only some bioavailability processes are considered explicitly, and assumptions made about other processes are not transparent. For example, there has been more focus on the absorption aspect of bioavailability (through the use of default values for dermal and oral relative bioavailability and BSAF values) while many of the other processes have been less explicitly examined. The default values used to represent certain bioavailability processes in risk assessment may not be protective and appropriate for all circumstances. Thus, replacing default values with site-specific information should be encouraged. It must be remembered that consideration of site-specific information on bioavailability processes may result in either an increase or decrease compared to the default value.

At present there is no legal recognition of "bioavailability" in soil cleanup, although bioavailability concepts are emerging for sediment management, and they have been more fully embraced for biosolids management and disposal. Formal recognition of "bioavailability" in state and federal regulatory contexts would eliminate at least some of the hesitancy and confusion on the part of risk assessors and managers regarding the acceptability of the concept.

There is no clear regulatory guidance or scientific consensus about the level and lines of evidence needed for comprehensive bioavailability process assessment. That is, it is not clear what threshold of knowledge is sufficient to be able to replace default assumptions about bioavailability with site-specific mea-

surements. Regulatory guidance from EPA is needed that addresses what information must be included in a bioavailability process assessment, its scientific validity, acceptable models of exposure, and other issues. This may help to guide research efforts that will further our mechanistic understanding of bioavailability processes.

BIOAVAILABILITY TOOLS

A myriad of physical, chemical, and biological tools has been used to evaluate bioavailability. These range from analytical techniques like spectroscopy that directly address where and how a chemical is associated with sediment or soil to techniques like extractions that operationally address form. Biological tools typically consider entry of the contaminant into the living organism (process D in Figure ES-1) without directly measuring processes A–C. However, processes A, B, or C might be manipulated by other means, with biological tools then being used to evaluate an organism's responses to those manipulations. The state of the science is such that little consensus exists about optimal approaches for measuring bioavailability.

A table is provided within this report (Table 4-2) that specifies generic strengths and limitations of many tools. The seven criteria used to evaluate the tools are (1) the tool's applicability to field settings; (2) its applicability to the solid phase; (3) whether it measures a single process vs. lumped processes; (4) its relevance to biouptake (bioavailability process D); (5) whether its results can be generalized to other sites; (6) its relevance to regulation; and (7) its usefulness as a research tool. The criteria reflect the committee's opinion that mechanistic approaches (that determine the form and associations of a contaminant) have the greatest potential for ultimately defining bioavailability processes and narrowing uncertainties, although they are less applicable at present. Regulatory and industry interests tend to prefer simplified approaches that are operational (e.g., extractions), that provide shortcuts to estimate mechanistic processes (e.g., equilibrium partitioning), or that estimate bioavailability indirectly via complex responses (e.g., toxicity bioassays). Because some of these approaches lack explanatory capability and have limited applicability, they should be employed cautiously in the current regulatory environment so as not to increase uncertainty or the degree to which actions seem arbitrary.

No one method achieves the highest rating in all categories, and none of these methods fails all criteria, illustrating that every tool has tradeoffs. Among the tests reviewed, some are appropriate for some situations, but most are not generally applicable to a wide spectrum of situations. It is important to recognize that most tools are still in development and few are fully validated by a body of work relating their predictions to independent measures from nature.

Techniques to Characterize Interactions among Phases

Mechanistic understanding of physicochemical phenomena controlling bioavailability processes requires knowledge of the geochemical compartments that contain the contaminant, the forms of the contaminant, and interactions of the contaminant within the compartment. Several new instruments that can help to develop this understanding are evaluated. For example, microscale surface mass spectrometric and infrared spectroscopic methods are capable of describing the occurrence and role of black carbon that may serve as an especially strong sorbent for organic contaminants. X-ray absorption spectroscopy can discern the distribution and bonding of metals in solids and provide data on element mineralogy for use in modeling the solubility of mineral assemblages. Owing to the sophisticated, specific nature of these instruments, most will remain research tools. However, detailed examination of selected environmental samples advances mechanistic understanding and thereby furthers the development of validated conceptual models for describing the chemical and kinetic factors controlling contaminant release, transport, and exposure.

Physical–Chemical Extraction Techniques for Measuring Bioavailability

A wide variety of simple, empirical extraction tests are used to estimate the bioavailable fraction of a contaminant pool. The tests involve chemical extraction for metal contaminants and extraction using organic solvents or solid phase adsorbents for organic contaminants. For human health risk assessment, extractions have been developed to mimic mammalian digestive processes, and thus measure the bioaccessible fraction of a contaminant bound to a solid phase. Most extractions used in ecological risk assessment account for contaminant release from the solid surface to pore water. Thus, they are most successful (i.e., predictive) when biological uptake is dominated by a pore-water pathway (e.g., plant uptake of metals). Extractions cannot account for other, more complicated uptake mechanisms that control an organism's overall dose, such as dietary exposure, acid extraction, removal by surfactants, ligand complexation in solution and on membranes, transport with amino acids, and enzymatic breakdown of organic chemicals.

Extraction procedures do not (with a few exceptions) remove metals or organic compounds from specific components of soils and sediments, nor can they explain the type or character of the sorbent phase to which an organic sorbate may be sequestered. Thus, they are operational, not mechanistic, methods for estimating contaminant availability. Such tests should be viewed as qualitative measures of reactivity that may be useful as screening tools. Validation of extraction tests (via correlation with a biological measure of bioavailability) is sparse, reflecting the difficulty and expense of bioassays using humans, ecologi-

cal receptors, or a surrogate. Certainly no one universal extraction procedure has been shown to consistently correlate with tissue concentrations in plants or animals across complicated environmental conditions.

Biologically Based Techniques for Measuring Bioavailability

Bioassays are employed to study influential biological processes themselves and as probes to study physical and chemical processes. Almost any technique that measures a biological response to contaminant exposure is suitable. However, interpreting the results from such experiments is not always straightforward because biological processes other than the one under investigation can affect the results. Tests that measure biological responses at levels of organization closest to contaminant transport across the membrane—such as assimilation efficiency and isolated organ tests—are easy to interpret from a mechanistic standpoint compared to responses that take place at more complex levels of organization. At the next level of organization is whole organism bioaccumulation, measured in feeding studies with invertebrates, fish, birds, and mammals. Bioaccumulation is not just the result of movement across the membrane, but also is influenced by how the organism encounters its environment and by species-specific internal processing mechanisms like digestion.

Other tests that measure more complicated biological responses or groups of processes reveal less about uptake and accumulation but are valuable for studying toxic effects. For example, biochemical responses to exposure at the cellular level can be measured with biomarkers such as P450. Toxicity tests (acute and sublethal) are widely used both in the lab and *in situ* to evaluate bioavailability, because they are practical, they depict responses of high relevance, and they are particularly useful for helping to understand the effect of contaminant mixtures. Because the number of potentially confounding factors grows beyond those relevant to whole organism bioaccumulation, toxicity tests are not optimal mechanistic indicators of bioavailability processes (as defined on page 2). Thus, there are tradeoffs between the biologically based tests available. In particular, those tests that directly measure biouptake provide unambiguous results about distinct mechanisms, but they may not capture the complexity of the environmental system nor speak to important effects that can be addressed by, for example, mesocosms and toxicity tests.

Biological tests are frequently used to validate the physical and chemical tools discussed earlier, or to provide complementary evidence about bioavailability processes in a system. For example, assimilation efficiency used in parallel with spectroscopy could reveal the properties of sediments that control bioavailability process A. Many of the tools discussed represent the state of the art or require additional research in order to reach their potential, especially molecular tools such as biomarkers and reporter systems.

Choosing Tools for Human Health and Ecological Risk Assessment

Prior to engaging in measurement of contaminant bioavailability from soils or sediments, it is critical to establish an accurate site conceptual model that describes the relevant exposure pathways, the receptors to whom the exposures are occurring, and the environmental conditions under which the exposures are occurring. This information is vital because all available tools for assessing bioavailability processes are receptor-, pathway-, and contaminant-specific, such that bioavailability data for a chemical for one exposure pathway are not necessarily applicable to another exposure pathway. The lack of an accurate site conceptual model can lead to measurement of the wrong endpoint or selection of an inappropriate bioavailability tool.

Regulatory acceptance of the tools used to generate bioavailability information in risk assessment is expected to be influenced by several factors, including the relevance of the tools to the site conditions and the extent of tool validation. Validation variously refers to the performance of a tool or approach in terms of reproducibility, reliability, and multi-lab calibration. An appropriate body of experimental work to validate a tool would (1) clarify where and when a tool yields a definitive response; (2) clarify that the tool can be linked to a biological response of a similar magnitude, and that the linkage stands up across a range of conditions in the type of environment that is being managed; (3) test the prediction of bioavailability using different types of experiments and field studies; (4) clarify which types of biological responses are best predicted by the approach; and (5) include critiques of the best applications and the limits of the tool, especially compared to alternatives. A tool that is well accepted and validated should be given greater weight than one that is new or experimental.

No single tool has been developed that can universally describe or measure "bioavailability," and approaches that have attempted this have failed. Thus, a complementary group of tools that characterize different bioavailability processes is a better choice than multiple tools that focus on only one step. Ideally, risk managers should consider processes influencing contaminant concentration, form, or transformation; biological processes affecting uptake; and linkages between internal concentrations and adverse effects in receptors. The complexity of this requirement illustrates the importance of a more comprehensive approach to exposure assessment as compared to a single-value regulatory approach in evaluating contaminant bioavailability. The corollary is that simple tests should be used cautiously. Simplification should only proceed once more mechanistic knowledge has become available, not in lieu of such information.

To avoid misapplying bioavailability tools it is important to understand the environmental setting for which a tool was designed and intended. The long-term success of implementing considerations of bioavailability in hazardous

waste management depends upon developing improved models and measurement techniques appropriate to site-specific conditions. Confusion in the regulatory process could result if tools intended for other purposes are misapplied to soil and sediment management.

An intensive effort to develop mechanistic tools or models based on mechanisms is critical to future development of bioavailability tools. Many operational tools (e.g., extractions, normalizations, and simple models) have proven ambiguous or shown large uncertainties in their estimates of bioavailability when rigorously tested. Such empirical tests cannot be extrapolated to other sites, nor can they be used with confidence to understand permanence or unforeseen conditions. They are poorly correlated across species and ranges of environmental conditions.

MOVING FORWARD WITH BIOAVAILABILITY IN DECISION-MAKING

The limitations in our understanding of bioavailability processes have important ramifications for site management. The most obvious is that lack of knowledge may inadvertently support poor decisions regarding exposure assessment and, subsequently, how much contamination should be cleaned up and at what cost. There are also treatment remedies that rely heavily on increasing or decreasing bioavailability, and without a better understanding of bioavailability processes it is difficult if not impossible to know if such treatments are effective.

Treatment technologies reported to "decrease bioavailability" generally impede transfer of a contaminant from the soil or sediment matrix to a living organism. Examples of such technologies include biostabilization (bioremediation to reduce contaminant mobility and toxicity of contaminated soils and sediments); sediment capping (reducing the ability of a bottom dwelling organism to get to the contaminant, and increasing mass transfer distance); vitrification or solidification (decreasing contaminant mobility by increasing mass transfer resistance out of the solid matrix); and chemical alteration (e.g., converting a compound to a low solubility form or redox state via amendment). Other technologies attempt to increase pollutant removal or destruction by facilitating bioavailability processes. These technologies increase mass transfer from the sorbed phase via physical or chemical means. Examples of the former include grinding or mixing to decrease diffusional paths, or increasing matrix temperature to increase mass transfer rates. Chemical means include the use of surfactants, co-solvents, or chelating agents to increase mass transfer by (1) increasing the apparent aqueous solubility of hydrophobic organic compounds or (2) mediating changes to the geosorbent matrix structure.

Determining whether these technologies are actually working to increase or decrease bioavailability is hampered by the plethora of different bioavailability

tools and measurements used whose relevance to treatment effectiveness is not clear. Indeed, there is no consensus on the tools or methods that should be employed to measure "bioavailability reduction" in the course of remedial technology selection or on how results from those tests should be incorporated into risk assessment. As a result, the state-of-the-practice consists of applying a battery of assays to the soil or sediment under investigation that all have some relationship (however ill defined) to contaminant bioavailability. Using biostabilization as an example, a review of remedies for hydrocarbon-contaminated soils found that a wide variety of surrogate measures of bioavailability were utilized. These included Microtox™ assays, reduction in the water soluble fraction, leachability evaluations, dermal uptake through human cadaver skin, absorption efficiency via feeding studies in mice, earthworm uptake and toxicity tests, desorption tests, and supercritical fluid extraction. Some of these correlative assays may aid in short-term decision making, but in the absence of better capabilities to measure bioavailability processes they must be applied with extreme caution to ensure that appropriate site management decisions are made. Further, the permanency of treatment technologies that aim to reduce or enhance bioavailability has not been addressed, in part because tools to assess bioavailability processes over long time scales and over a range of soil and sediment conditions are not yet developed.

Finally, site managers should be cognizant of treatment technologies that may unintentionally affect bioavailability. Especially for sediment dredging and for new technologies that have yet to be fully tested, like phytoremediation, there may be unanticipated side effects that result in undesirable changes in bioavailability to certain receptors.

Next Steps at Individual Sites

Various actions are needed to make progress in incorporating bioavailability processes in risk assessment and decision-making at individual sites, in acknowledging bioavailability processes in regulations and creating appropriate guidance, and in better understanding bioavailability processes on a mechanistic level. At individual sites, key issues that need to be addressed include (1) selecting appropriate measurement and modeling tools; (2) assessing and (when possible) reducing uncertainty in understanding, models, and parameters for particular bioavailability processes; (3) developing long-term monitoring plans that include monitoring of bioavailability processes critical to the risk-based remedial plan implemented; and (4) including community groups in remediation planning at early stages.

The development of tools relevant to bioavailability is a rapidly growing field, such that there can be considerable confusion regarding which tools and how many to choose in order for the results to be useful in decision-making. In

the face of limited information and imperfect tools, weight-of-evidence approaches may prove useful. That is, the results of tests should be combined to provide "multiple lines of evidence" about bioavailability processes at a site. This approach is especially needed to make near-term progress at sites where appropriate mechanistic tools are lacking, such that empirical tools must initially be relied on. (When it is possible to choose tools that will provide better mechanistic understanding, this opportunity should be exploited and not bypassed in favor of conventional empirical assessment approaches.) As more robust mechanistic methods evolve, the need for a multiple lines of evidence approach should diminish concomitant with our increasing ability to predict impacts, leading to greater acceptance of risk assessment that includes explicit consideration of bioavailability processes.

At the present time, many bioavailability processes are hidden within default assumptions that are both highly simplified and uncertain. More explicit, site-specific consideration of bioavailability processes in risk assessment can reduce this uncertainty. However, if there is substantial uncertainty associated with a bioavailability process that controls the ultimate estimated risk, there may be a tendency to not measure that process explicitly and instead to use conservative assumptions. Thus, it is important to recognize the uncertainty in each bioavailability process descriptor and the potential for propagation of error in risk assessment. The influence of bioavailability process uncertainty and variability on the overall risk can be assessed qualitatively, quantitatively through sensitivity analysis (deterministic risk evaluation), or through stochastic risk assessment.

The expanded consideration of bioavailability processes in the current risk assessment paradigm will likely alter both the prioritization of remediation efforts and the decisions pertaining to the remedial technology(s) chosen at individual sites. Whether these decisions provide long-term protection to humans and the environment will depend, in part, on how much is known about bioavailability processes over time. Thus, replacing default bioavailability assumptions with site-specific measurements must be accompanied by evaluations of future system states via newly focused long-term monitoring, including the potential for events to occur that might reintroduce unacceptable exposure conditions. Presently, there is almost no guidance on approaches for long-term monitoring that specifically target the *stability* of the contaminant "form" instead of total contaminant concentration.

Communities often have concerns about explicit consideration of bioavailability processes in risk assessment at hazardous waste sites. Bioavailability assessments may be viewed as a "do-nothing" or "do-less" approach, given that incorporating bioavailability information into risk assessment may raise acceptable contaminant concentrations in soil or sediment. Also, because bioavailability studies may not be conducted for the ultimate receptor of concern, or may yield results with considerable uncertainty, a community may not be confident that the

scientific evidence is adequate to apply the results within their community. Of the limited cases to date where communities have been presented with bioavailability information, the responses have ranged from strong support to acceptance to strong objection.

Because bioavailability processes for contaminated soils and sediments are inherently part of risk assessment, bioavailability does not present a unique risk communication problem. Thus, the public should be introduced to the concept of bioavailability as being a fundamental component of risk assessment no different from other exposure parameters or toxicity values. The technical components that should be part of any public communication program regarding bioavailability include (1) the factors that affect bioavailability from soils or sediments, (2) the concepts of absolute bioavailability and relative bioavailability, (3) the technical basis for the established toxicity values, (4) the selection of a model for bioavailability studies and why it was chosen, (5) how uncertainty was handled, and (6) how site-specific bioavailability information will be incorporated into the risk assessment. Finally, it should be acknowledged that rarely are bioavailability studies undertaken simply to improve the accuracy of a risk assessment. Rather they are performed to justify site cleanup goals that are more financially or technically feasible, and that involve leaving appreciable amounts of contaminant mass in place, while still being protective of public health and the environment.

Next Steps in the Regulatory Arena

The resistance in some regulatory domains to allowing site-specific measurements of some bioavailability processes to replace default assumptions stems from many factors, including uncertain methodologies and lack of validation, public anxiety and suspicion about motives, and lack of precedent. A viable way to move around these obstacles and achieve more widespread consideration of bioavailability processes in risk-based management of contaminated soils and sediments is to invoke an adaptive management approach, which embraces two ideas. The first is that there should be pilot studies to experiment with different tools and models. The second is that agencies should use the results from such efforts to develop a common systematic approach to determine how and when to incorporate bioavailability concepts into regulations in a consistent manner. Adaptive management concepts are not new, but rather are akin to the scientific method and engineering problem solving. An adaptive management example relevant to bioavailability is the approach recently recommended by EPA for determining the efficacy of dredging and how much PCB-contaminated sediment to dredge from the Hudson River. The plan involves evaluating risks over time and adjusting cleanup plans as performance monitoring data are acquired and analyzed.

Next Steps in the Scientific Arena

Expansion of bioavailability considerations into risk assessment and remedial decision-making requires improved scientific understanding and models for a number of key bioavailability processes. Investment in mechanistic understanding and models will prove more profitable in the long-term than reliance on empirical knowledge because models have greater predictive power for a broader range of situations. As part of this research effort it will be important to draw ties between mechanistic understanding and more operational tests for bioavailability with studies that, for example, quantitatively examine both the form of a contaminant and its biological uptake. Other areas in need of attention include contaminant–solid interactions (especially the nature and effects of aging on contaminant release rates), the feeding ecology of animals, and how organisms bioaccumulate and transfer contaminants to their predators. Better understanding of whether and when associations between contaminants and soils and sediments can be made permanent should be a future research goal. The results from such research are needed before bioavailability explanations can be used with confidence to determine the amounts of soil and sediment to be remediated.

Much information on bioavailability of contaminants comes from industry-funded studies at specific sites, particularly for human health risk assessments. Such studies are usually, and understandably, not conducted in a way that advances understanding of fundamental underlying processes. Over the last decade, EPA has supported studies on mobility of chemicals in the environment, uptake relevant to assessing ecological risks, and bioavailability processes that might affect bioremediation. Yet despite this research investment, progress in understanding these bioavailability processes is limited. Unless a greater commitment is made to fund bioavailability studies from a research rather than industry-driven perspective, progress in developing information that can be used to advance human health and ecological risk assessments will be slow.

1

Introduction

For the last 30 years, the nation has been trying to assess, remediate, and otherwise manage thousand of acres of soil and sediment[1] contaminated with chemicals produced during the industrial age. Of primary concern has been the risk that these contaminated media pose to humans and ecological receptors. Evaluation of exposure is a key component of chemical risk assessment, and understanding the factors that influence exposure enables decision-makers to develop solutions for addressing environmental contamination. This report of the National Research Council examines the bioavailability of contaminants in soil and sediment, focusing on those factors that influence the percentage of total contaminant levels to which humans and ecological receptors are exposed. The extent to which chemicals are bioavailable has significant implications for the cleanup of contaminated media.

National attention on bioavailability stems from a growing awareness that soils and sediments bind chemicals to varying degrees, thus altering their availability to other environmental media (surface water, groundwater, air) and to living organisms (microbes, plants, invertebrates, wildlife, and humans). It is also recognized that the physiological characteristics or "niche" of plant and animal species influence the availability of chemicals, such that exposure to the same contaminated material may be very different from one species to another. The altered availability of chemicals associated with soils or sediments has been variously described by such terms as *partitioning*, *reduced desorption rates*,

[1]The terms "soil" and "sediment" are defined in detail in Chapter 3.

reduced biodegradation rates, *geochemical binding*, *sequestration*, and *limited absorption through biological membranes*—to name but a few descriptors. While these descriptors may all involve different chemical, physical, and biological processes, they all describe the phenomenon that chemicals in soils and sediments behave differently than when those chemicals are present in other media, notably water and air.

"Bioavailability processes" are defined as the individual physical, chemical, and biological interactions that determine the exposure of plants and animals to chemicals associated with soils and sediments. One reason for adopting the term "bioavailability processes" in this document is the realization that "bioavailability" has been defined in different ways that are often discipline-specific. Instead of redefining the term "bioavailability," the committee has chosen to recognize the value of various definitions and to focus instead on the interacting biological, chemical, and physical processes particular to the presence of chemicals in soils and sediments that influence exposure. The term "bioavailability processes" captures this idea.

Currently, "bioavailability" is used in risk assessment most frequently as an adjustment or correction factor that accounts for the ability of a chemical to be absorbed by an organism—an approach that makes a number of assumptions regarding individual bioavailability processes. Unfortunately, contemporary risk assessment practice does a poor job of identifying and explaining these assumptions, such that it is generally not clear how bioavailability processes are incorporated into risk assessments. It can be difficult to know whether all of the relevant processes are addressed and whether assumptions are based on valid concepts and reliable data. In fact, there is ample reason to suspect that many bioavailability processes are dealt with inadequately or inaccurately. In order to improve this aspect of risk assessment, it will be necessary to identify relevant bioavailability processes in a more transparent way, to gain greater mechanistic understanding of these processes, and to evaluate the ability of various tools to offer information on bioavailability processes. Over the long term, such a process-based approach will improve exposure assessment, resulting in greater consistency, reliability, and defensibility in measurement, modeling, and prediction.

BIOAVAILABILITY PROCESSES FOR CONTAMINANTS IN SOILS AND SEDIMENTS

Several definitions for the term "bioavailability" are listed Table 1-1. Depending on the context, bioavailability may represent the fraction of a chemical accessible to an organism for absorption, the rate at which a substance is absorbed into a living system, or a measure of the potential to cause a toxic effect. Often, environmental scientists consider bioavailability to represent the accessibility of a solid-bound chemical for assimilation and possible toxicity (Alexander, 2000), while toxicologists consider bioavailability as the fraction of chemical

TABLE 1-1 Definitions of "Bioavailability" and Related Terms

Definition	Source
Bioavailability	
A chemical element is bioavailable if it is present as, or can be transformed readily to, the free-ion species, if it can move to plant roots on a time scale that is relevant to plant growth and development, and if, once absorbed by the root, it affects the life cycle of the plant.	Sposito, 1989
Generally used to describe the extent and rate of absorption for a xenobiotic which enters the systemic circulation in the unaltered (parent) form from the applied (exposure) site.	Hrudy et al., 1996
The availability of a chemical to an animal, plant, or microorganism. It may be assayed by measurement of uptake, toxicity or biodegradability.	Linz and Nakles, 1997
A concept that describes the ability of a chemical to interact with living organisms.	NEPI, 1997
The accessibility of contaminants to microbes from the standpoint of their metabolism, their ability to grow on these chemicals, to change cellular physiology, and perhaps modulation of genetic response.	Sayler et al., 1998
A measure of the fraction of the chemical(s) of concern in environmental media that is accessible to an organism for absorption.	ASTM, 1998
A measure of the potential for entry into ecological or human receptors. It is specific to the receptor, the route of entry, time of exposure, and the matrix containing the contaminant.	Anderson et al., 1999
The extent to which a substance can be absorbed by a living organism and can cause an adverse physiological or toxicological response.	Battelle and Exponent, 2000
Bioavailable: For chemicals, the state of being potentially available for biological uptake by an aquatic organism when that organism is processing or encountering a given environmental medium (e.g., the chemicals that can be extracted by the gills from water as it passes through the respiratory cavity or the chemicals that are absorbed by internal membranes as the organism moves through or ingests sediment). In water, a chemical can exist in three different forms that affect availability to organisms: (1) dissolved, (2) sorbed to biotic or abiotic components and suspended in the water column or deposited on the bottom, and (3) incorporated (accumulated) into the organisms.	EPA, 2000a
The fraction of an administered dose that reaches the central (blood) compartment, whether from gastrointestinal tract, skin, or lungs. Bioavailability defined in this manner is commonly referred to as "absolute bioavailability."	NEPI, 2000a

TABLE 1-1 Continued

Definition	Source
In the environment, only a portion of the total quantity of chemical present is potentially available for uptake by organisms. This concept is referred to as the biological availability (or bioavailability) of a chemical.	Casarett and Doull's, 2001
A measure of the potential of a chemical for entry into, or interaction with, ecological or human receptors. It is specific to the receptor, the route of entry, time of exposure, and the matrix containing the contaminant.	Lanno, 2001
A term used to indicate the fractional extent to which a dose reaches its site of action or a biological fluid from which the drug has access to its site of action.	Wilkinson, 2001
The degree to which a drug or other substance becomes available at the physiological site of activity after administration.	American Heritage Dict., 3rd Ed.
The degree and rate at which a substance (as a drug) is absorbed into a living system or is made available at the site of physiological activity.	Webster's Dictionary, 10th Ed.
Absolute Bioavailability	
The fraction or percentage of an external dose which reaches the systemic circulation, that is, the ratio of an internal dose to an applied dose. This ratio is called the bioavailability factor (BF).	Hrudy et al., 1996
The percentage of an external exposing mass that reaches the systemic circulation (the internal dose).	Paustenbach et al., 1997
The fraction of an administered dose that reaches the central (blood) compartment from the gastrointestinal tract. Bioavailability defined in this manner is equal to the oral absorption fraction.	Ruby et al., 1999
The fraction or percentage of a compound which is ingested, inhaled, or applied on the skin that actually is absorbed and reaches the systemic circulation.	Battelle and Exponent, 2000
The fraction of an administered dose that reaches the central (blood) compartment, whether from gastrointestinal tract, skin, or lungs.	NEPI, 2000a
Relative Bioavailability	
The absolute bioavailability of an external exposing mass divided by the absolute bioavailability of the chemical compound under the conditions used to derive the toxicity criterion.	Paustenbach et al., 1997

continues

TABLE 1-1 Continued

Definition	Source
Refers to comparative bioavailabilities of different forms of a chemical or for different exposure media containing the chemical (e.g., bioavailability of a chemical from soil relative to its bioavailability from water) and is expressed as a fractional relative absorption factor.	NEPI, 2000a; Ruby et al., 1999
A measure of the 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.	Battelle and Exponent, 2000
Other Definitions	
Bioaccumulation is the total accumulation of contaminants in the tissue of an organism through any route, such as food items as well as from the dissolved phase in water. **Bioconcentration** is accumulation of a chemical directly from the dissolved phase through the gills and epithelial tissues of an aquatic organism. **Biomagnification** is the process by which bioaccumulation causes an increase in tissue concentrations from one trophic level to the next from food to consumer.	Rand and Petrocelli, 1985; Schnoor, 1996; EPA, 2000a
Bioavailable fraction is that portion of the bulk concentration that is available to be accumulated into an organism under a defined set of conditions. For instance, for a metal it could be the freely dissolved ion of the metal. Other forms of the metal bound in precipitates or covalent or hydrogen bonded to other ions would not be available. The available fraction is a proportion ranging from 0.0 to 1.0. The available fraction determines the reactive portion of the total mass of material, much like the activity coefficient relates activity to concentration.	EPA, 2000a
Bioaccessibility describes the fraction of the chemical that desorbs from its matrix (e.g., soil, dust, wood) in the gastrointestinal tract and is available for absorption. The bioaccessible fraction is not necessarily equal to the RAF (or RBA) but depends on the relation between results from a particular *in vitro* test system and an appropriate *in vivo* model.	Paustenbach et al., 1997; Ruby et al., 1999
Relative absorption factor (RAF) describes the ratio of the absorbed fraction of a substance from a particular exposure medium relative to the fraction absorbed from the dosing vehicle used in the toxicity study for that substance (the term relative bioavailability adjustment (RBA) is also used to describe this factor.)	Ruby et al., 1999

TABLE 1-1 Continued

Definition	Source
Absorption describes the transfer of a chemical across the biological membrane into the blood circulation.[a]	Paustenbach et al., 1997
Biostabilization refers to the biodegradation of the more labile HOC (hydrophobic organic compound) fraction leaving a residual that is much less available and mobile.	Luthy et al., 1997

[a]In this report, "absorption" is used generically for non-mammalian organisms to be synonymous with "uptake."

absorbed and able to reach systemic circulation in an organism. Another view of bioavailability is represented by a chemical crossing a cell membrane, entering a cell, and becoming available at a site of biological activity. Others might think of bioavailability more specifically in terms of contaminant binding to or release from a solid phase. These different viewpoints of bioavailability create a semantic stumbling block that can confound use of the term across multiple disciplines—hence the reason that "bioavailability processes" is used in this report.

Figure 1-1 is a depiction of bioavailability processes in soil or sediment; it incorporates exposure by release of solid-bound contaminant and subsequent transport, direct contact of a bound contaminant, uptake by passage through a membrane, and incorporation into an organism. "A"—contaminant binding and release—refers to the physical and [bio]chemical phenomena that bind/unbind, expose, or solubilize a contaminant associated with soil or sediment. This may include geological processes like weathering and scouring, chemical processes like redox reactions or complexation, and biochemical processes through the action of biosurfactants or hydrolytic enzymes. Binding may occur by adsorption on solid surfaces, by absorption within a phase like natural organic matter, or by a change in form as in covalent bonding. "B" in Figure 1-1 involves the movement of a released contaminant to the membrane of an organism. Transport may result from diffusion and advection to target receptors such as microbes, plants, and humans. Thus, bioavailability processes A and B comprise exposure via various chemical and biochemical phenomena that affect release and subsequent transport of dissolved contaminants. "C" involves the movement of contaminants still bound to the solid phase, which can play a role in dermal contact of soils, oral ingestion of soil or sediment, or exposure to burrowing organisms in soil or sediment. It should be noted that processes A, B, and C can occur internal to an organism such as in the gut lumen, although they are depicted in Figure 1-1 as occurring in the external environment.

The bioavailability process depicted as D in Figure 1-1 entails movement across membranes. Here the contaminant passes from the external environment through a physiological barrier and into a living system. An example is transport

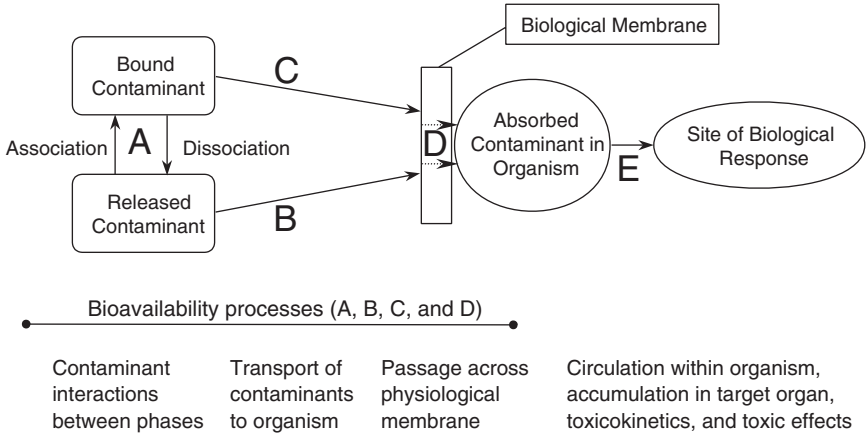


FIGURE 1-1 Bioavailability processes in soil or sediment. Note that A, B, and C can occur internal to an organism such as in the lumen of the gut.

through the gut membrane of an organism (e.g., the intestinal epithelium of a mammal). Exposure to both dissolved and solid-bound contaminants can lead to chemical interaction with the membrane of an organism and subsequent uptake or absorption (these terms are used synonymously). "E" in Figure 1-1 refers to paths taken by the chemical following uptake across a membrane. For example, after passage across a biological membrane the chemical can exert a toxic effect within a particular tissue (among many possibilities).

It should be noted that A, B, and C in Figure 1-1 are sometimes considered to be fate and transport processes (which they are) rather than bioavailability processes. On the other hand, process D is more traditionally associated with bioavailability in contemporary risk assessment. The committee's definition of "bioavailability processes" incorporates all the steps that take a chemical from being bound or isolated in soil or sediment to being taken up into an organism (A through D). Figure 1-1 makes it clear that soils and sediments can affect exposure in various ways, both external and internal to the organism. For example, solid phases influence the extent of contaminant transfer from one medium to another, thereby determining soluble chemical concentrations. There is also differential uptake of contaminants into animals and plants depending on whether they are solubilized or solid-bound. Although of great importance in determining the overall effect of a contaminant on an organism, E processes—the toxic action or metabolic effect of a chemical—are not defined as bioavailability processes per se because soil and sediment are no longer a factor. However, because E processes are often measured endpoints, they are described at length in Chapters 3 and 4.

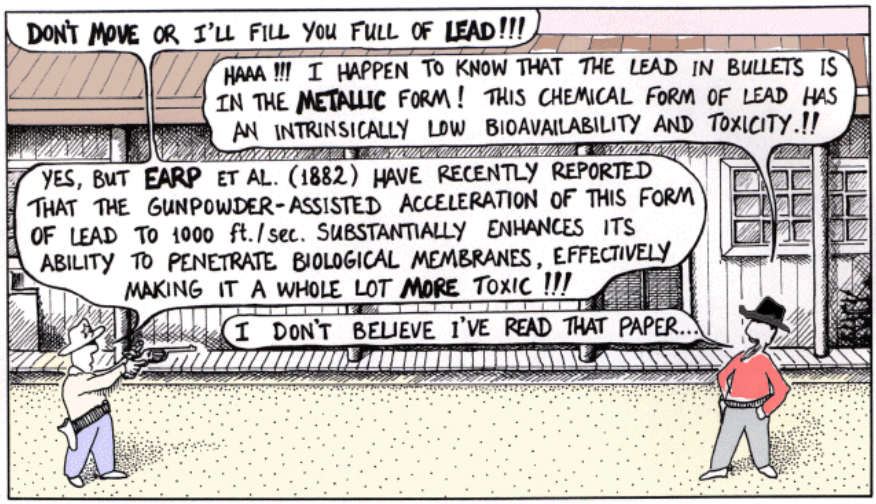
Bioavailability processes have definable characteristics that provide the foundation for this report. First, in the broadest sense, bioavailability processes describe a chemical's ability to interact with the biological world. Second, bioavailability processes are quantifiable through the use of multiple tools. Third, bioavailability processes incorporate a number of steps (see Figure 1-1), not all of which would be applicable for all compounds or all settings. Indeed, it is because the term implies several individual interactions and processes that the committee prefers the term "bioavailability processes" to "bioavailability." Fourth, there are barriers that change exposure at each step. Thus, bioavailability processes modify the amount of chemical in soil or sediment that is actually taken up and available to cause biological responses.

HISTORICAL PERSPECTIVE

That soils and sediments can impact chemical interactions with plants and pests has been known for some time by farmers and those involved in agricultural services (e.g., manufacturers of fertilizers, pesticides, and herbicides). However, in the past few decades the phenomenon has gained attention with respect to releases of hazardous chemicals to the environment. First, interest in bioavailability has been driven by a desire to reduce the uncertainties in estimating exposures as part of human and ecological risk assessment. That is, a better understanding of bioavailability processes could help identify sediment- or soil-specific factors that might influence exposure. A second impetus comes from the remediation of contaminated sites, including observations that the effectiveness of bioremediation and other treatment technologies can be limited by the availability of chemicals in soils or sediments. In some cases, the greatest opportunity for risk reduction may be to treat or contain the bioavailable fraction of the hazardous chemicals in soils and sediments and then to rely on natural attenuation approaches to treat the long-term, slow release of residual contaminants. Thus, there is considerable interest in setting cleanup goals based on the bioavailable amount rather than the entire contaminant mass. The brief history below acknowledges the varied use of the term and the extent to which bioavailability processes have been considered in different contexts.

Toxicological, Pharmacological, and Nutritional Use of Bioavailability

Although coinage of the term "bioavailability" is relatively recent, an appreciation of bioavailability concepts in the context of toxicology is ancient, particularly with regards to the treatment and prevention of poisoning. For example, pre-Columbian natives in South America were known to extract a powerful muscle-paralyzing agent—curare—from various *Strychnos* plants. They had no means of knowing that this alkaloid possesses a quaternary nitrogen atom, and that the charge on this nitrogen atom prevents its movement across the gas-



ENVIRONMENTAL SCIENTISTS IN THE WILD WEST

trointestinal epithelium. They understood quite well, however, that this poison was harmless when ingested, but very effective when injected. As a result, they could immobilize prey with curare-tipped arrows, dispatch the prey, and safely eat the meat.

From the fifth century BC to the fifteenth century AD, red clay from a specific hill on the Greek island of Lemnos was regarded as a sacred antidote for poisoning (Thompson, 1931). Called *terra sigillata*, it was considered effective against all poisons, no doubt acting as an adsorbent and preventing uptake in the gastrointestinal tract. The use of charcoal as an adsorbent to reduce the effect of poisons can be traced back to even earlier times, with its mention recorded in the Egyptian Papyrus of 1550 BC. In the nineteenth century, when toxicologists had the fortitude to serve as their own experimental subjects, P. F. Tourney demonstrated the effectiveness of charcoal before the French Academy of Medicine by ingesting ten times the lethal dose of strychnine combined with charcoal, and surviving (Holt and Holz, 1963).

One of the most fundamental concepts in toxicology is that an adverse effect is dependent upon the dose of the toxic substance (or *toxicant*) reaching a target organ or tissue. With the exception of chemicals that react with the organism on contact, such as corrosive agents, the toxicant must be absorbed into the systemic circulation to reach its biological target. From a toxicological perspective then, bioavailability implies movement of a chemical into the systemic circulation because to a large extent this is a good indication of the biologically effective dose. This view is reflected in the definition of bioavailability given in toxicology texts; for example, Casarett and Doull (2001) define bioavailability as the "frac-

tion of dose absorbed systemically." From the toxicologist's perspective, this definition applies to virtually all circumstances of chemical exposure, including exposure to chemicals in soils and sediments.

Because the disciplines of toxicology and pharmacology share many basic principles, this is essentially the same way bioavailability has been defined in medicine, except of course that the focus is on the absorption of drugs from dosage forms instead of chemicals from environmental media. The tenth edition of the classic pharmacology text, Goodman and Gilman (2001), defines bioavailability as "the fraction of dose of a drug reaching the systemic circulation or site of action."

Both toxicologists and medical doctors are cognizant of the importance of events outside the body and that physical–chemical properties of the toxicant or drug and its interactions with its surroundings can affect the rate and extent of absorption. In fact, much of what is termed *pharmaceutics* involves an understanding of these phenomena as they pertain to drugs and manipulation of drugs and their microenvironment to therapeutic advantage. Also, toxicologists are well aware that a variety of events in the environment can affect the rate and form in which chemicals are delivered to the body. Nevertheless, the defining aspect of bioavailability, as the term is used in both toxicology and medicine, is the movement of chemical from outside the body into the systemic circulation.

Bioavailability is also an important consideration in nutrition. Here the focus is on absorption of nutrients from the gastrointestinal tract, and the term bioavailability can have different meanings in different situations. For example, nutrients such as amino acids in proteins must be liberated through digestive enzyme activity in the gut. In this context, bioavailability may become synonymous with *digestibility*. Other nutrients, such as most vitamins, require metabolic activation in order to have nutritional value. For these substances, bioavailability is sometimes defined to include both absorption and the metabolic activation process. For still other nutrients that do not require digestion or metabolic activation, bioavailability is regarded simply as the process of absorption of the substance from the gut into the systemic circulation, as in toxicology and medicine.

In considering the toxicological use of the term, it is important to recognize that *systemic absorption* is not necessarily equivalent to general uptake or absorption into the body, particularly from the gastrointestinal tract. Mammalian anatomy is responsible for this complication. Chemicals absorbed from the gastrointestinal tract enter hepatic portal circulation and must pass through the liver before reaching the general circulation. The liver (and to some extent, the gastrointestinal epithelium) may metabolize the chemical, converting it to substances with greater, lesser, or qualitatively different biological activity. This view of bioavailability, in terms of what reaches the systemic circulation (as opposed to just crossing a biological membrane), includes both absorption and metabolism components, and components both internal and external to the body. It can also lead to some ambiguity in how bioavailability is operationally defined for a

particular chemical. Often bioavailability in toxicology is described in terms of the chemical itself, ignoring metabolites that are formed during the chemical's transit from the gut to the general circulation. However, in some instances it is important to describe bioavailability in ways that include metabolites, such as when metabolites are formed that contribute significantly to the biological dose of the chemical. This is analogous to the expanded definition of bioavailability in nutrition to include metabolic activation of vitamins. Regardless of how it is defined, a clear articulation of the basis for the bioavailability determination (with or without metabolites) is required in order to interpret the results.

Bioavailability in Agriculture

Nutrient Phytoavailability

The recognition that total soil concentration of a compound is not equivalent to bioavailable or effective concentration is well established in the agricultural sciences. This is well known not only for plant nutrients but also for water, where physical processes such as water tension or matrix potential control the fraction of total water that is plant-available. Attempts to maximize yields and optimize economic return have resulted in extensive research to describe the behavior of necessary plant nutrients in soil systems. Methods to determine total concentration as well as the plant-available ("phytoavailable") fraction of the 18 required plant macro- and micronutrients (including water) have been developed across a range of soil types (Bartels and Sparks, 1996). These have been validated with field trials for multiple crops under varied soil, climate, and moisture regimes.

The bioavailable nutrient pool varies significantly by soil type and by plant species (Chaney, 1994). This reflects the different complexing capacities of different soil orders as well as different plant mechanisms for accessing soil nutrients (Marschner, 1995). Availability can also depend on the source of the nutrient. For example, nitrogen can be added to soils as manure N, ammoniacal N, nitrate N, and N–P materials; each of these sources will have different release characteristics that vary by soil type, soil moisture, plant growth stage, and soil microbial activity (Pierzynski et al., 2000).

The range of factors that affect nutrient availability and the methods that have been developed to predict effective nutrient concentrations potentially can be used as a model for the development of appropriate protocols to assess bioavailability processes for contaminants in soils and sediments. Although the majority of these protocols have been developed to predict phytoavailability of nutrients in potentially deficient conditions, there is a direct correlation to the development of an understanding of the bioavailable fraction of soil contaminants. In many cases, however, plants are aggressively attempting to alter the rhizosphere environment to facilitate nutrient uptake, during which they may inadvertently access soil-bound contaminants.

While this research has significantly increased knowledge of bioavailability processes and led to the development of tools to measure the bioavailable fraction, it is not yet at the point where the phytoavailability of nutrients across a range of soils and crops can always be accurately predicted. Heterogeneity in soil colloids and adsorption surfaces and differences in soil pH, organic matter, and pore spaces preclude the ability to definitively predict the fate of nutrients in soil systems. This is further complicated by differences in uptake efficiencies across plant species. Nonetheless, the factors involved in nutrient uptake may help to clarify the processes that are involved in determining the bioavailability of contaminants in soil systems.

Pesticide Bioavailability

The concept of bioavailability also has a history in the application of pesticides, particularly herbicides, to agricultural soils. As with the uptake of nutrients by plants, the efficacy of an applied herbicide, fungicide, or insecticide depends on a range of soil properties, primarily soil organic matter content and texture. Specific properties of the pesticide will also affect its behavior in the soil system, including the size of the molecule, its structure and functional groups, its polarity, and resulting dissociation constants and partitioning coefficients (e.g., K_a, and K_{oc}). Thus, different application rates are recommended for different soil types and compounds. In addition, the potential for herbicide residues to damage successive croppings will vary because of changes in the persistence of the compound in different soils. This has been understood and incorporated in product development for several decades (Hance, 1967; Bailey and White, 1970; Walker et al., 1982).

Generally, herbicides must be dissolved in soil solution to be effective. As the soil organic matter concentration or soil clay content increases, the portion of the herbicide that is sorbed also increases (Stevenson, 1994). In soils of high organic matter such as peats, herbicides may be completely ineffective when applied at typical economic rates. For soils with very low organic matter concentrations, application may not be recommended because too much of the compound may be present in soil solution, increasing the potential for crop damage as well as leaching. Other factors, such as moisture content, soil texture, and timing of rainfall after application will also affect the efficacy of the compound (Mueller-Warrant, 1999). These factors have been sufficiently recognized within the industry that compound labels will generally recommend different application rates based on soil type. For example, application rate recommendations for S-metalochlor are based on soil texture and percent organic matter, with recommended rates varying from 0.8 kg active ingredient (ai) ha^{-1} to 1.4 kg ai ha^{-1} depending on specific soil characteristics (Blumhorst et al., 1990).

Bioavailability is also an issue when dealing with residues of agricultural chemicals applied in the past. In particular, the bioavailability of insecticides

BOX 1-1
Persistence and Bioavailability of Pesticides

Owing to the widespread use and economic importance of pesticides, their long-term persistence in soil has been studied for more than half a century. Methods to assess pesticide concentrations in soil have evolved to recover as much added compound as possible with ever-increasing precision and accuracy. Today there is a debate as to whether analytical methods designed to measure the total concentration adequately reflect the risk from such pesticides.

Early evidence showed that pesticides persist in soil for a long time. In 1949 and 1951, plots were established to study the long-term persistence and rates of disappearance of several chlorinated hydrocarbon insecticides applied to soil, including dieldrin, chlordane, and DDT (Nash and Woolson, 1967). Their results showed that 39 percent of the original DDT remained after 17 years. These soil plots gave an upper-limit persistence owing to the amount and means of pesticide added and management of the test plots with minimal tillage. Nash and Woolson's persistence data for DDT, heptaclor, dieldrin and five other pesticides are presented in semi-logarithmic fashion, implying long-term, steady decline. Alexander (2000) arithmetically plotted selected data sets of Nash and Woolson for DDT, heptaclor, and dieldrin to suggest gradual decrease in the rate of reduction of contaminant mass for which some latter data points do not change much with time. Thus, depending on one's presentation of such data, two views emerge—either a "hockey stick" curve where concentrations rapidly level off with time, or a first-order plot where concentrations progressively decline, albeit slowly. Other evidence for the long-term persistence of DDT and its residues in soil is presented by Boul et al. (1994), who report the longevity of DDT and its residues over a 30-year period for conditions typical for pastoral agriculture. Their data suggest gradual DDT decline since the last application in 1965, and that appreciable levels of DDT residues, mainly DDE, will remain.

Other studies have tried to demonstrate a link between persistence and bioavailability by measuring contaminant assimilation into animals or effects on crops for soils with aged compounds versus soils with freshly added compounds. For example, Morrison et

applied years ago has received attention in the environmental engineering arena, with the intent of determining whether these residues pose a present-day risk to humans or ecological receptors. Box 1-1 describes a series of studies on pesticide persistence in soil and resulting bioavailability.

Bioavailability in Evaluating and Managing Hazardous and Solid Wastes

The attention given to bioavailability in the environmental arena is relatively recent compared to disciplines like toxicology and agronomy. This attention has been driven in large part by hazardous materials and site cleanup legislation and concerns about the exposure to and risk from hazardous chemicals. For example, chemicals that are encapsulated, insoluble, or strongly bound to solids may not be prone to biological uptake or exert a biological response, while chemicals that are

al. (2000) studied earthworm assimilation from soils that had been treated with DDT and dieldrin in 1949, using the same soils prepared originally by Nash and Woolson (1967). Soil from Dahlgren, Virginia, contaminated with DDT approximately 30 years ago was studied as well. Comparison was made with soil freshly spiked with pesticide. Their data showed that although aging reduced uptake into earthworms, some of the pesticide was still assimilated by the earthworms even after an aging period of 49 years. For example, based on the differences in concentrations in the worms exposed to unaged and aged compounds with DDT and dieldrin spiked into unaged soil samples at concentrations found in the field after 49 years, 32 percent of the DDT and 28 percent of the dieldrin from the 49-year old samples was "available" relative to the unaged samples. Thus, while a compound's aging in soil reduced its uptake into earthworms, some exposure remains. In an analogous study, Robertson and Alexander (1998) showed a significant reduction in mortality of insects to DDT- and dieldrin-amended soils aged for 30 days compared to freshly added insecticides. Toxicity decreased with further aging for 180 or 270 days, showing no mortality. About 85 and 92 percent of the contaminant was recovered from the soil by extraction after 180 and 270 days, respectively. The authors concluded that pesticides residing in soil became "less bioavailable" with time.

Similar results are reported for herbicides, where the toxicity was less than anticipated based on total sample analysis. Scribner et al. (1992) assessed the bioavailability of simazine residues from a cornfield where simazine had been applied continuously for 20 years. Aged simazine residues were shown to be biologically unavailable to sugarbeets and to microbial degraders, whereas recently added simazine caused damage to sugarbeets and was substantially degraded by microbes.

In summary, pesticides can persist in soils for up to 50 years and perhaps much longer. Based on tests with microorganisms, worms, insects, and plants, pesticides may or may not exhibit greatly reduced bioavailability (as measured by degradation, uptake, or toxicity) over the long term.

dissolved may be readily available. Typically, modern analytical methods are designed to report the total amount of all forms of a compound present in a sample. Thus, the difference between the total amount of a compound detectable using modern analytical techniques and the bioavailable amount of the compound has become a central issue in the environmental arena.

The earliest studies of contaminant bioavailability from soil for the purposes of refining human exposure assessment focused on dioxins and furans in the mid-1980s (Bonaccorsi et al., 1984; McConnell et al., 1984; Lucier et al., 1986; Umbreit et al., 1986; Shu et al., 1988). These were soon followed by similar studies on the oral bioavailability of polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs) from soil (Fries et al., 1989; Goon et al., 1990). The dermal bioavailability of dioxins and furans, PCBs, and PAHs in soil was also under study during this time frame. Starting in the late 1980s, bioavailability

research for human exposures shifted to address inorganics, primarily lead because of the size of many Superfund mining sites in the Rocky Mountain West where childhood exposure to lead in soil was a significant concern. This prompted the development of lead bioavailability models in rats (Freeman et al., 1992) and swine (Casteel et al., 1997a), which were subsequently used to assess lead bioavailability from soil at approximately 20 sites. The success of this approach for lead resulted in the development of analogous models for arsenic in swine and monkeys (Freeman et al., 1995; Casteel, 1997b) and the use of these models to assess arsenic bioavailability from soil and house dust at more than ten sites. Mercury bioavailability also has been the subject of recent investigations (as reviewed in Davis et al., 1997; Paustenbach et al., 1997; Schoof and Nielsen, 1997). Several review documents compile the results from these site-specific bioavailability studies (Battelle and Exponent, 1999, 2000; NEPI, 2000a, b).

Despite this work, for many scenarios there is limited agreement on how to quantify all relevant bioavailability processes at hazardous waste sites, partly because too few compounds have been tested to make generalizations. A large body of information comes from empirical observations suggesting that bioavailability processes are important for assessing the risk of compounds in soil. In particular, for organic chemicals a pattern of chemical disappearance composed of a more rapid initial phase followed by a period in which little or no degradation of chemical can be detected is commonly observed (e.g., Linz and Nakles, 1997). In the case where the compounds are known to be biodegradable, the lack of disappearance in the second phase is taken to mean the compounds are unavailable to microorganisms. In addition, it is argued that the observed slowing in the biodegradation rate of organic compounds in aged samples imposes a limit on what may be achieved by bioremediation. Indeed, in many cases it has been observed that organic–solid partitioning or the aging of organic pollutants in soil and sediment systems results in residues that are recalcitrant to further microbial attack despite favorable environmental conditions (Mihelcic and Luthy, 1991; Alexander, 1995; Ramaswami and Luthy, 1997).

Beyond empirical observations, more quantitative attempts to document bioavailability processes at hazardous waste sites use a variety of techniques including mass transfer measurements, geochemical analyses, microbial responses, extractants that mimic the digestive action of organisms, accumulation or uptake tests (as in the lead model discussed above), and bioassays of acute and chronic responses (for a detailed discussion of tests see Chapter 4). Accumulation into earthworms (e.g., ASTM, 1998) is a relatively simple test that has been widely applied to contaminated soils and sediments for mainly ecological risk assessment purposes. Toxicity bioassays in use for ecological risk assessment (EPA, 1991; EPA/USACE, 1991; Ingersoll, 1995) have generally relied on acute toxicity tests. Where concern has focused on the potential risk associated with longer exposures to low levels of contamination, tests that measure sublethal endpoints such as growth and reproduction have been applied (Dillon et al., 1993;

Benoit et al., 1997; Moore et al., 1997). These approaches offer the advantage of providing a closer link to effects on higher levels of biological organization (e.g., populations and communities), which represent the focus of most ecological risk assessments (Suter, 1993; Bridges et al., 1996). Although bioassays of uptake and effect are most applicable to the test organism (usually microorganisms, clams, worms, and plants), the results may also be relevant to other animals and humans. There is not a long history of developing such surrogates; thus, it should not be surprising that bioavailability has infrequently "been considered in devising or interpreting toxicological tests of higher organisms or in assessments of risks from organic toxicants in soil" (Alexander, 1997).

In addition to the metals-contaminated mining sites mentioned previously, bioavailability also has been seriously considered at former manufactured gas plant (MGP) facilities, which made gaseous fuels from coal and oil prior to the widespread distribution of natural gas following WWII. These plants operated from 50 to 150 years ago, and wastes remain at thousands of sites around the world. Bioavailability processes have emerged as important for assessing environmental exposures and for remediating contaminated soils and sediments at MGP sites (e.g., Luthy et al., 1994; Stroo et al., 2000). The focus has been primarily on the bioavailability of coal tar constituents—specifically PAHs. The implications of bioavailability for biological treatment of these materials also have been evaluated. For example, some treatment technologies have focused on methods of increasing the availability of coal tar constituents (e.g., Ali et al., 1995). In other cases, the goal has been to demonstrate that contaminants in the treated soils or sediments are no longer in an available form and thus pose less risk. This is the case for MGP purifier waste, which contains elevated levels of cyanide compounds that happen to be much less bioavailable than simple cyanide salts (Ghosh et al., 1999). One state—Massachusetts—has tried to account for these differences by developing a method for determining the "physiologically available cyanide" present in soils (MA DEP, 2001).

Another area where bioavailability processes are a primary focus of environmental risk assessment is in the management of coal ash. Ash is one of the largest solid waste residuals associated with energy production from fossil fuels. The Electric Power Research Institute (1983, 1993) has conducted a substantial amount of the research associated with these materials, including development of geochemical models for predicting leaching and transport behavior of the metals in ash. These recent assessments include evaluations of exposure to ecological receptors and incorporate bioavailability processes as reflected by biological uptake factors.

Finally, bioavailability processes are an important component of U.S. Environmental Protection Agency (EPA) regulations concerning the beneficial use of biosolids, which are the residual materials generated by municipal wastewater treatment and applied to land for their fertilizer value. As discussed in greater detail in Chapter 2, the Part 503 Sludge Rule contains risk-based standards de-

signed for land application of biosolids. Initially, the proposed regulations called for limits on the amount of sludge that could be applied to land, based on metal toxicity to certain plants (Marks et al., 1980). As more studies using biosolids were conducted (e.g., Bingham et al., 1975; Dowdy, 1975; Latterell et al., 1978), evidence mounted to suggest that there may be concentrations below which there are no adverse effects from the metals or organics in biosolids—that is, below which the contaminants are not bioavailable (Page et al., 1987). For all exposure pathways other than human ingestion of biosolids, Part 503 regulations currently permit the use of data from such field studies to determine these concentration thresholds and set application rates of biosolids such that metal limits are not exceeded.

Bioavailability in Risk Assessment

Risk assessments provide the foundation for decisions about exposure to chemicals and cleanup of soils and sediments at contaminated sites. Bioavailability processes are important for evaluating exposures of humans and ecological receptors to persistent compounds. Indeed, risk management decisions related to judging the acceptability of dioxins in soils can be traced back to evaluations that explicitly considered bioavailability (Kimbrough et al., 1984). Since that time, some progress has been made in explicitly incorporating bioavailability concepts into risk assessment, particularly for lead contamination of soils and for dermal exposure pathways (see Chapter 2). In general, though, most bioavailability processes are not transparently dealt with during risk assessment, and are instead part of certain assumptions, adjustments, or correction factors, which may or may not be based on experiments. Following is a brief overview of how bioavailability concepts are incorporated into human health and ecological risk assessment. A more thorough examination of the topic is given in Chapter 2.

Human Health Risk Assessment

In human health risk assessment, the term "bioavailability" is specifically used in reference to systemic absorption. This is consistent with the toxicological use of the term "bioavailability," as explained previously, and is understandable given that human health risk assessment was developed from basic toxicological principles. Bioavailability processes leading up to absorption (processes A–C in Figure 1-1) are also included in human health risk assessments, but typically are not identified as such. Instead, they often are described using other terms, such as "environmental fate and transport" processes.

When bioavailability is considered as the fraction of the chemical that is absorbed into systemic circulation, two operational definitions are important—*absolute* and *relative* bioavailability. The amount of chemical that is ingested, lies on the surface of the skin, or is inhaled is called the *applied dose*. The amount

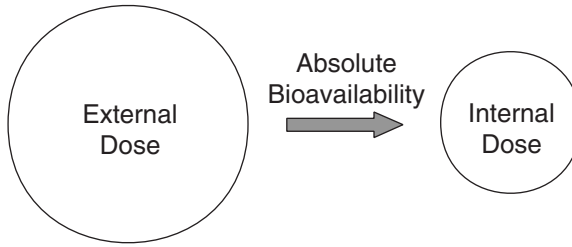


FIGURE 1-2 Absolute bioavailability determines the fraction of the external or applied dose that reaches the systemic circulation (internal dose).

that is absorbed and reaches the systemic circulation is called the *internal dose*; it is dependent upon the *absolute bioavailability* of the chemical, i.e., the fraction of the applied dose that is absorbed (Figure 1-2). Clearly, absolute bioavailability can never be greater than 100 percent.

Relative bioavailability represents a comparison of absorption under two different sets of conditions. Examples might include absorption of a chemical from two different routes of exposure, or from the same route of exposure but from two different types of environmental samples. Relative bioavailability says nothing directly about the amount of chemical absorbed into the body; it only describes the relationship between the amount absorbed under two different circumstances. For example, if a chemical is absorbed equally well through the skin as from the gut, the relative bioavailability (dermal versus ingestion) for these exposure routes is 100 percent, even though the fraction absorbed (absolute bioavailability) from each of the routes may be only 5 percent. Relative bioavailability can be greater than or less than 100 percent.

Human health risk assessment involves combining an estimate of exposure with a toxicity value to derive a risk. Most toxicity values are based on applied dose, meaning for example that an acceptable oral daily intake for a chemical is based on the amount ingested per day (usually per unit body weight). Although using applied-dose toxicity values is convenient, the disadvantage is that the toxicity of most chemicals is related more directly to their internal dose. As a result, comparing applied doses to gain inferences on risks can be misleading if the relationship with their corresponding internal doses is not consistent (i.e., if they have different absolute bioavailabilities).

Figure 1-3 shows two circumstances in which a comparison of applied (external) doses is not a valid reflection of the size of the internal doses because the relative bioavailability in each case is not 100 percent (that is, the absolute bioavailability in each case is different than in the test case). It is not difficult to imagine circumstances in which absolute bioavailabilities are not equal, for example when extrapolating from animals to humans, from fasted subjects to fed subjects, or from studies conducted with the test substance in a highly-absorbable

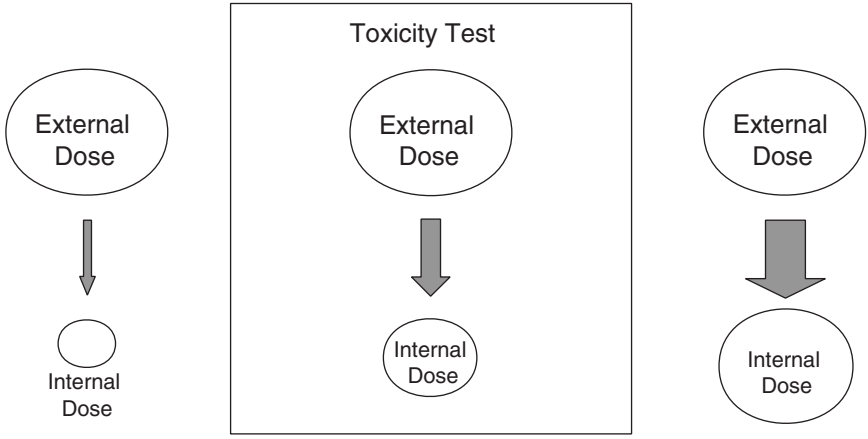


FIGURE 1-3 Comparing applied (external) doses can be problematic if relative bioavailability is not 100 percent. The size of each arrow indicates the magnitude of absolute bioavailability. The upper circles represent the magnitude of each applied (external) dose. If the conditions that prevailed in the toxicity test (center) are different than the actual situation such that relative bioavailability is much greater than (right-hand side) or much less than (left-hand side) 100 percent, comparing applied doses will lead to an overestimating or underestimating of the risk.

form versus environmental exposure to the chemical in a complex matrix. If the absolute bioavailability is less under conditions of environmental exposure than in the critical study used to develop the safe dose, the conclusion that a health risk exists would be in error, as indicated by the left side of Figure 1-3. Comparisons of applied doses still can be useful in these situations if a correction is applied in the form of a relative bioavailability term, which is the major reason that studies to determine relative bioavailability are much more common in human health risk assessment than absolute bioavailability studies. As described in Chapter 2, the results of relative bioavailability studies are used to refine risk calculations that would otherwise assume a relative bioavailability of 100 percent.

Chapter 2 discusses the methodology for estimating doses for ingestion, dermal contact, and inhalation routes of exposure, and the manner in which certain bioavailability processes have been explicitly included in exposure assessment for these routes. Incidental ingestion of contaminated soils is often the most important exposure pathway for human health risk assessment and drives many of the generic and site-specific cleanup criteria for soils contaminated with organic chemicals and metals. The exposure intake equation for incidental ingestion of soil invokes an adjustment factor if the absolute bioavailability for the case of concern is known to differ from the absolute bioavailability implicit in the toxicity value used (i.e., if relative bioavailability is something other than 100

percent). Such calculations are more difficult for dermal exposure pathways, because there are very few toxicity values available specifically for dermal exposure. This necessitates the use of toxicity values developed for other routes of exposure (ingestion or inhalation) and additional relative correction factors to account for bioavailability processes. Default assumptions for dermal bioavailability are often specified by EPA or state agencies (see Chapter 2). The inhalation pathway presents even more complexity because both the doses and the toxicity values are often expressed in terms of concentration in air, rather than an amount of chemical per unit body weight. There are few examples of situations where a bioavailability adjustment factor has been used to refine an inhalation risk assessment. In the absence of information on a specific chemical (which is exceedingly limited), absolute bioavailability from inhalation is usually assumed to be 100 percent.

Ecological Risk Assessment

Bioavailability processes are equally important to ecological risk assessment, and have actually been afforded greater attention here than in human health risk assessment because of the need to encompass an enormous number of exposure pathways. This is evident in long-standing ecotoxicology terms that are related to bioavailability, such as *bioconcentration* (i.e., accumulation of contaminants from the dissolved phase for aquatic organisms), *bioaccumulation* (i.e., accumulation of contaminants from all food sources as well as the aqueous phase), and *biomagnification* (i.e., increase in body burden of contaminants through the food web) (described in greater detail in Chapter 3). The science of ecological risk assessment has grown with extensive research on many bioavailability processes, such as transport of chemicals in the environment and environmental modeling. The number of factors that may alter exposure assessments is enormous, including species-specific criteria, interactions between competing organisms, ecosystem structure, interactions among communities of organisms, and other factors not specifically considered in human health risk assessment.

Bioavailability concepts can be explicitly considered in ecological risk assessments in many ways. With regard to the specific process of absorption, as with human health risk assessment there may be site-specific estimates of relative bioavailability that can be derived either from measurements, from modeling, or from a combination of the two and used in exposure assessment for certain pathways. A limited number of studies using highly bioavailable forms of chemicals have been conducted in organisms of interest. Exposure pathways for ecological risk assessment often involve food-chain models, particularly for bioaccumulative compounds such as PCBs, dioxins, pesticides, and methyl mercury. Thus, site-specific tests and models to determine the bioaccumulation of compounds into the tissues of plants and lower-order animals can be used to evaluate exposures to higher trophic levels such as fish and wildlife.

The importance of bioavailability processes is also acknowledged in screening-level ecological risk assessments. For example, it is standard practice to consider the partitioning of chemicals between sediment, sediment pore water, and animals when evaluating exposures to certain groups of organic chemicals (EPA, 2000b). The degree of partitioning is influenced by the organic content of the sediments, such that solid-phase chemistry data can be used to generate relative bioavailability factors or make other refinements to the ecological risk assessment. In the case of soils, EPA has recently decided to explicitly consider soil properties that influence bioavailability processes in setting screening levels for soil contamination (EPA, 2000c). The various methods by which bioavailability processes are explicitly included in exposure assessment for several common ecological risk assessment pathways are discussed in greater detail in Chapter 2.

IMPLICATIONS OF BIOAVAILABILITY PROCESSES

With regard to solid waste management, there is no doubt that interest in bioavailability processes has been fueled by the recognition that cleanup levels expressed as bulk concentrations in soils and sediments may not correlate with actual risk. The hypothetical example illustrated in Table 1-2 reflects the concern that many remedial engineers have about cleanup decisions based solely on bulk chemical measurements. In this table, contaminant bioavailability (as measured by an unspecified method) decreases in order from Site 1 to 5. Although Site 5 has the highest total contaminant levels, it has the lowest effective contaminant concentration because of limited bioavailability. This illustration shows that it is conceptually possible to reverse the order of importance for dealing with sites when the bioavailable chemical concentration rather than the total chemical concentration is considered.

A consideration of bioavailability processes offers the potential for reducing the volume of soil or sediment requiring remediation. If it can be demonstrated that greater levels of contamination can be left in soil or sediment without risk, decreased cost may be realized and an opportunity for less intrusive remedial approaches exists. Box 1-2 discusses the importance of quantifying the difference

TABLE 1-2 Hypothetical Illustration of How Bioavailability Processes Could Influence Exposure and Remedial Decisions

Site #	Total Contaminant Concentration (ppm)	Percent Contaminant Bioavailability	Effective Contaminant Concentration (ppm)
1	200	100	200
2	250	75	188
3	300	50	150
4	400	33	133
5	500	20	100

BOX 1-2
Total Concentration vs. Bioavailable Concentration: Metals in Sediment

It is important to understand the magnitude of error involved if bioavailability is not considered when evaluating sediment or soil contamination. Significant, even strong, correlations between bioavailability (measured by uptake into tissues or toxicity) and total metal concentration can be found among geochemically similar environments (Bryan, 1985) or within experiments using a single type of sediment (Lee et al., 2000). However, poor correspondence between total metal concentration and bioavailability is common when experiments are conducted with sediments or soils that differ widely in critical geochemical characteristics (Luoma and Jenne, 1977; DiToro et al., 1990, 1991).

For example, in a large data set from English estuaries, metal concentrations in fine grained surface sediments (judged to be oxidized by appearance) were compared to concentrations in the tissues of a bivalve and a polychaete that lived within the sediments and ingested sediments with their food (Luoma and Bryan, 1981; Bryan, 1985; Bryan and Langston, 1992). The estuaries included a wide range of physical, biogeochemical, and pollution conditions, and co-variance among geochemical variables was rare. Some sources of variability, such as particle size, large redox differences, or dilution of tissue concentrations by reproductive tissue, were carefully controlled. The results displayed the typical variability of correspondence between metal concentrations in organisms (bioaccumulation) and metal concentrations in sediments. For example, no significant correlation was observed between cadmium in sediments and in the polychaete *Neries diversicolor* or between copper in sediment and copper in the bivalve *Scrobicularia plana*. Bioavailability in these cases was completely unpredictable from total metal concentrations in sediments. In contrast, copper in sediments predicted over 50 percent of the variance in copper in the polychaete, and cadmium in sediment predicted over 50 percent of the variance in bivalve cadmium (Bryan, 1985). Silver and lead concentrations in sediments explained about half the variance in bioaccumulation in three species, especially when these elements were extracted from sediments with 0.1M HCl. Clearly, factors that influence bioavailability can differ among metals, species, and environmental factors, and differ with different combinations of these three variables.

In the above example, bioavailability processes add variance to the relationship between total concentration and bioaccumulated metal, so the importance of considering bioavailability depends upon how much variance is acceptable (Luoma, 1983, 1989; Landrum et al., 1992). In general, predictions of metal bioavailability from total concentration in sediment alone were outside the two-fold criteria for accuracy suggested by Landrum et al. (1992). If a higher threshold for variance is acceptable, then consideration of bioavailability is less important. Total concentration does appear to provide a first-order control on bioavailability. This control is (statistically) most evident if a large concentration gradient is considered. In the example, total concentration in sediment would be a feasible indicator of the exposure of deposit feeders to most metals if 2- to 50-fold uncertainty were acceptable (the implicit criteria employed by Long et al., 1995, for example). However, because the need to assure less than 50-fold uncertainty exists in many instances, much effort has gone into developing tools and techniques to better relate environmental concentrations and bioavailability.

between total and bioavailable concentrations of contaminants in soils and sediments, and also the variability of these differences and their dependence upon such factors as the geological materials, the contaminant species present, exposure pathways, and the potential receptors.

Despite the fact that bioavailability has gained popularity as a justification for leaving some contamination in place at hazardous waste sites, in fact the integration of bioavailability processes into risk-based cleanup has the potential to either *increase* or *decrease* currently accepted cleanup requirements for residual contamination. To understand this, it should be noted that the term "bioavailability" is often used to refer specifically to uptake or absorption. It is true that absorption efficiency can never be greater than 100 percent, and thus assessments that focus exclusively on absorption efficiency would seem to have the potential to measure only "reduced" bioavailability. However, when other bioavailability processes are taken into account, then it is possible for overall exposure to increase or decrease. That is, although one bioavailability process may suggest that less contaminant is available to a receptor, other bioavailability processes may act as counterbalances, such that the actual dose is not reduced. This is illustrated by the example in Box 1-3, where the overall dose received by an organism is dependent on many factors, including the presence of multiple exposure pathways, ingestion rates, total concentration, and other bioavailability processes. Thus, an examination of all relevant bioavailability processes may actually increase the cost of remediation or alter the remedial technology implemented.

A few points can be made with the example presented in Box 1-3 and Table 1-3. First, many definitions of "bioavailability" are limited to the term in the last column of Table 1-3 (uptake efficiency or absorption). This is somewhat analogous to the terms "absolute bioavailability" and "relative bioavailability" commonly used in human health risk assessment. In the absence of compound-specific data, assumptions about absolute and relative bioavailability are made, with a common assumption being that relative bioavailability is 100 percent (see Chapter 2). Part of the goal of this report is to suggest that experiments be conducted to better define the numbers used in the final column of such a table, numbers that often are based on limited data and may not be applicable in all situations. For example, the default for the relative bioavailability of soil-bound lead via oral ingestion is 60 percent, which may be too low or high in certain situations and for certain soils. Indeed, for most compounds and soil- or sediment-types, absolute and relative bioavailability numbers are not available.

Second, it should be clear from the above discussion that the committee's concept of bioavailability processes encompasses not only the uptake term in Table 1-3, but also the concentration term and the term dealing with ingestion rates. Gaining a better understanding of *all* bioavailability processes can help manage contaminated sediments and soils in a way that not only protects the environment but also considers other issues such as costs, permanence, future

land or water use, and community acceptance. As discussed in Box 1-3, management guidelines derived from the viewpoint of a single process can underestimate risk if other important processes are not considered, just as likely as they might overestimate risk.

TASK STATEMENT AND REPORT ROADMAP

Growing interest in bioavailability processes has generated numerous questions among scientists, engineers, risk assessors, managers, regulatory agencies, and other interested parties. It has highlighted a need for better understanding such processes in terms of specific pathways, contaminated media, biological receptors, and even routes of entry. This report seeks to address the most pressing issues and to contribute toward developing common frameworks and language to build a mechanistic-based perspective of bioavailability processes. Several key questions served to guide the work of the committee:

- What scientific understanding is missing that would provide confidence in the use of bioavailability factors for different contaminant classes? That is, what mechanisms and processes require better understanding? What are the highest priority research needs? For which contaminant classes, environmental settings, and organism classes are bioavailability assessments most important?
- What tools (biological, chemical, and physical methods) are available to characterize and measure bioavailability for different contaminant classes, and what new tools are needed? What criteria should be used to validate these tools?
- How do treatment processes affect bioavailability for different contaminant classes? How does bioavailability affect treatment processes that rely on microbial degradation of contaminants?
- How and when should bioavailability information be used? What are its implications for relevant regulations? How can information on bioavailability be reliably communicated, especially to the public?

This report assesses our current understanding of processes that affect the degree to which chemical contaminants in soils and sediments are bioavailable to humans, animals, microorganisms, and plants. Chapter 2 discusses how the bioavailability concept is used today in solid and hazardous waste management. The legal and regulatory framework for considering bioavailability during soil, sediment, and biosolids management is evaluated as well as the technical methods devised for use in human health and ecological risk assessment. Case studies are presented that illustrate where bioavailability adjustment factors have been used to refine risk assessment calculations.

Because the concept of bioavailability incorporates multiple physical, chemical, and biological processes that affect the concentration and transformation of chemicals in soils, sediments, and aquatic systems, Chapter 3 describes these processes in greater detail and weighs their relative importance in certain envi-

BOX 1-3
Multiple Bioavailability Processes Affect Contaminant Intake

Several environmental processes affect how a contaminant in soil or sediment is taken into an organism. Viewing bioavailability as a single factor, and then making implicit assumptions about the link between the single process and incorporation of the chemical into an organism, can lead to false conclusions. The example below illustrates how a mix of processes can be relevant to bioavailability of a contaminant in sediments, such as:

- the concentration the organism experiences (as influenced by the contaminant input, fate, and transport, and interactions between the organism and its environment);
- processes specific to the organism like the rate at which it feeds or the speed with which it passes water over an uptake surface; and
- processes (perhaps geochemical or biological) that affect the proportion of the total concentration that is incorporated into the tissues of the organism.

Influx rate at the membrane is an unambiguous indicator of incorporation into an animal. Mathematically, influx into an organism (say a sediment dwelling, deposit feeding animal) from a dissolved source is defined as:

$$\text{Influx}_{\text{water}} = C \times R \times A$$

where C is concentration in water (μg/g water), R is the rate at which the animal passes water across the gills ($g_{water}/g_{animal}/d$) and A is the absorption efficiency (what proportion of the total concentration is absorbed into the organism) (Wang et al., 1996). A similar equation defines other exposure routes such as from food, where C is concentration in food (μg/g), R is ingestion rate ($g/g_{animal}/d$) and A is the absorption efficiency (what proportion of the total concentration ingested is absorbed into the organism). This equation illustrates the interplay among contaminant concentration, biology, and factors modifying absorption, whatever the exposure route. The importance of considering all three in combination is illustrated in the table below.

Table 1-3 presents a hypothetical example using reasonable concentrations from a natural system. The goal is to compare intake from two sources with very different absorption efficiencies (often assumed to define bioavailability). The biological processes are typical of a sediment (deposit) feeding animal, like a bivalve. The feeding rate is 1 g sediment per g tissue per day; the filtration rate is 1000 g water per g tissue per day. The concentrations are typical of a moderate cadmium contaminated sediment: 4 μg Cd/g dry wt in sediment; 0.0002 μg $Cd/g_{pore\ water}$ in pore water (again, units are converted). Absorption efficiency from water is taken as 0.99 because it is often assumed that absorption from solution is highly efficient. Absorption efficiency from food is typical of cadmium availability for a bivalve

(20 percent). An analysis of the values could lead to the statement that cadmium is more "bioavailable" from water than sediment (because efficiency of absorption is much higher). But if all bioavailability processes are considered, intake is similar between the sources because concentrations are much higher in sediment. Filtration rate and feeding rates can also make great differences in the ultimate exposure.

TABLE 1-3 Hypothetical Intake Rates of Cadmium given Two Different Exposure Pathways

		Bioavailability Processes		
Exposure Pathway	Intake Rate (μg Cd/g_{tissue}/day)	Medium Concentration (μg Cd/g_{medium})	Medium Filtration or Ingestion Rate (g_{medium}/g_{tissue}/day)	Medium-specific Uptake Efficiency
Pore water	0.2	0.0002	1000	0.99
Ingested sediment	0.8	4	1	0.2

The point illustrated by this example has important implications for setting cleanup standards. Determination of the environmental toxicity of chemicals for regulatory purposes is typically based upon bioassay exposures of surrogate organisms to a dissolved chemical, under circumstances that maximize the efficiency of bioavailability process D in Figure 1-1. For example, selenium toxicity was first determined using exposure of fish or invertebrates to selenite in solution, recognizing that selenite is the "most bioavailable" of the oxidation states (the standard condition is assumed to be close to 100 percent absolute bioavailability). Tests typically reported selenite toxicities at concentrations > 70 μg/L (Lemly, 1998). The first case studies of selenium toxicity in nature, however, showed that selenium was responsible for the elimination of most fish species in Belews Lake, but that selenite concentrations were less than 5mg/L (Lemly, 1985). Clearly, in this system "bioavailability" was greater than predicted from the (originally implied) maximum bioavailability, and the standard test had underestimated risk. Interpretation of the lake data and later experimental studies showed that an additional *process* was responsible for the enhanced risk. Selenium exposure was found to occur primarily from diet, but dietary exposure was not considered in the tests that set the standard (Lemly, 1985; Luoma et al., 1992). The most recent analyses suggest that understanding of selenium risks in nature requires consideration of multiple additional processes (Lemly, 1995; Luoma and Presser, 2000).

ronmental settings. Solubility and sorption, burial and encapsulation, diffusion and advection, microbial transformation and degradation, and uptake into organisms are considered, among other processes.

Chapter 4 of this report describes and evaluates the myriad of methods and techniques for measuring different bioavailability processes for both metal and organic contaminants in soils and sediments. For each method, the report considers what bioavailability process(es) it addresses, for what chemicals and contaminated media it can be used, what endpoint is considered, its cost, and the extent to which it has been validated. Suggestions are given for improving our ability to quantitatively assess bioavailability.

The implications of more explicitly considering bioavailability processes in environmental cleanup constitute Chapter 5. In particular, the chapter discusses for which contaminants and environmental settings measurements of bioavailability are needed and likely to be most beneficial for the protection of human health and ecosystems. A section is devoted to exploring the complex relationship between contaminant bioavailability and success of bioremediation. Finally, it asks how more explicit consideration of bioavailability can be moved into the regulatory arena and also into practice. Because of the importance of regulatory and public buy-in prior to the refinement of risk assessment and the alteration of cleanup goals, the report discusses ways to effectively communicate bioavailability concepts.

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2

Current Use of Bioavailability in the Management of Contaminated Soil and Sediment

Cleanup of contaminated soil and sediment in the United States follows a risk-based paradigm that takes into account individual exposure pathways linking sources to potential receptors. Typical pathways include contaminant leaching from soil to groundwater, contaminant release from sediments to overlying water, ingestion of contaminated sediments or soils, direct dermal contact with sediments or soils, inhalation of particulate matter or vapors containing contaminants, and ingestion of food items that have accumulated contaminants from soils or sediments. Risk management decisions for soils or sediments focus on identifying relevant pathways of exposure that pose a risk to human health or the environment and then developing appropriate remedial measures that could include treating or removing sources or cutting off pathways or both. Many of the exposure pathways discussed above are affected by the bioavailability processes shown in Figure 1-1. Thus, bioavailability processes are an integral part of risk assessment and risk-based management of contaminated soils and sediments, although their consideration is not always obvious or explicit.

Risk-based cleanup approaches typically are characterized by a tiered methodology, in which a screening-level step is used initially to assess site conditions and potential actions, followed by one or more levels of site-specific assessment. The states have set many guidance values for use at the screening-level step. For example, there are state and federal soil screening levels for the protection of human health (that often differentiate between residential and industrial land use), the protection of groundwater, and the protection of ecological receptors. Sediment guidelines for protection of ecological receptors are often used to guide

cleanup. Because they are initial screening levels, they are typically developed to be conservative (i.e., to overestimate most exposures). Although there is continued debate about whether they are conservative enough, it is undisputed that the development of such screening levels requires that assumptions be made about certain bioavailability processes. In most cases, this has involved selecting default conditions or parameters regarding the environmental fate of the chemical as well as how it might enter a human or an ecological receptor. Examples include default assumptions about the relative amount of chemical that is absorbed via dermal contact or incidental ingestion, or the manner and degree to which an organic compound in sediment is bound to organic carbon. For some screening levels (in particular empirical sediment guidelines) bioavailability processes have not been explicitly considered but probably play a role.

Understanding how bioavailability processes have been considered at a screening-level stage is an important first step for evaluating how site-specific information might be used to refine exposure and risk assessments and reduce the uncertainties inherent in their outcomes. In some cases, this might involve developing site-specific information for a particular process that can be inserted into a risk equation. As discussed below, there has been considerable work in generating site-specific information on association/dissociation and absorption (bioavailability processes A and D in Figure 1-1) for certain metals in animal models that are applicable to humans. Another type of refinement could involve making site-specific measurements of contaminant release from soils. Still other site-specific estimates—such as those encountered in ecological risk assessments—could involve measurements of available contaminant pools or tissue levels in organisms. This information can be used to both refine a risk assessment calculation and help develop models of bioavailability processes that can be used at other sites.

This chapter first describes human health risk assessment to illustrate how bioavailability processes are considered in that arena, followed by an overview of the use of bioavailability processes in ecological risk assessment. The two sections describe the current state of the practice but do not represent an endorsement by the committee. Finally, the chapter describes how "bioavailability" is considered within legal and regulatory frameworks. As will become clear, the legal and even regulatory view of what is meant by "bioavailability" is narrower than the processes illustrated in Figure 1-1, in that the primary focus has been on absorption (particularly systemic absorption for humans) and thus on direct contact with soils via the oral and dermal pathways. This underscores the significance of semantic issues discussed in Chapter 1. What should be clear from this chapter is that bioavailability processes are an integral part of risk-based management of contaminated sites. They may be considered either implicitly or explicitly, and they may be dealt with either by using default values in risk assessment equations or by using site-specific data and information.

USE OF BIOAVAILABILITY IN RISK ASSESSMENT

Because bioavailability processes influence exposure of humans and ecological receptors to chemicals in soils and sediments, and because exposure is one aspect of risk assessment, measuring or modeling bioavailability is consistent with prevailing U.S. Environmental Protection Agency (EPA) and state risk assessment paradigms. The general framework used by EPA for human health risk assessments has four major components derived from NRC (1983):

- *Hazard Identification* is a systematic planning stage that identifies the major factors considered in the assessment and establishes its goals, breadth, and focus. It is essentially a scoping activity and is fundamental to the success of all subsequent components in the risk assessment. It consists of stating the objectives, developing the conceptual model, selecting and characterizing receptors, and identifying the endpoints of the assessment.
- *Exposure Assessment* estimates the magnitude of actual or potential human or ecological exposure to a contaminant of concern, the frequency and duration of exposure, and the pathways of exposure. Incorporation of bioavailability information often influences estimates of exposure.
- *Dose-Response Assessment* is "the process of characterizing the relation between the dose of an agent administered or received and the incidence of an adverse health effect." This step estimates the probability that an individual will be adversely affected by a given chemical dose, relying primarily on data obtained from animal studies. Information on bioavailability processes may influence measures of toxicity and other effects.
- *Risk Characterization* integrates the exposure assessment and dose-response assessment into a quantitative and qualitative expression of risk. This may include deterministic calculations, probabilistic methods, and professional judgement using various lines of evidence.

These four steps are similar in ecological risk assessment, with the following differences (EPA, 1992a; NRC, 1993). The first step is termed problem formulation, which determines the focus and scope of the assessment. Hazard identification and dose–response assessment are combined into an ecological effects assessment phase. And finally, dose–response is replaced with stressor–response to emphasize that physical changes make cause harm to ecosystems as well as chemicals (although for the purposes of this report, the focus is on chemical contaminants).

Although bioavailability processes can be considered explicitly in both human health and ecological risk assessments, there are some important differences. Unlike human health risk assessment, assessments of exposure and risk to ecological receptors consider various species ranging from invertebrates and plants to fish and wildlife. Some of these species are in intimate contact with soils

Both direct exposure via soil ingestion and indirect exposure via fish consumption are affected by contaminant bioavailability. Human health risk assessment often quantifies direct ingestion of soil (top photo), while ecological risk assessment frequently considers bioaccumulation of contaminants in animal tissues (bottom photo).

or sediments. Many are also exposed to contaminants exchanged from soils or sediments to the dissolved phase or through eating organisms that have accumulated contaminants from these media. Therefore, there are many exposure pathways and a larger number of bioavailability processes that may require simultaneous evaluation during ecological risk assessment as compared to human health risk assessment, where it is more feasible to evaluate one pathway at a time. A manifestation of this difference is that human health risk assessment often involves distinct exposure equations for the direct pathways of ingestion, dermal contact, and inhalation, within which a variable is included to account for absolute or relative bioavailability. This discrete consideration of bioavailability for individual exposure media and exposure routes is driven by the fact that human exposures can often be separated in time and space. For example, vegetables may be grown in a different section of a garden from where children play, and not all receptors have gardens. In contrast, in ecological risk assessment, at least for many receptors there is obligatory simultaneous exposure via multiple pathways and routes. Thus, ecological risk assessments include equations for some of the direct exposure pathways for wildlife (although this knowledge is not well-developed for most species) as well as many other types of measures and exposure models that differ from what is commonly employed in human health assessments. For ecological risk assessment, it is often not be possible to quantify bioavailability processes associated with each of these pathways separately, which is a primary reason for focusing on measures of bioaccumulation as an overall indicator of bioavailability.

A second important factor to consider is the acceptability of making measurements on organisms such as earthworms, plants, fish, and wildlife compared to humans. As described in Chapter 4, such measurements include toxicity tests as well as uptake or accumulation tests (determination of tissue residues of contaminants)—tests that for ethical reasons cannot be conducted in humans. Thus, there are more tools for quantifying bioavailability processes and the sum of multiple exposure routes using the actual receptor of interest during ecological risk assessment. This is not the case in human health risk assessment, where greater reliance is placed on default values and where it can be difficult to modify defaults on a site-specific basis.

Regardless of whether humans or ecological receptors are the concern at a particular site, some general criteria are useful when attempting to more explicitly consider bioavailability processes during risk assessment (Menzie et al., 2000). First, it is imperative to determine (as best as possible) the usefulness of incorporating new information on bioavailability in terms of altered outcomes at a site. Chapter 5 discusses the chemical and environmental settings for which bioavailability assessments are most likely to make a difference in site management. Second, a conceptual model of exposure for the site is critical to any bioavailability assessment. Because it is known that soils and sediments can alter contaminant bioavailability, relevant soil factors should be identified early. Fi-

nally, data on bioavailability processes should be collected using measures or models that are compatible with the risk assessment and risk management framework being used at the site.

Human Health Risk Assessment

In most situations, a quantitative assessment of risk to humans from exposure to contaminants in soils or sediments involves a comparison of the estimated magnitude of exposure with the measured toxicity of the chemical(s) in question. Bioavailability processes play a variety of important roles in these risk calculations. Although risk calculations for contaminated soils and sediments can sometimes be complex, there are three fundamental types of inputs: (1) the concentration of the chemical in soil or sediment at the point of contact with the individual, (2) variables related to the nature and extent of exposure (e.g., exposure frequency, amount of soil ingested, body weight), and (3) toxicity values for the chemical. Bioavailability processes can be reflected in all three types of inputs.

Soil concentration: Bioavailability processes A, B, and C in Figure 1-1 can influence the concentration of chemical reaching the exposed individual from its point of release or residence in the environment. Typically, these bioavailability processes are addressed either through direct measurement of soil concentration at the point of contact or through environmental fate and transport modeling.

Exposure variables: Numerical adjustments to account for bioavailability processes related to entry of soil or sediment contaminants into the body are typically included among the exposure variables. This is the usual means by which "bioavailability adjustments" are made in human health risk calculations. Clearly, the primary focus here is on bioavailability processes A and D (association/dissociation and absorption or uptake across a membrane) and to a lesser extent process E if systemic circulation is a measured endpoint.

Toxicity values: Toxic potency estimates are based on one or more critical studies which offer information on the relationship between dose of the chemical and toxic effects. Most toxicity values, in the form of cancer potency estimates or acceptable daily intake rates, are based on applied rather than absorbed doses. As a result, the toxicity value is a function, in part, of the rate and extent of absorption that occurred in the critical study. This bioavailability process—the absorption of the chemical into the body in the critical toxicity study—must be kept in mind when using toxicity values.

Human contact with contaminants in soils or sediments can occur through three direct routes of exposure: incidental ingestion, dermal contact, or inhalation of soil-derived particulates (dusts) or chemicals volatilized from soil. All three routes are usually relevant for human exposure to soils, while ingestion and dermal contact are the most likely exposure routes for sediments (see Figure 2-1).

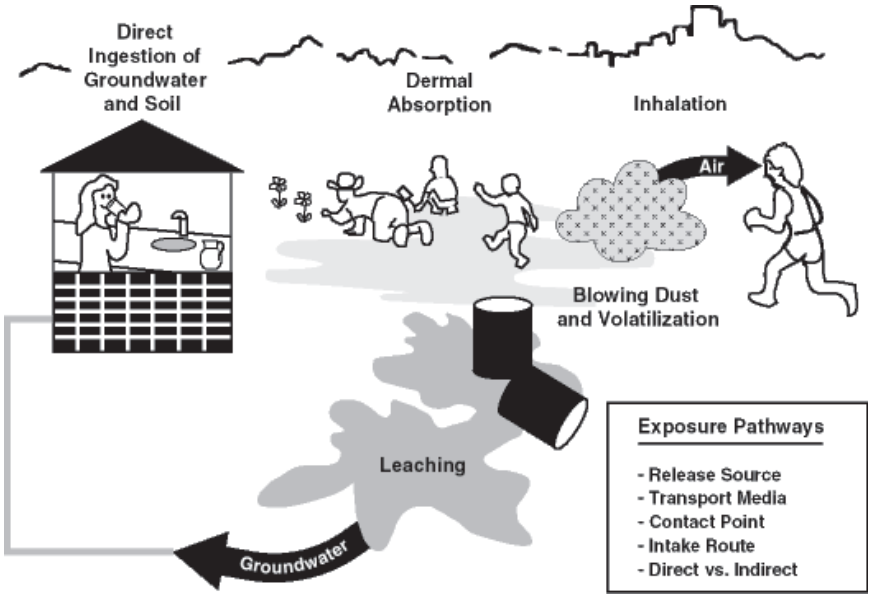

FIGURE 2-1 Major Exposure Pathways for Human Exposure to Contaminated Soils and Sediments. SOURCE: EPA Region 9 Preliminary Remediation Goals website (www.epa.gov/region09/waste/sfund/prg).

In addition to these three routes, there are other indirect pathways by which contaminants in soil and sediment can reach human receptors, notably leaching to groundwater and subsequent ingestion of well water. These routes of exposure are considered below, using contaminated soil (rather than sediment) as an example.

Incidental Ingestion

Incidental ingestion is often an important exposure route for contaminated soils in human health risk assessments. In its basic form, the intake equation for incidental ingestion of soils is:

$$Intake = \left(\frac{C_s \times IR \times RAF}{BW}\right)\left(\frac{EF \times ED}{AT}\right)$$

where:

C_s = chemical concentration in the soil at the point of contact
IR = incidental ingestion rate of soil
RAF = relative absorption factor

BW = body weight
EF = exposure frequency
ED = exposure duration
AT = period over which exposure will be averaged.

The chemical concentration in soil, soil ingestion rate, and body weight are used to determine the ingestion rate for the chemical per unit body weight. The exposure frequency, exposure duration, and averaging time are used to account for periods when exposure does not occur, and to develop an average intake over time. A correction for relative bioavailability can be introduced in the form of a Relative Absorption Factor (*RAF*). Usually, the *RAF* is expressed as a ratio:

$$RAF = \frac{F_s}{F_{sm}}$$

where F_s is the fraction of the dose of chemical absorbed from soil under circumstances of environmental exposure, and F_{sm} is the fraction of the dose absorbed from the study medium (e.g., food, water, or some liquid vehicle) used in the critical study upon which the toxicity value is based. The *RAF* may be an estimated or measured factor, and can be less than or greater than 1.0 (100 percent). If the absorption from soil is found or assumed to be the same as absorption in the critical study upon which the toxicity value is based, then the *RAF* is 1.0. Note that a *RAF* of 1.0 does not indicate that absorption is complete, but simply that absorption is known or estimated to be the same as that in the critical study. It is not uncommon for an ingestion intake equation to lack a *RAF* term. This simply means that the relative bioavailability is assumed to be 1.0.

Under some circumstances, the oral toxicity value might be expressed as an internal dose. In this situation, the *RAF* would be replaced by a term for absolute bioavailability from soil in order to permit an internal dose to be calculated for comparison.

Dermal Contact

A general form of the equation used to calculate the internal (absorbed) dose from dermal exposure to soil is:

$$Intake = \left(\frac{C_s \times SA \times AF \times ABS}{BW} \right) \left(\frac{EF \times ED}{AT} \right)$$

where:

C_s = chemical concentration in soil on the skin
SA = skin surface area
AF = soil adherence factor (how much soil covers a unit area of skin)
ABS = absorption factor from the soil into the body

BW = body weight
EF = exposure frequency
ED = exposure duration
AT = period over which exposure will be averaged

The soil concentration, surface area, adherence factor, and body weight terms allow calculation of an amount of chemical present on the skin per unit body weight. As with exposure by ingestion, the exposure frequency, exposure duration, and averaging time terms are present to allow determination of an average exposure rate over time. Usually, the absorption factor (*ABS*) is intended to reflect the absolute bioavailability of the compound from soil via the dermal route (dermal bioavailability) and is used to calculate the absorbed, or internal, dose of the chemical expected to result from dermal contact. Data on dermal bioavailability from soil are extremely limited or absent for most chemicals, although default assumptions have been specified by EPA and state agencies (see later discussion).

Once the intake has been determined from the equation above, it is compared with a suitable toxicity value for dermal exposure. Unfortunately, there are very few toxicity values available specifically for dermal exposure. Instead, if the toxicity is systemic in nature (i.e., doesn't occur through direct interaction with the skin) the applied-dose toxicity value from another route is converted to an internal-dose value in order to assess risks from dermal contact—a process known as route-to-route extrapolation. This requires knowledge or an assumption regarding the extent of absorption associated with the toxicity value. For example, an oral cancer potency value for a chemical based on a dietary study in laboratory animals could be converted to an internal dose equivalent for use in assessing risks from a chemical entering through the skin. This adjustment in the oral toxicity value would require some knowledge of the gastrointestinal absorption of the chemical in the critical study upon which the oral cancer potency estimate was derived. For cancer potency factors (such as EPA cancer slope factors), the adjustment is made by dividing the oral toxicity value by the known or inferred absolute bioavailability of the chemical from the gut in the critical cancer study. Thus, risks from dermal exposure commonly must rely on estimates of both dermal and oral absolute bioavailability of a chemical, with little supporting data for either.

An alternative approach is to compare dermal intake with an oral or inhalation toxicity value without adjustment of the toxicity value to an internal dose form. If this approach is used, the *ABS* term has a different meaning. Instead of representing the absolute bioavailability of the chemical through the skin, *ABS* is instead a relative bioavailability term, in this case quantifying the expected difference in absorption from the dermal route versus the absorption implicit in the toxicity value. If the toxicity value for comparison is based on the oral route, then the comparison point is the gastrointestinal absorption of the chemical in the

critical oral toxicity study. The example shown in Box 2-1 uses this approach. Similarly, if an inhalation toxicity value is used to assess dermal risks, then the *ABS* value will be based upon differences in dermal versus inhalation exposure to the chemical. Rarely are experiments conducted to generate these *ABS* numbers; rather they are the products of best professional judgment.

Inhalation

Calculating exposure from inhalation of contaminants from soils can be accomplished by measuring or estimating the associated concentration of the chemical in air. A simple form of inhalation intake equation is:

$$Intake = \left(\frac{C_a \times INR}{BW}\right)\left(\frac{EF \times ED}{AT}\right)$$

where:

C_a = chemical concentration in inspired air
INR = inhalation rate
BW = body weight
EF = exposure frequency
ED = exposure duration
AT = period over which exposure will be averaged

This equation calculates the average amount of chemical entering the respiratory tract per unit time and per unit body weight over a specified exposure interval. This intake value is in the form of an applied dose, and is analogous to chemicals entering the gastrointestinal tract after ingestion or coming in contact with the skin during dermal exposure. For exposure to chemicals in soils, the inhalation intake equation often uses the soil concentration and incorporates a model to calculate the corresponding air concentration of the chemical. This model can be viewed as representing the bioavailability processes that make a chemical in soil accessible to its site of entry into the body, which in this case is the lungs.

As with ingestion, risks from inhalation exposure are typically assessed through the use of estimates of applied doses resulting from exposure and of toxicity values based on applied doses. Unlike ingestion, however, both the doses and the toxicity values are often expressed in terms of concentration in air, rather than an amount of chemical per unit body weight. For example, a toxicity value for non-cancer health effects by inhalation exposure may be simply a safe concentration limit for the chemical in air. For estimating cancer risks from inhalation exposure, cancer potency can be expressed in reciprocal concentration terms, such that multiplication with the exposure concentration in air yields an excess cancer risk estimate (e.g., EPA inhalation unit risk values). In theory, if differ-

ences in pulmonary bioavailability are known to exist between the exposure situation and the critical study used to develop the inhalation toxicity value, this can be addressed through the use of a relative bioavailability or *RAF* term, as with exposure by ingestion. However, there are few obvious examples of situations where such an adjustment is required, and consequently it is rare in risk assessments. Instead, the implicit assumption is that the relative bioavailability associated with environmental exposure is 100 percent—that is, the pulmonary absorption of the chemical under environmental exposure conditions is equivalent to the pulmonary absorption that existed in the critical study used to derive the inhalation toxicity value.

Leaching to Groundwater

Leaching from soil to groundwater is another common pathway by which humans can be exposed to contaminants (see Figure 2-2). The calculation requires an estimate of the contaminant concentration in the infiltrating water and a determination of the dilution by mixing with underlying groundwater. Estimation of a soil concentration that will be "protective" of groundwater is achieved by working backward from the desired water concentration at the groundwater well (usually a water quality standard), via the dilution attenuation factor (*DAF*). The following equation for *DAF* is meant to account for the dilution by mixing with underlying groundwater:

$$DAF = 1 + \frac{Q_{gw}}{Q_l} = 1 + \frac{Kid}{IL}$$

where Q_{gw} is groundwater discharge per unit aquifer thickness over the mixing depth in the aquifer (d); Q_l is the leaching recharge [$L^3L^{-2}T^{-1}$]. The Q_{gw} depends upon the aquifer hydraulic conductivity (K), hydraulic gradient (i) and mixing depth (d). The Q_l depends upon the area covered by the contaminated soil (L) and infiltration rate (I).

The protective soil concentration for this pathway, C_s, is estimated by assuming equilibrium partitioning between the soil- and aqueous-phase contaminant concentrations in the soil pore water using the following equation:

$$C_s = C_w DAF \left[K_d + \frac{\left(\theta_w + \theta_a H'\right)}{\rho_b} \right]$$

where C_w is the water quality standard at the receptor (such as a maximum contaminant level or MCL); K_d is the sorption distribution coefficient for the contaminant; θ_w and θ_a are the volumetric air and water contents, ρ_b is the soil bulk density, and H' is the dimensionless form of the Henry's law constant or partitioning coefficient between the air and water phases at a specified temperature. C_s is then compared to the levels of soil contamination at a specific site to determine what actions should be taken next. Unlike the previous three pathways

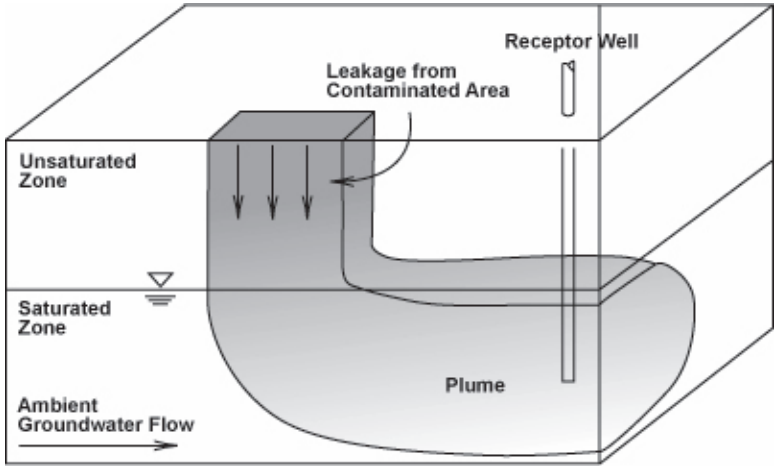

FIGURE 2-2 Conceptual View of the Leaching to Groundwater Pathway.
SOURCE: EPA (1996a).

described above, there is no explicit exposure intake equation used for the leaching to groundwater pathway. Rather, the intake equation—including dose, toxicity, and relative absorption values for ingestion of contaminated groundwater—is reflected in the water quality standard for the contaminant (C_w). For all practical purposes, the relative absorption factor for ingestion of contaminated water is assumed to be 100 percent.

Assumptions and Default Values

Direct Contact Pathways. Commonly, assessment of risks from direct contact with a soil chemical involves an evaluation of its intake from ingestion, dermal contact, and inhalation. As the preceding discussion indicates, this entails the need to make several assumptions regarding the absorption of the chemical by the various routes under different sets of conditions. Box 2-1 provides an example of these many assumptions that were made during the development of the soil cleanup criterion for the pesticide chlordane. It should be noted that assumptions also must be made about bioavailability processes A–C that lead to the chemical concentration used in the three intake equations, but these assumptions are not discussed here.

Data on the absorption of chemicals under conditions of environmental exposure are extremely limited. Also, information on absorption implicit in the toxicity values used in the calculations is required for determining absolute bioavailability. Unfortunately, the extent of absorption of a chemical that occurred as part of a critical toxicity study is almost never measured. Instead, the

BOX 2-1
Implicit Assumptions Regarding Bioavailability in Human Health Risk Assessments: Soil Cleanup Goals for the Pesticide Chlordane

Estimation of risks to humans from direct contact with contaminated soils requires several types of bioavailability assumptions, most of which are obscure to all but those familiar with the detailed mechanics of risk calculations. To illustrate "hidden" bioavailability assumptions, derivation of a risk-based soil cleanup goal for chlordane is used as an example. The procedure used to calculate chlordane soil cleanup goals and thus Preliminary Remediation Goals (PRGs) by EPA Region 9 is considered for this example, although the formula and assumptions vary among different regulatory agencies.

A PRG is a soil concentration thought to correspond to a specified risk level, given a set of default assumptions about the extent of exposure to soil. The PRG for chlordane in soil in industrial settings, based on a 10^{-6} excess cancer risk, is 11 mg/kg soil. Since chlordane is regarded as a carcinogen, the Region 9 PRG equation for direct exposure to carcinogens was used to develop this number. The equation for an industrial exposure scenario is:

$$C(mg/kg) = \frac{TR \times BW_a \times AT_c}{EF_o \times ED_o \left[\left(\frac{IRS_o \times CSF_o}{10^6\ mg/kg} \right) + \left(\frac{SA_a \times AF \times ABS \times CSF_o}{10^6\ mg/kg} \right) + \left(\frac{IRA_a \times CSF_i}{VF} \right) \right]}$$

where:

TR is the Target Risk (in this case, an excess cancer risk of 1×10^{-6})
BW_a is Body Weight for an adult worker
AT_c is Averaging Time, the total period over which exposure is averaged
EF_o is the Exposure Frequency
ED_o is the Exposure Duration
IRS_o is the incidental Soil Ingestion Rate for a worker
SA_a is the exposed skin Surface Area
AF is the soil Adherence Factor, or the extent of soil loading on exposed skin
ABS is the Absorption factor for skin, or the dermal bioavailability of the chemical
VF is the Volatilization Factor, which is used to estimate the air concentration resulting from volatilization of the chemical from soil
IRA_a is the Inhalation Rate
CSF_o is the oral Cancer Slope Factor, a measure of cancer potency for oral exposure
CSF_i is the inhalation Cancer Slope Factor, a measure of cancer potency for inhalation exposure.

This equation includes terms for intake resulting from incidental ingestion of soil, dermal contact with soil, and inhalation of chemical volatilized from soil. As the equation illustrates, development of an acceptable risk-based concentration for soil requires specific assumptions regarding several exposure parameters, including the exposure frequency, exposure duration, body weight, and incidental soil ingestion rate. With respect to bioavailability, a term for absorption of chemical through the skin, ABS, is specified;

however, there are a number of other bioavailability assumptions that are implicit in the calculation. To recognize these, it is important to understand the nature of the toxicity values—in this case the cancer slope factors—and how they are used in the equation.

To estimate cancer risk from chlordane, two cancer potency estimates (i.e., Cancer Slope Factors) are available from the EPA—one for oral exposure and one for inhalation exposure. The oral cancer slope factor is derived from a study in which mice fed chlordane in the diet developed liver tumors. No attempt was made to estimate the dose of chlordane absorbed by these animals, and the cancer slope factor is instead based simply on measurements of the amount of chlordane ingested daily. This is, therefore, an *applied-dose* toxicity value. Unless some adjustment is made, use of this cancer slope factor to estimate risks from ingestion of chlordane from soil or sediment assumes that the absorption from these media is the same as from food in the mouse cancer study. That is, the *relative bioavailability* is assumed to be 100 percent. If this assumption can be demonstrated to be incorrect, and the difference in absorption following ingestion from these two different sets of oral exposures can be quantified, a *RAF* can be introduced into the ingestion portion of the equation to correct for this. However, in this particular example, *RAF* is not in the equation, and it is consequently not obvious that a relative bioavailability of 100 percent is being assumed for the ingestion route of exposure.

No cancer data from inhalation exposure to chlordane are available, and so EPA uses cancer potency information from oral exposure to derive an inhalation cancer potency estimate. By using the same cancer potency estimate, without adjustment, for both routes of exposure, it is assumed that bioavailability from both routes is equivalent—that the relative bioavailability for inhalation versus ingestion exposure is 100 percent. Stated more precisely, it is assumed that the absorption of volatilized chlordane from the lungs is the same as the absorption of ingested chlordane from food in the critical oral cancer study. This assumption is discussed by EPA in technical support documentation for these cancer slope factors (Toxicological Review of Chlordane [Technical], EPA, 1997a), and data are presented in support of it. However, without investigating the basis for the chlordane inhalation cancer slope factor, the bioavailability assumption associated with this toxicity value would not be evident. If the relative bioavailability were something other than 100 percent, this could again be addressed by including a *RAF* term in the inhalation portion of the equation, although this is seldom done.

EPA does not produce toxicity values specific for the dermal route of exposure. As a consequence, toxicity values from oral or inhalation exposure must be adapted or utilized to address the contribution of dermal absorption to total risk from a chemical. In the equation above, the dermal component of the equation relies upon the oral cancer slope factor to estimate risks from dermal absorption. Here there is an explicit term for dermal bioavailability, *ABS*. What may not be clear to some is what this bioavailability term represents. Since it is used in conjunction with the oral cancer slope factor, which is an applied dose toxicity value, this value represents the relative bioavailability of chlordane from soil on the skin versus chlordane in the gut from food. Another approach that is commonly used in estimating risks from dermal absorption is to create a dermal cancer slope factor from either the oral or inhalation cancer slope factor. In order to do this, an

continues

BOX 2-1
Continued

internal dose version of one of these applied dose toxicity values must be derived. For chlordane, the oral cancer slope factor would be divided by its implicit absolute oral bioavailability; that is, the absolute bioavailability of chlordane from food in the gut. This internal dose version of the oral cancer slope factor could then be used with an estimate of the internal dose resulting from dermal exposure. Calculating the internal dose from dermal exposure would involve estimates of the amount of chlordane in soil on the surface of the skin and the absolute dermal bioavailability of that chlordane. Thus, assessment of risk can involve either an estimate of the relative bioavailability of chlordane by the dermal (versus oral) route, or separate estimates of both the absolute bioavailability of chlordane from soil through the skin and the absolute bioavailability of

extent of absorption must be inferred from absorption studies that may not duplicate well the conditions of the toxicity study. Overall, the situation is usually one in which a great deal of information is needed on bioavailability processes related to absorption, but almost no data exist specific to the exposure or toxicity study conditions of interest. These limitations are overcome to a large extent by conducting relative bioavailability studies at specific sites instead of attempting to determine absolute bioavailability.

Nonetheless, the paucity of absorption data, and the expense and difficulty associated with doing site-specific studies of relative bioavailability (see Chapter 4), have led to extensive use of simplifying or default assumptions regarding chemical absorption in human health risk assessments. Regulatory agencies have not discouraged this and, as a practical matter, often specify the defaults they regard as acceptable. The most prominent default assumption imposed is this: relative bioavailability is assumed to be 100 percent unless there is compelling evidence to the contrary and a scientifically defensible adjustment factor can be derived.[1] Criteria as to what constitutes an acceptable scientific basis to choose a *RAF* other than 1.0 have not been clearly articulated by regulatory agencies. As a result, the burden of proof required to depart from a default assumption of 100 percent relative bioavailability is poorly defined.

A default relative bioavailability assumption of 100 percent is often described as conservative. Occasionally this arises from a misconception that com-

[1]An example of this can be found in human health risk assessment guidance from U.S. EPA Region 4. They state, "Bioavailability questions arise as to potential differences in uptake levels under study conditions versus environmental exposure conditions, i.e., the matrix effect. Chemical-specific data is rarely sufficient to quantify this difference in bioavailability for all receptors under their varied exposure conditions. Therefore, Region 4 does not accept any adjustment in the 100 percent bioavailability default assumption in the exposure equation without extensive supporting data." (EPA Region 4, 2000).

chlordane from the gut. In the case of chlordane, and in fact for most contaminants, hard data on these bioavailability values are absent, and professional judgement must be used to generate estimates.

As shown in this example, when assessing risks to humans from contact with contaminated soils or sediments, each route of exposure requires at least one, and sometimes two or more assumptions regarding bioavailability. Most formulas for calculating risks do not include terms by which all of these assumptions are clearly shown. Even when a bioavailability term is present, the meaning is sometimes not obvious, that is, whether it is intended to represent relative or absolute bioavailability. As a result, the bioavailability assumptions incorporated into risk estimates are often obscure.

plete absorption is being assumed. There is certainly a reason to suspect that an assumption of 100 percent relative bioavailability is conservative in many instances, simply because most toxicity tests use forms of a chemical that tend to be readily absorbed. However, this is not always the case, and treatment with the chemical in diet, for example, may represent sub-optimal conditions for absorption. Under these circumstances, it is possible that exposure to the chemical in an environmental medium may entail greater absorption than during the critical toxicity study. In this situation, an assumption of 100 percent relative bioavailability will underpredict the toxic potential of the exposure.

As discussed above, there are many situations in which information on absolute bioavailability *is* needed. Examples include the extent of dermal absorption of a chemical for estimating intake by the dermal route, and the extent of gastrointestinal absorption of a chemical to convert an applied-dose oral toxicity value to its corresponding internal dose form. To facilitate locating absolute bioavailability information for various chemicals, compendia are available (see EPA, 2001a; Oak Ridge National Laboratory at http://risk.lsd.ornl.gov/cgi-bin/tox/TOX_select?select=nra). In many instances, the absolute bioavailability values represent chemical-specific information derived from studies with varying degrees of similarity to the conditions of interest. For example, information on the absorption of a chemical from diet might be sought in order to develop an internal-dose form of an oral toxicity value, but the only data available may be for oral absorption of the chemical from water.

There are several other sources of uncertainty associated with this absolute bioavailability information. For example, EPA has recommended absolute bioavailability values for the dermal absorption of 92 organic and six inorganic chemicals from soil (EPA, 2001a). Each value is from a study in which dermal absorption from soil was measured, but the number of soil samples examined was limited. Often these studies used uncontaminated soils to which the chemical of

interest was added, with or without subsequent aging. Dermal absorption of chemicals from soil could conceivably vary with soil type and with interactions between the chemical and soil. Consequently, even though the default values are based on simulated environmental exposure conditions, there is uncertainty regarding the extent to which these values are applicable to soils at contaminated sites.

For many chemicals, there is essentially no information on absolute bioavailability. For these chemicals, crude default assumptions are used based on simple chemical classifications. For example, in the absence of chemical-specific data, EPA Region 4 recommends an oral absolute bioavailability of 80 percent for volatile organic compounds, 50 percent for semi-volatile compounds, and 20 percent for inorganics. For dermal absorption of chemicals from soils, when chemical-specific data are not available, a default absolute bioavailability of 1 percent for organics and 0.1 percent for inorganics is recommended (EPA Region 4, 2000). Table 2-1 lists default absolute and relative bioavailability values for dermal and oral routes, respectively, used by EPA and the states.

The use of national default values for relative and absolute bioavailability and standardized exposure models has been most thoroughly developed for lead-contaminated sites. As mentioned in Chapter 1, mining sites were some of the first to receive attention as sites where the total amount of contaminant present may not be the best indicator of the actual human health risk. As explained in Box 2-2, EPA has developed an exposure model for lead contamination by direct contact (the Integrated Exposure Uptake Biokinetic or IEUBK Model) that focuses on the most sensitive receptor—children. It incorporates a value for the relative bioavailability of lead from soil of 60 percent (EPA, 1999a, 2001b). This value was then used to derive a national default value for absolute bioavailability of soil lead to children of 30 percent.

The IEUBK model allows for the use of more refined relative bioavailability values derived from site-specific data and information if they are available. This is actually an important feature of the model, because it has been shown that the relative bioavailability of lead in soil can vary by as much as two orders of magnitude with soil type. This variability is evident in Figure 2-3, which shows the results of 19 swine feeding studies on different soils contaminated with lead. Thus, despite having a national default value of 30 percent absolute bioavailability, there are clearly limitations with using this value in many circumstances. This underscores the limitations of default values for bioavailability processes in general. Indeed, it is because of the substantial variability with soil type observed in these studies that a significant portion of Chapter 3 is devoted to better understanding solid–contaminant interactions.

Leaching-to-Groundwater Pathway. Assumptions are also made with regard to the groundwater leaching pathway. Most important perhaps are the assumptions implicit in the MCL or water quality standard used to determine

TABLE 2-1 Examples of Default Values Used to Adjust Exposures to Account for Reduced Bioavailability of Compounds in Soil

Chemical	Dermal Absorption Factor (ABS)[a] [source]	Oral Relative Absorption Factor (RAF)[b] [source]
Benzene	0.08 [1], 0.0005 [2]	1.0 [1]
Ethylbenzene	0.2 [1], 0.03 [2]	1.0 [1]
Toluene	0.12 [1], 0.03 [2]	1.0 [1]
Xylenes	0.12 [1], 0.03 [2]	1.0 [1]
Volatile organic compounds	0.1 [5], 0.25 [6]	1.0 [5]
n-Hexane (for TPH)	0.5 [1]	0.91 [1]
Nonane (for TPH)	0.2 [1]	0.91 [1]
Eicosane (for TPH)	0.1 [1]	0.91 [1]
Pyrene	0.18 [1], 0.1 [2]	0.91 [1]
Acenaphthene	0.2 [1], 0.1 [2]	1.0 [1]
Anthracene	0.29 [1], 0.1 [2]	0.91 [1]
Benzo (ghi)perylene	0.18 [1], 0.1 [2]	0.91 [1]
Flouranthene	0.2 [1], 0.1 [2]	1.0 [1]
Fluorene	0.2 [1], 0.1 [2]	1.0 [1]
1-Methylnaphthalene	0.1 [1], 0.1 [2]	1.0 [1]
2-Methylnaphthalene	0.1 [1], 0.1 [2]	1.0 [1]
Naphthalene	0.1 [1], 0.1 [2]	1.0 [1]
Phenanthrene	0.18 [1], 0.1 [2]	0.91 [1]
Benzo(a)anthracene	0.18 [1], 0.1 [2]	0.91 [1]
Benzo(a)pyrene	0.18 [1], 0.1 [2]	0.91 [1]
Benzo(b)fluoranthene	0.18 [1], 0.1 [2]	0.91 [1]
Dibenz(a,h)anthracene	0.08 [1], 0.1 [2]	0.91 [1]
Indeno(123,cd)pyrene	0.2 [1], 0.1 [2]	0.91 [1]
Polycyclic Aromatic Hydrocarbons	0.15 [3], 0.05 [4], 0.01 [5], 0.13 [9] (0.1 for SVOCS [6, 9])	0.5 for SVOCS [5]
Lindane	0.04 [9]	
2,4-D	0.05 [9]	
Chlordane	0.04 [7, 9]	
PCB Aroclors 1254 and 1242	0.14 [7, 9]	0.5 [5]
DDT	0.03 [7, 9]	0.5 [5]
Pentachlorophenol	0.25 [7, 9]	

continues

TABLE 2-1 Continued

Dioxins	0.03 or 0.001 if OC >10% [9]	
Arsenic	0.03 [7, 9]	0.5 [5]
Cadmium	0.1 [7], 0.001 [9]	0.5 [5]
Lead[c]	0.3 [10], 0.12 [11][c]	
Inorganics	0.01 (qualitative screen only) [8]	0.5 [5]

[a]ABS equals the absolute bioavailability of the compound in soil via the dermal route.

[b]RAF equals the relative bioavailability of the compound (i.e., in soil vs. in the medium used in the toxicity study).

[c]Values for lead are absolute bioavailability.

SOURCES:

1. Massachusetts Department of Environmental Protection (1992).
2. EPA Region 3 (1998).
3. California Environmental Protection Agency (1993)
4. Illinois Environmental Protection Agency (1996).
5. Michigan Department of Environmental Quality (personal communication).
6. Ohio Department of Commerce (1992).
7. Wester et al. (1990); Wester and Maibach (1996).
8. Used by U.S. Environmental Protection Agency Region 1 (EPA Region 3, 1998).
9. EPA (2001a).
10. Value used for children in the EPA IEUBK Model (EPA, 1999a, 2001b).
11. Value used for adults in the EPA adult lead model prepared by the Technical Working Group (EPA, 1996b).

whether the water source poses an unacceptable risk to human health, which are similar to the assumptions discussed above regarding absorption and toxicity and thus are not discussed further here. In addition, there are numerous assumptions that go into the equations for determining the protective soil concentration, as discussed in greater detail in Box 2-3. One of the most common assumptions is that there is no dilution of the contaminant in groundwater as it travels from the source to the point of contact with humans. Partly because of this assumption, the leaching-to-groundwater pathway has been found to be the most sensitive exposure pathway for 86 of the 110 contaminants considered by EPA in setting soil screening levels (EPA, 1996a).

***

In summary, bioavailability processes are important in assessing risks to humans from both direct contact with soils and sediments and leaching of soil and sediment contaminants to water. The term "bioavailability," when used in a human health risk assessment context, generally refers to the relative or absolute absorption of the chemical from either ingestion, dermal, or inhalation exposure. Calculating risks from direct contact with contaminated soils or sediments typi-

BOX 2-2
Absolute Bioavailability of Lead in Soil:
The Integrated Exposure Uptake Biokinetic Model

National risk assessment guidance for lead is based on information that has been developed on the behavior of this metal in the gastrointestinal system, blood, and other organs. Lead is a compound for which there is a great deal of toxicological data. The disposition of lead is fairly well understood, as are the target organs, effects, and to some extent the mechanism by which lead exerts its adverse effects. Although lead has been shown to affect every system in the body, the most sensitive target organs are the nervous system in young children, the hematopoietic system, and the cardiovascular system—with the nervous system being by far the most sensitive.

For estimating child exposure to lead, EPA developed the IEUBK model, a pharmacokinetic model that takes into account multi-media exposures of young children (less than six months to six years old). This population is the most sensitive to the effects of lead, due in part to physiological conditions (e.g., efficient absorption and developing nervous system/blood brain barrier) and to behavioral conditions (e.g., hand-to-mouth contact and frequent ingestion of soils). The output of the IEUBK model is a predicted distribution of blood lead levels in children. From this distribution, the model calculates the probability that blood lead concentrations will exceed 10 mg lead per deciliter of blood (Centers for Disease Control, 1991).

The specifics of the IEUBK model are given in EPA (1994a). The IEUBK model can evaluate residential exposures to lead in soil, indoor dust derived from soil, ambient air, drinking water, and food. It does not evaluate exposures via inhalation of fugitive dust derived from soil. Dust exposures in the model are via ingestion of indoor dust derived, at least in part, from soil. Since dermal absorption of lead is very low (< 0.3 percent), this pathway is typically not evaluated. The model is implemented using an EPA software program.

The model includes two values for lead bioavailability in soils for incidental ingestion in children. The first is the relative bioavailability of lead from soil as compared to other exposure media (60 percent is recommended by EPA—EPA, 1999a, 2001b). This value is independent of the age of the subject. The second is the absolute bioavailability of lead in children (i.e., the amount of ingested lead that is subsequently absorbed through the gut). Because absorption is efficient in children, this value is quite high—50 percent. Combining the two factors yields an absolute bioavailability of 30 percent for lead in soil ingested by children, which is the national default value. These factors can and have been modified on a case by case basis when data from feeding studies or appropriate extraction measurements are available for site-specific soils.

The approach currently used to assess exposures of lead in soils to adults is the adult model described in EPA (1996b) and referred to as the EPA Technical Review Workgroup (TRW) Model. This is a biokinetic model that estimates uptake of lead ingested incidentally with soil. Like the IEUBK model, the TRW model also includes a value for the relative bioavailability of soil lead in the digestive track of adults, which presumably could be modified based on feeding studies and extraction studies performed on site-specific soils. The relative bioavailability of lead from soil (relative to lead in water) is assumed to be 60 percent. Because the absorption of lead from water into adults is assumed to be 20 percent, this equates to an absolute bioavailability of 12 percent for lead in soil ingested by adults.

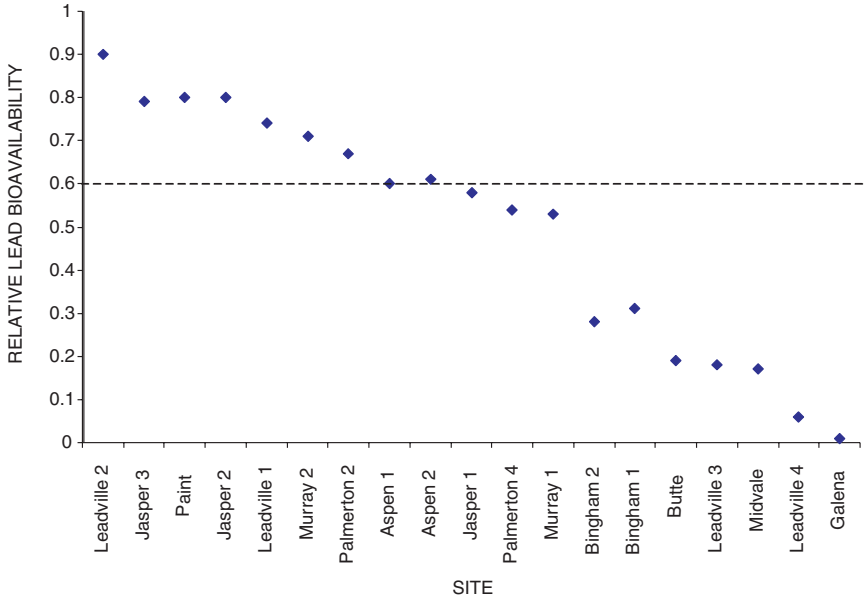

FIGURE 2-3 Swine feeding studies using 17 field soils contaminated with lead and two laboratory prepared soils (paint in soil and galena in soil). The dashed line represents the 60 percent relative bioavailability used to set the national default value for absolute bioavailability of lead in soil used by EPA. SOURCE: Reprinted, with permission, from Ruby et al. (1999). © (1999) American Chemical Society.

cally requires several bioavailability assumptions, many of which are not readily apparent. Currently, default assumptions are used extensively, although the opportunity exists to refine risk assessments by incorporating site-specific bioavailability process information using approaches described in Chapter 4.

Ecological Risk Assessment

Ecological risk assessment involves more complexity than human health risk assessment because of the types of species, physiologies, and physical/chemical processes that must be considered. Some organisms feed directly on soils and sediments and thereby access contaminants, and other species absorb dissolved chemicals across their external membranes. Still other species access contaminants that originated in soils and sediments by eating organisms exposed via the first two routes. There are also significant differences in what governs exposure between aquatic and terrestrial organisms.

Like human health risk assessment, information on bioavailability processes is generally utilized during the exposure assessment, but not always in an explicit

way. In general, the goal of the exposure assessment is to determine the concentration of each compound that will be accumulated into various levels of a food chain in the vicinity of contaminated soils or sediments—similar to determining intake in human health risk assessment. For a given exposure pathway, the most conservative approach is to assume 100 percent availability relative to the available tests of threshold toxicity. This might overestimate risk if all exposure pathways are adequately considered and toxicity tests are designed to maximize contaminant uptake. For example, compounds may be buried deep enough to be below the zone accessed by most organisms, or they may be bound to the solid phase in such a way as to be minimally available. It might underestimate risk if some important exposure pathways are missed or if toxicity tests are not conducted under conditions that maximize uptake.

Because there are many types of ecological receptors and because exposures to soils or sediments can include direct as well as indirect pathways, it is common practice to employ a conceptual model to illustrate the predominant exposure pathways. An example of a conceptual model of exposure to soil contaminants is given in Figure 2-4. There are multiple stressors and pathways—depending on the ecological receptors present as well as the spatial and vertical distribution of the contaminant—that vary in both time and space. Concentrations of individual compounds can change between compartments, including moving from water, sediment, or soils to biota, and between trophic levels. Thus, estimates of exposure can vary depending on the residue and system. Although plant and animal species use different depths within the soil system, most ecological risk assessments focus on surface soils (the upper few meters). Surface sediments, sometimes thought to be the upper 3 cm, are defined by an oxidized zone in which most animals live. However, the depth at which the animal is exposed to its microenvironment can vary from millimeters to tens of centimeters. Burrowing animals can interface with much deeper environments.

Intake equations require values for contaminant concentrations in the various compartments (solid, water, tissue), which can be either measured or predicted. To minimize uncertainties, ecological risk assessors have tried to minimize the length of pathways along which predictions are to be made. Ultimately, one would like to be able to link concentrations of contaminants in top predators to concentrations in the soils or sediments. In cases where top predators are the receptors of concern, such a linking would allow one to derive a proposed threshold concentration in soils or sediments, which would then be the cleanup criterion for a particular site.

Depending on which exposure pathways dominate, different bioavailability processes can be considered during ecological risk assessment. Table 2-2 considers where explicit bioavailability information has been typically used for four exposure pathways. To illustrate further how specific bioavailability processes are currently considered in ecological risk assessment and risk management, the following section focuses on direct contact of invertebrates with soils or sedi-

BOX 2-3
Assumptions Imbedded in the Leaching-to-Groundwater Exposure Pathway

Soil Screening Levels (SSLs) are generic values, established by the states and EPA, that are used in screening level assessments of contaminated soil. It turns out that for a large number of chemicals, the leaching-to-groundwater pathway controls SSL values. Thus, it is important to understand the assumptions about bioavailability processes A and B that play a role in this exposure pathway—assumptions that are not apparent from simply reading the list of numeric SSLs. A better understanding of the assumptions and the default parameters selected to obtain the numeric criteria can illuminate opportunities to improve bioavailability process assumptions via more site-specific evaluation of contaminated sites.

Two equations described earlier represent leaching of contaminants from the soil and subsequent mixing and dilution with underlying groundwater. Regarding the equation for the dilution attenuation factor (*DAF*), infiltration over the site area is presumed to be uniform and leached water is presumed to have uniform contaminant concentration. The contaminant is presumed to be uniformly distributed in the site soil, and the soils are assumed to be physically and chemically homogeneous. It is also assumed that there is no background concentration of the contaminant in the off-site groundwater. In order to generate generic SSLs, EPA established a "default" *DAF* of 20 to be used at all sites. This number was generated after applying the *DAF* equation to 300 selected groundwater sites across the country. Although the physical hydrologic properties of the subsurface soils vary from site to site, the default value is expected to be protective in most cases where the contaminants are above the water table and the site size is less than half an acre.

A number of assumptions are also found in the second groundwater leaching equation, which determines the protective soil concentration of contaminant. In order to obtain numeric estimates, default physical soil property values (θ_w, θ_a, ρ_b) are assumed. The *H′* constants are contaminant-specific properties and are tabulated in the literature

ments and exposure to wildlife feeding on soil invertebrates and plants—selected because they frequently drive ecological risk assessment efforts.

Direct Contact of Invertebrates with Soils or Sediments

Bioavailability processes A and D in Figure 1-1 (association and dissociation of the contaminant with the solid phase and absorption through a biological membrane) play an important role in this exposure pathway and are considered during ecological risk assessment in a variety of ways. One relatively simple technique has been to develop models that predict the partitioning of metals and organics between different phases—of which there are many levels of detail—and then incorporate these into exposure assessment. In the simplest formulations, thermodynamic partition coefficients are used to describe distributions of contaminants between various environmental compartments, with the contaminant in the aqueous or organismic phase usually assumed to be available. For

for most compounds of concern. For hydrophobic organic pollutants, the sorption distribution coefficient (K_d) is estimated as the product of the K_{oc} (organic carbon normalized sorption coefficient), a compound specific property that is also tabulated for many organic pollutants, and the fraction organic carbon content (f_{oc}), a soil-specific property. In order to determine default SSLs, a relatively low f_{oc} of 0.2 percent typical of a subsurface sediment is assumed for all calculations. Use of the K_{oc}-approach assumes that sorption is controlled by linear partitioning to "normal" soil organic matter (i.e., sorption to other types of carbonaceous solids, described in Chapter 3, is assumed negligible). For a select list of inorganic pollutants (including silver, copper, nickel), the K_d values are estimated using a geochemical model (MINTEQ) or empirical data. For the generic soil screening values, the estimated K_d values are derived based on assumptions about a number of soil properties, including circumneutral pH and sorptive clay-mineral coatings. For both organic and inorganic contaminants, it is assumed that the time to reach sorption equilibrium (contaminant concentrations in the dissolved and solid phases) is rapid compared to the rate of infiltration, which may not always be true.

In summary, determination of the generic soil concentrations that protect human health via the leaching-to-groundwater pathway relies upon a large number of assumptions about the soil and contaminant behavior. Some assumptions are more obvious because they are captured by "default" values. Other assumptions are less visible and underly the conceptual scenario established for the "generic" site. Clearly, collecting and applying site-specific information has the potential to reduce the uncertainty associated with using the more generic SSLs. By understanding where the default assumptions and parameters are in the leaching-to-groundwater pathway, opportunities for improving the rigor of the risk assessment via the collection of site-specific chemical and physical information are made obvious.

metals, their distribution in soils is assumed to be controlled by both the cation exchange capacity and the organic carbon content, while in sediments, the solubility of metals complexes (both inorganic and organic) and precipitates are assumed to determine the available fraction. As described below, a simple normalization technique known as AVS/SEM has been proposed to determine the fraction of metals that are bound to sediment phases or in pore water, based on what are assumed to be the canonical factors controlling availability. For organic compounds, partitioning between solid, aqueous, and organismal phases is assumed to be dependent primarily on the organic content of soils and sediments and the organism. Another simple and empirical test, known as BSAF, has seen increasing use in determining the distribution of organic contaminants in both soil and sediment systems. In both cases, these descriptors are useful for static or slowly varying systems, but are of limited utility in dynamic systems.

Estimates of the available fraction of a contaminant pool from the exposure assessment are used in ecological risk assessments directly by comparing the

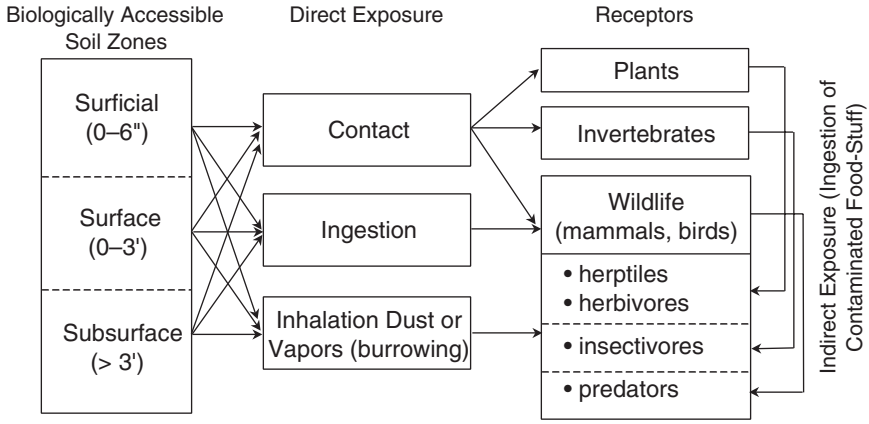

FIGURE 2-4 Hypothetical conceptual model for direct and indirect exposure of ecological receptors to soil contaminants. SOURCE: Adapted, with permission, from Menzie et al. (2000). © (2000) Journal of Human and Ecological Risk Assessment.

predicted available concentrations to threshold concentrations known to cause negative effects in invertebrates, if thresholds are known. (Such threshold levels are usually determined during simple, short-term, single media tests.) Or, as discussed in the subsequent section, estimates of the available fractions can be used to model contaminant concentrations in different phases and transfer to higher trophic levels.

Acid Volatile Sulfide Method for Metals in Sediments. A normalization technique developed for EPA to predict metal accumulation from sediment into aquatic organisms is based on redox conditions and equilibrium partitioning (EqP) theory. A redox boundary is common in aquatic sediments, although the depth of the boundary varies among sediments. Partitioning of metals between the forms typical of oxic vs. anoxic sediment is a first-order process in bulk sediments, because diffusion is the rate limiting process (Rand, 1995). For the anaerobic portion of sediments, the availability of metals is thought to be controlled, in part, by precipitation as insoluble sulfides, because the stability constants for most metal-sulfide associations are very high, and exchange from metal sulfides to water is low. Thus, it has been suggested that normalizing concentrations of metals in sediment by acid volatile sulfide (AVS) might provide a universal explanation of metal availability from sediments (DiToro et al., 1990; Hansen et al., 1996). The theory assumes that low pore water concentrations of metal translate into limited bioavailability. Because they are typically not anoxic, soils are not candidates for the AVS normalization.

TABLE 2-2 Where Bioavailability Information is Used in Ecological Risk Assessment

Exposure Category	Current Use of Bioavailability Information
Direct contact of invertebrates and plants with soils or sediments	This pathway refers to exposure through feeding, exposure to pore waters within sediments, or external contact of non-predator organisms. Bioaccumulation information is the basis for many guidelines and it is the starting point for evaluating indirect exposure to fish, wildlife, and humans (see below).
Release of contaminants from sediments to overlying water column	This fate and transport process (bioavailability process A in Figure 1-1) is commonly considered for exposures to water column organisms such as fish. Releases from soils to overlying air are rarely considered for terrestrial animals and plants.
Birds, mammals, and other predators feeding on plants or on soil or sediment invertebrates	Bioavailability processes are usually considered with regard to accumulation of chemicals into animals that are food for higher organisms. Bioavailability of contaminants in soils incidentally ingested by wildlife itself is rarely considered because of the difficulty in making such measurements.
Food web transfer of contaminants	Some bioaccumulative substances such as PCBs, mercury, and selenium are transferred up the food web. For these compounds, bioavailability processes occurring at lower levels (e.g., uptake into invertebrates and plants) have a great influence on exposure of higher trophic level animals.

Laboratory and field experiments have shown that if the ratio of AVS/SEM is greater than 1, where SEM is simultaneously extractable metal, there are likely to be no metals in solution. Most experiments were conducted with bulk sediments (e.g., Ankley et al., 1991a, b), but similar results are seen in more complex sediment typical of nature. For example, vertical redox gradients and sulfide were found to control concentrations of cadmium in lake pore water in a field setting in Quebec (Hare et al., 1994) and for cadmium, zinc, and nickel in an experimental setting (Lee et al., 2000a). There is also a body of work indicating that acute toxicity from sediments is not observed at ratios of AVS/SEM > 1, although this type of work has been mostly limited to traditional sediment bioassay approaches (i.e., dietary exposure is minimized or absent).

Despite these results, uncertainties remain about the use of AVS as the universal sediment normalizer. For example, studies to date have not defined how to determine biologically relevant AVS concentrations. Redox reactions, and thus sulfide concentrations, are heterogeneous on biologically relevant microscales within reduced sediments. AVS varies widely with depth in a different manner in every sediment, with time in the same sediment, and between the

outside and inside of animal burrows (see Luoma and Ho, 1993; Luoma, 1995 for reviews). So it is not clear how closely protocols for bulk sediment collection (which tends to homogenize samples) can account for the actual microenvironments to which relevant organisms are exposed (e.g., Kemble et al., 1994).

Mechanistic knowledge of sediment geochemistry suggests that factors in addition to AVS should influence the concentrations of metals in pore waters and thus metal availability from sediments. In sediments, a metal will distribute among iron oxides, manganese oxides, organic ligands, sulfides, and perhaps clay surfaces, depending upon (in simplistic terms) the balance of redox couples, the association constant with different types of binding sites, and the abundance of sites (Jenne and Luoma, 1977). In addition, most macrofauna have an obligate requirement for oxygen and therefore seek, or create, microenvironments where they can obtain oxygen. Macrofauna that burrow into sediment can irrigate their burrows with oxygenated water from above the sediment. Other macrofauna and meiofauna concentrate their activities in the oxidized zones of sediments (Rhoads and Boyer, 1983). In all these cases thermodynamics do not favor occurrence of sulfides. Samples of bulk sediment that mix microenvironments from the sediment column may misrepresent the influences of AVS and either overestimate or underestimate (more likely the former) the AVS that animals actually experience.

Finally, it is increasingly recognized that exposure to metals (and organic compounds as well) from sources other than pore water is important in many species–contaminant combinations. Indeed, a long history of study demonstrates direct uptake of metal, by some if not many species, after ingestion of the various metal forms found in sediments, including metal sulfides (Luoma and Jenne, 1977; Lee et al., 2000b). The AVS method assumes no contribution to exposure from dietary metal uptake, by ingestion of either sediments or other food sources. Lee et al. (2000b) showed that assimilation from diet was the best explanation for a disconnect between the measured cadmium, zinc, and nickel bioaccumulation by five different benthic species and the AVS/SEM predictions. While some experts promote the use of the AVS/SEM approach in risk assessment, others question its universality because of the confounding influences described above.

It should be noted that equilibrium partitioning methods similar to AVS/SEM have been developed for predicting organic compound distribution between solid phases and pore water (DiToro et al., 1991; Nichols et al., 1995). These methods assume that organic compounds are associated with organic matter in soils and sediments, that pore water concentrations vary depending on the octanol-water partition coefficient for the compound and the amount of organic matter present, and that the pore water concentrations of these contaminants determine bioavailability to invertebrates.

BSAF Values. The biota-sediment (or soil)-accumulation factor (BSAF) is another simple empirical method used to evaluate bioavailability of contaminants to invertebrates by direct contact. Rather than considering pore water contami-

nant concentrations like the AVS/SEM method above, these factors rely on measured contaminant concentrations in tissue. Organics and sediments are used as the examples in this section because of the existence of guidance material, but similar principles apply to metals (without the normalizations) and soils.

BSAF is an empirical ratio, defined as the chemical concentration in tissue (on a lipid-normalized basis) over the chemical concentration in sediment (normalized to the organic carbon levels in the solid) (Ankley et al., 1992; Cook et al., 1993; Tracey and Hansen, 1996).

$$BSAF = (C_t/F_l)/(C_s/F_{oc})$$

where:

C_t = contaminant concentration in the organism
F_l = the lipid fraction in the tissue
C_s = contaminant concentration in the sediment
F_{oc} = the organic carbon fraction in the sediment

Depending on the compound of interest and the organism, the numbers can range from much less than 1 to much greater than 1, with numbers greater than 1 indicating a compound that bioaccumulates. When predicting higher-order accumulations such as into birds that eat aquatic organisms, ratios referred to as Bio-Magnification Factors (BMFs) are used (Starodub et al., 1996; EPA, 1997b).

BSAF is a simple partitioning factor designed to account for the propensity of an organic chemical to partition into an organism vs. into the organic matter contained in sediment. Such values have the advantage of not assuming equilibrium between the sediment and benthic or pelagic species (Cook et al., 1993). BSAF is generally used to predict the potential accumulation of neutral organic compounds by benthic invertebrates from sediments, but has also been applied to accumulation by fish. For the direct ingestion pathway, BSAF is used mainly as a screening device; that is, a concentration measured in the sediment is multiplied by the BSAF to determine the amount in the organism, which is then compared to some value known to cause harm. As discussed later, BSAF values are also used as input to intake equations for wildlife exposure.

Because BSAF values are dependent on the chemical–physical properties of both the organic compound and solid as well as on the lipid content of the organism, they are site- and species-specific (Lake et al., 1990). Total organic carbon (TOC) values may be relatively constant among sediments. But other inorganic properties, the size of sediment particles, and how long the compound resides in the sediment can influence the BSAF value, especially for super-hydrophobic compounds that take a long time to come to steady state with both the sediment and biota matrices (Hawker and Connell, 1985). Indeed, the actual concentrations of organic compounds, such as polychlorinated biphenyls (PCBs), and the type of sediment and TOC content may be quite heterogeneous. Thus,

there can be substantial variation in BSAF values depending on the number of samples of TOC-normalized sediment contaminant concentrations that are used to estimate the denominator of the BSAF.

As an example of the application of the BSAF technique, BSAF values measured by Froese et al. (1998) were found to vary depending on whether they were calculated based on total concentrations of PCBs, the sum of non- and mono-*ortho*-substituted PCBs, or TEQ (toxicity equivalence, or the PCB congeners that cause TCDD-like toxicity) (Table 2-3). BSAF values calculated based on PCB_{total} normalized to TOC in sediments and to the lipid content of biota were between 8 and 11, while those based on non- and mono-*ortho*-substituted congeners ranged from 0.4 to 1.1. The average TOC-normalized total PCB concentration in sediments was 1.7 mg PCB/g TOC with a range of more than 34-fold between the least and greatest values, resulting in a range of as much as 35-fold for BSAF values calculated in this manner.

Although the BSAF method is empirical, it could be more mechanistically based (e.g., on fugacity theory—see Clark et al., 1988; Mackay and Paterson, 1991; Ling et al., 1993) through the use of several assumptions, including that the system is at steady state. Indeed, if the organic carbon in the sediment and the lipid in the animal tissues is equivalent as a solvent for the contaminant of interest, the BSAF should be 1.0 in systems at steady state (Hoke et al., 1994). However, this value is generally not observed in data collected from the field because the octanol-equivalent fat fraction for sediment dry weight organic matter is about 0.3 (Karickhoff et al., 1979; Sablijc et al., 1995). Thus, the BSAF is approximately 1.7 if it is calculated from organic carbon-normalized concentrations in the sediment and lipid-normalized concentrations in the tissues of the biota. Nonetheless, BSAF values for total PCBs are generally greater than would be expected based on the above assumptions. This may be related to changes in the organic matrix of the food within the guts of the invertebrates that promote further uptake. Similarly, anomalously high BSAF values have been observed for accumulation of some compounds from sediments by invertebrates (Eadie et al.,

TABLE 2-3 BSAF Values[a] for Various Matrixes Based on Total PCBs, the Sum of the Mono- and Non-*ortho*-substituted PCB Congeners, and TEQs

Matrix	PCB_{total}	non-, mono-PCBs	TEQ
Invertebrates	11	0.4	0.3
Tree Swallow Eggs	8.8	0.6	0.8
Tree Swallow Nestlings	9.3	1.1	1.0

SOURCE: Froese et al. (1998).

[a]Each value represents the ratio of lipid-normalized concentration in tissue to the organic carbon-normalized concentration in sediments.

1985; Landrum et al., 1989, 1992). However, BSAF values of 1 to 2 have also been reported for PCB_{total} (Ankley et al., 1992).

Numerous studies have calculated BSAF values for accumulation of PCBs from marine sediments by such organisms as mollusks (*Mercinaria mercinaria*) and polychaetes (*Neghtys incisa*) (Lake et al., 1990), the mayfly (*Hexagenia limbata*) (Boese et al., 1995; Drouillard et al., 1996), and the mussel (*Malacoma nasta*) (Landrum and Poore, 1988). Variation in BSAF is observed for individual species as well as for individual PCB congeners. In a compilation of previous studies, Tracey and Hansen (1996) reported that the mean of median BSAF values for various species is 2.10. Additional compilations of BSAFs are available for a range of ecosystems (Boese and Lee, 1992; Lee, 1992; Parkerton et al., 1993).

Interestingly, despite the variations observed, there have been calls to apply accumulation ratios (BSAFs or BMFs) from one location to another (Neely and Mackay, 1982; Velleux and Endicott, 1994). For example, for total PCBs in sediments, a global average BSAF value of 1.7 has been suggested for use in risk assessments for infaunal invertebrates where BSAF values have not been determined for a particular site (Landrum and Poore, 1988). Indeed, the BSAF approach has been proposed for use as a regulatory tool in risk assessment methodologies involving contaminated sediments (Parkerton et al., 1993), which would be useful if the values do not vary among locations or if an overall average value can be calculated for a region. However, the application of BSAF values determined at one location to other locations is limited (EPA, 2000). For example, at the Baird and McGuire Superfund site (a contaminated soil system) the upper-bound BSAF values taken from the literature were found to be three or four times higher than the site-specific measurements, which was probably explained by the high organic content of the soils (about 30 percent) that enhanced the soil binding of the pesticides (Menzie et al., 1992). Thus, it has been suggested that the method would be most useful as a first-level screening tool (Wong et al., 2001). The key concept should not be that there is a global correction, but that a site-specific correction can be made to account for certain bioavailability processes in ecological risk assessment.

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To summarize, the commonly used paradigms to incorporate bioavailability processes into assessments of exposure by direct contact have substantial uncertainties, and, at best, may capture only crude influences. The variability in empirical predictions of bioaccumulation (BSAFs) indicates that the degree of influence that bioavailability processes have on exposure can be large. But predictions of those influences from theoretical measures either have not been validated or can differ (sometimes substantially) from the observations in nature.

Exposure of Wildlife Feeding on Invertebrates and Plants

For a variety of reasons, the pathway of wildlife feeding on invertebrates or plants often drives ecological risk assessments. Wildlife that feed on terrestrial or aquatic invertebrates and plants can be exposed to chemicals accumulated into the tissues of these organisms as well as through the incidental ingestion of soils or sediments. The simplest form of the wildlife exposure model, assuming a soil environment, is shown below:

$$\text{Exposure Dose (oral, μg/g-day)} = [C_{food} \times I_{food}] + [RAF \times C_{soil} \times Soil_{diet} \times I_{food}]$$

where:

C_{food} = concentration of the contaminant of concern (COC) (μg/g) in the food (measured or estimated); this is the average concentration in the relevant exposure zone—an area determined by the size and locations of foraging areas. Estimates of C_{food} can be obtained by using the BSAF described earlier multiplied by the soil or sediment concentration to yield a concentration in the animals or plant. Estimates are also provided by models or actual measurements as described in considerable detail in Chapter 4;

I_{food} = amount of food ingested per day normalized to body weight (g/g-day) and usually expressed in terms of wet weight/wet weight;

RAF = relative availability factor for COCs in soil via incidental ingestion of soils;

C_{soil} = concentration μg/g in the relevant exposure zone; this is estimated as an average concentration in the exposure zone for chronic exposure and effects and as upper bound (e.g., maximum or hot spot concentrations) for evaluation of short-term or acute exposures;

$Soil_{diet}$ = fraction of soil in the diet; the product of this number and I_{food} yields an estimate of the amount of soil or sediment that is incidentally ingested.

This exposure model is similar in form to the one used for humans, and the two models share similar considerations regarding bioavailability processes. The relative amounts of invertebrates, plants, and soils or sediments that are ingested are species dependent. For example, a species that feeds on earthworms or invertebrates in sediments may ingest more soil or sediment than one that feeds on invertebrates that inhabit vegetation (e.g., grasshoppers). Beyer et al. (1994) have estimated the amounts of soil and sediment ingested by various species, and these data are frequently used in ecological risk assessments for wildlife.

The first term in the equation—exposure via contaminants in food—is often the most important source of exposure for wildlife. Thus, the accumulation of compounds in lower-order organisms is a primary concern, and some of the ways in which this is evaluated were described previously. However, the spatial and

temporal scales considered for wildlife are different than those used to evaluate exposure to invertebrate and plant communities. These scales also differ among wildlife species, such that the availability of chemicals and associated exposure will vary from species to species. This is usually taken into account by explicitly considering foraging areas (see Figure 2-5) in estimating exposure concentrations. The more sophisticated wildlife exposure models take into account the foraging behavior of individual animals in the population, food and habitat quality, and the spatial distribution of habitat and contamination (Hope, 2001).

Although it is recognized that wildlife may also be exposed via incidentally ingested soils or sediments (the second term in the equation), little effort has been spent determining *RAF* values because of difficulties in making such a measurement. (There has been considerable effort directed at the availability of lead in sediments ingested by waterfowl species—Beyer et al., 1997, 1998a, b, 1999.) Indeed, it is much easier to estimate or measure accumulation of contaminants into food items than it is to determine the bioavailability of soil-bound chemicals in the digestive systems of various wildlife species. Regardless of the species under consideration, the *RAF* value for food ingestion is typically assumed to be

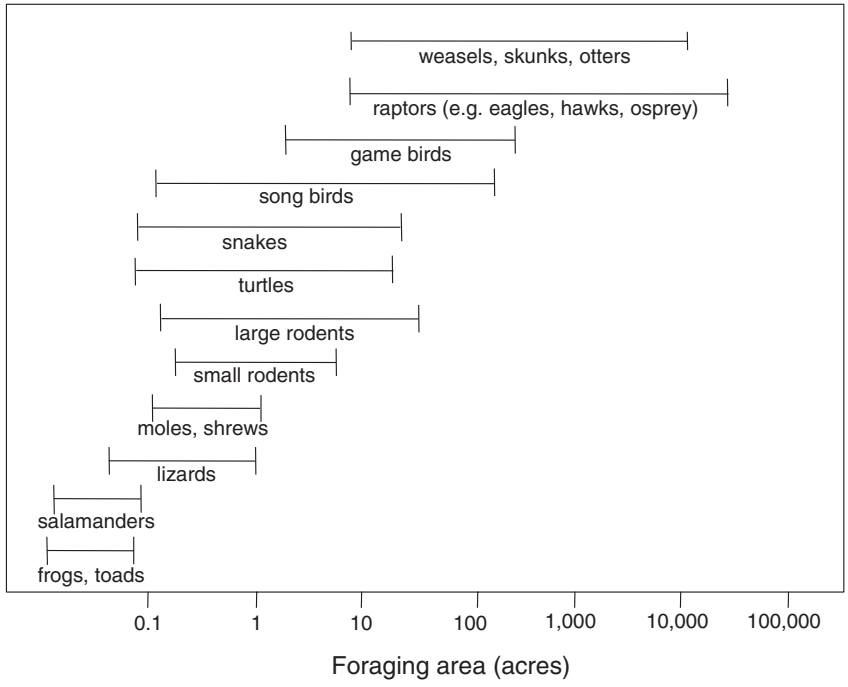

FIGURE 2-5 Examples of wildlife home ranges.

100 percent (with the exception of a few metals and organic chemicals and a few species of wildlife). This implies that predators absorb contaminants similarly from their food (unlikely given the very wide range of digestive physiologies), or that absorption is similar from all prey (also not likely), or both. Other than this assumption, there are few if any default values related to bioavailability that are commonly used in ecological risk assessment—unlike with human health risk assessment. Because of a lack of information, and because it is thought to be less significant than the food and soil ingestion pathways, dermal contact is rarely considered when estimating exposures of wildlife species, and therefore no default values for dermal absorption have been suggested.

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In summary, ecological risk assessments currently use a variety of empirical measures and relatively simple models to incorporate information on bioavailability processes, particularly bioaccumulation into invertebrates. There are similarities between the wildlife exposure models and human exposure models in that both contain terms for direct ingestion of soils and sediment that may employ a relative bioavailability value. However, for wildlife this pathway is not as important a source of contamination as is food.

The methods described here represent how bioavailability is currently considered in ecological risk assessment today. There are more innovative and mechanistic models of bioavailability processes on the horizon (such as dynamic bioaccumulation models), and these are discussed, along with the specific measurement tools used for bioavailability, in Chapter 4.

Case Studies to Illustrate Use of Bioavailability in Risk Assessment

Although bioavailability processes are implicitly a part of every risk assessment, those assessments that have been specifically labeled as "dealing with bioavailability" comprise only a small subset. For those involving human health, site-specific studies have been conducted to determine relative bioavailability, which reflects the difference between uptake of solid-bound contaminant vs. contaminant in the dosing medium used for the toxicity study (Table 2-4). Relative bioavailability results from such studies have been used to adjust the default value at sites where EPA is the lead regulatory agency and at sites where a state regulatory agency has the lead (e.g., California, Michigan, New Jersey, and Oklahoma). These adjustments have been supported by *in vivo* animal studies, *in vitro* testing, environmental health studies, studies of the chemical forms of contaminants in soil, or some combination of these methods (see Chapter 4 for a discussion of methods). To date, most relative bioavailability adjustments in human health risk assessment have been made for the oral route of exposure and for inorganic contaminants (arsenic, cadmium, lead, and mercury) in soil. This reflects the

importance of the oral pathway in human exposures to contaminants in soil and the relative ease of conducting a defensible bioavailability study for inorganics as compared to organics.

Most of the examples cited in Table 2-4 illustrate decreased relative bioavailability compared to the default assumptions, and thus numerically higher cleanup standards. Box 2-4 presents one of these cases in detail—the National Zinc Site, where the site-specific bioavailability of three metals was determined. However, in some cases bioavailability studies can support the default assumption or even demonstrate higher bioavailability than is reflected in the default. The best example is provided by lead, for which there is a national default assumption of 30 percent absolute bioavailability from soil to children. As described in Box 2-5, one such site is Palmerton, Pennsylvania, where the results from swine studies ended up supporting the default absolute bioavailability value for lead.

Bioavailability processes have also commonly been included in ecological risk assessments, although they have not been labeled as "bioavailability assessments or adjustments" per se. Nonetheless, there are certain pathways (e.g., sediments to invertebrates) and chemicals (persistent and bioaccumulative compounds) for which information on bioavailability processes is frequently sought and for which there has been greater regulatory acceptance (Table 2-5). Box 2-6 provides an example of where not all bioavailability processes were given equal consideration during ecological risk assessment, primarily because of the lack of acceptable measurement tools—with important implications for remediation efforts.

LEGAL AND REGULATORY FRAMEWORK

Management of contaminated soil and sediment in the United States is conducted on the basis of risk assessment, but with different levels of risk assessment employed depending on the regulatory domain and site type. As discussed previously, all risk assessments for soil and sediment contain implicit assumptions about bioavailability; the most common assumption has been that the contaminant is equally bioavailable from soil or sediment as from the medium used in the critical toxicity study. Other assumptions are also frequently made, e.g., prolonged human exposure, residential land use at a contaminated site, and direct consumption rather than dilution and attenuation during transport. Because of scientific uncertainty inherent in risk assessment and time and expense issues, the use of these generic assumptions during risk assessment has predominated over site-specific analyses. Many of these generic, default assumptions (which are often conservative) are now part of state and federal hazardous waste laws and regulations.

Research over the last ten years on hazardous waste cleanup has prompted site assessors, parties responsible for cleanup, and state and federal agencies to question the validity of the traditional generic approach in a variety of different contexts. A recent trend toward more site-specific risk assessments has led to an

TABLE 2-4 Examples of Relative Bioavailability Adjustments (RBA) in Human Health Risk Assessment

Site	Contaminant[a]	Test Used
Anaconda, MT	Arsenic	In vivo—monkey
	Arsenic (in house dust)	In vivo—monkey
Butte, MT	Lead	In vivo—rat
Carson River, NV	Mercury	Speciation
Jasper County, MS	Lead	In vivo—swine
Oak Ridge National Laboratory, TN	Mercury	In vivo, in vitro, speciation
Palmerton, PA	Lead	In vivo—swine
Rushton/North Tacoma, WA	Arsenic	In vivo—swine
Vasquez Blvd. & I-70 Site, Denver, CO	Arsenic	In vivo—swine
National Zinc Co. National Priorities List (NPL) Site, Bartlesville, OK	Lead	In vivo—rat, speciation
	Cadmium	In vivo—rat, speciation
	Arsenic	In vitro, speciation
Crego Park, Lansing, MI	Arsenic	In vitro, speciation
Almaden Quicksilver County Park, Los Gatos, CA	Mercury	In vitro, speciation
Hawthorne, NJ	Mercury	In vitro, speciation
Union Pacific RR, Sacramento, CA	Arsenic (in slag)	In vivo—swine
Former Coal Tar Manufacturing Site, Chicago, IL	PAHs	In vivo—mouse
Former MGP Site, Taunton, MA	PAHs	Literature value[g]
Former Koppers Wood Treating Site, Youngstown, OH	PAHs	Literature value[g]

[a]The contaminant was present in soil, unless otherwise indicated.

[b]Cleanup levels at all of these sites were increased due to the site-specific bioavailability adjustment, with the exception of the Palmerton, PA, site.

[c]Although studies generally determine the relative bioavailability of lead, the absolute bioavailability of lead in soil is used in the IEUBK model. The default value in this model is 30 percent absolute bioavailability.

Rel. Bioavail. Adjustment	Cleanup Level[b]	Regulatory Agency
18.3% 25.8%	250 mg/kg	EPA Region 8
24% (12% absolute)[c]	1,200 mg/kg	EPA Region 8
30%	80 mg/kg	EPA Region 9
60% and 80% (30% and 40% absolute)[c, d]	800 mg/kg	EPA Region 7
10%	400 mg/kg	EPA Region 4
60% (30% absolute)[c]	650 mg/kg	EPA Region 3
80%	230 mg/kg	EPA Region 10
42%	100 mg/kg	EPA Region 8
40% (20% absolute)[c] 33% 25%	925 mg/kg 100 mg/kg 60 mg/kg	Oklahoma DEQ
10%	68 mg/kg	Michigan DEQ
30%	300–500 mg/kg[e]	California EPA
6%	150 mg/kg	New Jersey DEP
<0.5%	No cleanup required[f]	California EPA DTSC
18%	RBA used; reduced area of remediation	EPA Region 5
29%	No cleanup levels calculated[h]	Massachusetts DEP
29%	No cleanup levels calculated[h]	Ohio EPA and EPA Region 5

[d]There are two numbers for each because more than one soil was analyzed. Both values were used in the risk assessment modeling.

[e]Cleanup goal varied in different areas of the park.

[f]Slag containing up to 1800 mg/kg arsenic was left in place.

[g]Based on Magee et al. (1996).

[h]RBA accepted by regulatory agency, and used to eliminate portions of the site from remediation.

BOX 2-4
Development and Use of Bioavailability Adjustments at the National Zinc Site

The National Zinc NPL Site in Bartlesville, Oklahoma, was home to a zinc smelter that operated continuously from 1907 until the early 1990s. Most of the soil contamination around the facility resulted from the period 1907–1976, during which the facility operated as a horizontal retort smelter. Facility emissions (stack and roof emissions, and windblown concentrate) resulted in elevated concentrations of zinc, lead, cadmium, and arsenic being deposited in soils, with the greatest concentrations downwind of the facility (prevailing wind direction is northerly). Residential areas lie primarily to the north and east of the facility (Figure 2-6), and these areas were of greatest concern for human exposures to metals in soil.

During the planning stages for the remedial investigation, it was concluded that site-specific studies of the oral bioavailability of lead, cadmium, and arsenic in soil would be beneficial. A detailed protocol for a study of the relative bioavailability of lead and cadmium in rats was prepared. (See Chapter 4 for a detailed discussion of whole animal uptake studies, *in vitro* studies, and mineralogical studies.) The rat model was selected because it had recently been published (Freeman et al., 1992) and had been used to assess oral lead bioavailability from community soils at the Butte, Montana, NPL site (EPA, 1994b). The protocol, which called for the study to be conducted in accordance with EPA's Good Laboratory Practice regulations (40 CFR Part 792), was provided to the Oklahoma Department of Environmental Quality (DEQ) for review, which had taken over from EPA as the lead regulatory agency. The protocol was also reviewed by a toxicologist selected by the community advisory group and by an expert in the field who was independent of any of the stakeholders. Comments from all of these reviewers were considered when revising the draft protocol. In addition to the feeding study, arsenic availability was also evaluated using mineralogical and chemical extraction (i.e., *in vitro*) studies.

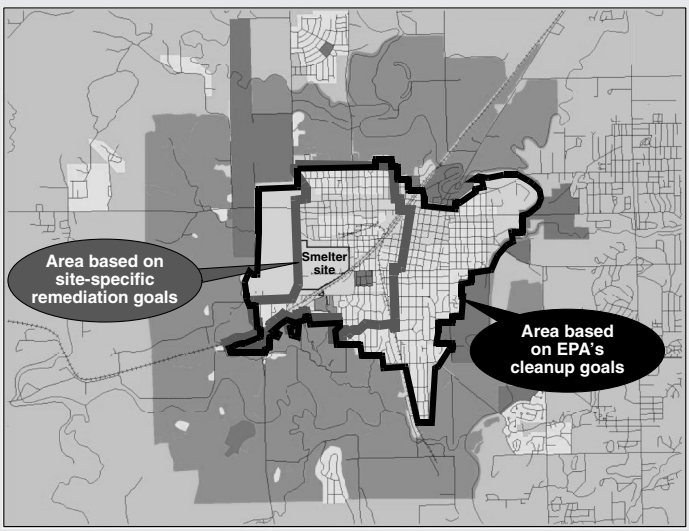

FIGURE 2-6 Site map showing the cleanup areas based on EPA goals and more site specific calculations.

The rat feeding study used a surficial soil composited from five residential lots in the vicinity of the historical smelter. The *in vitro* study used 11 surficial soils collected from residential lots. Electron microprobe analysis was used to identify the forms of lead, cadmium, and arsenic present in these samples. The *in vivo* study in rats involved dosing groups of five animals with either contaminated soil or lead acetate/cadmium chloride (the positive control) mixed in feed for a period of 30 days. Four dose groups spanning a 20-fold range in doses of lead and cadmium were used for both the soil and the positive control. On day 30, the rats were sacrificed, and samples of blood, liver, kidney, and bone were collected from each animal for analysis of lead and cadmium concentration. Relative bioavailability of lead was calculated from the amount of lead in blood and bone for the soil-dosed rats relative to the amount in rats dosed with lead acetate. Relative bioavailability of cadmium was calculated in a similar manner using data from kidneys, as this is the primary site of toxic action for cadmium. Relative bioavailability values for lead and cadmium determined in this manner were 40 and 33 percent, respectively, while the *in vitro* study supported a relative bioavailability value of 25 percent for arsenic. These values were incorporated into the human health risk assessments for residential, occupational, and recreational exposure scenarios (Oklahoma DEQ, 1994). These bioavailability adjustments, in combination with other site-specific factors, resulted in two- to three-fold numeric increases in risk-based cleanup goals over the values initially proposed by the EPA for this site (Figure 2-7). These revised cleanup levels greatly reduced the aerial extent of soils requiring remediation (Figure 2-6), reducing remediation costs by approximately $40 million (as estimated by the responsible parties). The bioavailability studies cost less than one-hundredth of this cost saving.

Critical factors in the success of the bioavailability studies at the National Zinc site included preliminary discussions of study design with the regulatory agencies, development of a detailed study protocol that was submitted for peer review to experts in the field, revision of the study protocol to address concerns raised during peer review, sharing of the study data, and detailed discussions of results and data interpretation methods.

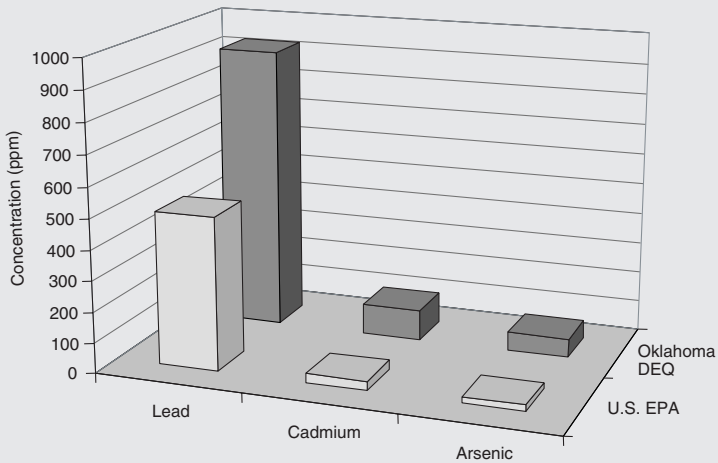

FIGURE 2-7 Changes in soil cleanup goals at the National Zinc, NPL site from those originally set by EPA to those later determined via bioavailability tests and agreed upon by Oklahoma DEQ.

BOX 2-5
Palmerton Zinc Pile Superfund Site Use of *In Vivo* Swine Tests to Assess Bioavailability of Lead in Soil

The Palmerton Zinc Pile Superfund Site encompasses the vicinity of a former large zinc smelting operation that had two separate smelting plants. The site is located in the Lehigh Valley of eastern Pennsylvania, next to Blue Mountain. One of the smelting plants, the "East Plant," was adjacent to Aquashicola Creek; the "West Plant" was on the Lehigh River. The smelting operations, which occurred from 1898 to 1980, resulted in long-term emissions of metals to the atmosphere and deposition of lead, cadmium, and zinc to the land near the site. The soil at many residences in the borough of Palmerton, located between the two former smelting plant sites, is sufficiently contaminated that the town itself was included as part of the Superfund site. Approximately 2,000 acres on Blue Mountain, adjacent to the smelter, were progressively defoliated, ultimately resulting in a barren mountainside that is also part of the site. Adding to the environmental impact was the disposal, over 70 years, of 33 million tons of slag at the site, creating a slag pile that extends for 2.5 miles and measures over 100 ft in height and 500 to 1000 ft in width. The name of the site derives from this pile.

In support of baseline risk assessment activities at the site, EPA used *in vivo* testing in juvenile swine to measure the oral bioavailability of lead in soil (Casteel et al., 1996). Soil from two locations was used, one denoted Location 2 (3,230 ppm lead) and the other Location 4 (2,150 ppm lead). The juvenile swine model, discussed in greater detail in Chapter 4, is considered by EPA to be the best method to measure the site-specific bioavailability of lead in soil (EPA, 1999a) because the gastrointestinal physiology and overall size of young swine are similar to that of young children, the population of principal concern for exposure to lead in soil.

Groups of five swine were given either doses of lead-contaminated soil or oral or intravenous doses of lead acetate for 15 consecutive days. The amount of lead absorbed by each animal was determined by measuring the amount of lead in the blood (measured on nine days during the trial), and the amount of lead in liver, kidney, and

bone (measured at study termination on day 15). The measured lead concentrations in blood and tissue samples from animals exposed to test soils were compared to those for animals exposed to lead acetate, and the relative bioavailability was calculated for each endpoint medium (blood, liver, kidney, bone). The relative bioavailability results for the two samples from the Palmerton site are given in Table 2-6.

TABLE 2-6 Relative Bioavailability (RBA) of Lead in Juvenile Swine for Palmerton Soils

Endpoint Medium	RBA—Location 2 Soil	RBA—Location 4 Soil
Blood Lead AUC[a]	0.74	0.58
Liver Lead	0.50	0.54
Kidney Lead	0.42	0.34
Bone Lead	0.47	0.39

[a]AUC = area under curve (cumulative lead absorption in blood)
SOURCE: Casteel et al. (1996).

In interpreting the results, Casteel et al. (1996) recommended emphasis on the blood lead data because they are less susceptible to random errors than the tissue lead data. They defined the "plausible range" to extend from the relative bioavailability based on blood AUC to the mean of the three tissues (liver, kidney, and bone). They defined the "preferred range" to be the interval from the relative bioavailability based on blood to the mean of all four relative bioavailability values. Their "suggested point estimate" is the mid-point of the preferred range. These relative bioavailability values are presented in Table 2-7.

TABLE 2-7 Aggregated Estimates of the Relative Bioavailability (RBA) of Lead in Juvenile Swine for Palmerton Soil

Type of Aggregate RBA Estimate	Aggregate RBA Estimate—Location 2 Soil	Aggregate RBA Estimate—Location 4 Soil
Plausible Range	0.74–0.46	0.58–0.42
Preferred Range	0.74–0.60	0.58–0.50
Suggested Point Estimate	0.67	0.54

SOURCE: Casteel et al. (1996).

Because soluble forms of lead are about 50 percent absorbed (absolute bioavailability) by a child, estimates of the absolute bioavailability of lead in soil can be determined by multiplying the relative bioavailability value by 0.5. This would result in absolute bioavailability values for the two Palmerton soils (EPA's suggested point estimate) of 0.33 (Location 2) and 0.27 (Location 4). This conversion is important because the IEUBK model (EPA, 1994a), which is used by EPA to estimate the effect of lead in soil on children's blood lead, contains a default value of 30 percent absolute bioavailability of lead in soil to children. The results of the Palmerton bioavailability study bracketed the default values used in the IEUBK model for predicting blood lead levels (Ioven and Hubbard, 2000). Thus, the juvenile swine testing served to confirm the oral bioavailability value used in risk assessment modeling for lead in soil at the Palmerton site, and no special adjustments for bioavailability were needed or used for this site.

TABLE 2-5 Examples of Including Bioavailability Processes in Ecological Risk Assessments

Exposure Pathways	Chemicals	Process and Method	Example Sites
Sediment to Invertebrates	Lead, Cadmium, Copper, Nickel, Zinc	These metals can be bound by sulfides, and their partitioning to pore water has been evaluated using the AVS/SEM methodology at a number of sites.	Lake Waban, Wellesley, MA; Neponset Reservoir, Foxborough, MA; Mill River, Fairfield, CT.
Sediment to Invertebrates	PAHs, PCBs	These organic chemicals can be bound by organic carbon in sediments, and their concentration in invertebrates has been evaluated using the equilibrium partitioning method.	PAH-contaminated sites including many manufactured gas plant sites and locations near refineries.
Soils to Invertebrates	Pesticides, PAHs, PCBs, metals	Bioaccumulation of these chemicals has been evaluated using various empirical or mechanistic exposure models as well as with site-specific measurements.	Baird & McGuire, Holbrook, MA, and Oak Ridge National Laboratory, Oak Ridge, TN.
Sediments to Waterfowl	Lead	A number of risk assessments have considered the relative bioavailability of incidentally ingested lead particles or contaminated sediments; other studies have examined lead shot.	Chesapeake Bay, MD, and Couer d'Alene River Basin, ID.
Soils to Wildlife	Mercury, PCBs, other chemicals	A number of food chain models that account for bioaccumulation into invertebrates and plants have been used to evaluate exposure to higher trophic levels.	Oak Ridge National Laboratory, Oak Ridge, TN; Baird & McGuire, Holbrook, MA; Rocky Mountain Arsenal, Denver, CO.
Sediment to Fish	Mercury, PCBs, other chemicals	A number of fate and transport and bioaccumulation models and measurements have been used to evaluate fish exposure to contaminated sediments.	Southern California outer continental shelf; Hudson River, NY; James River, VA; various dredged material disposal sites; San Francisco Bay.

interest in bioavailability processes, particularly contaminant uptake or absorption. However, these processes and the bioavailability concepts they represent have not often been explicitly acknowledged in laws or regulations for hazardous waste cleanup at the federal or state level. Assuming that adequate information is obtainable, an explicit consideration of bioavailability processes should lead to more scientifically accurate and cost-effective remediation, with no greater actual risk to human health or ecological receptors than under the traditional generic approach to cleanup.

The following discussion considers the current use of bioavailability as a concept in laws and regulation governing hazardous and solid waste cleanup, and it considers whether the law allows for the implicit assumptions currently made about bioavailability to become more explicit via site-specific risk assessment. Because legal and regulatory recognition of bioavailability narrowly targets how retention of contaminants by soils and sediments alters contaminant absorption into an organism, these processes are the focus of this section. Fate and transport processes (bioavailability processes B and C in Figure 1-1) are also an established part of the risk assessment paradigm, and some limited guidance has been developed for their consideration (e.g., partitioning models for soil-water exchange). They are not the focus of further discussion in this section.

Background

Federal and state environmental regulation and directives take a variety of forms, with differing legal impacts. At the federal level, statutes passed by Congress, such as the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) and the Clean Water Act (CWA), are binding nationwide on federal and state agencies as well as on private parties. Environmental statutes ordinarily designate an agency, often EPA, to oversee compliance with the statute. As part of this responsibility, Congress may delegate rule-making authority to the federal agency to regulate in more detail with the benefit of the agency's expertise, in accordance with the Administrative Procedure Act (APA). This process consists of proposing the rule in the Federal Register, having a comment period, and then producing the publication in final form in the Federal Register and ultimately in the Code of Federal Regulations (CFR). State agencies may administer their own complementary state environmental programs, assist in the administration of a federal environmental program, or assume responsibility for administration of a federal environmental program if the relevant federal statutory criteria are met.

As environmental issues have become increasingly complex, statutes and regulations (although voluminous) sometimes have lacked the comprehensiveness and detail necessary to put the regulatory requirements into practice. As a result, federal and state agencies have provided more detailed guidance in documents available to the public but which are not promulgated with the formality

BOX 2-6
Bioavailability Considerations during Ecological Risk Assessment at Clark Fork River Superfund Site

Since 1864, mining and smelting of copper and zinc ore have occurred in the headwaters of the Clark Fork River drainage basin at Butte, Montana. One of the world's largest smelters was constructed in 1900 in Anaconda, 40 km west of the mine. By the time the smelter was closed in 1980, over 1 billion megatons of ore and waste rock had been produced (Lang, 1988). The mining and smelting activities contaminated soils, sediment, groundwater, and surface water over an area one-fifth the size of Rhode Island (Moore and Luoma, 1990). The site is now the largest single Superfund site in the United States.

Like many mine sites, Clark Fork is affected by a legacy of historic waste inputs. Understanding more about the bioavailability of metal contaminants in soils and sediments in abandoned mine lands is important in restoring such areas. Sediment contamination is the most unambiguous sign of mining influences on the Clark Fork River. Maximum concentrations in fine sediments are >20 μg/g Cd and >2000 μg/g Cu compared to 0.2 μg/g Cd and 20 μg/g Cu in tributaries (Axtmann and Luoma, 1991; Cain et al., 1992; Farag et al., 1995). Metal contamination in sediments and resident invertebrates decline coincidentally away from the mine (Axtmann et al., 1997) and are enriched in both sediments and invertebrates as far as 550 km downstream. Signs of stress in the region include (1) vegetation and biodiversity losses in the floodplain; (2) reduced diversity of benthic communities in the river and histopathological lesions in trout consistent with contaminant effects (Cain et al., 1992; Farag et al., 1995); (3) reduced diversity of trout to mostly one species (brown trout) in the upper ~180 km of the river and absence of native bull trout (an endangered species); (4) reduced fish standing stock to about 20 percent that of trout in rivers with similar habitats elsewhere in Montana (Hillman et al., 1995); and (5) reduced abundance of mink, which are fish predators (Szumski, 1998).

An ecological risk assessment was aimed at identifying the extent to which the signs of stress in the system were the result of metal contaminants vs. other stressors like

necessary under the APA to be considered a legally binding rule or regulation (for example, EPA's Risk Assessment Guidance for Superfund). These guidance documents are of great practical importance and generally are assumed by regulated parties to state the methodology and criteria that must be followed to meet statutory and regulatory requirements. For example, if EPA, a regional EPA office, or a state environmental agency issues a guidance document on the use of bioavailability in risk assessment, the risk assessor generally assumes that any departure from that guidance will be closely scrutinized and questioned. Alternatively, lack of clear authorization or guidance on bioavailability would lead the risk assessor to conclude the approach is not favored or even prohibited.

Legal Recognition of "Bioavailability"

As a formal legal requirement, the term "bioavailability" currently receives little mention in any of the federal statutes and regulations governing environ-

nutrient runoff and dewatering of the river (Johnson and Schmidt, 1988; Hillman et al., 1995). In the river, sediment contamination was the clearest indicator of mine influences, and extensive, reliable data were available defining the levels of contamination. However the risk assessment ultimately relied more on smaller, less reliable data sets for water column metal concentrations and pore water concentrations to define risk (EPA, 1999b) and discounted the exposure pathway of direct ingestion where sediment concentrations play a large role.

One major reason for this decision was that different approaches and tools reached contradictory conclusions about the direct effects from metals in sediments. For example, a comparison to the sediment quality guidelines established by NOAA for benthic invertebrates showed all sediments from the Clark Fork exceeded levels of copper and zinc likely to cause toxicity. But toxicity tests using Clark Fork sediments and an amphipod (a surrogate species found at only one place in the Clark Fork) showed no toxicity. One study (from depositional zones) found AVS in excess of SEM at all Clark Fork sites, while another using sediments from riffle sites showed SEM > AVS at many sites. The risk assessors concluded that the inability to quantify the dietary pathway of exposure (invertebrates ingesting sediment) precluded drawing any conclusions about effects from direct ingestion of food organisms or sediments. Thus, the conclusions about effects on both benthos and fish were most heavily influenced by comparisons of waterborne concentrations to toxicity test effect levels. The risk assessment concluded that the major impact on fish was pulse inputs of dissolved metal to the river, rather than chronic impacts from the sediment contamination.

By discounting the sediment route of exposure, the risk assessment implicitly concluded limited bioavailability via the routes of direct contact and diet—a conclusion that will guide the approach to risk management. It is likely that management of risks from pulse inputs could be very different from management of contaminated sediment, and it may not target the true source of contamination.

mental regulation. The only statutory reference is a brief mention of the bioavailability of restricted metals in the Clean Water Act (CWA) Section 402 permit requirements for point source discharges into navigable waters. In contrast, there are 20 or more statutory references to "bioavailability" and "bioequivalence" requirements in the pharmacological context of food and drug regulation under the Food, Drug, and Cosmetic Act.

Conducting the same word search under the comments to the federal regulations, however, leads to a dramatically different result. The term "bioavailability" appears hundreds of times in the comments to the regulations, including comments to regulations under the major statutory programs outlined below. The incorporation of "bioavailability" into the more detailed, working guidance provided by the comments to the Code of Federal Regulations suggests that the *concept* of bioavailability, however denominated, is recognized and available for utilization far more frequently than the formal terminology of current laws and regulations would suggest.

EPA's only quasi-official explicit recognition of bioavailability in risk assessment is in Appendix A to the Risk Assessment Guidance for Superfund, Volume I, Human Health Evaluation Manual (Part A) (EPA, 1989a) [hereinafter "RAGS"], and there the term "bioavailability" is not even used. Instead, the Appendix refers to "adjustments for absorption efficiency." In other words, RAGS opens the door for consideration of information that absorption of a substance at a particular site may be more or less than typically presumed under the standard risk assessment paradigm. There is no agency-wide guidance on the data necessary to substantiate such an adjustment, however, leaving that critical determination to EPA regional offices, state environmental agencies, or the judgment of the risk assessors, risk assessment reviewers, remedial project managers, and risk managers to whom RAGS is addressed.

The fact that the term "bioavailability" does not appear in the laws and regulations, but does appear in the informal guidance of regulatory comments and guidance documents necessarily leads to confusion and even conflict over the acceptability of the concept in risk assessment between regulators and risk assessors. Regulators are unable to find authoritative authorization for explicit consideration of bioavailability, while risk assessors and others involved in the scientific aspects of risk assessment find that it is acknowledged in the more informal guidance, which is of more practical importance in the actual process of risk assessment. This difference of perception is exacerbated by scientific terminology that risk assessors and scientists may recognize as referring to bioavailability processes, but that regulators and others may not. Despite this disparity in perception, there is the potential for a more explicit analysis of bioavailability processes in any federal program utilizing risk assessment to determine an acceptable level of exposure to a contaminant.

The principal federal remediation programs for soils and sediment most susceptible to a better understanding of bioavailability concepts are (1) sediment quality assessment as regulated under multiple sections of the CWA; (2) sludge disposal programs; (3) the hazardous waste remediation programs; and (4) state and federal Brownfields programs.

Bioavailability in Regulation of Soil Remediation

This section analyzes the current use of bioavailability in national and state cleanup values for soil, as well as EPA regional guidance and state approaches to using bioavailability during soil remediation. As mentioned earlier, this discussion of bioavailability focuses on how retention of contaminants by soils and sediments alters contaminant absorption into an organism.

Regulatory Programs

The regulations discussed below set up a risk-based approach to cleanup, which requires (at least) an implicit consideration of bioavailability processes.

However, there is no explicit recognition of the concept or use of the term in any of the regulations.

RCRA. The Resource Conservation and Recovery Act (RCRA) regulates the generation, transportation, treatment, storage, and disposal of waste. Both RCRA and the Safe Drinking Water Act are designed to curtail the land disposal of untreated waste and to contain releases from any remaining land disposal. CERCLA, and to a more limited extent RCRA, also are directed toward cleanup of existing contamination.

The regulatory sections of RCRA focus on prevention of contamination. Only Section 7003 of RCRA addresses the problem of remedying contamination that has already occurred. Whenever past or present handling, storage, treatment, transportation, or disposal of any solid or hazardous waste "may present an imminent and substantial endangerment to health or the environment," the past or present owner or operator must take corrective action. RCRA and CERCLA use different "risk triggers" for specific types of response actions (Malone, 2002).

Under EPA's regulations, any significant increase in groundwater contamination by any of a list of designated pollutants, or any hazardous waste at the site, will require cleanup. Cleanup must continue until MCLs are met, or, if impractical, until alternate concentration levels are met (Novick, 2002). Section 7003 has somewhat lessened in importance since RCRA's regulatory expansion requiring prevention of contamination and CERCLA's creation of a fund for cleaning up contaminated sites.

CERCLA. The purpose of CERCLA is not to prevent soil contamination but to remedy contamination after it has occurred. Whenever there is a "release" of a hazardous substance, or substantial threat of a release of a hazardous substance, EPA may respond by taking a removal action or a remedial action. Procedures for both response and removal actions are set out in a National Contingency Plan (NCP). Both actions are designed to clean up contamination, particularly when no responsible parties can be found or required to do so.

In order to finance cleanup of abandoned hazardous waste, a revolving trust fund (the "Superfund") was established through CERCLA, funded by taxes on petrochemical feedstocks, crude oil, and general corporate income, and by general revenues. The fund may be reimbursed by "parties responsible" for the contamination; if responsible parties refuse to reimburse the fund, they can be sued by EPA. States, local governments, and private parties who conduct cleanups may also be reimbursed from the Superfund or directly by responsible parties. The hazardous waste sites in the Superfund program are on the NPL.

Over the last 15 years, EPA has produced numerous guidance documents both on risk assessment conducted under CERCLA and on remediation strategies for meeting cleanup goals. CERCLA specifies the factors to be considered in assessing treatment options and provides nine general criteria that must be considered:

1. overall protection of human health and the environment
2. compliance with the chemical-specific standards that are considered the statutorily required applicable or relevant and appropriate requirements (ARARs)
3. long-term effectiveness and permanence
4. reduction of toxicity, mobility, or volume through the use of treatment
5. short-term effectiveness
6. implementability
7. cost
8. state acceptance; and
9. community acceptance.

The preamble to the NCP makes it clear that EPA has a strong preference for treatment technologies over engineering and institutional controls, especially for "principal threat" wastes[2] (EPA, 1990). EPA does not encourage solutions in which institutional controls are the sole remedy, and prefers that such controls be used in conjunction with containment strategies. As mentioned above, the only mention of bioavailability in guidance documents created for CERCLA is found in Volume A of the Risk Assessment Guidance document for Superfund.

Brownfields. Brownfields are abandoned, idled, or under-used industrial and commercial sites where expansion or redevelopment is complicated by real or perceived environmental contamination (EPA Region 5, 1996). The goal of state and EPA Brownfields programs is the restoration of Brownfields so they can once again be used as a fruitful resource.

Incorporating bioavailability assessments into state and federal Brownfields programs would do much to alleviate businesses' concerns of unforeseen liability and lack of future profitability in property use or transfer. The purpose of any Brownfields program is to restore a site to a state of productive use. In most cases this does not mean restoration to the "highest and best" use of residential use, but rather to commercial development. Thus, an advantage of more explicitly using bioavailability tools to assess Brownfields restoration is to set the cleanup goal to the actual use and exposure levels that would occur at the site (and thereby limit cleanup costs). The more accurate the assessment of bioavailability, the more precisely tailored the future use of the property can be. Thus, granting legal recognition to bioavailability concepts as a method of improving the basis for evaluating cleanup goals has the potential to lower costs and lessen the potential liability of businesses.

[2]Principal threat wastes are broadly defined by the EPA as being liquid or solid wastes and soil containing hazardous substances that constitute a risk of 10^{-3} or greater. More detail is available in EPA, 1991.

EPA Guidance

At present, there is no national guidance or policy statement on the use of bioavailability in risk assessment, although a workgroup at EPA Headquarters has been preparing to issue such a statement for some time (P. Grevatt, EPA, personal communication, 2001). Nonetheless, measurements of bioavailability processes can be used to adjust and refine human health and ecological risk assessments, most readily with the authorization provided in EPA's RAGS (EPA, 1989a). As mentioned earlier, site-specific risk assessment is infrequently conducted because many states have specified generic, conservative screening levels for contaminated soil and groundwater that are used to quickly assess contamination. However, rather than using such generic cleanup levels, states can choose to do a site-specific risk assessment that would incorporate the full range of bioavailability processes. It should be noted that EPA's version of these generic screening levels, embodied in the Soil Screening Guidance (EPA, 1996a), provides not only initial screening levels but also a methodology for calculating risk-based, site-specific soil contaminant concentration levels in which bioavailability might be incorporated. Although the guidance covers only a subset of contamination problems (in terms of chemicals, land use, and exposure pathways), relative bioavailability adjustments could be a factor in some of the allowable scenarios (e.g., for direct ingestion of metal-contaminated soil).

In order to determine whether and how the EPA Regional Offices consider bioavailability in the hazardous waste programs they oversee, the committee heard presentations from and sent a brief questionnaire to each regional representative. The representatives were asked if the region, or the states in that region, had developed any default values for absolute or relative bioavailability, or any guidance material and policy statements regarding the use of bioavailability in environmental cleanup. Each regional office was also asked to identify any site-specific applications of bioavailability assessment for cleanup of metals or organics. The questions were phrased in terms of "bioavailability" specifically, rather than referring to the various processes (such as sequestration, mobility, leaching, etc.) that are often associated with bioavailability, in order to obtain a sense of more formal recognition of bioavailability by EPA administrators as a concept relevant to determining cleanup values. In addition, questions were directed toward human health risk assessment managers rather than ecological risk assessment managers. Table 2-8 summarizes responses to the questionnaire received from various EPA regional personnel.

The survey revealed that outright recognition, acceptance, and utilization of bioavailability factors in state and federal cleanup projects is limited at best, although the opportunity has clearly existed in the Superfund program since 1989. Several observations support this lack of acknowledgment. First, the regions are generally cautious in their recognition and utilization of bioavailability concepts. Second, there are wide variations among the regions in receptiveness to

TABLE 2-8 Formal Use of Bioavailability in Human Health Risk Assessment at the EPA Regional Level

Region 1	There is no formal regulatory guidance for the use of bioavailability, although Massachusetts and New Hampshire have policies that proscribe default values for absolute and relative bioavailability (see Table 2-1). There are several examples of site-specific calculations of absolute or relative bioavailability in cleanups in this region, indicating a willingness on the part of state agencies to consider the concept.
Region 2	There is no region-wide regulatory guidance, and there are no state policies within the region. Site-specific relative bioavailability factors for arsenic were calculated at two sites, but these were rejected by state regulatory agencies (Maddaloni, 2000).
Region 3	Neither the region nor any state in the region has developed guidance, although there is a list of absolute bioavailability default factors used for dermal exposure routes only. Changing the implicit assumptions about bioavailability in the baseline risk assessment is permitted only when a site-specific study has been performed (such as for lead and arsenic at the Palmerton Zinc Smelting site—see Box 2-5).
Region 4	Though there are two high profile examples of using site-specific bioavailability factors in Region 4 (mercury site in TN, arsenic site in GA), its guidance on risk assessment states: "Bioavailability questions arise as to potential differences in uptake levels under study conditions versus environmental exposure conditions, i.e., the matrix effect. Chemical specific data is rarely sufficient to quantify this difference in bioavailability for all receptors under their varied exposure conditions. Therefore, Region 4 does not accept any adjustment in the 100 percent bioavailability default assumption in the exposure condition without extensive supporting data."
Region 5	Region 5 has no formal guidance on bioavailability. Nonetheless, its interest is evident in that it has conducted a study on the site-specific relative bioavailability of PAHs in soil.
Region 6	Region 6 assumes a default relative bioavailability of 100 percent via ingestion. Other values can be used in site-specific situations if supporting scientific data are presented.
Region 7	Region 7 has no formal guidance but normally assumes a default relative bioavailability of 100 percent via ingestion. This region has also performed lead relative bioavailability studies at a few specific sites.
Region 8	Region 8 has no guidance other than the national default values for lead and the two national dermal values. However, the region has spearheaded many basic, site-specific studies of absolute and/or relative bioavailability primarily at large metal contaminated sites in the Rocky Mountain west.
Region 9	Region 9 has no formal policy or default values, but it has allowed the use of relative bioavailability factors on a site-specific basis. No state in the region has prohibited the use of bioavailability factors, nor have they formulated default values.
Region 10	There is limited guidance in Region 10 about bioavailability, including interim Region 10 guidance regarding default values for arsenic bioavailability in soil and a decision tree. State regulators rejected a proposal for assuming 40 percent relative bioavailability for arsenic, and instead choose 100 percent, noting that "it remains to be demonstrated that the results of any soil arsenic bioavailability study accurately represents bioavailability for humans or whether the results are more dependent on study conditions as opposed to actual differences in bioavailability." The guidance has been used at Region 10 federal Superfund sites.

the approach. Regions 2 and 7 appear to have given the concept limited consideration. Region 8, on the other hand, has been conducting extensive bioavailability studies, primarily because there are large metal-contaminated sites in that part of the country at which an explicit assessment of bioavailability could make a significant difference in cleanup costs. Regions 4 and 6 seem skeptical of explicit bioavailability assessments, although a prominent hazardous waste site in Region 4 embraced the concept for a mercury cleanup. Regions 1, 3, 5, 9, and 10 are actively exploring its use but also with varying levels of acceptability and actual utilization. These differences may be explained only partially by the regional differences in the nature, types, and costs of contaminated site cleanups.

Hesitancy to explicitly consider bioavailability processes during site-specific risk assessments, especially for human health, may reflect agency concern with costs (which can be very large for an *in vivo* bioavailability study), anxiety about public and community acceptance of the concept and the methods (see Chapter 5), and the absence of more formal national guidance that may lead to legal impediments or challenges. Other factors that contribute to caution on the part of regulatory agencies include lack of supporting data and concerns over available tools and study designs and their validation. Thus, despite the lack of legal impediments to its utilization, explicit bioavailability assessments are not currently a regular feature of site-specific risk assessment.

Considerations of bioavailability have been most common at large metal-contaminated sites in the West, such as where soil is affected by acid mine drainage.

State Recognition of Bioavailability

Although some information on state use of bioavailability was gleaned from the survey of regional EPA offices, there is less information about state practices. Recently, the Air Force Institute for Environment, Safety, and Occupational Health Risk Analysis surveyed state regulators to determine past use of bioavailability adjustment factors and the likelihood of utilization of bioavailability factors in the future in their state or region (unpublished data). Thirty-one (31) states responded to the survey, although three major states—California, Massachusetts, and Texas—did not. In general, the state environmental agencies do not have guidelines currently in place for the use of site-specific absolute or relative bioavailability adjustments in human health risk assessments and rely nearly exclusively on EPA risk assessment (RAGS) protocols (which as mentioned earlier are void of explicit guidance on bioavailability). Only West Virginia and Minnesota provided guidance documents addressing the use of site-specific bioavailability data (MPCA, 1999; WVDEP, 1999). New Jersey indicated plans to produce guidance that includes consideration of bioavailability. The report concludes "while there is little guidance, it appears that state regulators are willing to consider the use of bioavailability adjustments on a site-specific basis. However, it also appears that most states will follow the lead of EPA."

Other sources indicate that at least some states (like some EPA regions) have taken a more quantitative approach to bioavailability in the form of default values other than 100 percent for the absolute and relative bioavailability of certain compounds or classes of compounds. As discussed previously, these factors are primarily used to adjust exposure via the dermal and oral ingestion pathways. Such values are particularly noteworthy in the Northeast, led by Massachusetts and New Hampshire. In Massachusetts, the default values for four classes of chemicals are to be used "as a last resort" when the risk assessor is unable to find absorption efficiency data specific to the site and the chemical of interest. Michigan has set default relative bioavailability values by compound class for both oral and dermal exposures that are lower than EPA default values or any other state and are based primarily on the best professional judgment of Michigan Department of Environmental Quality scientists.

It should be noted that once an agency has established a default value, in regulation or guidance material, there is typically widespread acceptance and application of the value to a variety of sites. Because of a desire to maintain a level of standardization between sites, there can be reluctance to consider site-specific information in lieu of using default values. Nonetheless, there is also evidence that states are increasingly allowing the use of site-specific bioavailability adjustments. In Washington State, adjustment of "soil gastrointestinal absorption fraction" and "inhalation absorption percentage" is specifically mentioned in state regulations relating to contaminated sites. These are included in a longer list of exposure parameters which "may be changed where there is ad-

equate scientific data to demonstrate that use of an alternative or additional value would be more appropriate from the conditions present at the site" (from section 173-340-708, Human Health Risk Assessment procedures, in Proposed Amendments, Washington State Register, Issue 00-16, The Model Toxics Control Act Cleanup Regulation, Chapter 173-340-WAC). These additions, newly proposed in August 2000, are now accepted.

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In summary, although there is no legal recognition of the term "bioavailability" in soil remediation statutes, bioavailability processes can be encompassed by both the human health and ecological risk assessment paradigms used under CERCLA and RCRA. Several states and some EPA regions have specified default values (other than 100 percent) for absolute and relative bioavailability of contaminants in soil via the dermal and oral pathways that can be used during risk assessment. And in a few cases, the states and regions will allow these default values to be replaced by results from a site-specific bioavailability assessment. There are a number of sites that have successfully used site-specific bioavailability adjustments in human health risk assessments (predominantly for lead and arsenic), although state regulators appear to be waiting for more explicit approval and guidance from EPA before engaging in more widespread consideration of site-specific adjustment factors for bioavailability. Box 2-7 discusses the role that bioavailability plays in setting soil standards in a few select European countries.

Bioavailability in Regulation of Sludge Disposal

One of the most prominent and explicit uses of the bioavailability concept is its incorporation into the regulatory standards for biosolids (sludge) disposal. Biosolids are the residual material generated by municipal water treatment. They are commonly used as a fertilizer and source of organic matter in agricultural and forest soils. In addition, they are used, generally at high application rates, to restore or remediate mined soils. They contain measurable levels of trace metals, pathogens, and some trace amounts of synthetic organic compounds.

After the promulgation of the Clean Water Act, the amount of biosolids being generated increased dramatically along with concern over the potential detrimental effects of their use. As a result of this concern, EPA began to develop regulations (Part 503 Sludge Rule) that would establish standards for metals, toxic organics, and pathogens in biosolids (EPA, 1989b, 1993; Page et al., 1989). These standards were intended to assure that no adverse effects would occur as a result of land application of biosolids. In addition, because of the consideration of multiple exposure pathways in their evaluation of risk, the regulations are very comprehensive. Over time the regulations incorporated a great deal of research data, such that for all exposure pathways, other than direct human ingestion of

BOX 2-7
How Other Countries Use Bioavailability in Environmental Regulations for Soil Contamination

Some international regulations consider bioavailability in their assessment of soil contamination and remedial options. Examples include the British Contaminated Land Act (2000), the German Soil Protection Act (1998), the Swiss Ordinance Relating to Pollutants in Soil (1986) and the Ordinance Relating to Impacts on the Soil (1998), and the Contaminated Land Policy in Flanders (1995). Bioavailability is a factor in these laws in several different ways. It is considered for determining if the site is contaminated, determining if the presence of a contaminant is cause for concern, and in determining and evaluating remedial technologies.

The acts have some common characteristics. For all legislation, the presence of a contaminant is not sufficient proof that soil requires remediation. Several of the laws identify contamination by using the bioavailable rather than the total fraction of the contaminant. In Switzerland and Germany, the neutral salt extractable fraction of total soil metals has been used to determine whether a soil is contaminated and poses a threat to plants or to humans consuming the plants (VSBo, 1986; OIS, 1998). In Flanders, a microbial bioassay is used to evaluate metal bioavailability. Victor Dries of OVAM, the office in charge of implementing the Contaminated Land Policy in Flanders, notes that "for sites with a high ecological value, it is evident that [ecotoxicity] and bioavailability are the most essential parameters in deciding whether or not remediation is necessary."

The German Soil Protection Act defines "precaution" values, "trigger" values for initiation of investigations of the contamination, "action" values, and remediation requirements. The trigger, action, and precaution values take bioavailability into account both in the types of analysis that are required for determining the values as well as in the acceptable soil concentrations for different end uses. For example, trigger values for several inorganic ions for the soil-to-food plant pathways are based on the 1M NH_4NO_3 extractable concentration. This extract is classified as a neutral salt extract and is generally seen as reflective of the phytoavailable fraction of total metals (Hani, 1996). The elements whose trigger values are defined using this extract include cadmium, lead, copper, arsenic, and zinc. Organic matter content and particle size are also used in the German Soil Protection Act to modify these values.

Use of a pathway approach to evaluate whether a contaminant has the potential to cause harm is a common thread for all of these regulations. In Britain, for example, the local authority must first identify the contaminant, then a relevant receptor, and finally a pathway by which the contaminant is either causing or has a high potential to cause harm, before a "significant pollutant linkage can be established." The legislation in Britain, Flanders, Switzerland, and Germany lists a range of potential receptors including people, animals, plants, a group of living organisms or an ecological system.

biosolids, the bioavailable fraction rather than the total concentration of the compounds of concern formed the basis of the rule (Chaney et al., 1982; O'Conner et al., 1990). The body of research used was more extensive for certain elements

and certain pathways. For example, over 75 data points were used to determine the uptake slopes for soil cadmium by leafy vegetables, which were a component of the diet model used to predict total cadmium in humans (EPA, 1992b). The initial molybdenum limit was based on much less data and was subsequently challenged, and additional research was carried out to define an appropriate limit (O'Connor et al., 2001). The new proposed molybdenum limit is based on a soil-to-plant uptake coefficient derived from 29 field studies.

Multiple exposure pathways, and hence bioavailability processes, were used to formulate the regulatory requirements. For each of the pathways, a different highly exposed individual was identified—either humans, animals (soil organisms, soil organism predators, and grazing livestock), or plants. Regulators considered all potential direct routes of exposure (air, water, and soil) as well as indirect exposure pathways to humans or other higher-order animals via plants and lower-order animals. So, for example, a contaminant in soil could be viewed as a direct risk to a human who ingested the soil or an indirect risk to someone who consumed livestock that ingested soil. A range of potential receptors was also taken into account for each pathway. Thus, in the example given above the potential for direct harm to the livestock grazing on biosolids-amended soil would

Biosolids being applied in Leadville, Colorado.

also be evaluated. The most limiting pathway or concentration was then used to set the regulatory limit for each individual compound (Chaney et al., 1998).

The risk assessment calculation included the following steps. First, relative metal uptake coefficients (similar to absolute bioavailability factors) for a range of food crops were calculated, defined as the geometric mean of plant metal concentrations from multi-year field studies where high rates of biosolids had been applied. Next, a diet model (the same as an intake equation), accounting for changes in food consumption patterns over a lifetime, used the uptake coefficients that had been developed from all available field studies to calculate contaminant loading. Then, increased dietary intake as a result of consuming produce grown on biosolids-amended soils was combined with background intake from other sources to determine a maximum contaminant loading in biosolids that would not result in an adverse health effect.

A range of adverse health effects was used in the regulations. The scientists developing the regulation based their human health risk assessment on the potential for a highly exposed individual to have greater than a 10^{-4} risk of cancer. Non-cancer endpoints were also considered. The highly exposed individual was defined as a person who consumed up to 59 percent of their produce from a home garden that had been amended with biosolids containing the highest permissible metal loading rates for 70 years. Adverse effects for plants, when they were identified as the receptor of concern, were defined as a 50 percent yield reduction in vegetative growth (EPA, 1995a). In cases where animals were the endpoint of concern (e.g., commercial grazing animals or wildlife, soil organisms, and predators of soil organisms), toxicity was defined as unacceptably high metal accumulation in target organs potentially leading to mortality. The regulations relied on target tissue levels of contaminants in organs rather than on an observable lethal or sublethal toxic effect on the organism.

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The Part 503 regulations are unique because they attempt to protect a range of individuals from a wide number of potentially toxic agents based on their bioavailable concentration, rather than on their total concentration. The regulations strive to be protective of both chronic and acute toxicity. In developing the regulations, it was understood that in addition to the risks associated with the use of biosolids, benefits would also be derived.

Bioavailability in Regulating and Managing Sediment

The monitoring and management of contaminated sediments has recently become an area of considerable interest and activity and involves several federal agencies. EPA, the U.S. Army Corps of Engineers (USACE), the National Oceanic and Atmospheric Administration (NOAA), the U.S. Fish and Wildlife Ser-

vice, and the U.S. Geological Survey are required to do environmental monitoring and assessment of chemical contamination in sediments. For example, EPA and USACE have developed joint technical guidance for evaluating the potential for sediment contamination associated with the discharge of dredged material (1) in the ocean under the Marine Protection, Research, and Sanctuaries Act (EPA and USACE, 1991), and (2) in fresh, estuarine, and saline (near-coastal) waters under Section 404 of the CWA (EPA and USACE, 1998). In response to the Water Resources Development Act of 1992, EPA routinely conducts a national survey of sediment quality in the United States, making use of fish tissue residue data and bioaccumulation models (EPA, 1997b). Bioaccumulation testing and modeling play a role in several other CWA programs, notably Section 403 Procedural and Monitoring Guidance (EPA, 1994c, 1995b), and the Section 320 National Estuary Program (EPA, 1992c). The varying methods used in these cases are intended to link the level of a contaminant(s) in sediments to adverse effects in aquatic life or water quality, which involves an explicit consideration of bioavailability processes.

Aside from some conceptual similarities, however, the different agencies' approaches for defining sediment quality and the links to bioavailability and

Contaminated sediments at the Wingate Road Incinerator Superfund Site.

biological effects are different. NOAA researchers have developed an empirical, statistical approach for screening sediment quality that does not explicitly address bioavailability processes at all. EPA has taken a more theoretical approach by developing criteria for protecting ecosystems from sediment toxicity using equilibrium partitioning theory to explain how certain sediment characteristics are thought to affect bioavailability. The USACE uses an experimental approach that tests the toxicity of every sediment (for disposal of dredge spoils), and thereby implicitly considers bioavailability on a sediment-by-sediment basis.

The following sections discuss three approaches for setting sediment quality criteria, which refer to recommended concentrations of contaminants in a sediment sample. All three methods take certain bioavailability processes into account, particularly association/dissociation and absorption. Sediment quality criteria are basically analogous to standards for water quality; however, sediment quality criteria are not legally enforceable. Possible exceptions are the Great Lakes sediment criteria, which are used to set enforceable water quality standards. Section 118(c)(2) of the CWA (as amended by the Great Lakes Critical Programs Act of 1990) requires EPA to publish guidance on minimum water quality standards, antidegradation policies, and implementation procedures for the Great Lakes. The resulting guidance (EPA, 1995c) incorporates bioaccumulation factors into the derivation of sediment quality criteria and values to protect human health and wildlife.

EPA Approach

EPA has formulated sediment quality criteria to be consistent with previously established standards for water quality using an approach referred to as equilibrium partitioning. Recently, these sediment quality criteria have been renamed equilibrium partitioning sediment guidelines (ESGs) by EPA (see EPA, 2001c). The approach assumes that contaminants partition between the aqueous and solid phase as a function of sediment composition and contaminant type. Sediment contamination above a concentration that results in an aqueous phase level greater than water quality standards is not acceptable and thus determines the value of the ESG. The water quality standards are based upon toxicity bioassays with benthic invertebrates, dissolved contaminants, and aqueous conditions that maximize uptake. Thus, the ESGs are described as EPA's best estimate of the concentrations of a substance that may be present in sediment and still protect benthic organisms from direct toxicity in that sediment. EPA has conducted efforts to develop and publish ESGs for some of the 65 toxic pollutants or toxic pollutant categories (EPA, 2000, 2001c).

ESGs incorporate research that identified some of the chemical factors that influence partitioning from sediments to the dissolved phase, and thus directly address an important bioavailability process (particularly A in Figure 1-1). ESGs

can be used to address site-specific issues, and they are quantitative. Note that ESGs do not protect against synergistic or antagonistic effects of contaminants or bioaccumulative effects of contaminants, and they are not protective of wildlife or human health endpoints. ESGs are not regulations and do not impose legally binding requirements. In addition, EPA does not recommend the use of ESGs as stand-alone, pass-fail criteria for sediments. Instead, they are intended for use as screening levels.

As for other strengths and limitations, it is worth noting here that the guidelines may not be applicable where digestive uptake is a significant exposure pathway, where multiple pollutants occur, or where the bioavailability is controlled by physicochemical factors not considered in the EqP approach. The measures are more applicable for instances of acute toxicity or toxicity via direct contact to gills or surfaces of the organism as compared to chronic toxicity. Given such limitations, these measures are best used for those cases where extreme sediment contamination immediately kills fauna or flora.

NOAA Approach

In contrast to the ESGs used by EPA, numerical sediment quality guidelines (SQGs) have been suggested by researchers at NOAA (Long et al., 1995, 1998). In this approach, contaminant concentrations in sediment are correlated with large data sets of observed biological effects (usually done with toxicity tests using field-collected sediments). To date, corresponding chemical and biological effects data have been compiled from one thousand or more studies. A SQG is defined as a range between a lower and upper concentration limit. The lower limits are intended to represent concentrations below which adverse effects were not frequently expected. The upper limit values are the concentrations above which effects had a high probability of occurrence. The range between the lower and higher values can vary but is typically between factors of 2 and 10.

One limitation of this approach is that sediments often contain more than one contaminant, but the majority of studies showing biological effects were conducted by evaluating contaminants individually. Bioavailability processes are not explicitly considered in this approach at an individual site, but they have implicit influences. For example, results from sediment toxicity tests can encompass both sediment chemistry and species-specific effects. The authors state that "numerical values were not intended as regulatory criteria" (Long et al., 2000). Nevertheless, this approach is sometimes used at the local and state level, at least informally, to screen or characterize sediment contamination problems, perhaps because the guidelines are simple to apply. Because this approach is confounding with respect to bioavailability processes, it is not suggested even for screening-level assessments of sites.

USACE Approach

A third approach to sediment quality criteria is that of the USACE for managing dredge spoils. As part of its navigation dredging, the USACE disposes of contaminated sediments in confined disposal facilities for inland sites or in ocean disposal sites. The law dictating Corps activities in this regard (The Marine Protection, Research and Sanctuaries Act) has been interpreted in EPA and Corps' guidance documents as requiring bioaccumulation testing and other bioassays for purposes of determining which materials are environmentally acceptable for ocean dumping (EPA and USACE, 1998).

Using this approach, dredged soils are first tested for bulk chemical concentrations (Tier I). If these tests indicate contamination, then the sediment elutriate is tested for concentration and toxicity via benthic bioassays (Tier II). One of the assumptions of the Tier II water column analysis is that all contaminants present in the sediment will be released to the water column during disposal and emplacement, although it is acknowledged that this assumption is highly conservative due to the tendency of many contaminants to remain associated with the sediment.

Third and fourth tier testing involve advanced site-specific toxicity and bioaccumulation experiments and bioassays with a deposit feeding bivalve. Tests are conducted during a 28-day exposure time for organisms that are selected based on their ability to metabolize the target analyte and to survive the exposure test. The first two tiers of this approach are widely used; the bioaccumulation tests in Tiers III and IV are less frequently needed because the earlier tests are pass–fail.

The approach of USACE is empirical, site-specific, and more biologically based than are the other two approaches. The tiered treatment of site-specific sediments considers different bioavailability processes at different times. However, the measures cannot be extrapolated to other circumstances, nor are the relative influences of different bioavailability processes quantified.

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The methodology and approaches used for sediment quality criteria differ among the three agencies in fundamental ways, not the least in how certain bioavailability processes are assessed and taken into consideration. These differences could serve as a point of confusion for practitioners hoping to better quantify the risks involved in various sediment management scenarios, and they reflect the lack of consensus among environmental managers about how to deal with bioavailability processes.

CONCLUSIONS AND RECOMMENDATIONS

Considering the processes that influence bioavailability is entirely within the human health and ecological risk framework. Bioavailability processes should not be considered as "something new" that falls outside of the basic risk-based approach to hazardous waste cleanup that has been adopted in the United States. The goal of bioavailability analysis is to reduce uncertainty in exposure estimates and thus improve the accuracy of the risk assessment.

Although consideration of bioavailability processes is inherent to risk assessment, usually only some of the relevant bioavailability processes are considered explicitly, and assumptions made about the other processes are not transparent. All risk assessments contain implicit, and usually conservative, assumptions about many bioavailability processes. However, different users have chosen different processes to consider explicitly. For example, EPA has focused on the absorption aspect of bioavailability (through the use of default values for dermal and oral relative bioavailability and BSAF values) while many of the other processes have been less explicitly examined. Because of this variability, it is important to use parameters containing the word "bioavailability" (such as absolute bioavailability and relative bioavailability) only with very clear definition of the parameter and its role in the entire spectrum of bioavailability processes. The lack of mechanistic understanding and description during risk assessment precludes the development of technically sound exposure models, especially those that could incorporate temporal changes in physical, chemical, and biological factors.

Explicit consideration of bioavailability processes is more common in ecological risk assessment than in human health risk assessment. This is because it is easier and more acceptable to make measurements on ecological receptors (e.g., worms, small mammals, birds, fish) than it is on humans, and because risk managers are usually willing to manage uncertainty in ecological risk assessments (including the incorporation of bioavailability processes) differently. In addition, during ecological risk assessment there is a greater focus on how bioavailability processes influence bioaccumulation into various wildlife food items. The burden of proof is often higher for adjusting exposure estimates for human receptors than it is for ecological receptors.

There is a misconception that the default values representing bioavailability processes in risk assessment are protective and appropriate for all circumstances. The tendency to standardize regulatory risk assessment has led to the use of certain default factors (e.g., equilibrium partitioning for organic chemicals, relative bioavailability values, dilution attenuation factors) typically considered to have wide applicability across a variety of sites. Although determining these default values required explicit consideration of bioavailability processes

with adoption of a conceptual model and incorporation of quantitative assumptions, the values are sometimes based on only a few studies and may not be applicable to the site of interest. Thus, replacing default values with site-specific information should be encouraged. It should be noted that consideration of site-specific information on bioavailability processes may result in an increase or decrease compared to the "default value."

At present there is no legal recognition of "bioavailability" in soil clean-up, although bioavailability concepts are emerging for sediment management, and they have been embraced more fully for biosolids management and disposal. The fact that the term "bioavailability" does not appear in the laws and regulations, but does appear in the informal comments to regulations and guidance documents, necessarily leads to confusion and even conflict over the acceptability of the concept. More formal recognition of "bioavailability" in state and federal regulatory contexts would eliminate at least some of the hesitancy and confusion on the part of risk assessors and managers. The lack of clear authorization or guidance on using bioavailability in site-specific risk assessments from EPA has generally led to the perception that the approach is not favored.

There is no clear regulatory guidance or scientific consensus about the level and lines of evidence needed for comprehensive bioavailability process assessment. That is, it is not clear what threshold of knowledge is sufficient to be able to replace default assumptions about bioavailability with site-specific measurements. All of the decisions made at the limited number of case histories have been unique and variable. Regulatory guidance from EPA is needed that addresses what information must be included in a bioavailability process assessment, its scientific validity, acceptable models of exposure, and other issues. This may help to guide research efforts that will further mechanistic understanding of bioavailability processes.

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3

Processes

This chapter summarizes the physical, chemical, and biological processes that together comprise the science of contaminant bioavailability in soils and sediments. These processes are strongly influenced by a range of site-specific variables, such as soil or sediment composition, contaminants of concern, and available human or ecological receptor(s), as addressed in detail throughout this chapter. While there is substantial understanding of many of the processes that determine contaminant bioavailability, quantitative models are lacking for most.

The schematic presented as Figure 1-1 is repeated here to emphasize how physical, chemical, and biological processes interact as part of the bioavailability concept. As illustrated in this figure, contaminants may reside in a bound form (associated with soil or sediment particles), a released form (dissolved in a liquid or gas phase), or associated with a living organism. Contaminants become bound to solids as a result of chemical and physical interactions with soils or sediments (A in Figure 1-1). For example, heavy metals in soil or sediment are usually associated with ionic groups of soil surfaces. The strength of association will determine the extent to which contaminant–solid interactions can be disrupted, allowing the contaminant to become more bioavailable. Thus, understanding contaminant–solid interactions is a necessary first step to assessing bioavailability.

To appreciate the importance of this interaction, it is worth noting that for many chemicals of concern the fraction of contaminant mass that resides in the released form is orders of magnitude less than that which may be present in the bound form. For example, in Lake Michigan only 3 percent of the total polychlorinated biphenyl (PCB) pool is dissolved in the water column, with the bulk bound in bottom sediments (Pearson et al., 1996). In contrast, Lake Superior,

which is situated in a less industrialized area than Lake Michigan and receives most of its PCB inputs via the atmosphere, has a much higher fraction (67 percent) of PCBs in the aqueous phase (Jeremiason et al., 1994). The rate and extent to which bound-phase contamination can be released (or transported directly) to an organism are often the controlling factors, such that understanding contaminant release is critical to the establishment of bioavailability-based cleanup levels and soil or sediment quality criteria. As discussed in Chapter 1, contaminant release can occur far from the receptor, directly on skin surfaces, or within the lumen of the gut.

Following release from the bound state, a contaminant enters a dissolved aqueous state or a gas state (B in Figure 1-1), where it is subject to transport processes such as diffusion, dispersion, and advection. These processes combine to move contaminant molecules through the liquid or gas phases and may result in the reassociation of the contaminant with the soil or sediment (i.e., a return to the bound state), or they may carry the contaminant to the surface of a living organism. Transport of bound contaminants (C in Figure 1-1) via similar processes can also bring contaminants within close proximity of potential receptors. Because exposure of an organism to contaminants is strongly influenced by transport processes, contaminant transport is an important bioavailability component. However, in cases where the contaminant has been released directly on the skin or within the gut, transport processes (other than movement of the organism itself into the vicinity of the contaminated material) may be negligible.

Once the contaminant comes into contact with an organism (either externally or internally in the gut lumen), it is possible for the contaminant to enter living cells and tissue (D in Figure 1-1). Because of the enormous diversity of organisms and their physiologies, the actual process of contaminant uptake into a

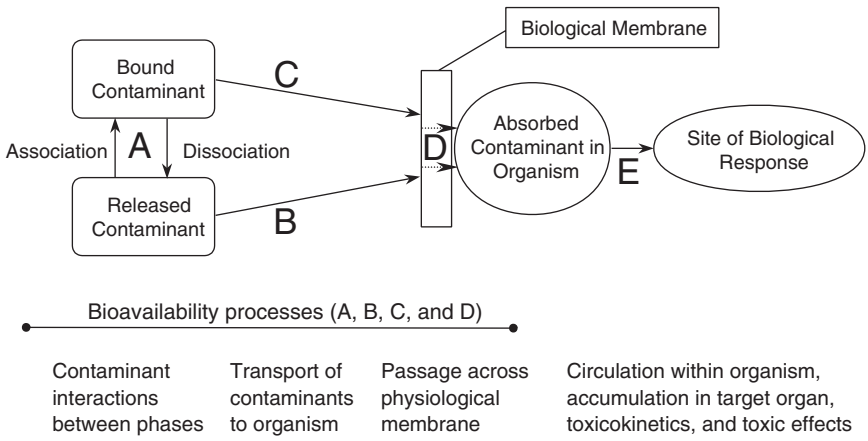


FIGURE 1-1 Bioavailability processes in soil and sediment.

cell—or factors that may impede or facilitate uptake—varies depending on receptor type. One common factor among all organisms is the presence of a cellular membrane that separates the cytoplasm (cell interior) from the external environment. Most contaminants must pass through this membrane before deleterious effects on the cell or organism occur. (In some instances, it is possible for contaminants to exert a toxic effect without penetrating the cell membrane such as β-lactam antibiotics, which damage bacterial cell walls and cause cell lysis.) Uptake generally requires contaminant transfer to and through a released state. In the case of bacteria, physical features (e.g., the cell wall) can isolate their cellular membrane from contact with particulate material, such that contaminants must be dissolved in the aqueous phase before they can be taken up. However, there are exceptions to the notion that bioavailability is directly dependent on solubility. For example, contaminant-laden particles that undergo phagocytosis can be delivered directly into some cells (although within the cell the contaminant may eventually need to be solubilized to reach its site of biological action). How contaminants in the bound or released state interact with the surface of a living organism constitutes the final step that defines the concept of bioavailability.

Once absorbed, contaminants may be metabolized, they may be excreted, or they may cause a toxic effect, among other things. Although these pathways are discussed in this chapter (and shown as E in Figure 1-1), they are not considered bioavailability processes.

SOLIDS PRESENT IN NATURAL ENVIRONMENTS

An important step that limits the bioavailability of contaminants is their retention onto solids that compose soils and sediments. A wide range of solids exists in natural systems that vary in their reactivity toward organic and inorganic contaminants. Before discussing retention processes themselves, it is useful to review the types of solids in soils and sediments and to define how the terms *soils* and *sediments* are used in this report.

Box 3-1 provides comprehensive definitions of soil and sediment that acknowledge the richness of these materials as ecosystems. For the purposes of this report, however, simpler more operational definitions are adequate and used throughout the chapter. Soils are usually considered to be unconsolidated (organic and mineral) material on upland landscapes and thus well aerated. As a result, their organic matter content is generally less than 5 percent, and oxidized materials define their mineralogy. Sediments, in contrast, are generally referred to as material having an overlying stratum, either water or soil. Aquatic sediments are saturated with water, and their aeration status depends on the redox conditions of the water column; they often achieve very anoxic states due to limited diffusion of molecular oxygen through sediments. Subsurface sediments underlie soils, often contain very low organic carbon content, and may be aerated or anaerobic depending primarily upon the carbon content in the formation. For

BOX 3-1
Different Perspectives on Soil and Sediment

Although the operational definitions of soil and sediment are adequate for the purposes of this report, soils and sediments are characterized by intricate associations of biological, chemical, and physical processes that impart functionality in these systems. Furthermore, scientists, engineers, and policy makers define these terms quite differently.

Soil

Soil is an elaborate ecosystem that encompasses secondary mineral matter derived from the weathering of geological material in association with detrital and living organic matter. A rich community of micro- and macroorganisms resides within and acts upon soils, an aspect not well captured by the operational definition of soil as simply unconsolidated matter at the earth's surface. As a result, contaminants in soil may undergo complex reaction pathways involving microbial degradation, plant assimilation, or binding to multiple phases ranging from mineral to organic in structure.

Soil is a term used frequently by many groups whose definitions of these media often differ greatly. Farmers and plant scientists may consider soils a medium for plant growth. Geologists may consider them as the "skin" on the geologic body. Structural engineers might envision soils as material for supporting roads and buildings, while environmental engineers consider soils as filtration media. From a soil science perspective, soils are defined as "dynamic natural bodies having properties derived from the combined effects of climate and biotic activities, as modified by topography, acting on parent material over periods of time" (Jenne, 1968). Thus, soils are not just inert material on the surface of the earth but rather a complex ecological system, with biological functionality and undergoing continual evolution.

the purposes of this report, the term *sediment* when used alone refers to aquatic sediments unless otherwise noted. The contrasting physical environments for soils and sediments can lead to very different solids—and thus properties with regard to contaminant retention (i.e., both strength and magnitude of retention).

Common Materials within Soils and Sediments

Solids within both soils and sediments are a composite of inherited material termed primary minerals (which are minerals formed by geological processes) and solids developed in place (authogenic). Such solids also have a balance of inorganic and organic fractions. This section discusses both primary and authogenic minerals, focusing mainly on clay minerals and organic compounds which are often the most reactive phases and thus most important for influencing bioavailability.

Sediment

Aquatic sediments are an open, dynamic, structured biogeochemical system typically composed of an oxic zone overlying anoxic materials (Fenchel, 1969; Chapman, 1989; Luoma, 1983, 1989). A variety of organisms ingest aquatic sediments or particulate detritus as food or live within the upper few centimeters of sediments, maintaining contact with the oxic zone to satisfy their oxygen requirements. The depth of the boundary between oxic and anoxic zones is affected by the diffusion rate of oxygen into the sediment compared to the consumption of oxygen by microbes in addition to complex interactions between deposition and erosion, geochemical reactions, and physical and chemical effects of the benthos (Aller, 1982; Myers and Nealson, 1988). Biologists consider sediment to be a medium within which benthos live. Engineers might be concerned about its physical properties with respect to supporting a building or describing the stability of a slope. Hydrologists might be interested in the water holding characteristics of aquatic sediment. These various definitions may assume dimensions that differ from the operational definition used in this report.

Geologists define sediment as a solid material that is produced by the weathering, erosion, and redeposition of preexisting rocks (referred to previously as "subsurface sediment") (Blatt et al., 1980). Sediments can be formed either by erosion and deposition by water (such as beaches), air (such as dunes), or ice (such as glacial moraine deposits) (Gary et al., 1974). The materials that form sediments can be derived from any preexisting rock type, including previously formed sediments, or accumulated by other "natural agents," such as organic matter that settles after being formed in suspension by organisms. Sediments become generally more compacted and altered chemically (consolidated and lithified) when they are buried within the subsurface. Broadly, the present composition of a sediment depends upon the source materials, the transport processes that occur, the redeposition environment, and any post-depositional processes. Thus, the geologist's description of sediments tends to focus on factors that identify the sediment formation process.

Inorganic Materials

Greater than 90 percent of the Earth's crust is composed of silicate (silicon and oxygen framework) minerals (Hurlbut and Klein, 1977), and as a result these minerals constitute a large fraction of soils and sediments. More specifically, quartz and feldspars make up the greatest fraction of coarse materials (those having particle diameters greater than 0.05 mm) and can also be appreciable in finer (< 0.05 mm) materials of soils and sediments (Allen and Hajek, 1986; Huang, 1989). With the degradation of primary minerals, smaller particles (< 0.002 mm in diameter) develop. This smallest size fraction is typically dominated in volume by secondary (authogenic) minerals composing a mineralogical class known as the clay minerals (a chemical definition of layered aluminosilicate minerals). Although they do not generally constitute the greatest abundance, the high surface area reactivity of clay minerals (as well as organic or carbonaceous

Soil profile at Oak Ridge National Lab showing the intricate and complex nature of soils.

components—see below) causes them to be one of the most important classes of materials controlling contaminant–solid interactions.

Clay minerals are layered silicates in which sheets of silicon coordinated by oxygen anions are bound with sheets of aluminum and/or magnesium coordinated by hydroxyl anions. Individual layers then stack to form the clay mineral. Kaolinite, a material of alternating silicate and aluminum sheets, is probably the most ubiquitous clay mineral in the world. The physical and chemical properties of soils and sediments in temperate climates are usually dominated by smectite and vermiculite minerals, organic matter, or metal (e.g., iron, aluminum, and manganese) hydrous oxides. Smectite and vermiculite are aluminosilicate minerals containing a permanent negative charge that originates from cations of lesser charge substituting for Si^{4+} or Al^{3+} within the sheet structure (commonly Al^{3+} substitutes for Si^{4+} and Mg^{2+} for Al^{3+}). The extra negative charge associated with

the defect structure is then satisfied by hydrated cations within soils and sediments, and the degree of negative charge is denoted as the cation exchange capacity (CEC).

A multitude of additional phases may be present in soils or sediments at much lower concentrations, and such phases are termed accessory minerals, most of which are authogenic. Despite their low levels, many accessory phases exert a strong influence on the chemical-physical properties of natural environments owing to their high reactivity, their ability to form coatings on other minerals, and their high surface area. Hydrated oxides of iron and aluminum are the most prevalent accessory minerals within aerated environments (i.e., soils); manganese oxides, while less abundant, have a very high reactivity. Collectively, these phases are termed hydrated metal oxides, and they often control the dissolved concentrations of inorganic contaminants such as lead or arsenic through reaction with ionizable surface functional groups.

Conditions within anaerobic sediments lead to the destabilization and dissolution of iron and manganese oxides. If sulfur is prevalent in such an environment, e.g., as for marine systems, this can lead to the precipitation of minerals such as pyrite or other iron sulfide phases (Morse et al., 1987). Elevated levels of carbon dioxide within waterlogged sediments can also lead to conditions favorable for the precipitation of carbonate minerals, particularly at alkaline pH values, that may include calcite, dolomite, and siderite. All of these solids have a defined reactivity toward contaminants that is addressed further below.

Organic and Carbonaceous Materials

Organic matter in surface soils and many sediments is principally from detrital material of plants and animals or their degradation products, as well as thermally altered and geologic forms of organic matter, such as kerogen, coal, soot, charcoal, and black carbons. Organic matter in solids tends to be highly reactive toward ionic and polar contaminants because ionizable functional groups within natural organic matter (e.g., carboxylate, phenolate, sulfhydral, amino, and phosphate groups) have a propensity to bind metal ions. In addition, aromatic moieties and hydrophobic micropores within organic matter promote the sorption of many hazardous organic compounds.

Because plant and animal residues degrade rapidly in aerated environments of temperate and tropical regimes, soils typically contain less than 5 percent organic matter (Brady and Weil, 1999). Nevertheless, owing to the reactive nature of organic matter, even just a few percent of such material can impart dominant physical and chemical characteristics to soils (Buol et al., 1997). Sediments, on the other hand, are often characterized by anaerobic conditions, and thus tend to accumulate carbon over time. Indeed, wetlands, including estuarine environments, can accumulate an organic fraction well in excess of 20 percent and have their physical-chemical characteristics completely dominated by this material.

Degradation products of plant and animal matter are often broadly categorized based on operational definitions of their solubility. Nondetrital organic matter that is insoluble in acid or base is termed *humin*, while that which dissolves in base is classified as *humus*. Humus can further be broken into fractions that are insoluble in acid (*humic acids*) and those that are soluble in acid (*fulvic acids*). Although these definitions are based on extraction procedures, the properties of organic matter are well represented by this methodology. For example, fulvic acids are small molecular weight organic molecules (generally less than 2000 daltons) and have a high proportion of functional groups that make them extremely reactive. Humic acids are larger molecular weight compounds with less functionality than fulvic acids. Despite differences in the degree of reactivity, all natural soil and sediment organic matter has appreciable effects on contaminant retention and therefore bioavailability.

Black carbon—particularly noteworthy because of its high reactivity towards nonpolar organic pollutants and its ubiquitous occurrence in sediments (Schmidt and Noack, 2000)—is a product of combustion/pyrolysis of either vegetation or fossil fuel. Post-1900 sediments and soils contain oil- and coal-derived black carbon as well as residues derived from plant combustion prior to 1900. Black carbon is condensed and highly aromatic in structure and composition. Because it is extremely resistant to weathering processes, it persists in the environment.

Along with black carbon, other forms of thermally altered carbonaceous material (coals, kerogens) appear to dominate hydrophobic organic compound sorption and desorption in some systems and potentially dominate bioavailability, even when they make up a small proportion of total carbon. These types of carbonaceous materials arise from geologic processes such as sediment burial and associated elevated temperature that (1) make the material more condensed and aromatic, (2) reduce its oxygen and hydrogen contents, and (3) increase its carbon content (Tissot and Welte, 1978). Under conditions of regional metamorphism, graphite can be formed. Coals, which by definition contain greater than 50 percent organic matter (Hutton, 1995) from primarily terrestrial plant material, are created through "coalification" (peat, lignite, bituminous coal, anthracite) that also results in more condensed and structured organic matter. Below the depth of soil formation, there is evidence that these older and more resistant forms of carbonaceous material can form the bulk of the observable carbon in at least some circumstances (Keller and Bacon, 1998). As explained in Box 3-2, the different types of organic matter discussed above bind contaminants to varying degrees, which may influence bioavailability.

Table 3-1 provides the chemical composition and characteristics of some representative forms of carbonaceous material that occur in soils and sediments. To briefly summarize, humic substances (humic and fulvic acids and humin) generally contain more oxygenated functional groups and less aromatic character and turn over more readily than more condensed, thermally altered forms of

BOX 3-2
Differing Sorptive Capacities of Organic Materials

Different types of solid organic carbon retain hydrophobic organic contaminants (HOCs) to different degrees. In particular, coal-derived and coaly, particulate sorbent media are significantly more efficient in sequestering HOCs compared to natural sediment organic matter (Karapanagioti et al., 2000). Gustafsson et al. (1997) reported for Boston Harbor sediments that polyaromatic hydrocarbon (PAH) sorption coefficients for carbonaceous residues from pyrogenic sources like soot may be two to three orders of magnitude greater than that for biogenic organic matter. Similarly, Grathwohl (1990) has shown that partition coefficients for HOCs on coals and shales may be approximately two orders of magnitude higher than that for HOCs on soil organic matter, such as humic acids.

Reported values of sorption coefficients for different sorbent carbons are illustrated in Figure 3-1 for trichloroethylene (TCE). The H/O ratio of the carbonaceous material indicates its polarity and provides a general indication of the structural characteristics of the material. The figure indicates that more condensed organic phases, such as coals and kerogenic shales, result in higher equilibrium TCE sorption. Similar behavior has been observed for phenanthrene (Gustafsson et al., 1997; Huang et al., 1997). It is evident that soot, coals, and shale-derived carbonaceous materials found in soils and sediment have nearly two orders of magnitude higher sorption capacities compared to humic substances and plant materials that are commonly predominant in modern surficial soils. Thus, from purely equilibrium considerations, the presence of even low proportions of diagenetically or thermally altered carbon solids in sediments should result in a substantial reduction in aqueous equilibrium or pore-water concentrations of the sorbed contaminants. To the extent that exposure and bioavailability are proportional to the aqueous concentration of HOCs, the presence of soot, coal, and charcoal may reduce toxicity and accumulation in comparison to humic or fulvic acids.

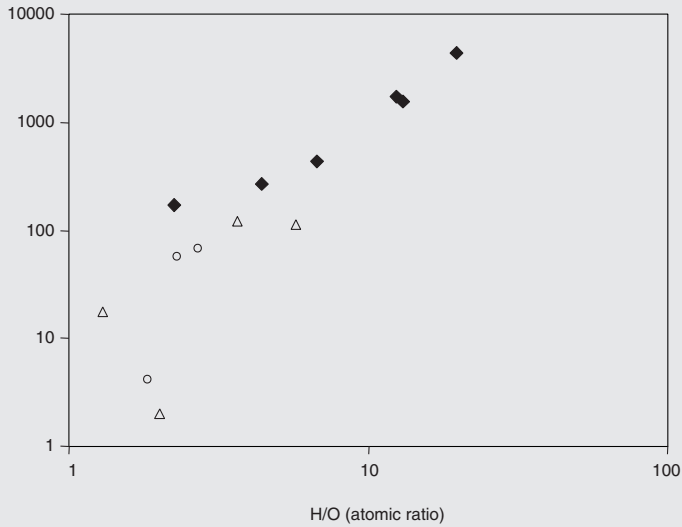


FIGURE 3-1 Reported partition coefficient values for trichloroethylene (TCE) on different types of carbon materials that can occur in soil and sediment. SOURCE: Reprinted, with permission, from Grathwohl (1990). © (1990) American Chemical Society.

TABLE 3-1 Representative Characteristics of Organic and Carbonaceous Materials

Material	Approximate Age (yr)[a]	MW (Da)[b]	C%[b]	H/C[c]	O/H[c]
Soil fulvic acid	10^2–10^3	~10^3	46	2.20	1.19
Soil humic acid	10^2–10^3	10^4–10^5	56	1.95	0.84
Humin	10^3	10^4–10^6			
Kerogen (in shales)	10^4–10^6	10^4–10^6	66	1.3	0.1
Coal	10^4–10^6	10^5–10^6	80		
bituminous				0.78	0.06
anthracite				0.32	0.02
Soot, char[d]	10–10^6		48–97[e]		

[a]From Weber et al. (2001) for all materials except soot/char.

[b]As cited in Weber et al. (2001) except for soot and char (Allen-King et al., 2002).

[c]As cited in Grathwohl (1990) for example materials.

[d]Soot and char contain a high proportion of C and a highly aromatic structure (Schmidt and Noack, 2000; Allen-King et al., 2002).

[e]Black carbon is predominantly elemental C and has an extended, aromatic network structure.

NOTE: Values shown are for particular well-characterized example materials typical of the characteristic compound described.

carbonaceous material such as soot, shale-derived kerogen, or hard coal. Although humic substances are usually the dominant form of carbonaceous material in soils and modern sediments, they have much lower sorption capacity for hydrophobic organic contaminants than the more condensed carbon forms. The methods used to identify and, when appropriate, quantify the forms of carbonaceous matter in soil and sediment are described in Chapter 4.

The prevalence and reactivity of solids—both organic and inorganic—found in soils and sediments are summarized in Table 3-2. The surface reactivity of the solids is broadly grouped into three categories: chemical, electrostatic, and hydrophobic reactivity. Surfaces having reactive functional groups (coordinatively unsaturated sites on mineral surfaces) are deemed chemically reactive. Electrostatic reactivity results from the development of charge, whether it be from isomorphic substitution in phyllosilicate minerals or from ionizable surface functional groups. Organic material having non-polar sites provides the possibility of hydrophobic bounds and thus is classified as having "hydrophobic reactivity." The probability of the material reacting with inorganic or organic contaminants is broadly classified, such that there are exceptions to the generalizations. Finally, those solid fractions with higher specific surface area (e.g., clays) tend to have higher reactivity.

TABLE 3-2 Prevalence and Dominant Reactivity of Solids Common to Soils and Sediments

Material	Type of Reactivity[a]	Occurrence	Reactivity with Inorg. Contamin.	Reactivity with Org. Contamin.
Fulvic acid	Chemical, Electrostatic, Hydrophobic	Soils, Aquatic sediments	High	Moderate
Humic acid	Chemical, Electrostatic, Hydrophobic	Soils, Aquatic sediments	High	Moderate
Humin	Hydrophobic	Soils, Aquatic sediments	Moderate	Moderate
Kerogen	Hydrophobic	Soils, Aquatic sediments, Subsurface sediment	Low	High
Coal	Hydrophobic	Soils, Aquatic sediments, Subsurface sediment	Low	High
Soot	Hydrophobic	Soils, Aquatic sediments, Subsurface sediment	Low	High
Clay minerals	Electrostatic, Chemical	Ubiquitous	High	Low
Metal oxides	Chemical, Electrostatic	Soils, Subsurface sediment	High	Low
Metal carbonates	Chemical, Electrostatic	Alkaline environments	Low to moderate	Low
Metal sulfides	Chemical, Electrostatic	Aquatic sediments	High	Low

[a]Chemical reactivity denotes material having functional groups that tend to form bonds with contaminants through the sharing of electrons (covalent/ionic bonds). Electrostatic reactivity relates to the creation of a charged surface. Hydrophobic reactivity results from the presence of non-polar surface groups.

Aggregates in Soils and Sediments

Within soils and sediments, various "glues"—organic and inorganic polymers—bind individual particles together forming larger clumps of matter termed aggregates that can greatly affect overall reactivity with contaminants. Aggregation can fundamentally alter water infiltration and transport and consequently bioavailability; in general, infiltration and translocation of water are enhanced by aggregate formation because larger channels are formed between particles. For these reasons, aggregation is one of the primary factors controlling soil structure.

Aggregation is initially promoted by high ionic strength, which allows particle flocculation (or the bridging of individual precipitates). Organic matter invariably promotes aggregation of small assemblages produced by flocculation within aerobic and anaerobic environments as manifested by increased hydraulic conductivity and water movement. Inorganic polymers such as hydrous ferric oxides, mineral carbonates (principally calcite), and silica (typically as an amorphous phase) may also promote aggregation. However, inorganic polymers may undergo hardening within soils upon dehydration (Buol et al., 1997), leading to conditions in which water flow (and penetration by soil organisms) is restricted.

The chemical properties of soils are also influenced by aggregation and heterogeneous precipitation, since it is the composite material and not its separate components that dictates overall reactivity. As depicted in Figure 3-2, in a natural soil environment, mineral grains such as kaolinite have an integral assemblage of secondary material deposited on their surface. Commonly deposited precipitates include (hydr)oxides of iron and manganese, organic material, and metal carbonates. The complexity of natural soil solids was recently illustrated for iron oxides, which are typically not pristine minerals as commonly depicted but rather an association of iron oxide and silica bound by organic matter (Perret et al., 2000). As one would expect, the reactivity of natural iron oxides, in terms of contaminant attenuation or reductive dissolution, is dramatically different than for pristine mineral phases.

Aggregates of particles in soils and sediments can be broken up through physical and chemical perturbations, such as increased fluid shear, a decrease in ionic strength, a change in electrolyte compositions from divalent to monovalent cations, the introduction of a reductant, or a change in pH (Bunn et al., 2002). When this occurs, small particles or colloids initially present in the aggregates may be mobilized, carrying with them any associated contaminants. This can fundamentally alter the percentage of contaminant mass thought to be bioavailable, particularly if organisms can take up and be adversely affected by particle-bound contaminants. Certain extraction techniques discussed in Chapter 4 can be used to determine what percentage of the total mobile contaminant mass of interest is colloid-bound as opposed to dissolved in the aqueous phase. The potential for colloid-enhanced contaminant transport and organismal uptake of colloids depends on many factors, as discussed later in this chapter.

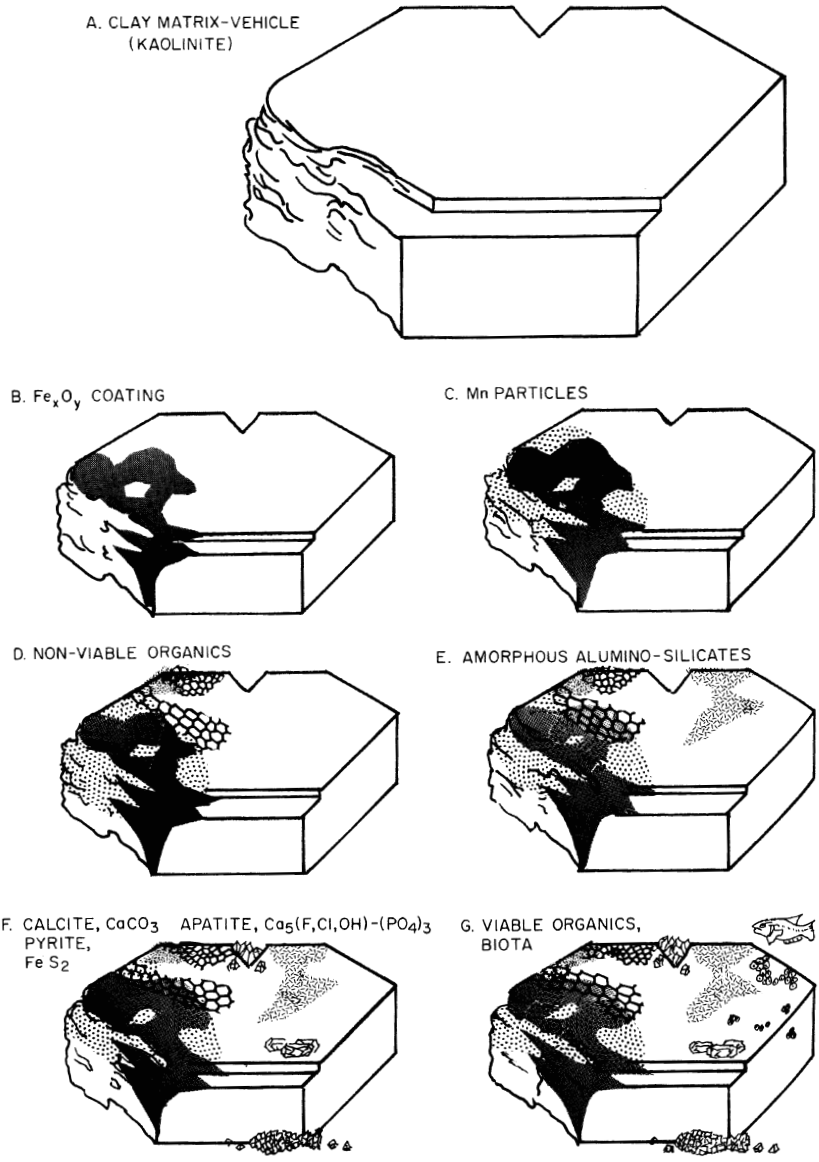


FIGURE 3-2 Diagrammatic representation of the important trace element sinks on the surface of an idealized kaolinite crystal. SOURCE: Reprinted, with permission, from Jenne (1977). © (1977) Dekker Publishing.

CONTAMINANTS

The contaminants discussed in this section are those for which bioavailability considerations are expected to be important (see Chapter 5 for more in-depth discussion). That is, they are persistent and tend to bind strongly to soils and sediments in natural settings. In addition, they tend to exist as mixtures that may have widely varying properties that affect bioavailability, such as solubility. Organic and inorganic compounds are differentiated for two reasons. First, the bioavailability of organic compounds over time tends to decrease as these compounds diffuse into soil and sediment particles. Metals, on the other hand, may experience increased or decreased bioavailability over time depending on the form of the metal originally deposited in soil or sediment. Second, some organic compounds can be microbially degraded to harmless products in the subsurface, while metals can only be transformed to a different metal species. The susceptibility of organic compounds to degradation is closely related to their bioavailability.

Organic Contaminants

The United States produces and consumes enormous quantities of organic and inorganic chemicals, some of which enter the environment through accidental or purposeful releases. Approximately eight million synthetic and naturally occurring organic compounds have been widely disseminated since the late nineteenth century (NRC, 1994) through their uses in fuels, solvents, food additives, and other products. Many organic pollutants released into the environment are found associated with soils and sediments, where they can persist for decades.

Classes of contaminants commonly found in soils and sediments are listed in Table 3-3. Because many of these compounds bind strongly to solids, the movement of the particulate phases, rather than the advective flow of water or air, can dominate their transport in soil and sediment systems. Depending on the receptor, association of these contaminants with the solid phase may also reduce the potential for their transport into living cells that come in contact with a contaminated matrix.

Polycyclic aromatic hydrocarbons (PAHs) exhibit persistence in soils and sediment due in part to their tendency to sorb strongly. PAHs are created from or used in combustion processes, petroleum refining, wood treating operations, and natural processes. Sites contaminated with PAHs over a century ago are still routinely found to contain soils and sediments containing high levels of these pollutants despite long-term weathering and natural attenuation processes. Other contaminants persistent in soil and sediment systems are PCBs and certain pesticides such as DDT. PCBs were once used in a variety of industrial materials including electrical transformers, and they tend to accumulate in aquatic sediments. Pesticides are widespread in the subsurface primarily as a result of com-

TABLE 3-3 Organic Contaminants, their Frequency, and their Sources

Compound Class	Examples of Compounds[a]	Sources
Polycyclic aromatic hydrocarbons (PAHs)	Naphthalene Phenanthrene Benzo[a]pyrene Pyrene	Combustion of coal, oil and wood Asphalt, creosote Automobile emissions, fuels, lubricating oils Coal tar[b]
Nitroaromatics	2,4,6-trinitrotoluene (TNT) Trifluralin Benefin Ethalfluralin Methyl parathion	Military installations Bombing ranges Bactericides Pesticides
Phenols, anilines	Pentachlorophenol Phenylamide herbicides: phenylureas, phenylcarba-mates, and acylanilides	Wood preservative Biocide Dyestuff wastewater Phenylamide herbicides
Halogenated aromatics	Polychlorinated biphenyls (PCBs) Dioxins[c]	Hydraulic oils, capacitor dielectric Pesticide application Incineration of medical/municipal sludge Forest fires and volcanic eruptions Cement kilns and boilers Petroleum, coal, and tire combustion Draft black liquor boilers Secondary lead smelting
Halogenated aliphatics	Chloroform Bromomethane Carbon tetrachloride Vinyl chloride 1,1-dichloroethylene Trichloroethylene (TCE) Tetrachloroethylene (PCE)	Degreasing solvents Former dry-cleaning facilities Plastics manufacturing
Pesticides[d]	Alachlor Aldicarb Atrazine BHC Carbofuran Chlordane 2,4-D Toxaphene DDT, DDD, DDE	Agriculture Residential and industrial pest control

continues

TABLE 3-3 Continued

Compound Class	Examples of Compounds[a]	Sources
Petroleum hydrocarbons	Benzene Xylenes Toluene Ethylbenzene Alkanes	Oil recovery and refining industry Automobiles and other forms of transportation Oil tankers, pipe lines, and other modes of transporting oil Industry

[a]Compounds given are examples and are not all-inclusive.

[b]Coal tar is a liquid byproduct of coal gasification that was commonly disposed of in burial pits at gaswork sites.

[c]Dioxins is a term used to collectively refer to the congeners of polychlorinated dibenzodioxins and dibenzofurans.

[d]Note that some pesticides are also halogenated aliphatics.

SOURCE: Adapted from NRC (1994, 2000).

mercial agriculture and residential application. Nitroaromatics, another class of recalcitrant compounds in soil, are used for a range of applications from explosives to biocides to polymer-precursors in synthetic chemical production. One example commonly associated with soils at military facilities is 2,4,6-trinitrotoluene (TNT). TNT has been found to persist for decades, partly because it is relatively resistant to microbial degradation.

Several other classes of contaminants are frequently detected in soil, sediment, and groundwater, but do not display the long-term persistence of the previous examples. This may be due to several factors, including the compound's biodegradability, its tendency to partition into water, or its volatility. For example, the gasoline components benzene, toluene, ethylbenzene, and xylene (BTEX) are widespread contaminants of the subsurface, but are reasonably water soluble and tend to biodegrade rapidly. Thus their potential to be highly persistent in soils and sediments is generally less than for hydrocarbons such as PAHs.

Inorganic Contaminants

At least nine of the top 25 most frequently detected hazardous substances in groundwater are inorganic compounds, primarily metals (NRC, 1994). Nitrate is the most commonly detected inorganic contaminant in groundwater, while the most frequently detected metals are lead (Pb), chromium (Cr), zinc (Zn), arsenic (As), cadmium (Cd), copper (Cu), nickel (Ni), and mercury (Hg). These elements plus antimony (Sb), beryllium (Be), selenium (Se), silver (Ag), and thallium (Tl) constitute the "priority pollutant metals" established by the U.S. Environmental Protection Agency based on potential hazard to human health.

Inorganic chemical contamination in soils and sediments is the result of multiple commercial, industrial, and military uses, including mining, metal refining, battery recycling, fertilizer application, and weapons operations. Radionuclides (primarily uranium, technetium, strontium, and tritium) generated during the manufacture of nuclear weapons are a significant threat at Department of Energy hazardous waste sites.

Table 3-4 lists the classes of inorganic chemicals that are major environmental contaminants. As many of these contaminants occur in multiple chemical forms, the most important isotopes in terms of toxicity, mobility, and bioavailability are noted.

Inorganic contaminants can exist in soil and sediment systems in the aqueous phase, as part of a precipitated mineral, or adsorbed on the surface of a mineral. The phase association of an element is very important in determining its availability to plants and animals. For elements that have at least moderate solubility in the aqueous phase, the tendency to bind on other minerals is often the factor that controls mobility and hence bioavailability. Most of the inorganic contaminants listed in Table 3-4 bind strongly from water onto surfaces of soil and sediment components depending on solution conditions, with pH and ionic composition being primary determining factors (Sposito, 1989; Dzombak and Morel, 1990; Langmuir, 1997). Exceptions are the chemical species that occur in water primarily as hard monovalent anions (e.g., nitrate and perchlorate).

The speciation of inorganic compounds also plays a dominant role in determining their bioavailability and other processes such as toxicity. Depending on

TABLE 3-4 Inorganic Contaminants and their Sources

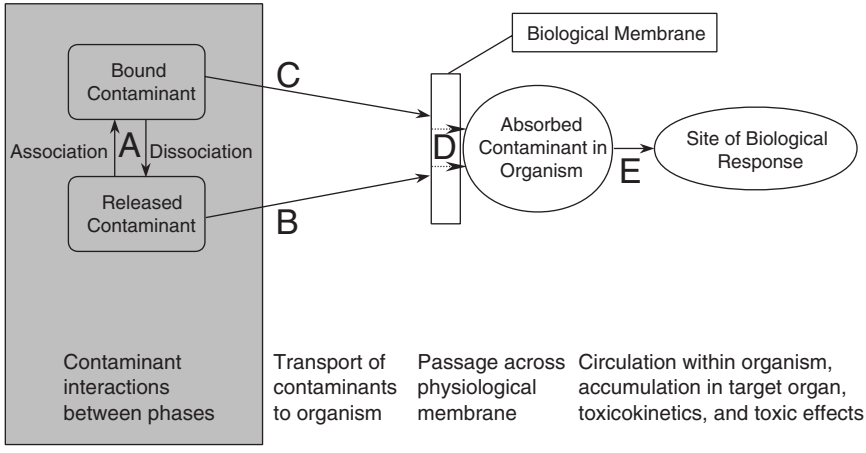
Chemical Classes	Example Contaminants[a]	Sources or Applications
Metals	Cr, Cu, Ni, Pb, Hg, Cd, Zn, As, Se	Mining, leaded gasoline, batteries, paints, fungicides, pesticides, irrigation drainage
Nonmetals	Ammonia Nitrate (Per)chlorate Phosphate	Fertilizers, paper manufacturing, disinfection, aerospace
Organometallics	Tributyltin Methylmercury	Paints, chemical manufacturing
Radionuclides	^{3}H, $^{238, 239, 240}Pu$, $^{235, 238}U$, ^{99}Tc, ^{60}Co, ^{137}Cs, ^{90}Sr	Nuclear reactors, weaponry, medicine, food irradiation

[a]Contaminants given are examples and are not all-inclusive.
SOURCE: Adapted from NRC (1994, 1999, 2000).

the compound and the receptors of concern, certain species of both metals and non-metal inorganic compounds are more or less mobile and/or toxic. Cyanide, for example, is extremely toxic in its free form or when weakly complexed with metal cations such as zinc, while strong metal-cyanide complexes, e.g., iron or cobalt cyano complexes, render the cyanide much more inert with respect to toxicity (Ghosh et al., 1999). Mercury and arsenic are examples of where the complexed (in this case methylated) metal is more toxic than the free ion form (e.g., to fish). For plants in particular, the concentration of the free ion of metals is thought a key parameter that determines their biological effects (Lund, 1990; Stumm and Morgan, 1996; Parker and Pedler, 1997). However, exceptions to this concept have been demonstrated for both plants and aquatic species, indicating that complexed ions are also bioavailable (van Ginneken et al., 1999; Parker et al., 2001). The transformation processes that bring about changes in inorganic compound speciation are discussed in a subsequent section.

CONTAMINANT–SOLID INTERACTIONS

An important factor affecting bioavailability of contaminants is their interaction with solids in soils and sediments, as shown in the grey highlighted section of Figure 1-1 below. Such interactions are termed association (retention) and dissociation (release) in order to be inclusive of the multitude of mechanisms that may be operational. The association reactions of organic and inorganic contaminants may differ appreciably. Inorganic contaminants associate with solids through physical or chemical bonding or through the precipitation of a new solid phase. Organic contaminant binding may involve hydrophobic partitioning or the



formation of chemical or physical bonds with the solid surface. The terminology used to describe contaminant–solid interactions for both organic and inorganic contaminants is provided below:

Association, Retention, or Sorption: The binding of a species without implication to the mechanism (which may include adsorption, absorption, precipitation, and surface precipitation).

Adsorption: The binding of an ion or small molecule to a surface at an isolated site—a two-dimensional surface complex. Binding can be electrostatic, chemical, or hydrophobic.

Absorption: The uptake of a species within another material (analogous to water uptake into a sponge).

Partitioning: The distribution of a population of molecules of a given compound between any two phases, determined by the compound's relative compatibility with each medium (Schwarzenbach et al., 1993).

Precipitation: The formation of a three-dimensional structure without the association of a substrate (sorbent) material. This process occurs in solution directly and leads to discrete particles. Surface precipitation, a heterogeneous mechanism, refers to nucleation on previously existing particles. Both are important processes for metal and metalloid retention but generally do not contribute to organic compound retention in soils and sediments.

Retention of Inorganic Contaminants

Unlike organic molecules, inorganic species cannot be degraded. They can, however, be retained on mineral and organic surfaces or they can form discrete precipitates; in either case they are removed from the aqueous phase and their bioavailability is consequently restricted. The predominant components of soils and sediments that retain inorganic compounds are clays and oxides of iron, aluminum, and manganese. These components bind ions from solution through electrostatic attraction and through short-range chemical bonding interactions, with the retention strength dependent on the given mechanism. The principal associations of inorganic contaminants with solids, as defined above, are depicted in Figure 3-3.

There are many different processes responsible for the removal of an inorganic species from solution, and each has a different binding strength. The strength of association (or degree to which the contaminant will resist release from the solid phase) depends both on the solids within the system and the contaminant itself. Associations can be predicted by understanding the chemical

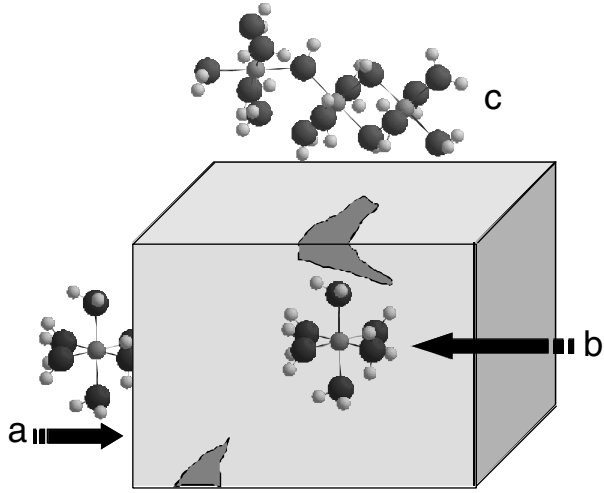


FIGURE 3-3 Ion retention mechanisms illustrating (a) adsorption, (b) absorption, and (c) precipitation reactions on a mineral surface.

reactivity of the contaminant (Table 3-5) and solids (Table 3-2). For example, chemical interactions, described below, should arise if the contaminant has a high reactivity and the solid has ionizable functional groups (hydrous metal oxides or organic matter). Additionally, conditions conducive to the precipitation of a contaminant (Table 3-5) will also lead to a strong association with the solid phase. If chemical interaction or precipitation is not operable, then associations via a physical attraction of an ion and surface of opposite charge may arise. The following sections discuss different association mechanisms and their retention strengths, including information about the current state of knowledge of inorganic solute retention mechanisms and models. Our current understanding of mechanisms and processes is limited to relatively simple systems, such as sorption mechanisms on pristine minerals or soil/sediment isolates. Retention under native conditions—in particular rates of release from the solid-phase—are much more poorly understood.

Adsorption

Adsorption refers to an ion associated with a surface (organic or mineral) either by (1) chemical interactions through a sharing of electrons (covalent or ionic bonding) or (2) electrostatic attraction involving an ion and surface of opposite charge (see Figure 3-4). The energy of adsorption includes contributions from both electrostatic and chemical interactions (Dzombak and Morel, 1990; Stumm, 1992). It is important to note that even if the ion and surface have like

TABLE 3-5 Inorganic Contaminant Reactivity[a] and Conditions Conducive for Precipitation

Class	Contaminant	Chemical Reactivity	Precipitation Conditions
Metal cations	Cr^{3+}, Al^{3+}	High	pH > 5
	Pb^{2+}, Cu^{2+}, Co^{2+}, UO_2^{2+}	High[b]	pH > 7
	Cd^{2+}, Zn^{2+}, Ni^{2+}	Moderate[c]	High carbonate or sulfide
	Sr^{2+}, Ca^{2+}	Low	High carbonate
	Cs^{+}	Low[d]	Limited
Oxyanions	AsO_4^{3-}, AsO_3^{3-}, PO_4^{3-} SeO_3^{2-}	High	High dissolved Al or Fe
	SO_4^{2-}, CrO_4^{2-}	Moderate	Limited
	NO_3^{-}, ClO_4^{-}	Low	None

[a]Contaminant reactivity is a necessary factor for chemical adsorption.
[b]Limited reactivity for U when carbonate complexes form.
[c]High for Cd and Zn in anaerobic environments.
[d]Binds strongly to vermiculite and illite clays.

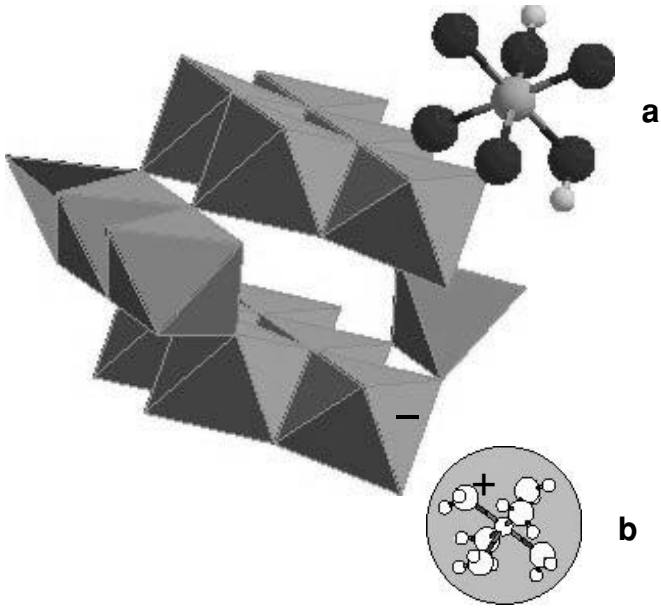


FIGURE 3-4 Adsorption reactions illustrating (a) chemical interactions and (b) electrostatic (physical) associations.

charges, the chemical affinity of an ion for the surface can override the electrostatic repulsion (i.e., if there is a sufficiently strong chemical interaction, a positively charged ion can adsorb on a positively charged surface).

Ions retained strictly by electrostatic forces are generally easily displaced by ions of like charge and are thus termed exchangeable. Exchangeable ions are essential for maintaining plant nutrient levels, but are not typically strong enough to immobilize environmental pollutants over a prolonged time period. The affinity of a charged surface for an exchangeable cation is principally based on the ion's charge-to-size ratio. As a result, ion charge will be the primary factor controlling the electrostatic retention force, with ion size having a secondary role. The greater the charge and the smaller the hydrated radius, the greater the affinity.

Chemically retained ions form very strong associations with solids that are often considered to be irreversible (McBride, 1994). As a result, chemically bound ions will have a diminished potential for release and should therefore pose a lower risk than ions held strictly by electrostatic forces. A transition from an electrostatic to a chemical association with increased reaction time, as discussed below, will modify the availability of the contaminant.

Inorganic contaminants vary considerably in their tendencies to bind on soil and sediment components, even with similar solution conditions. Figure 3-5, for example, shows data for the adsorption of various metal cations on iron and aluminum oxides as a function of pH. This figure illustrates that lead binds appreciably across a wide pH range, while other metal cations such as strontium bind less extensively than lead at similar pH values. Note that for either electrostatic or chemical associations, cation adsorption will generally increase with increasing pH while anion adsorption will generally increase with decreasing pH. Electrostatic binding increases as a result of greater charge on ionizable functional groups; chemical binding is facilitated by the formation of better leaving groups on the contaminant or surface.

Precipitation

Precipitation reactions result from a solution being oversaturated with respect to a solid phase. Solubility constants for precipitation in bulk solution are tabulated in many textbooks. Using these constants, one can use the saturation index (SI) to determine if a solution is undersaturated ($SI < 0$), oversaturated ($SI > 0$), or in equilibrium ($SI = 0$) with a solid:

$$SI = \log (IAP/K_{sp})$$

where IAP is the ion activity product and K_{sp} is the solubility constant for the specific reaction. Precipitation is the underlying mechanism assumed for the acid volatile sulfide (AVS) method used to assess the bioavailability of many metals in sediments (see Chapter 2).

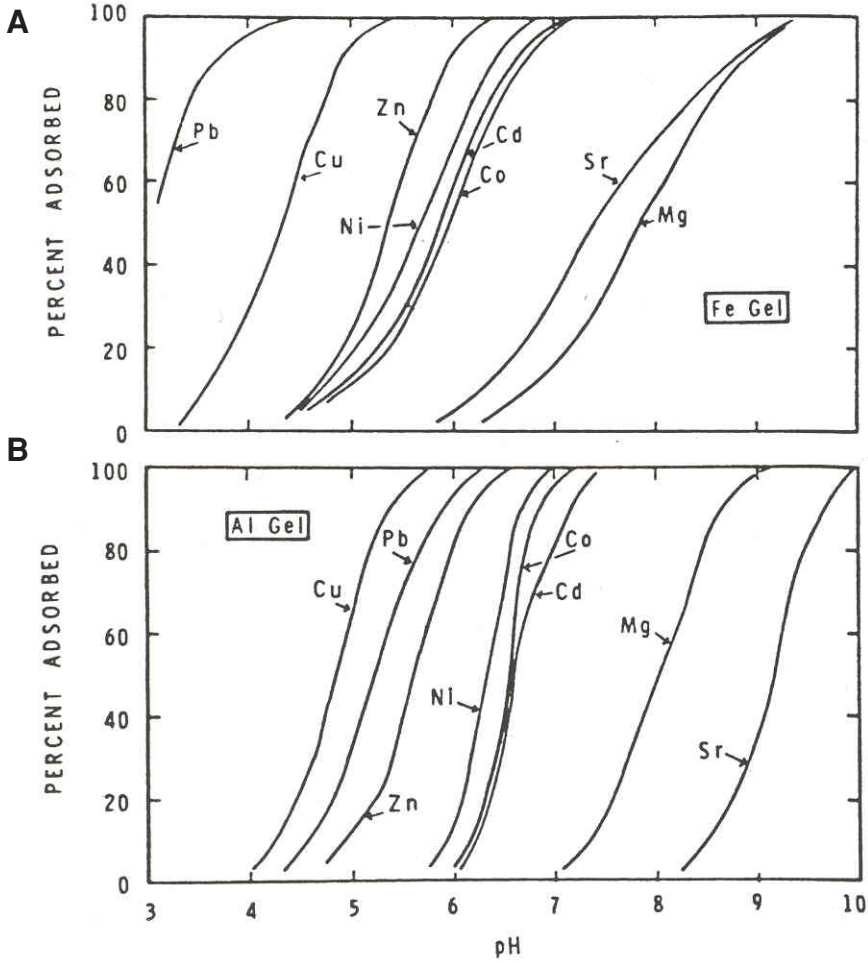


FIGURE 3-5 Retention behavior of eight divalent metal cations as a function of solution pH. For each experimental system (data point), Me_T = 0.125 mM in 1M $NaNO_3$ background electrolyte. (A) Adsorption data for freshly precipitated ferric hydroxide, Fe_T = 0.093 M. (B) Adsorption data for freshly precipitated aluminum hydroxide, Al_T = 0.093 M. SOURCE: Reprinted, with permission, from Kinniburgh et al. (1976). © (1976) Soil Science Society of America Journal.

While the SI is a convenient means for assessing the thermodynamic possibility of precipitation, it does not reveal whether the reaction will actually happen—only if it is possible. Kinetic factors usually govern the phase that forms over a short period of time, which is primarily dictated by the activation energy or energy barrier of a reaction. Generally, large well-crystallized particles have a

lower K_{sp} and higher activation energy. Consequently, amorphous particles are frequently found in soils and sediments due to their meta-stable conditions. Given sufficient time, these amorphous phases will transform into more crystalline solids (a process called "ripening"), which are thermodynamically more stable (i.e., they have a lower solubility). Additionally, existing surfaces often provide a catalytic role in precipitation and lead to surface (or heterogeneous) precipitates.

Recent evidence has revealed the potential for mixed metal phases to form as precipitates on mineral surfaces, providing the resulting phase has a lower solubility than the parent substrate. Association of transition metals with unstable aluminosilicate clay minerals, such as pyrophyllite, may lead to the release of aluminum from the clay and incorporation of the transition ion in a takovite-like solid; such phases have been noted recently for cobalt (Thompson et al., 1999), nickel (Scheidegger et al., 1996), and zinc (Ford and Sparks, 2000). Upon aging, silicon appears to be reincorporated into the precipitate leading to the neoformation of a transition metal-bearing clay mineral. Moreover, the stability of the phase increases with age and thus will lead to diminished dissolved concentrations of transition metal contaminants.

***

In summary, association of inorganic contaminants with solids in soil or sediment is typically dominated by adsorption processes. However, depending on the specific contaminant and site conditions, precipitation may play a large role in governing aqueous metal concentrations, particularly in anaerobic sediment environments where high concentrations of sulfide can result in the precipitation of metal sulfides.

Retention of Organic Contaminants

Organic contaminants can be retained on different components of soils and sediments, as illustrated in Figure 3-6. Nonpolar organic compounds are usually retained on organic components of soils and sediments such as condensed humic material or soot particles. Polar and ionizable organic compounds, in contrast, can associate with soils and sediments primarily through interaction with reactive sites on the mineral components (Sposito, 1989; Schwarzenbach et al., 1993). As described below, the primary retention mechanisms for organic compounds are absorption (partitioning) and adsorption.

Low Polarity Organic Compounds

Low polarity organic chemicals, which have had widespread use, generally associate with carbonaceous components of soils and sediments, although retention on mineral surfaces may be important in materials rich in high-surface-area

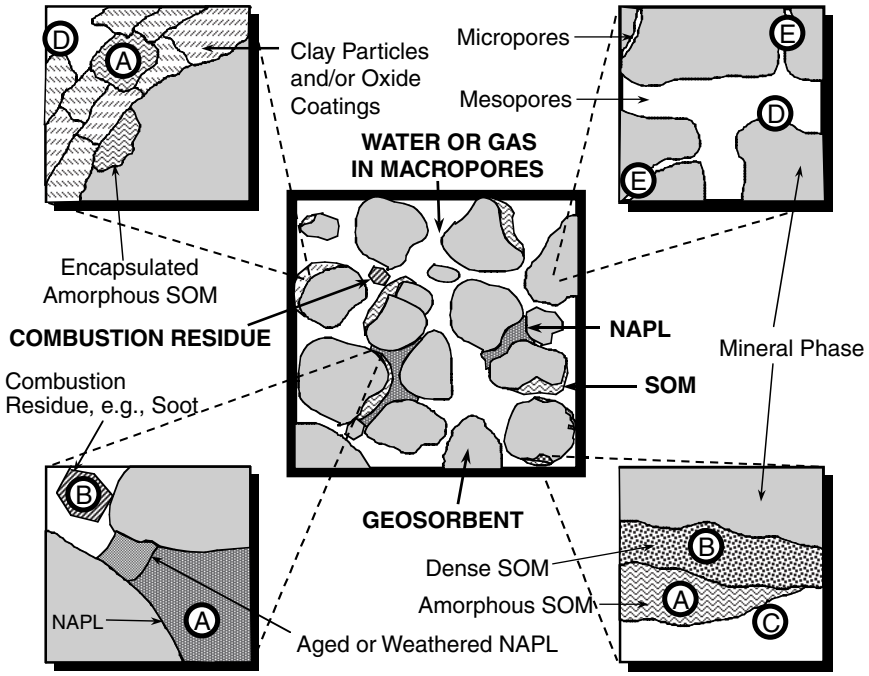


FIGURE 3-6 Conceptual model of association and dissociation of *hydrophobic* organic compounds with soils and sediments. The geosorbent domains include different forms of sorbent organic matter (SOM), combustion residue particulate carbon such as soot, and anthropogenic materials including nonaqueous-phase liquids (NAPLs). Retention processes denoted within the diagram are (A) absorption or partitioning into amorphous or "soft" natural organic matter or NAPL; (B) absorption or partitioning into condensed or "hard" organic polymeric matter or combustion residue (e.g., soot); (C) adsorption onto water-wet organic surfaces; (D) adsorption to exposed water-wet mineral surfaces (e.g., quartz); and (E) adsorption into microvoids or microporous minerals (e.g., zeolites) with porous surfaces at water saturation < 100 percent. SOURCE: Reprinted, with permission, from Luthy et al. (1997a). © (1997) American Chemical Society.

clay compounds with extremely low carbon content (Schwarzenbach et al., 1993). Although progress has recently been achieved in understanding these processes, substantive debate over specific mechanisms and models continues.

Organic pollutants can undergo both solvent partitioning and adsorption mechanisms (Karickhoff, 1984; Weber et al., 1992). Two-domain models have been proposed that capture these two empirical functionalities:

$$q = q_p + q_a$$

where q_p and q_a are the solvent partitioning and adsorption contributions to total retention, respectively (see Figure 3-7). For recent reviews of such models and

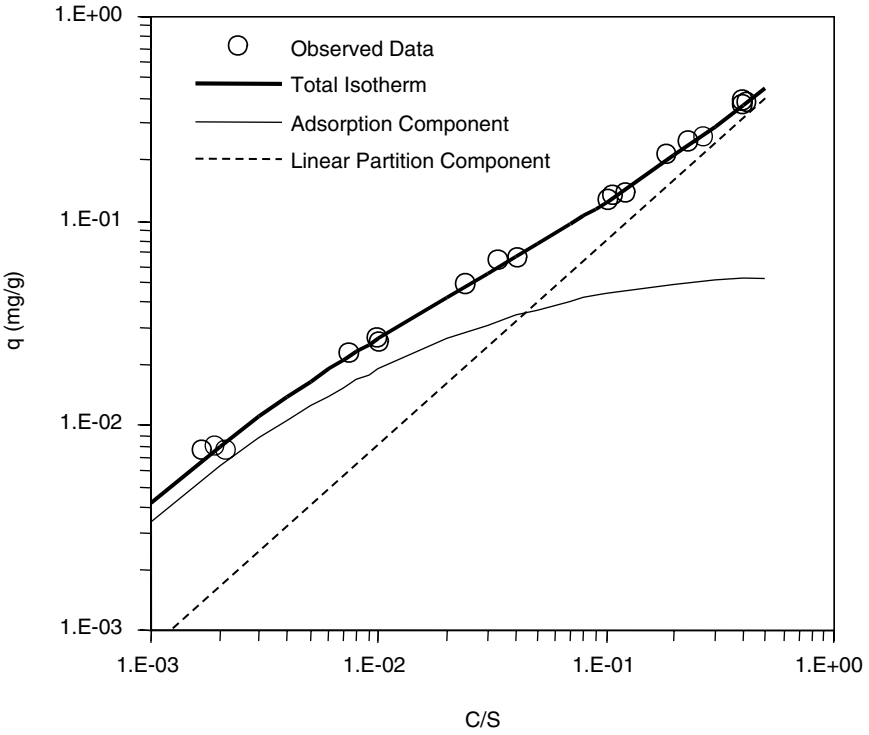


FIGURE 3-7 Example of a model fit of pyrene retention to a silty/clayey aquitard material, showing contributions of adsorption and partitioning to total retention. On this plot, aqueous concentration (C) is normalized by solubility (S). Note that for this example material, the adsorption component dominates at lower solution concentrations while the partitioning component dominates at the highest solute concentrations. The magnitude of the contribution of each of the two components depends upon the relative abundance of the types of carbonaceous materials present in the sediment. SOURCE: Data reprinted, with permission, from Xia and Ball (1999). © (1999) American Chemical Society. Model lines reprinted, with permission, from Allen-King et al. (2002). © (2002) Elsevier Science.

the underlying mechanisms see Xia and Ball (1999), Weber et al. (2001), and Allen-King et al. (2002).

Solvent Partitioning. Partitioning is often found to be linear for low polarity compounds (Chiou et al., 1983). It is an absorption process in that the sorbate exists and is essentially "dissolved" within the complex organic matrix. The solvent partitioning coefficient (K_p) that defines the extent of this behavior has been modeled as the product of two parameters:

$$K_p = K_{oc} f_{oc} \quad \text{or} \quad K_{oc} = K_p / f_{oc}$$

where K_{oc} is the organic-carbon normalized partition coefficient and is intended to be a compound-specific parameter and f_{oc} accounts for the sediment or soil properties by simply quantifying the organic carbon content. Similar formulations exist that use the organic matter (OM) content of the sediment as the normalizing parameter instead of organic carbon. For many sediments and soils, the K_{oc} of individual compounds (or K_{om} in the case of OM normalization) is essentially constant (Schwarzenbach et al., 1993). Furthermore, K_{oc} can be correlated to physicochemical properties, such as the octanol-water partitioning coefficient or inverse of water solubility, for a variety of low polarity organic compounds (e.g., Karickhoff, 1981). PAHs exhibit a large K_{oc} compared to other nonpolar solutes, apparently because of structural compatibility with aromatic components of soil organic matter (Chiou et al., 1998).

The organic-carbon (or organic-matter) normalized partitioning concept has been the paradigm applied to virtually all neutral organic compounds. It appears to explain retention behavior best when (1) the solute is present at a high concentration relative to compound solubility (Chiou et al., 1998; Xia, 1998) (see Figure 3-7) and (2) when humic substances are the dominant carbonaceous material (Kleineidam et al., 1999). In practice, samples with organic carbon contents greater than ~0.5 percent exhibit dominantly solvent partitioning behavior (Xia, 1998). The organic matter in sediments is less polar than in soils and exhibits approximately two-fold greater retention of low polarity compounds than soils (Kile et al., 1999).

Adsorption. Low polarity organic compounds may also bind through adsorption mechanisms, which result in greater binding coefficients relative to partitioning and also nonlinear behavior. Thermally or diagenetically altered forms of carbonaceous materials such as coals, kerogen from shales, soot, and charcoal (Grathwohl, 1990; Weber et al., 1992; Binger et al., 1999; Bucheli and Gustaffson, 2000; Karapanagioti et al., 2000) have particularly high binding coefficients and nonlinear adsorption behavior. The carbon-normalized Freundlich sorption coefficients (at 1 μg/L for comparison) reported for these materials are as large as 50 to 250 times greater than typically reported K_{oc} values (Grathwohl, 1990; Binger et al., 1999; Kleineidam et al., 1999; Bucheli and Gustaffson, 2000). The attributes of these carbonaceous materials that may account for the observed behavior include a greater H/O ratio, greater aromaticity, and a "more structured" form. In these studies, particles variously labeled as coaly particles, a charcoal-like substance, soot, kerogen, and coal/wood particles are responsible for the majority of compound retention even though they constitute a small portion of the total sediment mass (Binger et al., 1999; Ghosh et al., 2000) or a small proportion of the f_{oc} (Gustafsson and Gschwend, 1997; Chiou et al., 2000; Karapanagioti et al., 2000). Ghosh et al. (2000) provides the only direct

measurements that retention by "coal/wood" particles within a sediment is greater than retention by humic substances associated with silicate mineral grains.

Although various forms of black carbon (coal particles, soot) have been implicated in retention for some field sample–compound combinations, the specific properties of carbonaceous material responsible for this effect have yet to be identified. For example, it is not clear whether the enhanced retention associated with the carbonaceous material in shales results from geologic thermal alteration (due to elevated pressure and temperature associated with sediment burial) or is attributable to the presence of combustion products (e.g., char) within the original sediment. A better understanding of the operative mechanisms will be important to understanding the relative importance of adsorption versus partitioning of nonpolar organic compounds onto these solids.

Polar and Ionizable Organic Compounds

Compared to nonpolar organic compounds, polar and ionizable organic compounds are involved in more diverse binding mechanisms, which for ionizable compounds are similar to those outlined for inorganic contaminants. For organic compounds that have one or more ionic groups in their structure, electrostatic attraction–repulsion and bonding at specific surface sites can contribute to compound retention. Organic compounds that are polar but nonionizing exhibit sorption characteristics that span those of hydrophobic compounds and ionizing compounds. Sorption can occur primarily through hydrophobic interactions with organic matter rather than site-specific reactions, depending on the nature of the chemical (Schwarzenbach et al., 1993). In general, the more polar a compound, the less important is hydrophobic partitioning.

Polar and ionizable substituents on organic compounds can either enhance or inhibit the extent of retention relative to related neutral, nonpolar compounds, depending on the characteristics of the molecule and the extent of ionization. For example, Evanko and Dzombak (1998) studied the binding of five ionizing carboxylic acids on the iron oxide goethite, ranging from benzoic acid (one carboxyl group on the benzene ring) to mellitic acid (six carboxyl groups on the benzene ring). As the number of carboxyl group substituents on the benzene ring increased, retention increased and extended over a wider pH range (Figure 3-8).

The association of organic acids (a very common class of ionizable organic compounds) with the solid vs. the aqueous phases is strongly affected by the state of protonation of the compound. This is reflected in a strong dependence of retention on pH, which is illustrated in Figure 3-8. Organic acids generally are retained most strongly to oxidic minerals at lower pH values, and desorb as the pH increases. Thus, many organic acids will be more bioavailable at higher pH values where association with the aqueous phase is favored.

A very specific adsorption interaction has been documented between nitroaromatic compounds and clays. It seems that the aromatic nucleus of the

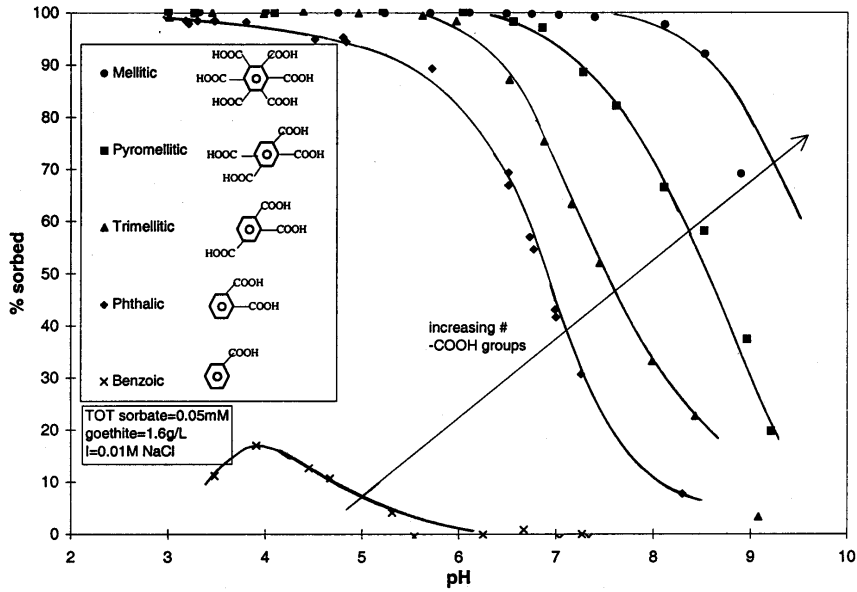


FIGURE 3-8 Fractional retention or sorption of carboxylic acids to the iron oxide goethite as a function of pH and number of carboxylic functional groups. SOURCE: Reprinted, with permission, from Evanko and Dzombak (1998). © (1998) American Chemical Society.

nitroaromatic compounds engages in electron donor/acceptor interactions with the oxygens of the external siloxane surface of the clays (Weissmahr et al., 1997). Such interactions are extremely fast and reversible, apparently independent of pH and ionic strength, and a strong function of the exchangeable cation (Haderlein et al., 1996). However, Sheremata et al. (1999) showed that the extent and reversibility of binding on actual sediments (consisting of mixtures of organic and inorganic phases) of TNT and several of its biodegradation products differed substantially. That is, the amino product compounds such as 2,4-diamino-6-nitrotoluene were more strongly retained than TNT, suggesting that the clay-based adsorption mechanism was insignificant in this scenario. Clearly, although the binding of many polar and ionizable organic compounds can be readily reversible, the extent and kinetics can vary significantly depending on the compound and the solid phase.

Overall, the retention of polar and ionizable compounds such as trinitrotoluene, chlorinated phenols, and other common compounds on soils and sediments is governed by a complex set of physical-chemical processes making it difficult to generalize about trends in behavior. The retention of ionizing organic compounds is much more dependent on solution chemistry than is the case for nonpolar compounds.

Aging Effects on Retention

An important aspect governing the bioavailability of solid-phase contaminants is time. With aging, a contaminant is generally subject to transformations that yield a more stable solid-associated compound. This in turn leads to a decrease in the bioavailability of the contaminant with increased reaction time in both soils and sediments.

Inorganic Contaminants

The state of an inorganic contaminant bound to the solid phase may change on a micropore scale with increasing reaction time. Depending on the solid, the contaminant, and solution conditions, various mechanisms may account for such changes. Contaminants that undergo a rapid uptake on organic or inorganic solids via electrostatic adsorption will gradually undergo a secondary transformation that may lead to the development of an inner-sphere complex (Sparks, 1989). The latter species is more stable than the former and thus decreases the availability of the contaminant. In addition, metal contaminants may actually become incorporated within the lattice structure of solids over time in such a way as to limit subsequent release. For example, Ainsworth et al. (1994) observed increasing desorption hysteresis for cobalt and cadmium, but not lead, upon increasing incubation time with hydrous iron oxide; they speculated that their results reflected contaminant incorporation into the lattice structure, the rate of which corresponded with ionic radii. Intraparticle surface diffusion, a third mechanism, may be a rate-limiting step that leads to the sequestration of metals within microporous solids such as hydrous iron, aluminum and manganese oxides, and some types of organic matter (Aharoni and Sparks, 1991; Axe and Trivedi, 2002).

As noted in the preceding section, mixed metal hydroxides occur extensively on a number of clay minerals. Aging results in new solids being formed, each having progressively decreasing solubilities (a ripening effect) and further retarding the dissolution of a sequestered contaminant (Ford and Sparks, 2000). For common clay minerals such as montmorillonite, nickel retention has been noted to continually increase even beyond a 206-day reaction period owing to the "neoformation" of a nickel phyllosilicate clay (Dahn et al., 2002). A final process that may account for diminished availability of inorganic contaminants over time is simply physical occlusion by deposition of organic or inorganic matter. As a result of the microscale burial, contaminants become sequestered within the solids and have minimal contact with surrounding aqueous solutions. Some of these aging processes are illustrated in Figure 3-9 using lead as an example. Many of these processes are considered irreversible (e.g., occlusion) or reversible only over very long time periods (e.g., surface diffusion).

Although laboratory investigations have clearly established that availability to the aqueous phase may decrease with aging of inorganic contaminants in soils

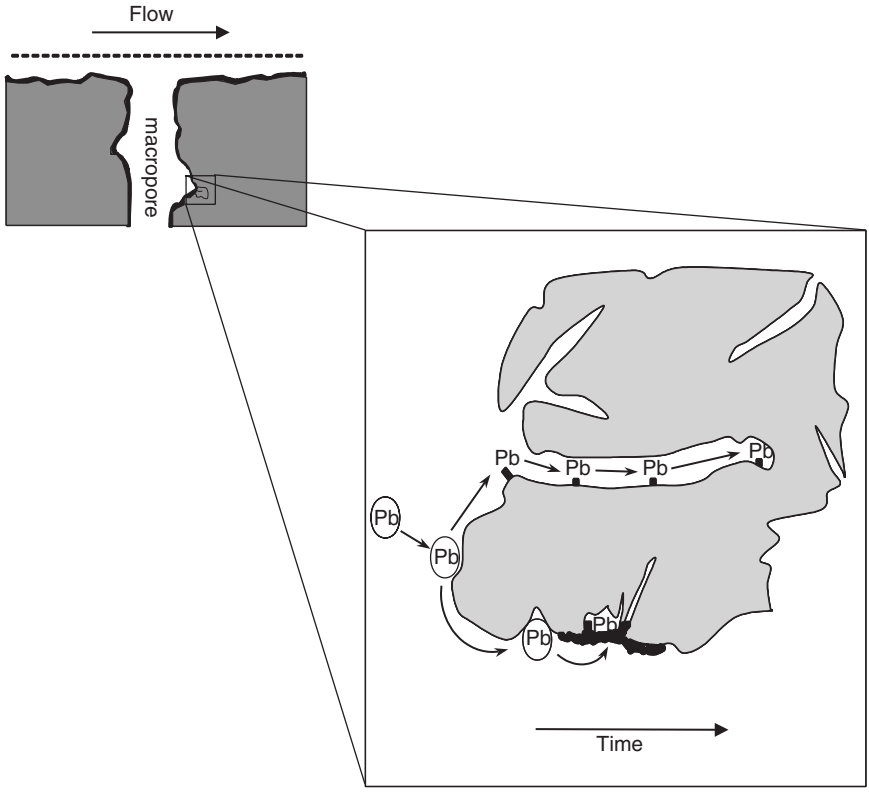


FIGURE 3-9 An example of the effects of aging on Pb^{+2} retention. The initial step in adsorption is film diffusion and the formation of an electrostatic bond. With increased reaction time, a chemical bond may develop between the ion and surface functional group. Despite the strong retention, the ion may migrate along the surface (surface diffusion) into the interior of the particle (upper pathway). It is also possible that once within the micropore, addition material (mineral or organic) may coat the particle and occlude the micropore (bottom pathway). In either case, contaminants become less susceptible to release into the aqueous phase.

or sediments, field observations of contaminant distribution upon aging have been variable and dependent on specific site conditions—often for the same metal. For example, within smelter-contaminated soils in France and at Leadville, Colorado, lead predominated as a surface complex on organic matter and hydrous oxides of iron and manganese (Morin et al., 1999). Lead was also noted within the organic fraction of garden soils proximal to an alkyl lead production plant (Manceau et al., 1996). In contrast, lead silicates were observed within soils associated with a former lead battery reclamation facility (Manceau et al., 1996).

A consistent theme from studies of natural materials is that surface phases of organic matter and hydrous metal oxides can have a pronounced effect on inorganic contaminant sequestration (Bertsch and Seaman, 1999). Retention within the lattice structure of such solids is also likely, including the precipitation of secondary aluminosilicates (Manceau et al., 1992; Ford and Sparks, 2000).

Organic Contaminants

Although the aging processes that affect the retention of organic contaminants to solids over time are less well understood than for inorganic contaminants, there are two general types: diffusional or reaction processes of the organic solute, and diagenetic processes that change the properties of the soil or sediment sorbent. Solute-based aging processes include chemical oxidation reactions that lead to solute incorporation into natural organic matter (Richnow et al., 1994; Burgos et al., 1996; Karimi-Lotfabad et al., 1996); slow diffusion into very small pores (similar to Figure 3-9 for lead) (Carroll et al., 1994; Hatzinger and Alexander, 1995; Weber and Huang, 1996; Pignatello and Xing, 1996; Cornelissen et al., 1998); and absorption into organic matter (Nam et al., 1998). Diagenetic alterations of the sorbent are caused by various physical, chemical, and biological processes. For example, soil organic matter becomes more aromatic in character with time as continued biochemical transformation of degrading plant matter occurs. This greater aromaticity of natural organic matter results in greater sorption capacity for hydrophobic organic contaminants. Grathwohl (1990) demonstrated that the sorption capacity of soil constituents is related to the age of the soil organic matter. Simulated diagenesis of peat has shown that aged peat had increased sorption capacity for phenanthrene (Johnson et al., 2001).

In general, the longer the contaminant is in contact with the sorbent, the greater is the extent to which aging processes advance. The slower rate of release or greater propensity for retention the longer an organic compound is in contact with soil or sediment may be manifested by extremely slow diffusion rates and high desorption activation energies (e.g., Ghosh et al., 2001). In addition, hysteresis (or an irreversibility of sorption processes) may be observed between the sorption and desorption isotherms (Chen et al., 2000).

It has been demonstrated that the movement of molecules during aging into the micropores of soils and sediments can result in their inaccessibility to even the smallest of microorganisms (Nam and Alexander, 1998). For example, the rate and extent of phenanthrene mineralization by bacteria in silica declined as the percentage of the pollutant in nanopores within silica particles increased (Hatzinger and Alexander, 1998). Further examples of the role of aging in organic compound bioavailability are given in Bosma et al. (1997), Kelsey and Alexander (1997), Alexander and Alexander (1999), White et al. (1999), and Morrison et al. (2000)—with an interesting counterexample provided by Reeves et al. (2001). Taken together, these studies point to the need for improved mecha-

nistic understanding of the aging processes that determine organic contaminant bioavailability.

Contaminant Release

Physical-Chemical Release Processes

Contaminants can be released (the opposite of retention) to water or gas in contact with soil or sediment by a variety of physical and chemical processes. These releases occur in response to changes in water saturation of the soil or sediment, to changes in water and gas chemistry, and to changes in soil or sediment surface properties. Rates of release can be relatively fast (minutes to hours) or extremely slow (many years) depending on the contaminant, solid phase, and fluid properties.

Dissolution of solids in water can lead to the release of contaminants existing as part of, or entrapped in, a solid structure. Metal ions, for example, can be released into water by dissolution of a metal oxide or carbonate solid. Dissolution processes usually have a large role in determining the chemistry of natural waters (e.g., Morel and Hering, 1993; Langmuir, 1997). For those contaminants bound to the surfaces of soil and sediment particles by adsorption or partitioning, desorption can occur in response to changes in water chemistry or surface properties. In addition to releases into the aqueous phase, volatile contaminants may be transferred to the gas phase (Lyman et al., 1990; Lorden et al., 1998). The rate of contaminant volatilization from soil or sediment to a gas phase depends not only on the specific contaminant but also on environmental factors such as temperature.

Contaminant release to the bulk aqueous phase of pore water or surface water involves multiple steps as the chemical moves through different soil or sediment compartments. Some of the inter-compartment transfers occur rapidly while others are slow. This multi-step process may be seen in Figure 3-10 where the release of a biphenyl molecule from sediment particles to the water column in a river is shown. In accordance with current understanding, release from sediment to the water column is considered to involve three steps: (1) contaminant desorption from the river sediment to the pore water until equilibrium is achieved (note that equilibrium may never happen given the following coupled processes); (2) diffusional transport of the contaminant in the macropores of the sediment toward the sediment–water interface; and (3) diffusional transport across the boundary layer at the sediment–water interface and into the river water (Formica et al., 1988; Wang et al., 1991; Ortiz, 1998).

Because these steps are sequential, the slowest step will control the overall rate of contaminant release to the water column. For many strongly retained compounds, the rate of desorption controls the rate of release to the aqueous or gas phases in contact with soil or sediment. In general, overall release rates are

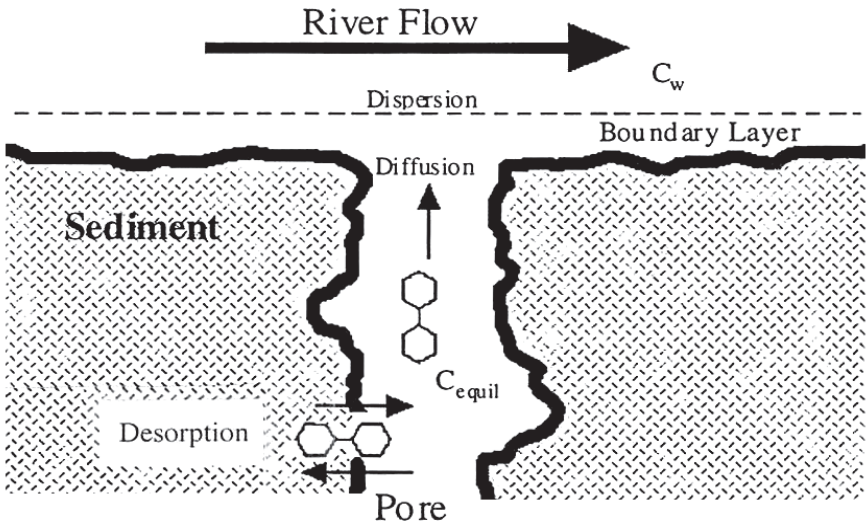


FIGURE 3-10 Schematic illustrating the desorption of a PCB molecule from sediment into porewater, and diffusive transport of the PCB molecule through the sediment macropore to the sediment–water interface. After the molecule moves across the interface it will be transported with the flowing river water. Note: the scale of this figure is significantly larger than the right-hand portion of Figure 3-9. SOURCE: Reprinted, with permission, from Ortiz (1998). © (1998).

controlled by the combined effect of the solid and the contaminant. In the case of retained organic contaminants, the release rate into water is often, but not always, strongly dependent on particle size (Wu and Gschwend, 1986; Ball and Roberts, 1991). However, no effect of particle size on the rate or extent of desorption of organic compounds from natural soils or sediments has been noted in other cases (Pavlostathis and Mathavan, 1992; Pignatello et al., 1993; Carroll et al., 1994).

Biologically Mediated Release Processes

A variety of biological processes within soils or sediments may alter contaminant retention and release and thus impact bioavailability. The most prominent example is contaminant desorption from soil or sediment particles mediated by the digestive tract—the mechanisms of which vary considerably across species. Within the gut, acid extraction, removal by surfactants, ligand complexation in solution and on membranes, transport with amino acids, and enzymatic breakdown of organic chemicals are all operative. Extraction tests developed to mimic these processes are discussed in detail in Chapter 4. Other biologically induced release processes include chemical transformations brought about by microbes and plants, as discussed below. Such biologically induced transformations need

to be appreciated because they often underlie strategies for remediating hazardous waste sites.

Microbial Surfactants. Hydrophobic organic compounds have low aqueous solubility, which when coupled with strong binding onto solids may limit their biodegradation. Surfactants produced by some microbes (biosurfactants) have the potential to increase the amount of sparingly soluble organic compounds in the liquid phase via incorporation into surfactant micelles or aggregates. Some microorganisms growing on essentially insoluble alkanes or oils secrete surface-active or emulsifying agents (microbial surfactants) that convert the hydrocarbon to droplets or particles with diameters of 0.1–1 microns (Einsele et al., 1975). These surfactants increase the apparent solubility of organic molecules and can account for their utilization by microbes (Goswami and Singh, 1991; Alexander, 1994). Microbial surfactants have been characterized as polysaccharides, polysaccharide-protein complexes, or glycolipids (Rosenberg, 1986). These and related compounds produced from the enzymatic degradation of starch and other materials have been studied to determine whether they may significantly increase bioavailability and thereby enhance the biodegradation of low-solubility organic compounds. Representative research has shown, for example, that two forms of a biosurfactant, a monorhamnolipid and a dirhamnolipid, and a cyclodextrin increased the apparent solubility and biodegradation of phenanthrene (Zhang et al., 1997; Wang et al., 1998). While biosurfactants can certainly enhance mobilization and biodegradation of organic compounds that exist as a separate organic phase (Herman et al., 1997), it is less clear whether they can enhance desorption and biodegradation of predominantly solid-associated organic compounds. Thus, at this time a clear understanding of surfactant effects and the linkages between solubilization, bioavailability, and biodegradation in systems comprised of hydrophobic organic compounds and soils or sediments is lacking.

Plant and Microbial Effects on Contaminant Release. Plants can also influence contaminant release from solid surfaces. In order to access required macro- and micronutrients, plant roots have the ability to alter the environment directly adjacent to them. Some of the parameters that may be altered as a result of plant activity include pH, redox status, ionic strength of the soil solution, macronutrient concentration and nature, and concentration of organic ligands (McLaughlin et al., 1998). The extent of alteration of the rhizosphere environment will vary by plant species and cultivar as well as by the nutrient status of the soil. The examples below illustrate the extent of modifications that are commonly observed as well as their implications for the bioavailability of contaminants.

In cases of phosphorus deficiency, plants can secrete organic acids along with H^+ to solubilize soil phosphorus. One side effect of this is that in arsenic-contaminated soils, phosphorus deficiency will induce elevated arsenic uptake and potential phytotoxicity, because both elements share the same uptake system

(Lee, 1982). Plant uptake of lead and zinc are also elevated in cases of phosphorus deficiency, potentially via plant-induced dissolution of lead and zinc phosphate precipitates (Laperche et al., 1997, Brown et al., 2003) or via dissolution of iron oxides. This may occur as a result of rhizosphere acidification or root proliferation and secretion of organic acids.

The mechanisms by which plants access solid phase soil iron can also influence the release of other contaminants in a soil system. At biological pH, the maximum amount of uncomplexed iron in solution is no greater than 10^{-18} M. Yet, most aerobic microorganisms and all plants need iron for growth. Plants follow one of two strategies to solubilize iron (see Figure 3-11). The roots of Strategy I plants (dicots and non graminaceous monocots) may induce reducing conditions in the rhizosphere with NAD(P)H electron donors located on root cells' plasma membranes. These plants may also secrete reducing or chelating compounds (often phenolic compounds). Proton pumps located on the surface of root cells can decrease solution pH by up to 2 units. In addition to solubilizing iron, these alterations can inadvertently solubilize a range of cations, particularly ones bound to iron mineral surfaces, that may be detrimental to the soil system.

Strategy II plants (grasses) release phytosiderophores (from the Greek: plant "iron carriers")—low molecular weight compounds that have a high affinity for ferric iron (Marschner, 1995). Many microorganisms also synthesize and secrete siderophores. Most siderophores that have been characterized belong chemically to the catecholates, the hydroxamates, or the polyhydroxycarboxylates, or they are polyfunctional. These molecules can compete successfully with the hydroxyl ion for Fe(III). Most microbial siderophore uptake systems involve an outer membrane receptor (Neilands, 1984) and a transport system consisting of a periplasmic binding protein, an integral membrane component, and an energy-providing membrane-bound ATPase (Winkelmann, 1991). Siderophore production is regulated by iron availability (Neilands, 1995), and formation constants for iron chelates are very high ($>10^{30}$). Coincidentally, gallium and elements from the actinide series, as well as other heavy metals, can be tightly bound to siderophores (Winkelmann, 1991). In plants, the uptake mechanism for iron chelates is specific enough to prohibit entry of cations other than iron. However, there is the potential for their uptake through other, less specific mechanisms.

In some cases, plants have evolved specific mechanisms that permit them to survive in potentially phytotoxic soils by *reducing* contaminant bioavailability to plant tissue. For example, wheat roots exude malate to complex and detoxify aluminum in acid soils (Papernik and Kochian, 1997), although the relevance of this mechanism is limited because aluminum is generally not considered a contaminant. A very limited number of metal hyperaccumulator or excluder plant species have been identified that are able to tolerate excess concentrations of metals in soil solution by highly specialized exclusion mechanisms (Baker, 1987; Kramer et al., 1996; Reeves et al., 1999). Such species are generally found only on historically contaminated soils.

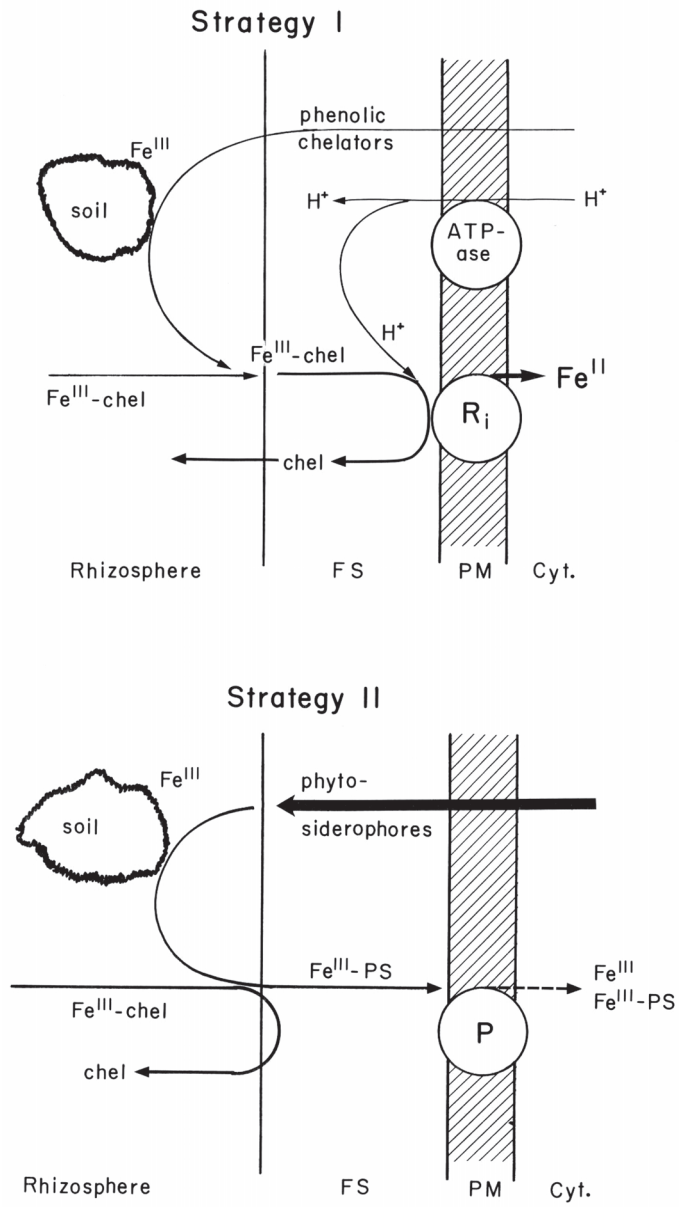


FIGURE 3-11 Plants utilize two distinct strategies to access solid phase soil Fe. Strategy I plants secrete phenolic chelators that can induce reducing conditions as well as hydrogen ions to lower rhizosphere pH, leading to reduction of Fe (III) via membrane-bound reductases. Strategy II plants secrete highly specific phytosideraphores (iron chelates) into the rhizosphere. SOURCE: Courtesy of David Parker, University of California, Riverside.

In addition to plants and microorganisms altering the soil and sediment environment in order to better access compounds for themsleves, their activities can also gratuitously affect the bioavailability of compounds to other receptors.

BOX 3-3
Arsenic in Bangladesh: Microbially Mediated Release

Arsenic is a toxic trace element that is rather ubiquitously distributed throughout the world. Owing to its toxicity and accumulation, even low concentrations of arsenic in drinking water can pose a serious health threat. Bangladesh and West Bengal serve as examples of the serious health impacts arsenic can impose and the role of microbes in increasing arsenic mobility, transport, and bioavailability.

In order to eliminate the potential for disease via surface water pathogens, the use of groundwater as the primary source of drinking water within Bangladesh and West Bengal has been promoted by government and world heath organizations. The shallow aquifers used are within sediments derived from upland Himalayan catchments and are laden with arsenic. Nearly 28 percent of the shallow wells in the region have arsenic concentrations exceeding 50 μg/L (the drinking water standard of Bangladesh) (Smedley and Kinniburgh, 2002). As a consequence, between 30 and 35 million people in Bangladesh alone have been exposed to water exceeding allowable arsenic levels. An estimated one million people have been projected to be impacted by arsenocosis with incidence of cancer in the tens of thousands (Chowdhury et al., 2000; Anawar et al., 2002).

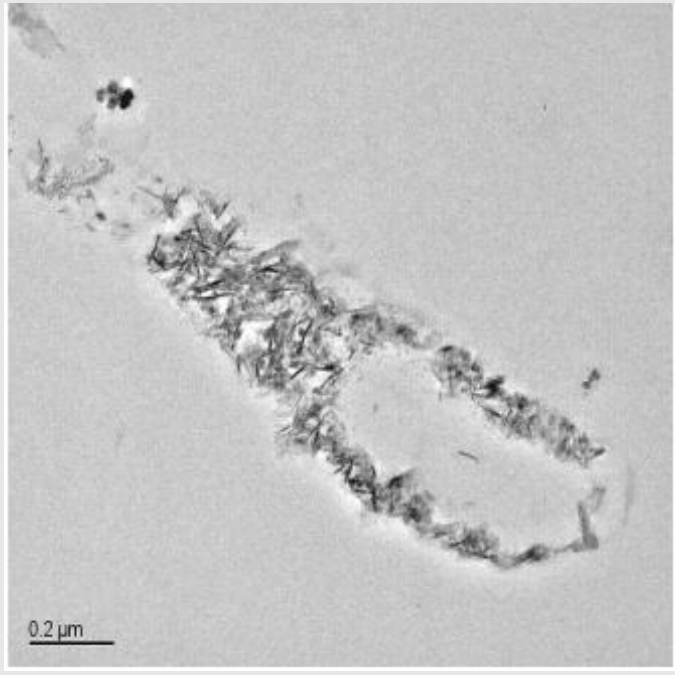
Although the solid concentrations of arsenic in the region (typically less than 6.5 mg/kg—Smedley and Kinniburgh, 2002) do not exceed world average concentrations for river sediment (Martin and Whitfield, 1983), and are on the order of one-tenth to one-hundredth those of mining-impacted sediments or soils (Harrington et al., 1988; Moore et al., 1988), the dissolved concentrations remain high. The reason why arsenic is partitioned to the solution and not the solid phase is because of redox conditions present in the subsurface. Arsenic is a redox active element that generally exists in either the +3 or +5 oxidation state. Both oxidation states lead to oxyanions—As(III) as arsenite and As(V) as arsenate, although As(III) may also be coordinated by sulfur ligands in sulfide-rich environments. Arsenate dominates in aerobic environments while arsenite persists in anaerobic systems. With the exception of its redox activity, arsenate is an analog to phosphate and generally binds tenaciously to solids within soils and sediments, particularly hydrous oxides of ferric iron. Arsenite also forms strong complexes on iron (hydr)oxides and iron sulfide minerals but it has a narrow adsorption envelop centered around pH 7, and it does not partition extensively on Al-hydroxide or aluminosilicate minerals (e.g., kaolinite). Thus, in non-sulfidic systems where ferric (hydr)oxides are absent or undergoing degradation, or where the pH deviates appreciably from neutrality, one can expect arsenic to partition to the solution phase.

Unfortunately, the subsurface sediments of Bangladesh and West Bengal support anaerobic conditions leading to the formation of arsenite, and the sediments are not enriched in reactive iron sulfide phases. In addition, ferric (hydr)oxides are absent or undergoing degradation because of anaerobic microbial respiration. That is, Fe(III) is serving as an electron acceptor for dissimilatory iron reducing bacteria (DIRB), which are ubiquitous within surface and subsurface material and account for the vast majority of iron (hydr)oxide reductive dissolution (Lovley, 1991). Microbially mediated degradation of ferric solids by DIRB has, in fact, been demonstrated as a release mechanism for

As discussed in Box 3-3, the microbial reduction of iron in sediment (which has led to the release of iron oxide-bound contaminants) has contributed, along with other important processes, to serious arsenic exposure to humans in Bangladesh.

retained arsenic (Cummings et al., 1999; Zobrist et al., 2000)—a pathway that accounts for the majority of arsenic within anaerobic waters.

While various hypotheses have been given for the release of arsenic within sediments of Bangladesh, microbial reductive dissolution of ferric hydr(oxides) and the concomitant release of arsenic is a probable mechanism. Carbon introduced from surface runoff laden with animal and human excrement may episodically stimulate DIRB activity and lead to reductive dissolution of the ferric solids. Alternatively, or possibly in concert, detrital organic matter (predominantly as peat) residing in the sediment may allow for slow but sustained reduction of ferric (hydr)oxides. In either case, the unfortunate outcome of the aquifer conditions and biologically induced solid-phase alteration is that arsenic is placed in a bioavailable form to which millions of people are exposed.



Goethite encrusting the a cell of the dissimilatory iron-reducing bacterium Shewanella putrefaciens.

Bioturbation. It has long been recognized that the presence of macrofauna can change the physical and chemical properties of sediments (Aller, 1982; Rhoads and Boyer, 1983). Bioturbation is the mixing that occurs when biota move sediments from one location to another (usually vertically) by ingestion and defecation or by activities such as burrow construction. Bioturbation and resuspension can change the release of contaminants and consequently their bioavailability. Another common effect is to mix surface material into the sediment column or to move sediments from depth to the surface. In aquatic environments, resuspension can be caused by currents generated by tides, winds, and high velocity flows. Metals, for example, are released slowly from sediments in general, but rates are faster from oxidized than from anoxic sediment. When resuspension or bioturbation move sediment from an anoxic microenvironment (e.g., at depth) to an oxic environment (e.g., at the sediment surface), desorption of metals can accelerate (Giblin et al., 1986). The opposite can also occur if surficial contamination is buried.

The dramatic influences of bioturbation by the lugworm *Arenicola marina* on uptake and distribution of cadmium in a marine sediment was recently demonstrated by Rasmussen et al. (1998) using laboratory sediment cores. In cores without lugworms, all cadmium was found in the surface sediment over 16 days of exposure. In cores containing lugworms, cadmium was found dispersed throughout the sediment column to 15 cm depth (the feeding depth of the worm) after 16 days. The presence of lugworms more than doubled the rate of removal of cadmium from solution to sediment due, at the least, to increased turnover of sediment (from feeding activity) and increased contact of cadmium-labeled water with potential binding sites in the sediment.

Bioturbation does not always lead to increased removal or transformation of contaminants. For example, burial by bioturbation slowed the degradation rates of fluoranthene (Kure and Forbes, 1997). Bioturbation depths differ considerably, and short-lived isotopes of atmospheric origin can be employed to determine how deep the sediments are mixed (Fuller et al., 1999). In the San Francisco Bay, mixing occurred over a 30-cm depth in some locations during a six-month period (Fuller et al., 1999).

Summary

For inorganic contaminants, a variety of mechanisms exist by which ions associate with the solid phase. This mechanism will in turn determine the extent to which the contaminant is bioavailable. Ions retained by electrostatic forces (physical adsorption) can easily be displaced by other ions and thus will have a high probability of being rapidly released. Thus, formation of such complexes would not be expected to appreciably retard the bioavailability of a contaminant (i.e., the contaminant remains available for release into solution and for subsequent biological uptake). In contrast, compounds that form strong chemical inter-

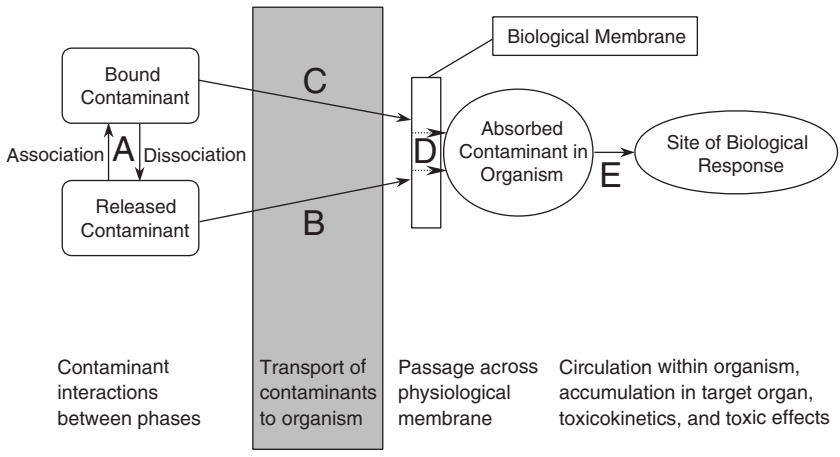
actions with the solid will not be easily displaced. Often they can be considered irreversibly bound and thus their potential for release (desorption) into solution is minimal. Similarly, contaminants forming precipitates on existing mineral surfaces or as discrete phases will be rendered immobile and unavailable for plant or animal uptake via the surrounding solution, provided that conditions maintaining a stable solid (i.e., low solubility) prevail.

Polar organic compounds will undergo adsorption processes similar to those noted for electrostatic interactions of inorganic ions; they bind to charged functional groups on minerals and particulate organic matter. Nonpolar organic compounds, however, are usually retained on organic components of soils and sediments such as condensed humic material or soot particles. Owing to the porous nature of organic matter (or at least a large fraction of it), molecules may diffuse into the interior portion of the particles. Within these confines their potential for release is dramatically diminished. Furthermore, pores may become occluded, thus entrapping contaminants within the particle and helping to minimize their bioavailability.

Rates of desorption for both organic and inorganic contaminants from soils and sediments are highly variable and dependent on the mode of uptake, the time of reaction (aging), and on the current solution conditions.

CONTAMINANT TRANSPORT

Inorganic and organic contaminants associated with soils and sediments can be transported to biological receptors by a variety of pathways in environmental systems. As highlighted in the grey box below, the contaminant may be transported on the soil or sediment particle with which it is associated, or it may be released from the soil/sediment particle to water or a gas phase (e.g., soil gas or



air) and transported in that medium. In some circumstances, contaminants may be transported in liquids other than water, such as oil or gasoline, but this is most relevant to a spill scenario for which considerations of bioavailability are secondary. The particular transport pathway depends on the initial location of the contaminant (such as occurrence in deep or shallow soil or sediment), the properties of the contaminant (such as volatility and aqueous solubility), and on environmental properties (such as degree of water saturation in the soil and near-sediment water velocity).

Transport of Contaminants on Particles

Contaminants on soil and sediment particles can be transported along with the particles themselves, via entrainment in moving water or air. This allows transport of contaminants that are strongly associated with the particles and have little potential for release in soluble form to water or in vapor form to air.

Soil-borne Contaminants

There are three major transport pathways for soil particles and associated contaminants to reach receptors that are not in their immediate vicinity: entrainment in air, suspension in water, and colloidal movement in groundwater. Soil particles at the soil–air interface can be entrained in air flows moving over the ground surface, or they can be suspended in surface runoff following precipitation. These contaminant-bearing particles may be transported directly to receptors, e.g., through inhalation by animals or deposition on plants, or to other environmental media, e.g., via atmospheric deposition or runoff to surface waters. In addition, solid-bound contamination can be transferred to receptors via colloid movement in groundwater. Colloid movement is notable because generally soil particles below the ground surface are immobile, and thus serve to keep any affiliated contaminants immobile. However, the finest (< 10 μm) soil particles can be mobile in coarse-grained porous media under some conditions (Figure 3-12). These colloids have potential to move with groundwater through the near-surface unsaturated zone to the deeper, saturated zone and then to pumping wells, discharge areas, plant roots, and other receptor locations. Significant contaminant transport by colloids in the subsurface appears to be possible only under special conditions, such as when contaminant adsorption is strong and not readily reversible, and when concentrations of mobilized colloids are high (Ryan and Elimelech, 1996; Roy and Dzombak, 1997, 1998). While there has been much study of the association and transport of contaminants with soil particles, including colloids, there has been much less study of the availability of these particle-associated contaminants to human and ecological receptors. What is known about the extent of uptake of colloid-bound contaminants during oral ingestion and inhalation by mammals and invertebrates is discussed in a subsequent section.

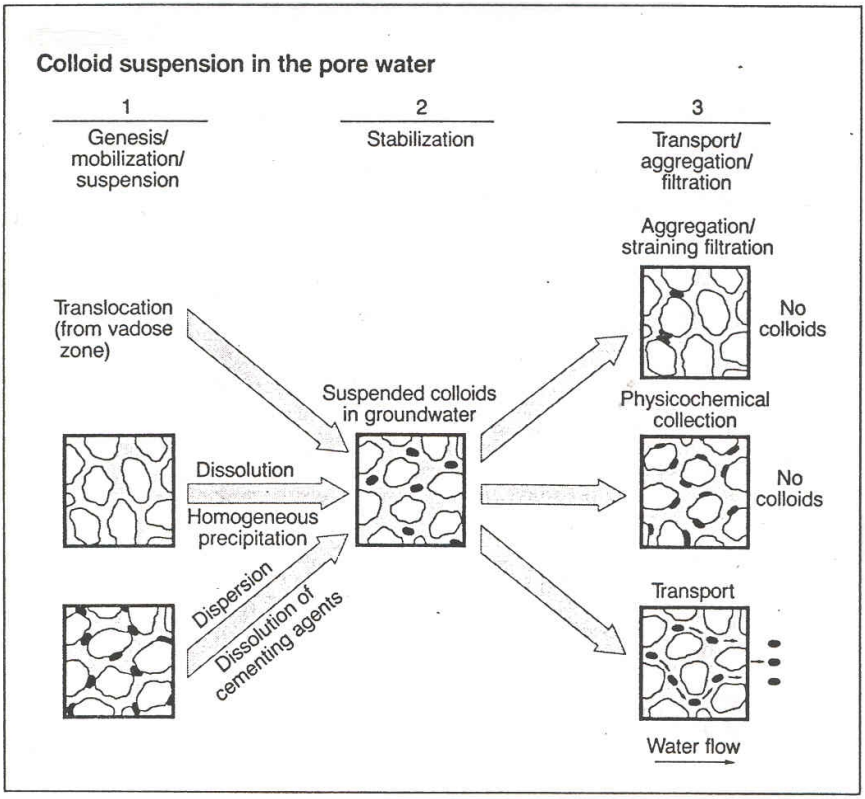


FIGURE 3-12 Colloid suspension in soil pore water. SOURCE: Reprinted, with permission, from McCarthy and Zachara (1989). © (1989) American Chemical Society.

Sediment-borne Contaminants

Contaminated sediment particles at the sediment–water interface can be transported via resuspension in water flows moving along the sediment surface (Figure 3-13). Due to their size, larger and heavier particles may be suspended for just a short period of time, resulting in their deposition after lateral transport for a short distance. This process, known as bed load transport, often can be repeated many times in sequence, resulting in the downstream movement of the larger, heavier particles (Figure 3-13). Downstream bed load sediment transport occurs at a slower rate than is the case for smaller, lighter particles, which tend to remain suspended in flowing water. The amount of material transported downstream is an exponential function of flow velocity, so large events (floods) are responsible for a large proportion of the sediment transport in most systems. In contaminated rivers this means that floods can move contaminated sediments onto floodplains. In the Clark Fork River, Montana, contaminated sediments of many meters depth

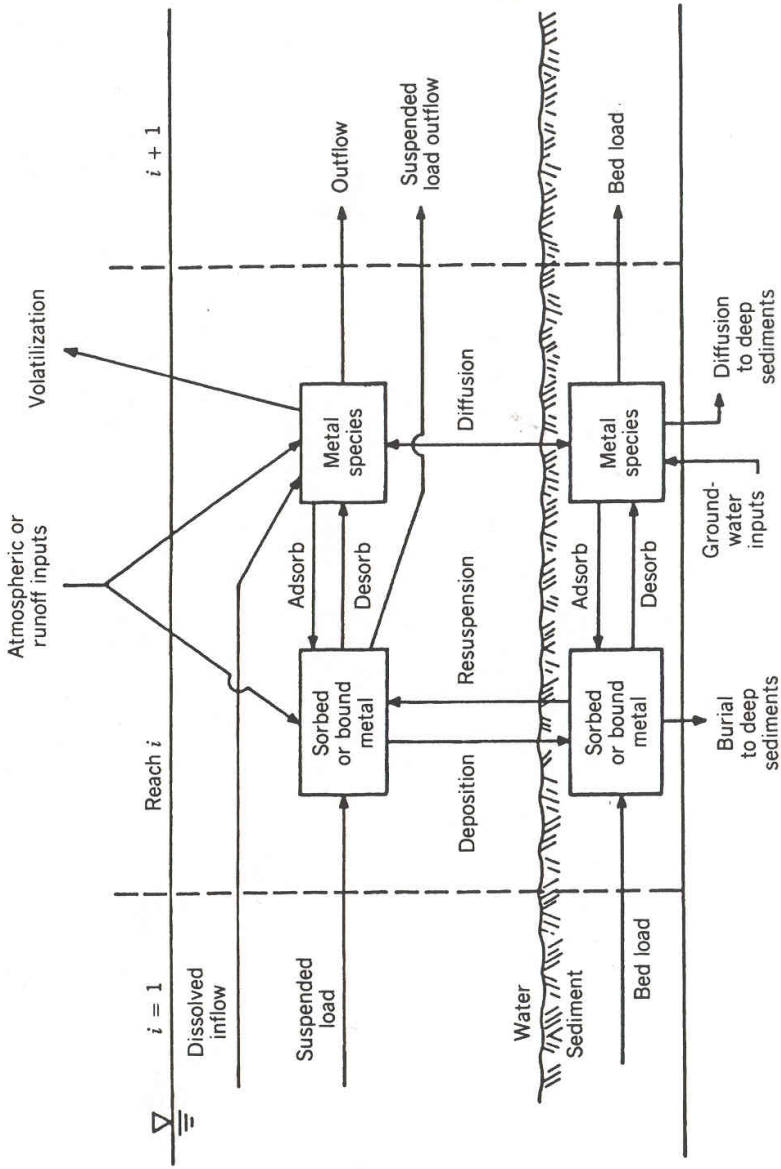


FIGURE 3-13 Schematic representation of metal transport in a stream or river showing suspended and bed load transport of dissolved and retained particulate material. Note that the suspended load contains particles of all size including colloids. SOURCE: Reprinted, with permission, from Schnoor (1996). © (1996) John Wiley and Sons, Inc.

occur across the entire floodplain near the mining district, and substantial contamination is deposited in floodplains more than 200 km downstream from the mining district (Moore and Luoma, 1990). Most of the contaminated sediment probably was moved onto the floodplains during a few floods. The implication is that, as the river cuts new banks, over centuries, it continually cuts into the contaminated sediments present in the floodplain, creating a downstream, secondary source of additional contamination. Contaminated floodplains thus add to the complexity of remediating contaminated rivers.

Sedimentation and burial are also important transport processes than can effect the bioavailability of sediment-bound contaminants. The rate of sedimentation is dependent on the particle size and density and on the physical-chemical conditions in the system that determine the rate and extent of particle aggregation. Whether particles that are deposited on the bed of the surface water undergo burial or are resuspended and moved downstream depends on the hydraulics of the surface water, the size and density of the particle, and the magnitude of the suspended particle load. Within a single water body there usually are locations where particles tend to settle and accumulate and locations in which particles reside in the sediments for only a short period of time. Connolly et al. (2000) describe sections of the Hudson River in which particle deposition and burial occur and sections in which particle resuspension is the norm.

Transport of Released Contaminants

Compared to our understanding of contaminant–solid interactions, our current understanding and ability to model contaminant transport in fluid phases (water, air, or soil gas) are fairly well advanced. Once contaminants are released to water, air, or soil gas, they are transported in those phases by the movement of the fluid, or advection. This is illustrated in Figure 3-14 for the mobilization of a contaminant from near-surface, unsaturated soil. Infiltrating water moves through the unsaturated zone to soil in which the pores are completely filled with water (i.e., the saturated zone). Input of the contaminated infiltration water to the saturated zone results in establishment of a contaminant concentration (C_o) in the volume directly beneath the contaminated soil. Groundwater flow in the saturated zone (from left to right as indicated in Figure 3-14) transports (advects) contaminant mass "downstream," resulting in a plume of contaminated groundwater emanating from beneath the site. A similar process occurs for any contaminant mass that is volatilized and moves up and out of the soil in soil gas. As shown in Figure 3-14, when this contaminant mass enters the air flowing over the contaminated soil area, it will be transported with the air in the prevailing wind direction—sometimes for long distances. The long-range transport and atmospheric deposition of PCB congeners, for example, has been found to add to the chemical burden of animals far from where the chemicals were used or disposed. Using butter as a sampling matrix to reflect global-scale distribution of PCBs and DDT,

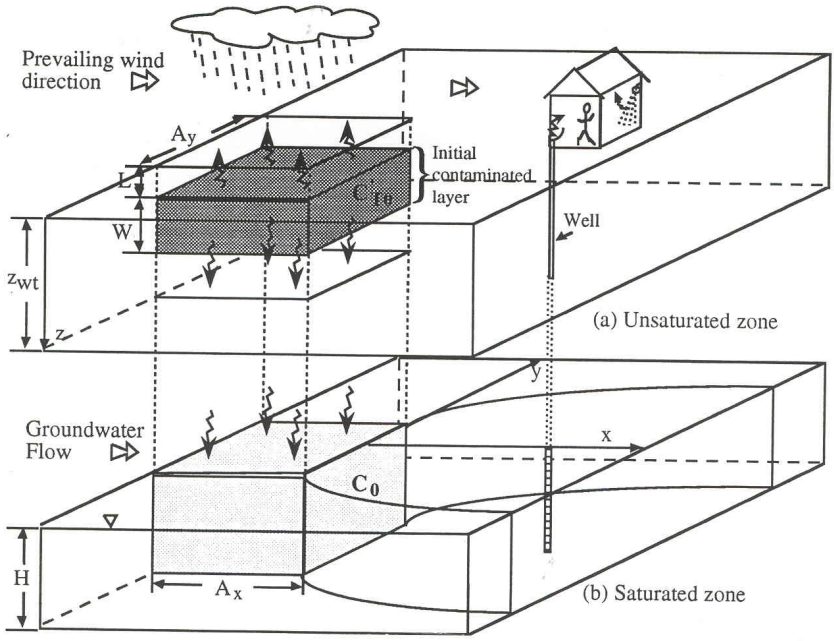


FIGURE 3-14 Schematic illustrating potential transport pathways for contamination in a soil layer at ground surface. SOURCE: Reprinted, with permission, from Labieniec et al. (1996). © (1996) Journal of Environmental Engineering.

Kalantzi et al. (2001) found PCBs in butter in remote areas, while the levels of DDT, which is not as volatile as PCBs, where highest in areas of current use. This illustrates the importance of accounting for bioavailability processes that operate both locally and remotely.

If the fluid into which the contaminant is released is not flowing or flowing only at very slow rates, such as groundwater in low permeability soil or porewater in fine-grained sediments, molecular diffusion will be the primary means of transport. An example is again provided in Figure 3-10, which shows the release of a biphenyl molecule into the porewater of a fine-grained sediment, and the diffusive transport of that molecule to the sediment–water interface. Subsequent transport of the molecule to flowing river water would result in advective transport of the molecule. Once in a flowing system, molecular diffusion and nonuniform velocities in the fluid cause mixing of the contaminant mass in the fluid volume, a process known as dispersion. Dispersion causes the contaminant mass to become distributed nonuniformly in a flowing fluid, even one that is moving in a uniform, steady state manner. Advective processes, including resuspension and

upwelling of groundwater currents, and bioturbation dominate over diffusion processes in more dynamic systems, with dramatic impacts on bioavailability.

Contaminants undergoing transport in water, air, or soil gas are subject to immobilization reactions and processes that result in the contaminant not being transported with the fluid indefinitely in its original state. Some important immobilization processes for transport in water include sorption on solids (such as aquifer material, river sediments, or settlable particles), precipitation, and physical entrapment in micropores or immobile zones; each of these has been discussed previously.

Transformation of Released Contaminants

As contaminants are being transported to receptors upon release from soils and sediments, they can undergo transformation of chemical form by means of various chemical and biochemical processes. These include biotransformation, oxidation–reduction reactions, reactions with water (hydrolysis and acid–base reactions), and photochemical transformation. These transformations, relevant and important for both inorganic and organic contaminants, can affect greatly the bioavailability and toxicity of the contaminant.

Many different chemical forms of a particular element can exist in aqueous systems. These different forms can have vastly different properties, affecting their reactivity, toxicity, and fate in the environment. Transformation processes fundamentally alter the chemical form of inorganic contaminants. Microorganisms can mediate the transformation of species of elements from one form to another, for example the transformation of dissolved Hg^{2+} to extremely toxic methylmercury (CH_3Hg^+) and the conversion of selenate to organoselenium, elemental selenium, and highly toxic methylated selenium. Varying chemical conditions can cause redox-active elements such as arsenic and selenium to change oxidation states, e.g., the oxidation of dissolved Cr^{3+} to the much more toxic CrO_4^{2-} form in which chromium exists in the +6 oxidation state. Many elements react with water; dissolved mercury hydrolyzes to form the hydroxy species $HgOH^+$, $Hg(OH)_2^o$, and $Hg(OH)_3^-$. These complexes dominate mercury speciation across a wide pH range and are sufficiently strong that they can inhibit mercury retention in soils and sediments (Dzombak and Morel, 1990). Photochemical reactions can also affect inorganic contaminants. For example, compounds of copper with organic molecules having carboxylate and amino functional groups are photoreactive (Morel and Hering, 1993). Light absorption by such compounds can result in their decomposition and subsequent redox transformation of the metal or the organic moiety.

Arsenic illustrates the potential complexity of inorganic contaminant transformations. Arsenic is typically in the pentavalent oxidation state in aerated environments, forming the arsenate oxyanion $H_xAsO_4^{x-3}$. Upon anaerobiosis, arsenic is reduced to the trivalent state that often forms the arsenite anion,

$H_xAsO_3^{x-3}$. As discussed in Box 3-3, arsenite is more toxic than arsenate, and conditions conducive to its formation (such as the reduction of ferric iron solids) tend to enhance the mobility of arsenic. If a sediment is reduced to the point of being sulfidic, arsenic may form soluble sulfur complexes (e.g., $H_2As_3S_6^{2-}$) or insoluble phases such as the mineral orpiment (As_2S_3).

Organic compounds can also undergo a wide range of biochemical, thermochemical, and photochemical transformations, resulting in wholly different compounds. PCBs provide good examples of the diversity of transformation processes. PCBs can undergo biotransformation under aerobic and anaerobic conditions, though the pathways and extent of these reactions are compound specific. Complete mineralization of less-chlorinated PCBs can be achieved by many aerobic organisms (Bedard, 1990; Furukawa, 1994). Di- and tri-chlorobiphenyls can be degraded by aerobic cometabolic processes using biphenyl or 4-monochlorobiphenyl as carbon and energy sources. More specialized microorganisms are capable of degrading tetra- and higher chlorinated biphenyls (Bopp, 1986). Formation of intermediates during PCB degradation is common, particularly chlorobenzoates, which may be more recalcitrant than the original PCB (Sylvestre et al., 1985; Seeger et al., 1997). Environmental conditions, including pH, affect the rate and extent of aerobic PCB biodegradation (Williams and May, 1997).

Under anaerobic conditions such as typically found in PCB-contaminated sediments, reductive dechlorination can occur resulting in an increase in less-chlorinated PCBs, that is, mono-, di-, and tri-chlorobiphenyls (Brown et al., 1987, 1988; Natarajan et al., 1996) and a decrease in the highly chlorinated (tri-, tetra-, and higher substituted) congeners (Mohn and Tiedje, 1992; Berkaw et al., 1996; Quensen and Tiedje, 1997). From these observations it has been inferred that reductive dehalogenation of PCBs can occur, although no axenic cultures of anaerobes reductively dehalogenating PCBs have been obtained so far (Wiegel and Wu, 2000). Different sediment systems appear to have different populations of dechlorinating organisms (Quensen et al., 1990; Sokol et al., 1994; Bedard and Quensen, 1995), and dechlorinating organisms show specific congener preferences (Rhee et al., 1993; Sokol et al., 1994). The less-chlorinated congeners of a PCB mixture are substrates for cometabolic transformation by organisms expressing biphenyl oxidation pathways (Fetzner and Lingens, 1998; Billingsley et al., 1999; Bruhlmann and Chen, 1999; Seah et al., 2001). Interestingly, the final congener distribution may vary widely from the parent PCB material due to combined aerobic and anaerobic transformations. Although the daughter material may exhibit reduced toxicity, it may have increased mobility due to the inverse relationship between chlorine substitution and aqueous solubility (Opperhuizen et al., 1988; Mackay et al., 1992).

PCBs are subject to other kinds of reactions that affect their fate and transport. The effective solubility of PCBs can be enhanced, for example, in aqueous systems with high dissolved natural organic matter (Brownawell and Farrington, 1985, 1986) or with significant quantities of miscible organic liquids. Conversely,

PCB solubility in water is diminished in systems with oil or other immiscible organic liquids (Luthy et al., 1997b). Hydrolysis or photolysis are only significant for PCBs under non-environmental conditions (e.g., Zhang and Hua, 2000).

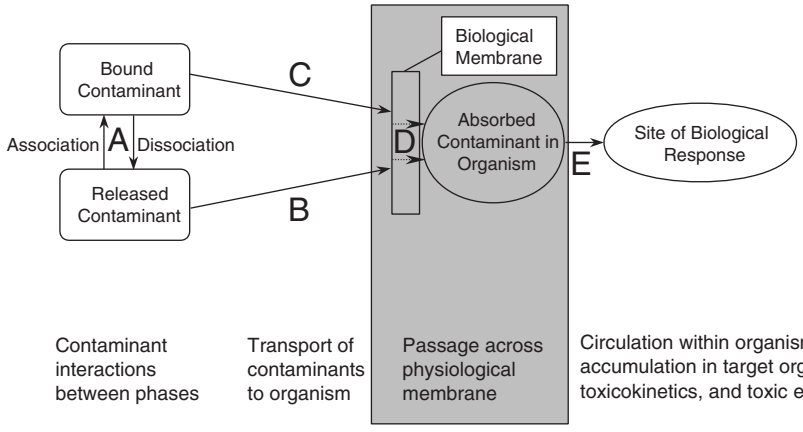
***

In summary, the chemical form of the contaminant released from soil and sediment, the geochemical environment where the release and transport take place, and the fluid properties of that environment will determine the form, delivery route, and delivery rate of contaminant to biological receptors. All of these factors must be considered in assessing the availability of a soil or sediment contaminant to biological receptors.

CONTACT AND ENTRY

The terms *contact* and *entry* are often used to describe how contaminants (typically in their released—i.e., dissolved or gaseous—state) interact with and pass through a biological membrane and into a cell. This section provides basic information on the mechanisms that cells employ to take up chemicals from the environment and how these mechanisms differ between tissues and organisms.

Because a range of receptors—microorganisms, plants, animals, and humans—and a range of exposure routes are of interest in contaminant bioavailability, it is difficult and perhaps dangerous to generalize the process of contact and entry. It is possible, however, to represent the processes conceptually, and to describe how an organism's physiology and the mode of contact can influence the extent of contact and entry that may occur. Contact and entry steps are highlighted by the grey box in the figure below.



Movement Across Cellular Membranes

The organization of biological systems depends, in part, on the presence of membranes that serve to separate biological compartments within an organism as well as separate the organism from the outside world. In order to be functional, biological membranes must allow some substances to move through them while resisting the passage of others.

The ability of membranes to serve as selective barriers is a function of their structure. Biological membranes are composed primarily of phospholipids arranged in a bilayer, with the hydrophobic portion of the molecules oriented toward the middle of the membrane and the hydrophilic portion toward the outside (Figure 3-15). Thus, the surface of the membrane, which interfaces with water, is hydrophilic, while the center of the membrane is lipid in nature. Proteins are embedded in the lipid bilayer membrane, some of which play a role in the movement of chemicals across the membrane, either by creating pores in the membrane through which small chemicals can move, or by serving as carriers. There are four fundamental processes by which chemicals can move across biological membranes, described below.

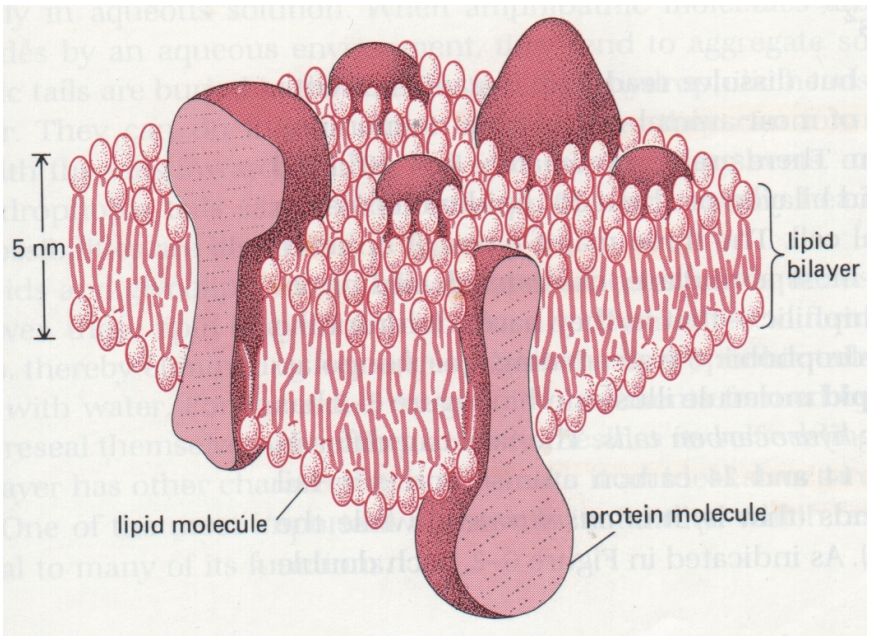


FIGURE 3-15 Basic structure of membranes. SOURCE: Reprinted, with permission, from Alberts et al. (1989). © (1989) Garland Science Publishing.

It is important to recognize that many organisms maintain other structures outside of the cell membrane that may influence the ability of a contaminant to reach the cellular membrane. For example, in some bacteria (excluding the *Mycoplasma*) a cell wall exists, and in most cases an external outer membrane or addutibak layers (e.g., S-layer, exopolymeric substance layer) are present. Both structures represent a potential barrier to contaminant uptake across the cell membrane.

Passive Diffusion

During passive diffusion, chemicals move across a membrane in the direction of their concentration gradient. Pores in the membrane offer one pathway for movement, but their size is usually small (< 4 nm), and they are consequently accessible only to molecules with molecular weights of a few hundred Daltons or less. Nonetheless, pores are an important means of passage for small hydrophilic molecules. Passive diffusion for larger molecules necessitates moving through the lipid membrane. The rate at which these chemicals cross a membrane by passive diffusion is determined by their lipid solubility and molecular size. Greater lipid solubility allows a chemical to penetrate the lipophilic core of the membrane more easily, and small lipophilic molecules are able to move through membranes by passive diffusion more quickly than large ones. The rate of movement of a chemical across a membrane increases as a function of the concentration gradient and, in terms of mass movement across a membrane, also with increasing surface area. Nonionic species (organic contaminants) typically diffuse through the cellular membrane, such that the microbial uptake of many hydrophobic solvents (e.g., alkanes, mono- and polynuclear aromatic hydrocarbons) is often a simple passive diffusion process (Bateman et al., 1986; Sikkema et al., 1995; Bugg et al., 2000). However, ionized groups on certain chemicals can greatly impede passive diffusion. Thus, for example, the diffusion of heavy metals across the membrane is typically limited. For weak acids and bases, the extent of ionization is controlled by pH, and pH is therefore an important determinant in the absorption of these chemicals.

Facilitated Diffusion

In facilitated diffusion, chemicals move in the direction of their concentration gradient (as with passive diffusion), but movement of the chemical across the membrane is assisted by carrier proteins. The chemical binds to the carrier protein and is carried through the membrane through a process that requires no cellular energy. There is some specificity to the carrier protein binding, and so this process is applicable only for selected chemicals. For example, transport of some essential metals across membranes may be facilitated by carriers or pores specific to the element (Nies and Silver, 1999). It also appears to be common that

metals are transported on carriers designed for elements of similar physicochemical characteristics (e.g., manganese and copper may share a carrier in some phytoplankton; Nies and Silver, 1999). For microbial uptake of heavy metals, the process involves diffusion across the outer wall through porins, and then facilitated diffusion across the cytoplasmic membrane via the relatively unspecific magnesium uptake system that involves a membrane integral protein and is driven solely by chemiosmotic gradients (Nies and Silver, 1999; Rensing and Rosen, 2000). One way to identify facilitated diffusion experimentally is to demonstrate that the influx rate can become saturated (i.e., demonstrate that a finite number of carriers exist).

Movement by passive or facilitated diffusion does not preclude cellular accumulation of contaminants (and other chemicals) to concentrations higher than in the external media. If the chemical is rapidly transformed by complexation (as is the case for many metals), conjugation, or conversion to a stable compound (e.g., selenium), then an inward diffusion gradient can be sustained. Equilibrium-based exchange between the converted form and the form crossing the membrane will ultimately determine the steady state concentration that the chemical will attain. Internal contaminant concentrations can reach levels 10^3–10^6 higher than in the external medium if robust transformation reactions occur.

Active Transport

Active transport uses carrier proteins to move chemicals *against* their concentration gradient, which requires cellular energy in the form of adenosine triphosphate (ATP) or a proton motive force. As with facilitated diffusion, there is specificity in the binding of chemicals to these carrier proteins. Active secretion of organic acids and bases by the kidneys, for example, utilizes membrane active transport processes. Physiologists use strict criteria to differentiate active transport from facilitated diffusion, primarily based upon energy dependence. Although the term "active transport" is occasionally employed for hazardous chemicals, little evidence exists that transport of any organic contaminant is energy dependent. Rather, passive or facilitated diffusion followed by transformation is sufficient to explain most organic contaminant uptake. However, for microbes there can be active *export* of certain contaminants. For example, several solvent-resistant bacterial strains exhibit an active efflux system for organic solvents to regulate their intracellular concentration (e.g., Kieboom et al., 1998; Bugg et al., 2000) because extensive accumulation of hydrophobic solvents can deteriorate a membrane's physicochemical properties. Similarly, because elevated extracellular metals concentrations necessarily result in elevated intracellular concentrations, many microbial cells have developed metal-ion homeostasis mechanisms, which often involves active (ATP or proton gradient-driven) heavy metal export (Nies and Silver, 1999).

Phagocytosis and Pinocytosis

Other processes also exist to bring substances across membranes and into cells. Large particles can be internalized into cells through phagocytosis, during which the plasma membrane of a cell surrounds and engulfs a particle that is outside the cell. The membrane closes around the particle, creating a vesicle that then detaches within the cell. Macrophages use phagocytosis to remove damaged tissue components, destroy microorganisms, and process antigens. Cells of the reticuloendothelial system also use phagocytosis to clear particulates from the blood. Pinocytosis is similar to phagocytosis, except that it involves surrounding and internalizing an external volume of fluid rather than a particle. Pinocytosis and phagocytosis are well known in mammals. Uptake of iron particles by phagocytosis has been demonstrated in marine mussels (*Mytilus edulis*) (George et al., 1978), but the quantitative importance of this specific process is difficult to demonstrate.

Animal Uptake

Three types of uptake into animals are discussed that correspond to the three pathways of direct exposure evaluated in risk assessment—direct ingestion, dermal contact, and inhalation.

Absorption from the Gastrointestinal Tract

Because the gastrointestinal tract is the principal site of nutrient uptake, it is a prime location for uptake of chemical contaminants as well. A colloid- or particle-bound contaminant can reside in the gastrointestinal tract for hours to days—plenty of time for the unique environment of the gut to affect particle–contaminant associations. Although the membrane transport processes described above are universal, digestive processes result in more complicated membrane transport phenomena than occur, for example, across the gill in aquatic organisms or across the skin in mammals. This complexity is illustrated by absorption of metals from the gastrointestinal tract. A prevailing assumption is that metals must be in a free ion form before they can be transported across a membrane. But in the gut this is not necessarily the case. The gut is designed to transport simple organic compounds as well as elements, such that absorption of contaminants can be facilitated by their association with specific amino acids. Within the organism classes discussed below, gut characteristics of different species vary greatly with regard to the types of enzymes present and their concentration, the presence of organic-rich fluids, pH, and redox potential.

Invertebrates. Invertebrate digestion is complex, with transport mechanisms in the gut receiving limited study. It is known, however, that invertebrates, like

bivalves, digest materials in the gastrointestinal tract both externally (in the intestinal lumen) and intracellularly (within cells that, presumably, engulf materials in the "digestive gland"). Intracellular digestion is more rigorous in that animals that employ this mechanism can take up metals otherwise predicted to be unavailable (Decho and Luoma, 1991). For example, marine bivalves with strong capabilities for intracellular digestion can assimilate insoluble Americium with about 30 percent efficiency (Luoma et al., 1992); they can assimilate otherwise unavailable Cr(III) from bacteria with about 90 percent efficiency (Decho and Luoma, 1996); and they appear to assimilate metals that are not in solution from algal cells (Wang et al., 1995, 1996; Schlekat et al., 2000). (The tool used to measure uptake in these cases—assimilation efficiency—is discussed in detail in Chapter 4.)

Compounds in gut fluids play a role in determining what contaminants are available for transport into the organism. In particular, high concentrations of amino acids (>1M) and surfactants can occur in the gut fluids (Mayer et al., 1997) and are very effective in solubilizing sediment-associated metals and organic contaminants (e.g., PAHs), respectively. Indeed, metal and PAH concentrations in the gut fluids of marine polychaete worms (*Arenicola marina*) can be orders of magnitude higher than predicted from seawater–solid partitioning (Mayer et al., 1996). The relationship between metals and amino acids in the invertebrate gut is particularly intriguing. Among 35 deposit- and suspension-feeding invertebrates, metal and amino acid concentrations differed widely and yet correlated strongly. Enrichment factors in the fluids also followed the Irving-Williams series, among metals, consistent with soft ligand complexation (Chen and Mayer, 1999). Metal-to-amino acid ratios in tissues and gut also agreed with each other to within one order of magnitude. Such results do not directly elucidate the transport mechanisms responsible for bringing the contaminant from the gut into the tissue, but the relationship between gut fluids and tissues suggests that transport of the amino acid-bound metals occurs.

For soil invertebrates, the relative importance of gut ingestion of contaminants vs. soil pore water as a source of exposure depends on the physical characteristics of the animal (soft or hard bodied) and the physiology of the gut. Soft-bodied animals such as earthworms and some insect larvae are thought to be exposed mainly by the soil pore water (Saxe et al., 2001; Scott-Fordsmand et al., 2002). Those covered with a hard cuticle or carapace (adult forms of many beetles, insects, and crustacea) are thought to be exposed more through food and soil ingestion routes (Smit et al., 1998). The physiology of soil invertebrate digestive systems also influences the bioavailability process of gut uptake of contaminants in soils. Because many sediment and soil invertebrates are related taxonomically, the discussion above provides insight into some of these processes.

As with sediment invertebrates, mechanisms of uptake in soil invertebrates are not fully understood, although there have been attempts to model *Eisenia andrei* body concentrations of cadmium, copper, lead, and zinc as a function of pH, metals, and soluble organic carbon (SOC) (Saxe et al., 2001). In this case, the

model assumed that metals soluble at bulk soil pH were available for dermal exposure, while gut exposure was estimated by determining the soil metal in solution near neutral pH. This was based on evidence that the optimal pH for earthworm enzymes associated with digestion is near neutrality (Merino-Trigo et al., 1999), and that pH in several earthworm species' guts is buffered near neutral pH (Michel and DeVillez, 1978; Doube, 1997). The model, which combines relevant soil chemistry characteristics with certain biological phenomena thought to influence metal bioavailability to earthworms, awaits further refinement and validation.

Mammals. Gastrointestinal absorption in higher species, and particularly in mammals, has been studied in much greater detail. While the stomach and even the oral cavity can be sites of absorption for a number of chemicals, most gastrointestinal absorption occurs in the intestine. The contents of the intestine are well mixed overall, but there is a layer of watery content adjacent to the intestinal wall that is relatively stationary. This layer, termed the unstirred water layer, is about 30–100 nm thick, and chemicals must diffuse through it to be absorbed. Between the unstirred water layer and the outer membrane of the epithelial cells lining the intestine (sometimes termed *enterocytes*) is another very thin layer, which forms a microacidic environment. This layer is significant for the absorption of weak acids and bases, because the pH here determines their extent of ionization and consequently their ease of passive diffusion across the apical or brush border membrane of the enterocyte.

Generally, chemicals can be absorbed from the intestine by either passing through or around the enterocytes, which comprise the intestinal villi that line the intestine (Figure 3-16). In order to pass through the cells, they must first cross the apical membrane. This can occur by passive diffusion, by carrier-mediated transport (active transport or facilitated diffusion), or by pinocytosis, depending upon the chemical. The chemical then passes through the basolateral membrane of the enterocyte, through the basement membrane, and into the subepithelial space of an individual villus called the *lamina propria*. Movement across the basolateral membrane can also occur by diffusion, transport, or pinocytosis. Enterocytes are connected by tight junctions, but these form an imperfect seal. Water and small molecules can move readily through channels between cells, cross the basement membrane, and reach the lamina propria.

Another means to bypass movement through endocytes is termed persorption. Enterocytes are rapidly and continuously produced, migrating from the base of the intestinal villi, where they are formed, to the tip of the villi. Once they reach the tip of the villi, they are sloughed off (Figure 3-17). During the sloughing, a temporary break in the junctions between enterocytes is formed. Large particles have been observed to enter the circulation through these breaks. Once a chemical reaches the *lamina propria*, it can enter the circulation by passing through the membrane of one of the numerous capillaries there (see Figure 3-16).

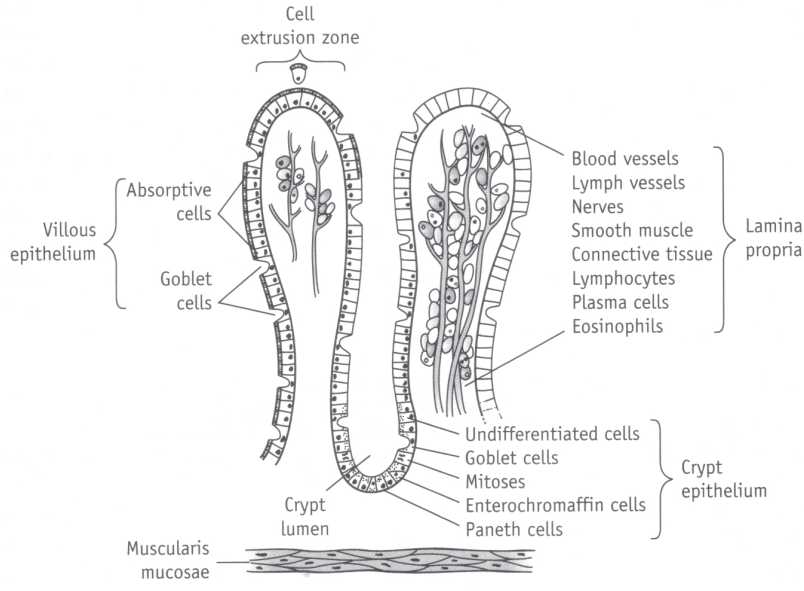


FIGURE 3-16 Structure of the intestinal villus. Individual cells of the villi, noted as absorptive cells here, are termed epithelial cells or enterocytes in the text. SOURCE: Reprinted, with permission, from Aranda-Michel and Giannella (1999). © (1999) Current Medicine, Inc.

Chemicals that cannot readily penetrate the capillary membrane enter the circulation by a more circuitous route through the lymphatics. For example, studies in both dogs and sheep have shown absorption of PCBs into intestinal lymphatic drainage following oral administration (Ziprin et al., 1980; Busbee et al., 1985). When flow from the intestinal lymphatics to the vascular circulation was interrupted by cannulation of the thoracic lymph duct, appearance of PCBs in the plasma following an oral dose was prevented (Busbee et al., 1985), indicating that virtually all of the PCB dose in the gut entered the bloodstream via the lymphatic route.

The gastrointestinal absorption of most environmental contaminants probably occurs by passive diffusion, but there appear to be many exceptions. Many inorganics are nutrients, and specialized transporters exist to regulate and facilitate their absorption from the gastrointestinal tract. For example, DMT1, a divalent metal transporter, is located in absorptive epithelial cells of the intestine. It has broad specificity, and has been shown to transport Fe^{+2}, Zn^{+2}, Mn^{+2}, and other ions (Canonne-Hergaux et al., 2000). Copper absorption in mammals is thought to involve active transport across the basolateral membrane (Linder, 1991). There is considerable evidence that the intestinal uptake of lead occurs

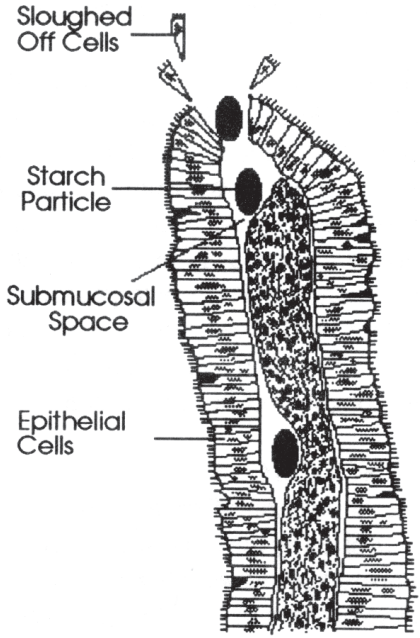


FIGURE 3-17 Persorption of particulates by the intestinal villus. SOURCE: Reprinted, with permission, from Wilson et al. (1989). © (1989) Ellis Horwood Ltd.

through a capacity-limited process, implying a transport mechanism. Competition for the transporter could explain the ability of a variety of substances to interfere with lead absorption, including iron, zinc, calcium, phosphorus, and magnesium (Conrad and Barton, 1978).

While it is often assumed that chemicals must exist in solution to be absorbed, there has been clear demonstration of the intestinal absorption of small particulates including colloids. Much of this research aimed to develop microparticulates as oral drug delivery systems. Using microspheres of varying size and composition, rapid uptake and distribution to the liver, spleen, and bone marrow have been reported (Jani et al., 1990; Mathiowitz et al., 1997). Evidence exists for at least four mechanisms of small particulate absorption: (1) persorption, described above; (2) endocytosis by enterocytes; (3) phagocytosis by intestinal macrophages; and (4) uptake by the M cells of the Peyer's patches[1] (O'Hagan, 1996). Persorption has been observed in a number of species, including humans, involving particles up to 100 μm. Other processes appear to be restricted to much smaller particulates, typically 1 μm or less. Observations suggest that uptake of microparticulates can occur both by passing through and around epithelial cells.

[1]Peyer's patches are areas of lymphoid tissue on the mucosal surface of the small intestine.

Particulates absorbed from the gut appear rapidly in the mesenteric lymphatics and are ultimately delivered to the portal circulation of the liver (Thomas et al., 1996; Mathiowitz et al., 1997). Particle size and composition, age of the animal, and dietary composition all appear to influence particulate uptake (Simon et al., 1994, 1997; Seifert et al., 1996; O'Hagan, 1996). Although gastrointestinal absorption of soil microparticulates has not been explicitly demonstrated, it is reasonable to suspect its occurrence. In a study of arsenic-bearing mine tailings that had been sieved to a small particle size (< 20 μm) and dosed to 12-day old mouse pups, arsenic was found primarily in the liver (Golub et al., 1999). This observation is consistent with uptake of soil microparticulates in the gut and delivery via lymphatics to the liver.

Absorption Through the Skin

In contrast to the gut and the lung, there is no mechanism for absorption of chemicals attached to soil or sediment particles through intact skin. Consequently, dermal absorption requires dissociation of the chemical from the soil or sediment matrix.

Mammalian skin is comprised of three layers. The outermost layer of skin is called the epidermis, which consists of the stratum corneum and the viable epidermis (see Figure 3-18). The stratum corneum overlies the viable epidermis and in humans consists of several layers of flattened, keratinized, dead cells called corneocytes. Corneocytes are stacked together like over-lapping plates and bound together by adherent structures (called corneodesmosomes). The water content of corneocytes is usually relatively low, particularly for cells near the surface, which may be only 15 percent water by weight. Spaces between the corneocytes are filled with intercellular lipid. The structure and composition of the stratum corneum make it an effective barrier, not only against escape of water from the body, but also against entry of microbes and chemicals.

Cells on the outermost surface of the stratum corneum last about two or three weeks before they are sloughed off and replaced by cells moving up from deeper layers. Stratum corneum cells originate from the underlying viable epidermis, which also contains pigment cells (melanocytes). The second layer, the dermis, lies beneath the epidermis and comprises most of the thickness of the skin. A network of connective tissue in the dermis gives the skin its strength and elasticity. Unlike the epidermis, the dermis contains an extensive vascular network, and some portion of a chemical that penetrates the epidermis can be absorbed into the circulation here. The third layer, the hypodermis, is below the dermis, and consists of a loose fibrous network and fat cells. The hypodermis is responsible for much of the insulating and mechanical cushioning properties of the skin. Like the dermis, this layer is extensively vascularized.

Hair follicles extend from the surface of the skin through the epidermis, with the base in the dermis or hypodermis. Sebaceous glands secrete sebum, a lipid

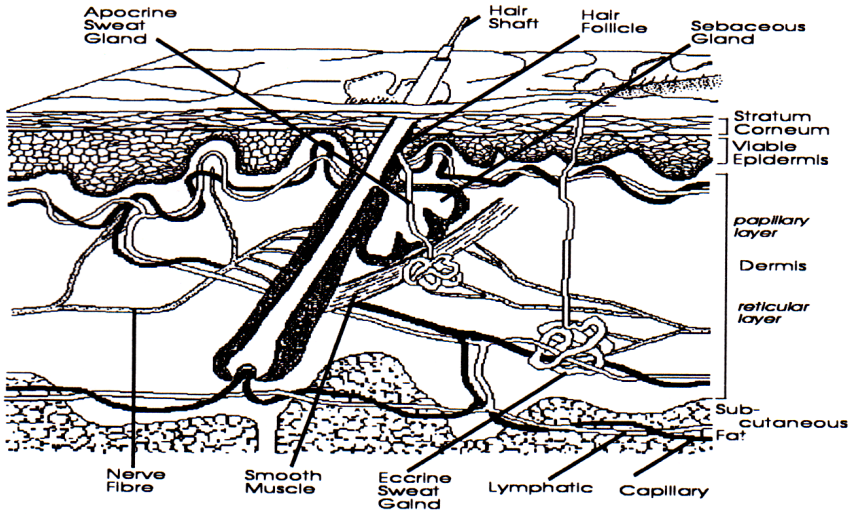


FIGURE 3-18 Structure of skin. SOURCE: Reprinted, with permission, from Washington and Washington (1989). © (1989) Ellis Horwood Ltd.

substance, into the hair follicle. There are two types of sweat glands, eccrine and apocrine. The more numerous eccrine sweat glands, located in the dermis, deliver an aqueous secretion directly to the skin surface through a coiled duct. Apocrine sweat glands are fewer but larger and secrete their fluid into the hair follicles.

The stratum corneum represents by far the greatest barrier to absorption of chemicals through the skin. Chemicals can traverse the stratum corneum by traveling through the corneocytes and interstitial spaces (called the transcellular route) or by traveling around the cells through the lipid-containing interstitial spaces (called the intercellular route). Lipid soluble chemicals are thought to favor the latter route, although this convoluted pathway greatly limits their rate of absorption. The transcellular route is generally envisioned as more suitable for water and hydrophilic chemicals, although some experimental evidence argues against separate routes for polar and nonpolar chemicals (Zatz, 1993).

Once a chemical has traversed the stratum corneum, the viable epidermis, the dermis, and the hypodermis offer little additional resistance to absorption. However, the high lipid content of the hypodermis can act to delay absorption of lipophilic chemicals. Lipophilic chemicals that are not readily taken up by the vasculature of the dermis and hypodermis may partition into the lipids, with the adipocytes serving as a reservoir of chemical that has permeated the skin, but not yet reached the circulation.

Hair follicles and eccrine sweat glands offer pathways for chemicals to reach the dermis and hypodermis without having to cross the stratum corneum. Within the hair follicles, the space surrounding the hair shaft is filled with sebum, through

which lipophilic compounds can presumably readily diffuse. The aqueous secretions of the sweat glands offer a pathway of entry for hydrophilic chemicals, although diffusion would have to occur against their direction of flow. Hair follicles and sweat glands, although offering means for chemicals to circumvent the stratum corneum barrier, have usually been regarded as minor pathways for dermal absorption because they comprise a very small percentage of the surface area of the skin. However, experiments using rat skin where hair follicles and sweat gland pathways have been eliminated suggest that, at least in some circumstances, their contribution to dermal absorption may be substantial (Zatz, 1993).

Several factors can influence the absorption of chemicals through the skin. One is the age of the individual. Neonates do not possess a fully developed stratum corneum, and thus chemicals can be absorbed more readily through their skin. Pre-term infants are particularly vulnerable. In the elderly, the stratum corneum becomes thickened and more dried, reducing dermal absorption. Another factor is the anatomical location of the skin. In general, permeability of skin follows the order: genitals > head > trunk > limbs (Zatz, 1993). Hydration of the stratum corneum can reduce its barrier function considerably, particularly with respect to hydrophilic compounds (Behl et al., 1980). Swelling of the corneocytes as their water content increases may disrupt the organization of the stratum corneum, increasing both the size and the hydrophilicity of the spaces between the cells. Similarly, disease and mechanical injury can disrupt or remove the stratum corneum, increasing the permeability of skin. Psoriasis, ichthyosis, inflammation, sunburn, and thermal burns all have been shown to increase skin permeability (Frost et al., 1968; Spruit, 1970; Behl et al., 1980). Stratum corneum disruption can also occur from chemical exposure. Contact with chemicals with surfactant properties or solvents in particular are associated with increases in skin permeability.

Absorption from the Respiratory Tract

Chemicals can enter the respiratory tract as gases, vapors, or particulates. Chemicals in gas or vapor form could arise through volatilization from contaminated soils or sediments. Inhalation of particulates is important when contaminated soils give rise to respirable dust.

When air is inhaled through the nose, it passes through the nasal turbinates. These ridge-like structures create turbulence in the air flow, causing large particulates to come in contact with the mucosal lining. Nasal mucous drains into the oral cavity where it is swallowed, carrying with it particulates trapped in the nasal cavity. Although absorption of airborne environmental contaminants directly from the nasal mucosa has not been well studied, it is apparent from observations (such as the carcinogenicity of inhaled formaldehyde in rodents) that significant absorption can occur there. The importance of nasal absorption probably varies with species because the structure and complexity of the nasal turbinates differ

substantially among species, with rodents, for example, having much more intricate structure than humans do. This offers both greater opportunity for deposition of particulates and a larger surface area for absorption. Also, rodents and many other species are obligate nose-breathers, whereas some portion of inspired air in humans enters through the mouth, bypassing the nasal mucosa.

From the nasal cavity or the mouth, air is conducted into the lungs through the larynx, trachea, bronchi, and non-respiratory bronchioles. These conducting airways are lined with epithelial cells and mucous-secreting cells. The upper airways contain numerous ciliated cells. Movement of the cilia assists in creating a flow of mucus up the airway toward the nasopharynx. Particulates coming in contact with the walls of the upper airways adhere to the mucus and are swept upward and eventually swallowed. In the bronchioles, the numbers of ciliated cells is greatly diminished. Clara cells are found in increasing numbers as the bronchioles become progressively smaller. Their function is not known with certainty, but they appear to be secretory. Pulmonary architecture of the lower respiratory tract varies somewhat with species, but in all cases the respiratory pathways terminate with small sac-like alveoli.

Most of the surface area of the alveoli (over 90 percent) is lined with flattened epithelial cells called Type 1 cells (Figure 3-19). The remainder of the surface area is occupied primarily by cuboidal Type II cells, which secrete a surfactant fluid. This surfactant fluid reduces surface tension in the alveoli, preventing their collapse. To facilitate exchange of oxygen, carbon dioxide, and other gases between the blood and the alveolar space, capillary circulation is

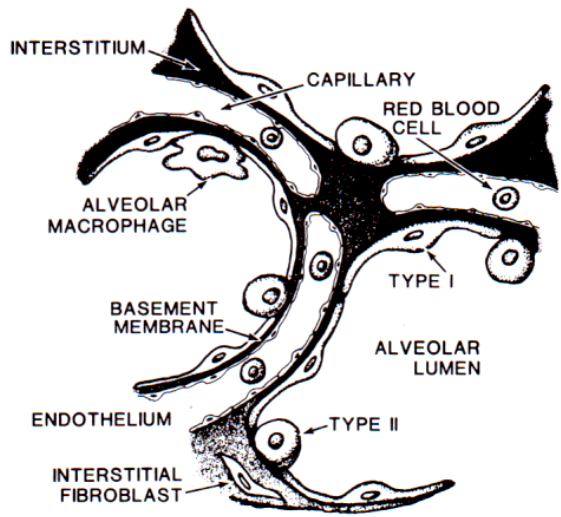


FIGURE 3-19 Structure of the alveolus. SOURCE: Reprinted, with permission, from Sabourin (1994). © (1994) Appleton & Lange.

quite close to the alveolar lumen. In fact, in some locations, membranes of the endothelial cells lining the capillaries have become fused with membranes of Type 1 cells lining the alveolus to form a thin basement membrane. This creates a very short diffusion distance for absorption from the alveoli, about 0.4 μm. Macrophages are found in the lumen of the alveoli, where they remove particulates and microorganisms by phagocytosis.

Potential sites of absorption of inhaled chemicals within the respiratory tract depend in part on the characteristics of the substance. Water soluble gases tend to dissolve into the mucus lining the upper airways and reach the lower airways and alveoli only when present in high concentrations in air. Lower solubility gases such as ozone reach the lower airways more readily. Because the structure of the alveolus favors rapid diffusion of gases between the alveolar space and the capillary blood, gases reaching the alveolus are usually readily absorbed. The rate of uptake of the gas into the blood will depend upon both its concentration in air and its solubility in blood.

The depth within the respiratory tract reached by inhaled aerosols and particulates depends upon the size of the particles, with smaller particles better able to remain suspended in air and reach the alveoli. Particles greater than 5 μm are usually deposited in the nasopharyngeal region. Particles deposited in mucus in the anterior portion of the nose may be removed by sneezing, nose-blowing, etc. Particles deposited more deeply in the nasopharyngeal region will follow the flow of mucus to the oral cavity and be swallowed. As such, the site of absorption for chemicals bound to these particulates may include both the nasal mucosa and the gastrointestinal tract. Particles between 2 and 5 μm will reach the tracho-bronchiolar region. The flow of air slows here, allowing particles in this size range to settle on the mucus-covered membranes. Trapped particles are carried by ciliary-assisted upward movement of mucus and are eventually swallowed. Particles that are 1 μm or less are able to reach the alveoli. There they may deposit and be carried by the flow of alveolar fluid up to the ciliated mucosa, and then transported up through the conducting airways and cleared as described above. They can also be phagocytized by alveolar macrophages, which are then cleared upward by mucociliary action and swallowed. Particles in the alveoli may be absorbed directly into the lymphatics because the endothelial cells lining the alveolar lymphatic capillaries are porous, allowing relatively large molecules to enter. Finally, partial or complete dissolution of the particle in the alveolus can result in absorption into the blood or lymphatics, primarily through passive diffusion. Aqueous membrane pores assist the movement of hydrophilic chemicals, with the rate of diffusion inversely proportional to molecular size.

Plant Uptake

In plants the most common route of exposure is through the roots. Ions and organic molecules contact roots via the transpiration stream, diffusive transport,

and microbially facilitated transport. Once at the root surface, soluble contaminants have the potential to enter into root tissue through the transpiration stream or through a range of mechanisms that are designed to facilitate nutrient uptake. In general, it is thought that only uncomplexed, free ionic species of cations and ions can be taken up by roots; this has been described using a free ion activity model (FIAM) (Lund, 1990; Parker and Pedler, 1997). However, exceptions to this model have been identified. Ionic or organo-metal complexes that increase the total concentration of elements at the root surface have been correlated with increased uptake, either through disassociated ions or through uptake of intact complexes (McLaughlin et al., 1994: Parker et al., 2001). In addition, it is not clear how well plants can distinguish between ions of similar size and charge. The size of solid particles precludes their entry into plant roots, even for very small particles like colloids, such that contaminant release from the solid phase is a prerequisite regardless of the underlying uptake mechanism.

Plant uptake of macronutrients is much better understood than uptake of micronutrients or contaminants, with the primary work on uptake of micronutrients focusing on iron (Welch, 1995). Different mechanisms have been identified that control macronutrient uptake by plants; these mechanisms may provide a means through which contaminants can enter root tissue (Figure 3-20). One mechanism (Figure 3-20A) involves altering pH through efflux of H^+ ions, which sets up an electrochemical gradient that facilitates transport of cations and anions. Such proton pumps often require cellular energy in the form of ATP. Ion channels (Figure 3-20B) also exist as a means of entry, although their role in uptake has been more clearly defined for plant shoot rather than root tissue. Ion channels are thought to facilitate uptake of divalent cations and to mediate uptake and release of K^+; when open they are capable of rapidly transporting ions. Specific channels have been identified for Ca^{2+}, K^+, H^+ and Cl^-. There is also evidence for carrier mediated active transport (Figure 3-20C) of K^+, SO_4^{2-}, NO_3^-, and Mg^{2+} that uses ATP as an energy source as well as specific binding sites. ATP driven pumps are located at both the plasma membrane and the tonoplast (Marschner, 1995).

With regard to micronutrients, there are chemical reduction mechanisms present at the plasma membrane to facilitate uptake of iron that may play a role in uptake of other cations (Welch, 1995). This is because the selectivity of many of these mechanisms is limited, so that ions or compounds of similar charge and radius may be indistinguishable from nutrients. For example, root exudates in iron-deficient barley and wheat plants are associated with increased uptake of zinc, copper, and manganese in addition to iron, although cadmium uptake is unaffected (Fan et al., 2001). Other examples where lack of selectivity has lead to increased contaminant uptake include mechanisms that gratuitously transport cadmium along with zinc, lead along with calcium, and selenate in addition to sulfate (Oliver et al., 1994; Huang and Cunningham, 1996; Feist and Parker, 2001). The factors controlling plant uptake of cadmium have been extensively

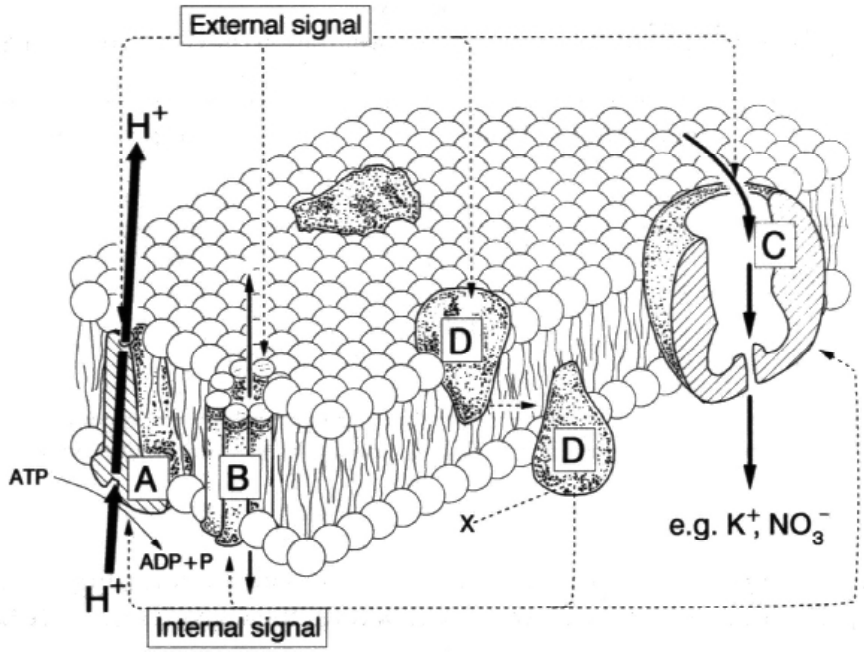


FIGURE 3-20 The primary mechanisms of ion transport across plant root membranes: (A) H^+ pump using ATP; (B) ion channel; (C) carrier facilitated transport; and (D) proteins for signal perception and transduction. SOURCE: Reprinted, with permission, from Marschner (1995). © (1995) Academic Press.

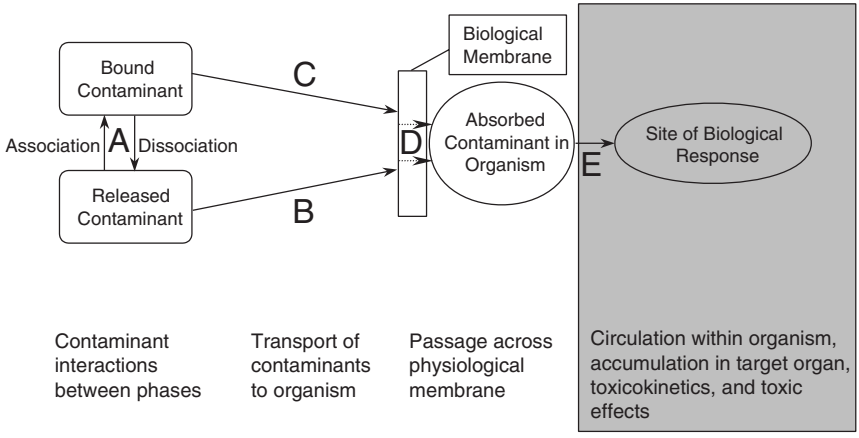
studied because consumption of plant tissue with elevated cadmium at levels below phytotoxic thresholds has resulted in human fatalities. Plant zinc concentrations, soil temperature and moisture status, soil solution chloride concentration, pH, total and extractable cadmium concentrations, and plant species and cultivar have all been found to affect plant uptake of cadmium (McLaughlin and Singh, 1999).

In general, less is known about specific uptake mechanisms for organic compounds, where the primary research has focused on herbicides. Although many herbicides' mode of action is through direct contact with leaf tissue, several are delivered to plant roots through soil and the transpiration stream. However, only smaller, more soluble organic compounds are able to enter root vascular tissue (Hsu et al., 1990). More lipophilic compounds enter plant tissue through diffusion into root cells (symplasmic pathway) (Little et al., 1994). The mode of action of many herbicides centers on the destruction of root cell membrane integrity (Devine et al., 1993; Holtum et al., 1994; Koo et al., 1997) and it is possible that this mechanism may result in root exposure to other xenobiotics as well.

ACCUMULATION AND EFFECTS

Although chemicals such as caustic agents can damage an organism simply by coming in contact with it, most chemicals exert their biological effects from within organisms. After contact and entry into an organism, chemicals interact with one or more cellular constituents to alter biological functionality. Because soil and sediment play no role at this stage, accumulation and subsequent effects are not considered bioavailability processes per se. However, they are influenced by other bioavailability processes and thus are indicators of bioavailability, they are frequently measured endpoints, and they are of great concern to some stakeholders.

The fate of a chemical once it enters the organism can be complex. Its binding to different constituents within the organism, the actions of various enzymes on the chemical, and the efficiency of excretion mechanisms can all profoundly influence the concentration and form of the chemical reaching its biological target. Since the magnitude and the nature of the effect will be determined in part by the form and concentration of the chemical at its active site(s), consideration of these factors is critical to an overall understanding of the health consequences of exposure to environmental contaminants. If concentrations of the chemical achieved at the biological targets are too low, or if the chemical has been converted to a form that no longer interacts with the target, no effect will be observed. On the other hand, exposure may lead to concentrations that are sufficiently high so as to be lethal. Between these extremes is the potential for nonlethal, yet deleterious effects such as reduced metabolic activity, impaired reproduction, and increased sensitivity to physical or chemical stresses. The events that act upon a chemical after contact and entry, the interaction of the chemical with its biological targets, and the consequences of those interactions, are represented by the gray box in the figure below.



Fate of Contaminants that Enter the Organism

Distribution, Accumulation, and Sequestration

The ability of a chemical to move about within an organism will depend, to some extent, on the same factors that influenced its uptake. Chemicals with attributes that allow them to readily diffuse across membranes will tend to be distributed widely in an organism. Chemicals with limited ability to cross membranes may be confined to localized areas unless carriers or transporters exist to facilitate their movement.

A chemical moves within an organism in either a free or bound form. Usually a chemical will spend much of its time in an organism bound to some other compound. In order to move in an aqueous environment such as blood or lymph, strongly lipophilic molecules must attach themselves to a water-soluble compound. For example, PCBs and organochlorine pesticides exist in the blood in association with lipoproteins (Kenaga, 1975; Lawton et al., 1985). Metal ions are also bound to proteins, and some proteins (e.g., metallothionein, transferrin, ceruloplasmin) seem to function primarily as transporters for certain metal ions (e.g., Scott and Bradwell, 1983). Even relatively hydrophilic organic chemicals can be bound to plasma proteins such as albumin. This binding is nearly always reversible, but nonetheless it affects where, how rapidly, and to what extent a chemical will distribute to different parts of the organism, and even how rapidly it will be eliminated.

Some chemicals tend to accumulate at target sites within an organism, creating storage sites or depots. If the affinity of the chemical for a storage site is high, as is the case for lipophilic chemicals and fatty tissue, this can lead to profound accumulation of the chemical. For example, the presence of comparatively high concentrations of lipophilic chemicals such as PCBs and organochlorine pesticides (e.g., DDT) in adipose tissue of numerous species has been well documented (Dix, 2001). Because lead may become substituted for calcium in bone, the skeleton is an important storage site for lead in the body, over time accounting for 95 percent of the lead body burden (Gordon et al., 2002). These storage sites can act as sinks by pulling chemicals away from biological target sites, thereby reducing the effects of the chemical. However, chemicals held within these storage sites are generally inaccessible to normal elimination mechanisms such as metabolism and excretion (discussed below), making them persistent in the body. Slow release of the chemical from these storage sites can result in protracted "exposure" within the body even when external exposure has been reduced or eliminated.

This accumulation of chemicals in biological tissues is called *bioaccumulation* (usually measured as a tissue concentration—mg/kg). The term encompasses both direct and indirect contaminant accumulation. That is, organisms can be exposed to contaminants directly from abiotic media—such as soil,

sediments, water, or air—or indirectly through their diet. Thus, aquatic organisms can bioaccumulate waterborne contaminants through their gills during respiration or by consuming contaminated prey (Farrington, 1991). *Bioconcentration* refers specifically to accumulation from direct exposure. When the ratio of body mass to surface area of an organism exposed to contaminants is small, as it is for many primary producers, bioconcentration of contaminants from environmental media is of primary importance. For organisms higher on the food chain that have a higher body mass to surface area ratio, there is a shift in the processes contributing to the body burden of contaminants from bioconcentration via direct contact to bioaccumulation via dietary intake.

The term sequestration is used when compounds are accumulated from the environment but are inactivated in the tissues of the plant or animal. These sequestered contaminants may become available at some point to organisms that eat the plant or animal in which the contaminants are sequestered. Plants often "store" metabolites or conjugates in vacuoles, which can be thought of as exterior to the cell's ongoing metabolic processes. While this initial process of compartmentalizing the contaminant (or its metabolites) is analogous to bioaccumulation in other organisms, further processing of the contaminant (or metabolite) is often observed with the eventual covalent binding of the contaminant into the lignin of the plant (Zenk, 1996; Hall, 2002; Susarla et al., 2002). Carbon in this form is not readily broken down or reused by the plant; thus, long-term sequestration results. In most instances, this incorporation of a contaminant into the lignin of the plant transforms the compound to a state in which it is no longer bioactive. Similarly, animals can bind both inorganic contaminants such as metals and organic compounds in such a way that the compounds are not available to interact with critical structural or functional biomolecules.

Metabolism and Biotransformation

Biotransformation processes are common to all forms of life. The term metabolism frequently is used to capture these processes. However, metabolism generally refers to the transformations of natural substrates necessary for life rather than the transformation of contaminants. Consequently, the term *xenobiotic* metabolism, while more cumbersome, is better suited to a discussion of metabolic reactions involving environmental contaminants. Technically, xenobiotic metabolism eliminates the contaminant from the body by converting it to a different chemical species. The products of these reactions are termed metabolites. From a toxicokinetic perspective, the contaminant has been eliminated as soon as it has been changed into the initial metabolite. In most instances, the toxic effect of a contaminant is inversely proportional to the extent of its metabolic detoxification and subsequent elimination. The more efficient the removal of the contaminant, the less of it that will be available at the site of toxic action.

Xenobiotic metabolic steps are largely enzymatic transformations that confer increased water solubility, which will afford easier elimination of the contaminant in urine or bile of animals or the translocation to leaf-tissues of plants. They can also change the configuration of the chemical such that its structural attributes responsible for toxicity (i.e., that allow it to interact with its biological target to produce an effect) are lost. Both processes—increasing the ease of excretion and decreasing the inherent biological activity of the chemical—contribute to its detoxification. On the other hand, xenobiotic metabolic transformation can convert some classes of contaminants into more active and toxic products—a type of reaction that has attracted considerable attention from toxicologists because it is crucial in a number of important types of toxicity. For example, most environmental contaminants designated as carcinogens are thought to produce cancer through conversion to toxic metabolites (Hietanen et al., 1997).

The processes by which plants and animals metabolize xenobiotics share similarities. In both cases, xenobiotic metabolism is divided into primary (Phase I) reactions and secondary (Phase II) synthesis (Grant, 1991). Primary metabolism refers to biotransformations that alter basic chemical structure. Examples of Phase I reactions include oxidations, reductions, and hydrolysis. A classic example of Phase I metabolism is the stepwise oxidation of the methyl group of toluene to benzyl alcohol, benzaldehyde, and benzoic acid (Williams, 1959).

Phase II metabolism is often referred to as conjugation. It involves modification of existing reactive functional groups by combining either the original or an altered molecule with sugars, amino acids, or other compounds. In keeping with the above example, this might include the conjugation of the Phase I product benzoic acid with glycine to form hippuric acid (i.e., benzoylglycine). The preferred types of conjugation reactions vary somewhat with species. In the preceding example, glycine conjugation with benzoic acid would be expected in most species except birds and reptiles, where ornithine conjugation would occur instead (Bridges et al., 1970). Because most of the conjugates are ionized at physiological pH, Phase II conjugation is usually successful in increasing the water solubility of the xenobiotic compound and hence its excretion via the kidneys or the bile. Also, particularly in the case of attachment of bulky groups such as glucuronic acid, Phase II reactions substantially change the overall structure of the chemical, which usually dramatically reduces the toxicity of the chemical (though there are exceptions).

In general, the metabolites formed by Phase II reactions are excreted rapidly and are not further metabolized. However, metabolites from Phase I reactions can either be excreted without further metabolism, undergo additional Phase I metabolism, or undergo Phase II metabolism. If the metabolite undergoes another Phase I metabolic reaction, the same options apply to its metabolite. As illustrated in the benzoic acid example above, Phase I reactions can occur as a series of metabolic steps. As a result, it is not uncommon for a single chemical entity to be converted to several metabolites, and literally dozens of metabolites have been

identified for some compounds. Although the presumed objective of these reactions is detoxification, the reality is that many of these intermediates may retain some biological activity, and, as mentioned above, may even be more toxic than the parent molecule. This compels consideration of not only the chemical itself, but also its metabolites when trying to understand mechanisms of toxicity.

Excretion

Excretion is the removal of a contaminant from the blood and its return to the external environment (Rozman and Klaassen, 2001). In contrast to metabolism, which is a chemical mechanism for eliminating the toxicant, excretion is a physical mechanism. The route and speed of excretion depend largely on the physicochemical properties of the contaminant. Substances may be excreted as parent compound, Phase I metabolites, or Phase II conjugates.

The major excretion route for most chemicals (especially low molecular weight, polar chemicals) is via the kidneys (Wilkinson, 2001). Water-soluble chemicals in the plasma not bound to proteins can appear in the urine through glomerular filtration. In theory, passive diffusion of chemicals from the plasma to the urine can also occur in the renal tubules, although this mechanism is probably a minor contributor to overall urinary excretion because concentration gradients typically favor reabsorption more than excretion (Wilkinson et al., 2001). (Organic acids and bases, which at certain pH values are significantly ionized in the urine and, thus "trapped", are exceptions.) Some chemicals may be substrates for the organic anion and cation transporters in the renal proximal tubules that actively secrete organic acids and bases into the urine. Depending upon the physicochemical properties of the toxicant or its metabolite (e.g., lipophilicity/hydrophilicity), some portion of a chemical that appears in the urine may be reabsorbed in the tubules through passive diffusion. Also, reabsorption of some small proteins filtered in the glomerulus occurs in the renal tubules; a chemical bound to one of these proteins can escape excretion by being reabsorbed along with the protein. An often-noted example of protein-bound reabsorption is the small protein metallothionein, which carries bound cadmium with it from the tubular lumen into proximal tubular cells, where the cadmium produces toxicity (Dorian et al., 1992).

Excretion in the feces is a second key pathway for elimination of toxicants; it is generally more complex and less well understood than urinary excretion. Some ingested xenobiotics pass through the gut unabsorbed, especially metals. Other materials are transported from the liver into the bile and excreted into the gut (i.e., biliary excretion). In such cases if the chemical is not reabsorbed from the gut, it is eliminated with the feces. Biliary excretion is the major contributing pathway to fecal excretion (Wilkinson, 2001). This reflects the liver's ability to extract, transform, and eliminate orally ingested toxicants prior to systemic distribution.

The mechanisms of xenobiotic transport from plasma to hepatocyte and hepatocyte to bile are largely unknown, with no less than four transport systems having been identified (McKinney and Hosford, 1992; Takikawa, 1995).

For several xenobiotics (e.g., dinitrobenzamide and hexachlorobenzene) neither intestinal non-absorption nor biliary excretion can explain the concentration of toxicant found in the feces. In these cases direct passive diffusion from the blood has been proposed as the mechanism for fecal excretion (Dayton et al., 1983). Thus, fecal excretion is an important route of excretion, especially for high molecular weight chemicals and their conjugated metabolites found in bile.

Volatile chemicals such as solvents and metabolites may be eliminated from the lungs in expired air (Feingold, 1977). This is thought to occur simply through passive diffusion from alveolar capillaries into the alveolar space. Chemicals can also escape the body by excretion into sweat, hair, nails, and saliva (Wilkinson, 2001). These routes of excretion are typically insignificant from the standpoint of mass excreted but sometimes form the basis for tests to indicate exposure (e.g., the measurement of arsenic in hair and fingernails; the measurement of pesticides in saliva).

Excretion of chemicals into breast milk is important, not only as a means of elimination of the chemical, but also as a source of exposure for the nursing young. The percent fat content of milk varies with species, but is often substantial, allowing lipophilic chemicals such as PCBs, DDT, and dioxins to be carried from the mother to the infant (e.g., Cavaliere et al., 1997; Czaja et al., 2001). Metals, such as lead, and pesticides have also been detected in milk.

In plants, the term *excretion* is not typically used to describe the loss of contaminants or their metabolic products. However, various processes take place that result in the elimination of these materials from a plant. Volatilization through the stomata is important for volatile compounds (Schonherr and Riederer, 1989; Kesselmeier, 1992). Chemicals that are translocated to leaves will be lost during shedding (Ernst et al., 1992).

Effects of Contaminants after Entry

Changes to Cellular Activity

Contaminants affect cells adversely by one of the following means: cellular dysfunction or impairment of internal or external cellular maintenance, and inappropriate repair. These in turn can lead to altered cell function, mutation, or death.

Cellular Dysfunction. The reaction of a contaminant at the molecular site of action may result in impaired cellular function. The type of cellular dysfunction caused by the contaminant depends on the role of the affected target molecule. If the target molecule is involved in cellular regulation, then dysregulation of gene

expression and/or dysregulation of momentary cellular activity will occur. However, if the target molecule is involved predominately in the cell's internal maintenance, then the resultant dysfunction potentially impacts cell survivability. In addition, reactions of a contaminant with targets that serve external functions influence the processes of other cells and thus the organ or organ system.

Although contaminants can induce a variety of cellular dysfunctions, among the more important is dysregulation of gene expression. Dysregulation of gene expression may occur at elements that are directly responsible for transcription, at components of the signal transduction pathway, and at the synthesis, storage, or release of the signaling molecules. For example, transcription of genetic information from DNA to mRNA is controlled largely by interplay between transcription factors and the regulatory or promoter region of genes. While a variety of natural compounds, (e.g., hormones, vitamins) influence gene expression, some contaminants mimic these natural ligands.

An interesting example is the disruption of estrogenic activity (Kavlock, 1999; Taylor and Harrison, 1999). A number of classes of environmental contaminants, including the hydroxylated metabolites of PCBs, are known to be estrogenic (Waller et al., 1996) in that their structure resembles the natural ligand 17ß-estradiol (Shi et al., 2001). Many PCB congeners are metabolized *in vivo* to more polar compounds that can further disrupt normal estrogen system activity (Bergman et al., 1994; Koga et al., 1992; Schultz et al., 1998). The net result can be inappropriate cell division, apoptosis, or altered protein synthesis.

Disruption of Cellular Maintenance. All cells must synthesize endogenous molecules; assemble macromolecular complexes, membranes, and cell organelles; maintain the intracellular environment; and produce energy. Contaminants that disrupt these functions impact survivability. Because both impairment of oxidative phosphorylation and a sustained rise of cytoplasmic Ca^{2+} have consequences that are detrimental to cell survivability, these events are regarded as common ultimate mechanisms for lethal cellular toxicity.

Synthesis of ATP is a complex, multi-step process consisting of hydrogen and oxygen delivery to the electron transport chain, electron transport itself, and ADP phosphorylation. Alteration in any step (e.g., uncoupling of oxidative phosphorylation) will result in impaired synthesis. The impairment of oxidative phosphorylation is detrimental to organisms not only because of the depletion of ATP but also because the failure of ADP to rephosphorylate results in an accumulation of ADP and other breakdown products. Among the better-known soil contaminants that disrupt oxidative phosphorylation are phenols with multiple halo moieties (e.g., pentachlorophenol) (Stockdale and Selwyn, 1971). In eukaryotes, these toxicants act at the level of the mitochondrial membrane by inhibiting the coupling between the electron-transport chain and phosphorylation reactions without affecting the respiratory chain (Mitchell, 1966; McLaughlin and Dilger, 1980; Terada, 1981).

Contaminants may induce elevation of cytoplasmic Ca^{2+} levels by promoting Ca^{2+} influx into or inhibiting Ca^{2+} efflux from the cytoplasm. Sustained elevation of intracellular Ca^{2+} can result in depletion of energy reserves, dysfunction of microfilaments, and activation of hydrolytic enzymes. Other cellular mechanisms that cause death include direct damage to membranes, destruction of the cytoskeleton, and disruption of protein synthesis. Moreover, contaminants also may interfere with cells that are specialized to provide support to other cells and tissues; contaminants acting on the liver demonstrate this type of hazard.

Inappropriate Repair. Repair occurs at the molecular, cellular, or tissue level of organization, with molecular repair involving proteins, lipids, or DNA. An example of contaminants disrupting molecular repair are those that oxidize protein thiols to protein disulfides, protein-glutathione mixed disulfides, and protein sulfenic acids (Caldwell and Mills, 2000). Thiol groups are essential for the function of numerous proteins. At a higher level, the active removal of damaged cells (apoptosis or programmed cell death) can be disrupted by chemical contaminants. PAHs have been demonstrated to induce apoptosis in several cell types (Burchiel and Luster, 2001; Yoshii et al., 2001; Tithof et al., 2002).

Resulting Impairments

If cell function is altered, the cell is present and viable but no longer performs as it should to maintain the normal physiology of the organism. The consequences of this depend upon the cell type affected and how it is affected. For example, altered function in immunocytes could lead to immune system compromise and increased susceptibility to infectious disease, or it could lead to a hyperresponsive immune system and autoimmune disease. Usually, cell function is restored if exposure to the chemical is removed.

Cells that have undergone mutation may express a different phenotype. The principal concern is mutation leading to uncontrolled growth of cells. The resulting benign or malignant neoplasms can produce morbidity and mortality, usually by interfering with the function of other cells. In some situations, the progression of a mutated cell to a malignant cell can be influenced by the continued presence of the chemical. However, once a malignant transformation has taken place, it cannot be reversed by removing exposure to the chemical. PAHs are noted for their genotoxic and tumor-initiating effects (Upham et al., 1998; Rummel et al., 1999).

Cell death results in loss of cell function, the consequences of which will depend upon the number of cells affected and their function. The difference between this and "altered" cell function, other than perhaps the severity of effects, is in the prognosis for recovery if exposure is terminated. If cell death occurs in a situation where repair is rapid and complete, recovery may be complete. On the other hand, if replacement of the dead cells is slow or incomplete,

the tissue may undergo changes in its architecture that result in lasting impairment. The liver provides an example of both situations. Acute poisoning with the drug acetaminophen can destroy a significant percentage of liver cells. However, provided the individual can survive the toxic insult, the dead cells are usually replaced within a short time with no apparent lasting consequences. With chronic liver injury from alcohol and other agents, dead cells are often replaced with fibrous tissue, leaving scars in the liver. With the accumulation of these connective tissue scars, the number of viable cells is diminished and their normal arrangement in the tissue is distorted. Over time, the liver will begin to fail irreversibly. Some tissues, such as the nervous system, characteristically have limited ability to replace dead cells. In these situations, effects can persist long after exposure has been eliminated.

At the organism level, the impairment of cellular activities can lead to acute or chronic effects. Acute effects occur rapidly (within a few hours or days) and are relatively severe. The most common acute organism effect is lethality; other acute effects include weight loss, lethargy, behavioral modifications, and general morbidity. Chronic effects may be lethal or sublethal, and they sometimes alter growth, reproduction, or both. PAHs may induce pathologic changes in the blood vessel wall, including endothelial cell injury—an event that is critical in the pathogenesis of vascular disease (Sbarbati et al., 1991). Moreover, benzo[a]pyrene has been shown to be a promoter of atherosclerosis in animal models (Penn and Snyder, 1988). Other chronic effects include behavioral changes.

HIGHER ORDER PROCESSES

The physical, (bio)geochemical and biochemical processes described above are commonly considered the dominant forces influencing bioavailability. But biological processes operating at the level of the whole organism can also be important, both directly and indirectly, in determining exposure to a contaminant—particularly for ecological risk assessment. If simple uptake from solution were assumed to be the only important route of exposure to contaminants, then biological differences among species might have a relatively small effect on contaminant bioavailability. However, when differences in how species interact with soils and sediments, how they feed, and food web structure are added to the considerations, the biological and ecological attributes of the organism become increasingly significant. Thus the conceptual model guiding exposure assessments must include not only first-order geochemical and biological principles but appreciation of higher order biological and ecological processes as well.

In order to capture processes important for higher order organisms, Figure 1-1 can be made more detailed to show food web transfer of contaminants from prey to predators and other higher order organisms (see Figure 3-21). In fact, food chain transfer is probably a more important exposure pathway to contaminants in soils and sediment for higher order animals than is direct ingestion of the soil or

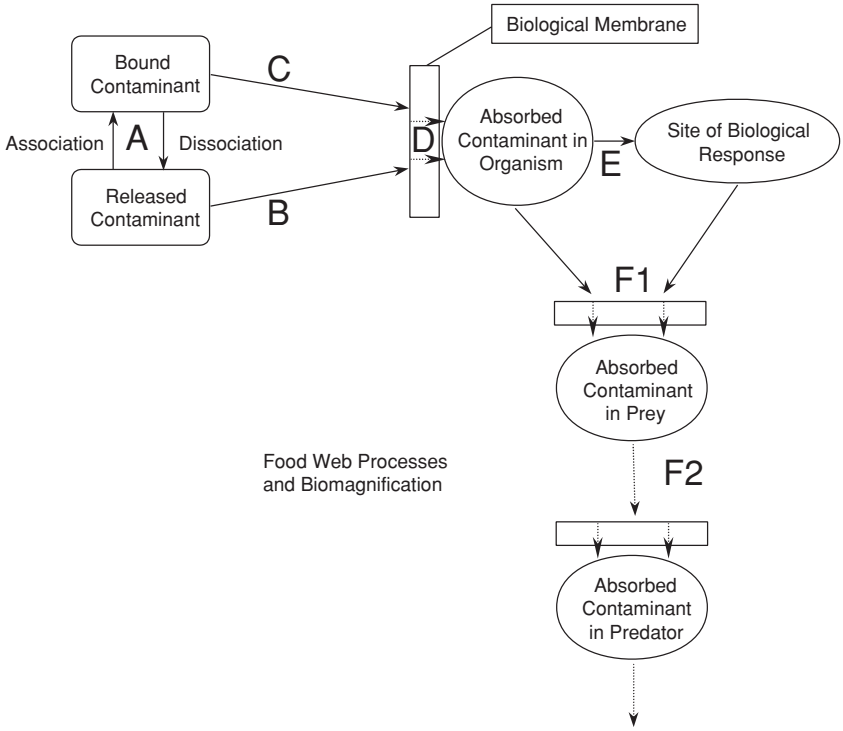


FIGURE 3-21 Bioavailability processes in soil or sediment, focusing on those between prey and predator that affect higher-order animals (denoted F1 and F2).

sediment. Figure 3-21 captures some additional processes that control the bioavailability of contaminants in soils and sediment to higher order animals, in particular the extent of contaminant uptake through the biological membranes of each successive organism and the resulting bioaccumulation in each organism. If there is sufficient biomagnification through the food web, higher organisms can be exposed to contaminants that originated in soils and sediments at concentrations high enough to cause adverse effects. Issues such as feeding ecology, food chain transfer, and biomagnification that control contaminant bioavailability to higher-order organisms are discussed below. Given the wide areal range over which exposure can occur to some higher-order animals, these processes may spread contamination far from its initial release site.

Feeding Ecology

Studies with invertebrates provide examples of some of the feeding ecology processes that can be important to bioavailability. As discussed in Chapter 2, the

bioavailability of certain metals in sediment is partially controlled by the presence of sulfides that bind to these metals and take them out of solution. Indeed, the AVS method for determining metal bioavailability in sediments is based on this reaction. However, benthic species (which are in continual contact with sediments and are often an ecological receptor of concern) obtain oxygen and nutrients differently from sediments and are exposed to different microenvironments. Some oligochaetes feed "head-down" in reduced sediments and "breathe" by periodically returning to the oxidized surface of the sediments (G. Lopez, SUNY Stony Brook, personal communication). These organisms would be predominantly exposed to sulfide-rich, reduced sediments, and would be directly impacted by the influences of sulfides on metal bioavailability. In contrast, most meiofauna are restricted to oxidized layers of sediments where metals sulfides occur in low concentrations. Sulfides are much less likely to be a consideration in this microenvironment. Many macrofauna bury into the reduced layers of sediments, but use tubes or burrows to feed and obtain oxygen from the oxidized sediment surface. The influences of sulfides are probably limited for such species. These differences have been borne out in experiments by Hare et al. (1994) and Warren et al. (1998) that showed how different lake benthos responded to cadmium-contaminated sediments.

Generically, different species ingest different foods from sediments, and feeding can change within species in response to their environment or life stage. The availability of food is also an important factor controlling exposure to contaminants. Lee and Luoma (1998) found that as benthic microalgae were added to sediments, the uptake of cadmium, zinc, and chromium to bivalves increased because the living fraction of the sediment material had grown and more algae were being ingested. Similarly, organisms may select to ingest only specific types of particulate material within a sediment, which can bias uptake towards certain geochemical forms of metals. For example, Luoma and Jenne (1977) and Harvey and Luoma (1985) showed that bivalve uptake of cobalt, cadmium, zinc, and silver from ingested sediments varied by 10-fold or more depending on whether the metals fed to the clams were bound to iron oxides, manganese oxides, detritus, carbonates, or organic coated iron oxides. All of these forms can occur in natural sediments (Jenne, 1977).

Similar processes also affect exposure of soil invertebrates to contamination. The feeding ecology of soil invertebrates concerns where the animal is located within the soil as well as the extent to which it will engulf soil particles as part of its diet. Earthworms in particular are represented by groups that live and feed at the surface (epigeic), that live in soil burrows but feed at the surface (anecic), and that live and feed below the surface (endogenic). Most studies have worked with anecic or epigeic species because they are thought to be more abundant and important in food webs (Diercxsens et al., 1985).

For those soil invertebrates that ingest particles, selective feeding on particular fractions (e.g., particle size and soil type) may be an important bioavailability

Invertebrates are an integral part of soil and sediment food chains and are the frequent target of bioavailability measurement tools.

process. Selective feeding has been found to affect the composition of material within invertebrate digestive systems compared to the surrounding soil (Edwards, 1997). For example, Diercxsens et al. (1985) found that PCBs were enriched in earthworm gut contents as compared to the surrounding soil, probably because the worms were ingesting the more organically rich soil components to which PCBs are more strongly associated.

From the above it is clear that although geochemical processes may have broad effects relevant to bioavailability, biological processes determine how each organism is exposed to that geochemical milieu, and substantial differences in that exposure are possible among species and among contaminants. It is not practical to understand all biological factors for all species in the near term (e.g., contaminant assimilation from all combinations of food sources available to all benthos). But understanding, for example, assimilation of the most common food items, and generalizing about how biology and ecology affect exposures for key species, may be necessary for reliable exposure assessments. Such understanding could also be critical in evaluating whether some species might be more vulner-

able to contaminants than others because of the way that they experience their environment.

Food Web Concepts

Bioavailability processes vary greatly between predators, prey, and degraders within an ecosystem (Kim et al., 2002). As mentioned earlier, organisms can be exposed to contaminants either from soil, sediments, water, or air, or through their diet. Invertebrates that bioconcentrate PCBs from sediment can be eaten by other wildlife, allowing the compounds to bioaccumulate in their tissues. Eventually, an entire food chain, which refers to the sequential feeding of a series of organisms, can be affected (Hebert et al., 2000). *Biomagnification* refers to the process by which tissue concentrations of bioaccumulating contaminants increase via the food chain as they pass from one trophic level to the next. Biomagnification results in exposure to higher contaminant levels in top predators of some ecosystems and, consequently, greater bioavailability (Fisk et al., 2001). Thus, important bioavailability processes are not limited to exposure to contaminants at the first trophic level; higher-order food transfers can be extremely relevant.

The susceptibility of compounds to bioconcentration, bioaccumulation, or biomagnification is a characteristic of the food web, the compound of concern, and the status of the system in terms of steady state. Biomagnification is generally observed for nonpolar or lipophilic contaminants that have low solubility, high log K_{ow}, and are recalcitrant in the environment and in the organism (Fraser et al., 2002). Biomagnification is generally not as great a concern for metals, except for those which biotransform to organic forms that are toxic (e.g., tin, selenium, mercury, and plutonium).

The food web concept defines interactions of interrelated food chains and takes into account species participation in multiple food chains over different trophic levels (see Figure 3-22) (Sharpe and Mackay, 2000; Fisk et al., 2001). Food web models can be used to elucidate the presence or potential for contaminant bioconcentration, bioaccumulation, and biomagnification. This can be done by direct measurements from within the food web or, alternatively, it can be predicted by utilizing empirical data (e.g., BSAF data—see Chapter 2) in conjunction with food web models. For each of these methods uncertainties can be minimized by reducing the length of pathways along which predictions are to be made (Fisk et al., 2001).

Even in instances where the food web concept coupled with a predictive model accurately assess compound bioconcentration, bioaccumulation, and biomagnification, the limitations of such predictions must be understood. Different organisms within the same food web can have vastly different toxic responses to a given chemical (Russell et al., 1999). For example, aquatic emergent insects display a low level of sensitivity to PCBs, while higher organisms within the

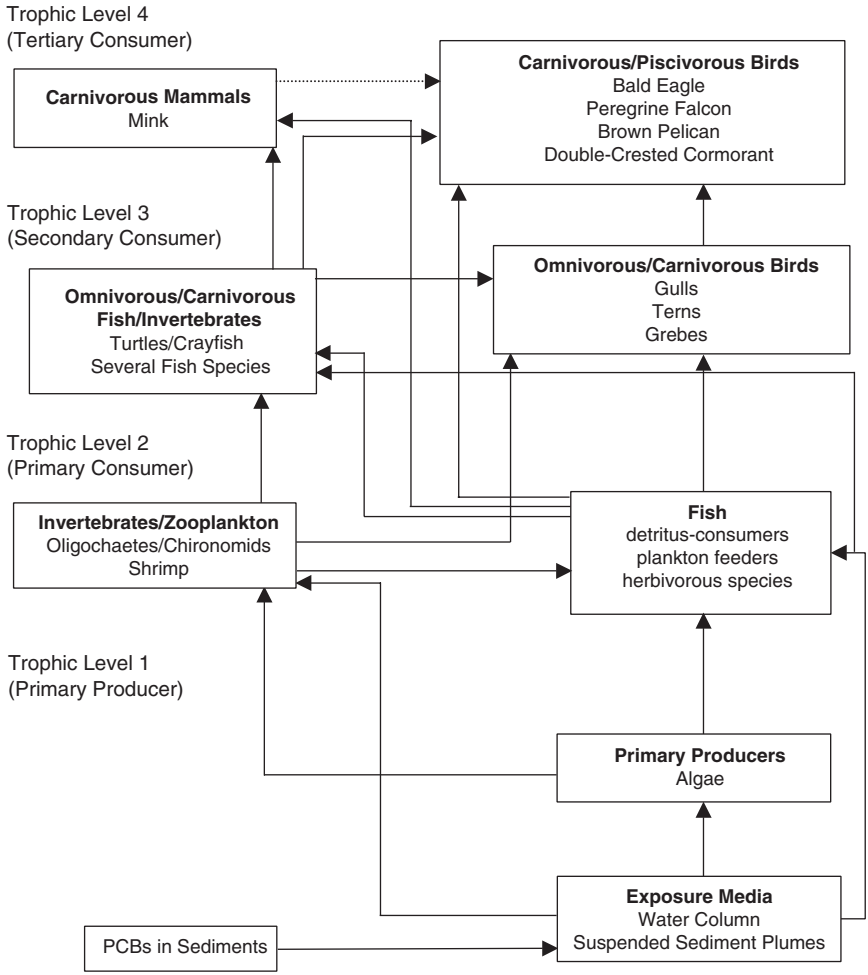


FIGURE 3-22 Food web with examples of representative species. This figure illustrates the complexity of determining whether contaminants are bioavailable to higher-order (trophic level 2 or greater) organisms. The solid lines represent primary pathways of exposure by predators consuming prey, while dotted lines represent possible exposure routes that are less likely.

same food web such as mink and bald eagles are considered highly sensitive (Olsson et al., 2000). A second limitation is that the toxicity of individual components of contaminant mixtures can be vastly different. Finally, even when the total contaminant concentration is predictable, the relative concentrations of individual components may change due to processes such as weathering, bio-

accumulation, and metabolic processes as the chemicals move from one trophic level to the next (Fisk et al., 2001). This is described in Box 3-4 for the case of PCBs, in which certain congeners are more or less bioavailable depending on what trophic level is being considered.

BOX 3-4
Bioavailability of Different PCB Congeners Up the Food Chain

One of the major concerns about predictive food web models is their ability to describe the movement of complex mixtures across trophic levels. Contaminant mixtures such as PCBs can contain between 60 and 85 different congeners with different chemical-physical characteristics. Environmental weathering changes the relative concentrations of PCB congeners due to differential solubilities, volatilities, and sorption coefficients (Mackay et al., 1983). In addition, metabolism by microorganisms (Bedard, 1990) and animals (MacFarland and Clarke, 1989) can cause relative proportions of some congeners to increase while others decrease (Boon and Eijgenraam, 1988; Borlakoglu and Walker, 1989). The resulting degree and position of chlorine substitution on the biphenyl rings influence not only the physicochemical properties but also toxic effects (Williams and Giesy, 1992; Quensen et al., 1998).

When concentrations of individual PCB congeners and total PCB concentrations were examined in the sediments of Saginaw Bay and after bioaccumulation into different animals, it was found that the absolute and relative tissue concentrations of individual congeners change as a function of trophic level (Froese et al., 1998). Individual PCB congeners and total PCBs were measured in sediments, emergent aquatic insects (primarily *Chironomidae*), and eggs and nestlings of tree swallows (*Tachycineta bicolor*). First, average lipid-normalized PCB_{total} concentrations were not different among the invertebrates, eggs, or nestlings. The average organic carbon-normalized PCB_{total} in sediments was about an order of magnitude less than tissue values. This suggests that there is no net biomagnification of PCBs at these trophic levels. Furthermore, this observation indicates that the changes in relative concentrations of individual PCB congeners, while significant, did not have a great influence on the total mass of PCBs predicted to occur in tissues of higher trophic levels. In addition, these results suggest that the concentrations of total PCBs in the tissues of the tree swallow eggs and nestlings were near steady state.

The results for individual congeners were quite different. In this instance the critical toxicants to which wildlife are exposed are the congeners that are structurally similar to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Ludwig et al., 1996). Concentrations of "TEq" represent the total potential of the dioxin-like PCB congeners to cause TCDD-like toxicity. Froese et al. (1998) found that lipid-normalized concentrations of TEqs increased with increasing trophic level. The greatest increase, as measured by the ratio between trophic levels, was from invertebrates to the tree swallow eggs, with a lesser increase from the eggs to the nestlings. These results illustrate that bioaccumulation and biomagnification processes are species- and chemical-specific.

To summarize, although geochemical factors will impart a contextual framework on bioavailability, higher order biological and ecological processes can determine ultimate exposure within specific environments. Food chain transfer is probably the most important exposure pathway to soil and sediment contaminants for higher order animals and must be considered a primary bioavailability process (Sharpe and Mackay, 2000).

CONCLUSIONS AND RECOMMENDATIONS

The bioavailability of contaminants present in soils and sediments is governed by a wide range of physical, chemical, and biological processes. Within this chapter we have described the individual processes impacting bioavailability. While it is instructive to consider these processes in isolation, it is imperative to realize that they occur in concert and often are interdependent. In fact, bioavailability is the integrated result of a number of complex, site-specific, chemical-specific, and organism-specific processes. Bioavailability of a contaminant to a receptor will be determined by the combined effect of these processes, as well as by the properties of the soil or sediment, the contaminant, and the receptor of interest. In particular, the heterogeneity of soils and sediments has a profound effect on bioavailability processes.

Although the number of specific processes involved in bioavailability is invariably large, typically a few steps will be most restrictive and thus impart the greatest impact on total bioavailability (i.e., for a given situation, a select few processes are expected to dominate contaminant bioavailability). In planning a bioavailability assessment, which typically will involve measurement of various physical-chemical properties and some kind of biological response, the objective should be to characterize only the most critical features of the system using tools appropriate for measuring bioavailability (described in Chapter 4). The challenge is to understand the system well enough (i.e., mechanistically) so that the measurements taken sufficiently address key aspects, and the aspects not studied experimentally are well known (or their uncertainty is recognized). To meet this need, a multi-disciplinary team approach is essential.

At a given site, bioavailability must be evaluated through measurements and conceptual modeling of exposure pathways, similar to that done during human health and ecological risk assessment. At present, it is possible to form conceptual models and identify some important processes. Nevertheless, our level of understanding regarding these processes is highly variable. For example, our understanding of contaminant speciation in solution is generally well developed, but contaminant retention by various types of organic matter remains unresolved. Important aspects of feeding ecology remain unknown for certain species but are well recognized for others. Free-ion uptake is well described, but the effects of metal complexation with humic materials and anthropogenic chelating agents on bioavailability are not well understood. In general, our understanding of the fate,

transport, and uptake of dissolved contaminants is substantially greater than for solid-bound (including colloid-bound) contaminants. And, finally, very little is known about bioavailability processes for contaminant mixtures, which are common to almost all contamination scenarios. There are sure to be synergisms and antagonisms that affect how contaminants in mixtures bind to subsurface solids and how they are taken up into organisms. (For example, it is known that cadmium uptake into plants is affected by zinc and calcium.) In order to provide accurate assessments of contaminant bioavailability as part of quantitative risk assessment, we must seek to fill the voids in our knowledge and better understand how the various different processes are linked.

The following specific recommendations address the most pressing knowledge gaps deemed necessary for better understanding, predicting, and measuring bioavailability processes.

An improved understanding of contaminant–solid interactions is needed, especially regarding the nature and effects of aging on contaminant release rates. It is presently recognized that contaminants may become less available for biological uptake with aging in soils or sediments. However, in many situations quantitative descriptions and physicochemical understanding of the mechanisms responsible for reduced release rates over time are lacking. Without this knowledge predictions about changes in bioavailability over the long term are not feasible.

Mechanistic knowledge of bioavailability processes at the field-scale is needed. A reductionist approach has been commonly taken to decipher the mechanisms of individual processes. Although important information has certainly been gleaned from such studies, scaling up to the complexity of natural systems has generally assumed a linear coupling of the isolated processes. In reality, the interdependence of different processes and the shear complexity of natural environments (including the presence of contaminant mixtures) likely translate into non-linear effects in scaling. As a consequence, processes need to be understood within the complexity of their natural states.

Improved understanding is needed for some of the biological processes that can most influence bioavailability. For example, it should be a goal to identify generally operative and quantifiable mechanisms of uptake in the gastrointestinal track that might hold across multiple species. The feeding ecology of animals is critical to better understanding exposure of those animals, given the wide differences in assimilation efficiency observed when animals select different types of food from soils and sediments. The bioavailability of contaminants associated with particles such as colloids—including what fraction of the contaminant pool is bound, how gut and lung environments promote contaminant–colloid dissociation, and the extent of particle uptake across biological mem-

branes—needs to be much better understood. How consumer organisms bioaccumulate and transfer contaminants to their predators is essential to understanding the broad effects of some types of soil and sediment contamination.

Quantitatively descriptive models of bioavailability processes are critical and at present lacking. Such models are integral to accurately predicting the fate of contaminants and describing links between bioavailability processes. For example, well tested models of the association–dissociation processes which account for the heterogeneous nature of soil and sediment and the various retention mechanisms operating at different contaminant concentrations are needed to accurately predict bioavailability process A in Figure 1-1 for a spectrum of field settings. Similarly, knowledge of the dynamic properties of contaminant uptake (focusing on D in Figure 1-1) would allow development of species-specific bioaccumulation models that could incorporate factors that affect bioavailability (e.g., food type). Data for model development and validation are generally scarce and yet essential for accurate bioavailability assessment.

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4

Tools

This chapter describes the physical, chemical, and biological tools that have been used to evaluate bioavailability, and it assesses their scientific basis. In general, understanding contaminant bioavailability from soils and sediments requires studying the processes illustrated in Figure 1-1. A first-order need is to identify the contaminant of concern and determine its form, concentration, and distribution (which can correlate with understanding bioavailability process A). These characteristics can be inferred from the soil or sediment matrix or determined directly with operational or mechanistic measurements. Some analytical techniques like spectroscopy can directly address where and how a chemical is associated with sediment or soil, while techniques like extractions operationally address form. Biological tools typically consider entry of the contaminant into the living organism (D in Figure 1-1) without directly measuring processes A–C. Of course, processes A, B or C might be manipulated or measured by other means, with biological tools then being used to evaluate an organism's responses to those manipulations or measurements. One class of biological tools addresses complex responses like toxicity (E in Figure 1-1), for which bioavailability is only one of several possible influences. This chapter does not discuss tools applicable to processes B and C, like fate and transport models, as there are numerous other reports dealing with fate and transport. Rather, the tests that are part of this chapter mainly deal with bioavailability processes A, D, and E; such tests usually assume a constant transport condition.

SUMMARY TABLES

In illustrating the range of physical, chemical, and biological approaches that have been used to evaluate bioavailability processes, this chapter reflects the existing state of knowledge. It is not meant to be an exhaustive list from which one can choose the ultimate tool, nor should it be read as a list of approved approaches for explicitly considering bioavailability. The state of the science is such that little consensus exists about optimal approaches. Among the tests reviewed here, some are appropriate for some situations, but most are not generally applicable to a wide spectrum of situations.

Table 4-1 summarizes the characteristics of the tools covered in the chapter, including what process the tool studies, the approximate cost, and the status of the tool in terms of its future use. It is important to recognize that most tools are still in development and few are fully validated by a body of work relating their predictions to independent measures from nature. Almost all of the tools are broadly applicable to both soils and sediments. Where a test is specific to one or the other, it is mentioned in the description of that test, rather than in the table.

Table 4-2 specifies some generic strengths and limitations of each method and thereby illustrates that every method has tradeoffs. The criteria used for Table 4-2 are:

1. Application to the field. Some methods can be employed in complex natural settings (score 3), some can be used on materials collected from the field (score 2), and some require experimental manipulations such as contaminant spiking (score 1).

2. Application to solid phase. A method that directly addresses processes in the solid phase of sediments or soils, such as a method that evaluates contaminant form in the solid, would score 3. In contrast, a method that requires measurement of the properties of an extract scores 1. A biological test that addresses the solid phase *in situ* scores higher (a field bioaccumulation survey) than a method that takes the solid phase out of context for the evaluation (a lab sediment bioassay), which scores higher than a test that uses an extract (pore water, Microtox or elutriate bioassay).

3. Single vs. lumped processes. Methods that measure a single process are most likely to illustrate a specific mechanism at work. For example, some physical-chemical methods directly evaluate metal form, while other methods measure one mechanism instrumental to bioavailability such as initial biouptake. These score 3. Speciation can be inferred from some methods, as can biouptake from methods like whole organism bioaccumulation (score 2). Other methods that measure a mixture of processes are more operational and less mechanistic (score 1). For example, extractions remove contaminants from an unknown suite of forms without quantifying any processes. Biological methods like toxicity tests are influenced by biouptake plus other processes that influence toxicity.

TABLE 4-1 Characteristics of Tools for Measuring Bioavailability

Tool	Process Studied[a]	Cost[b]	Status[c]
Physical/chemical characterization of the solid phase			
General characteristics • Organic carbon content • Particle/grain size • pH • CEC • Humic and fulvic acid content	Chemical characteristics of the solid (except particle size which is a physical characteristic).	$	Standard protocols available
Specific structures • Characterization of carbonaceous and other solid phases using NMR, petrography, EA, IR/FTIR	Molecular characteristics of solid substrate.	$$ to $$$	Currently research grade for contaminated site application
Specific forms of contaminant bound to solids • XRD and SEM • XAS • μL^2MS • SIMS • NMR • EPR • XPS	Association and dissociation processes, including the roles of surface morphology, oxidation state, and compound or element location.	XRD, SEM —$$ All others —$$$	XRD, SEM—Standard protocols available; all others are research grade
Extraction of soils and sediments for inorganic contaminants			
Extracts that change the solid phase • Conventional • Sequential • TCLP, SPLP	Dissociation from the solid phase. Sequential extracts attempt to differentiate between forms of elements associated with different components or phases of the particle.	$	Some extracts in use and in regulations and thus standardized, but sequential extracts at research stage or in development
Passive approaches • Passive extracts • Pore water measurements with ASV or ion-specific electrode • Exchangeable resins	Passive extracts determine dissociation from the solid phase. ASV and electrodes measure pore water concentrations. Exchange resins measure dissociation from the solid phase and physical flux to aqueous phase.	$ (but need ICP-MS for exchangeable resins)	Research grade, no standard protocols developed; exchangeable resins better developed for sediments

TABLE 4-1 Continued

Tool	Process Studied[a]	Cost[b]	Status[c]
In vitro tests to mimic human intake for both organics and inorganics	Dissociation from the solid phase that mimics physiological fluids.	$ to $$	Variable: validated for lead, but in various stages of development for others
Extraction and other tests of soils and sediments for organic contaminants			
Fluid-phase extractions • Mild solvents • SWE • Supercritical CO_2 extraction • PTD	Dissociation from the solid phase.	$	Mild solvents have standardized protocols; supercritical CO_2 and SWE are in development
Solid phase and membrane-based extractions • Tenax • C-18 • SPME • SPMD • DGT	Dissociation from the solid phase and physical flux to aqueous phase by capturing desorbed contaminant on highly sorptive matrix or gel device.	$	Standard protocols for using these methods for measuring contaminants *in water*; for soils and sediments, all of these techniques are in development
Other desorption tests • Gas purge • Desorption kinetics and activation energy	Dissociation from the solid phase.	$$ to $$$$	In development
Normalizations			
Organic and inorganic correlations • Ratios and models • AVS/SEM • EqP	EqP and AVS/SEM assume reactants control dissociation from the solid phase; other ratios are determined empirically from regressions in field data.	$	Research grade; varies with evaluator
Biological approaches to measuring uptake			
Assimilation efficiency	Biological uptake across the gut wall.	$$	Research grade
Mineralization/ assimilation assays for microorganisms	Integrated measure of bacterial uptake and metabolic degradation.	$ to $$	Research grade

continues

TABLE 4-1 Continued

Tool	Process Studied[a]	Cost[b]	Status[c]
Bioassays: cell cultures and isolated organs/ tissues	Biological uptake into cell or organ.	$$	Research grade
Bioassays: whole organism bioaccumulation • Plants • Invertebrates • Fish • Birds and mammals	Biological uptake into whole organism. Various endpoints are measured, including tissue, blood, etc.	Plants—$ to $$ Earthworm test—$$ Mammals—$$$	Standard protocols for plants, invertebrates, and birds; research grade when plants and other animals used as surrogates
Field survey: whole organism bioaccumulation	Biological uptake into whole organism in field.	$$	Research grade
Biological approaches to measuring organismal response and toxicity			
Reporter systems	Integrated measure of dissociation from the solid phase, bacterial uptake, and effect on gene expression and subsequent events.	$$$	Research grade
Biomarkers	Integrated measure of uptake and response at a subcellular level.	$ to $$$$ (gene expression)	In development
Toxicity tests: spiked • Plant • Invertebrate • Fish • Mammal, bird	Integrated measure of uptake and toxic effects.	$ to $$	Standard protocols available for fresh and saltwater sediments
Toxicity tests: site-specific materials	Site-specific integrated measure of uptake and toxic effects.	$ to $$$$	Standard protocol available
Microbial community bioassays	Integrated measure of uptake, toxic effects, and community interactions.	$$	Research grade
Ecosystem level mesocosms	Integrated measure of many processes including ecosystem level processes like food web transfer.	$$$$$	Standard protocols available

TABLE 4-1 Continued

Tool	Process Studied[a]	Cost[b]	Status[c]
Environmental exposure studies	Integrated measure of many processes including measurable effects in humans.	$$$$$	Research grade

[a]Process Studied: How does this tool address the physical, chemical or biological processes that influence bioavailability?

[b]Cost: $ to $$$$$: Costs in approximate order of magnitude, with $ equal to $100s.

[c]Status: standardized protocol, research grade, or in development.

4. Immediacy or relevance to entry of contaminant into living cell (biouptake). Entry of a contaminant into a living cell across a biological membrane is the process most immediately relevant to determining bioavailability. Some biological methods involve direct determination of transport or biouptake (score 3). Some measure many processes including biouptake, or a process tangential to biouptake like toxicity, or they mimic biouptake as with certain extractions (score 2). Some physical-chemical methods are unrelated to biouptake (score 1).

5. Ability to generalize. Although site-specific tests are essential to managing an individual site, methods that allow predictions (or development of predictive capabilities) without measuring all processes are ultimately a desirable approach. Methods that are predictive, like some models or some tests that determine a mechanism that can be unambiguously compared from site-to-site, score highest in this category (score 3). Methods that are predictive but not yet of proven reliability score 2. Approaches that are of value at a site but do little to explain how the bioavailability processes at that site are comparable to other sites score 1.

6. Relevance to regulation. The relevance of a method to the pressing concerns at a site has led to the use of certain tests for regulatory purposes (like toxicity tests or direct evaluations of human health). Also, methods that are simple and practical to employ, or methods that yield a single value, are most likely to have been applied in the regulatory setting. Thus, methods that managers or decision-makers can interpret or have interpreted as directly relevant to their needs score 3. Methods that have seldom been used in a regulatory setting or have limited potential for such use score 1.

7. Usefulness as a research tool. Relevance as a research tool is just as important as relevance to regulation because of the great need for better understanding the processes that govern bioavailability. Methods that are of use in explaining processes in specific circumstances or in mechanistic detail score highest (score 3), even if they are of limited use in applications. Methods that are of use in a correlative fashion in experimental studies score 2. Methods of limited use in research score 1.

TABLE 4-2 Rankings of Bioavailability Tools According to Seven Criteria Used to Assess Their Strengths and Weaknesses

Technique	Application to the Field	Application to Solid Phase	Single vs. Lumped Processes
Physical/chemical characterization of the solid phase			
General characteristics • Organic carbon content • Particle/grain size • pH • CEC • HA/FA	2 Can test field samples in the laboratory.	3 Directly relevant to solid phase *in situ*; necessary to understand solid phase reactions.	2 Measures are the outcome of lumped processes, but can be used to interpret single processes.
Specific structures • Characterization of carbonaceous and other solid phases using NMR, petrography, EA, IR/FTIR.	2 Can test field samples in the laboratory.	3 Directly measures the solid phase.	2 Determines nature of the phase but not contaminant-phase interactions.
Specific forms of contaminant bound to solids • XRD and SEM • XAS • μL^2MS • SIMS • NMR • EPR • XPS	2 Some methods hard to use on natural particles. Detection limits of equipment can cause problems in natural settings.	3 Directly applicable to solid phase.	3 Uniquely suited to identify mechanisms of association.
Extraction of soils and sediments for inorganic contaminants			
Extracts that change the solid phase • Conventional • Sequential • TCLP, SPLP	2 Can extract field soils and sediments, but must remove from field for test.	2 Concentration extracted is qualitatively or operationally related to associations (form) in the solid phase.	1 Operational measure that lumps different association/ dissociation processes.

Immediately Relevant to Entry into Living Cell	Ability to Generalize	Relevance to Regulation	Usefulness as a Research Tool
1 Characteristics alone are not predictive of biouptake, but are necessary for inferences about other measures and models.	2 Leads to generalization, but by themselves such measures are not predictive of bioavailability processes.	2 Regulators sometimes use such information in normalizations.	3 Essential to understanding contaminant form and links to biouptake *in situ*.
1 Characteristics alone are not predictive of biouptake, but are necessary for inferences about other measures and models.	2 Leads to generalization, but by themselves such measures are not predictive of bioavailability processes.	1 Seldom used for soil/sediment criteria. May be useful eventually.	3 Potential for contributing to mechanistic understanding.
1 Requires inference about link between specific form and biouptake.	2 Will eventually be essential to generalizing about bioavailability processes.	1 Complicated and consequently of limited use in regulatory environment.	3 Potential to understand what controls bioavailability processes.
2 Extracted concentrations are linked to biouptake by correlation. Best developed for use in particular conditions (e.g., restricted soil series; nutrient deficiency).	2 Generalizations are correlative and some are useful in the appropriate context.	2 Some extracts are in regulatory guidelines, mainly for use as screening tool (e.g., TCLP). Used where groundwater is focal point. Not for sequential extracts.	2 Better accepted for soils. Contentious for use in sediments. Relationships are correlative rather than mechanistic.

continues

TABLE 4-2 Continued

Technique	Application to the Field	Application to Solid Phase	Single vs. Lumped Processes
Passive approaches • Passive extracts • ASV • Pore water measurements with ASV or ion-specific electrode • Exchangeable resins	2 Extracts miss *in situ* influences because you must remove materials from field setting. *In situ* pore water measurements are difficult to make and thus limited.	2 Passive extracts mimic solid phase exchange reactions, at equilibrium. Measures in pore water determine actual outcome of solid phase reactions and dissolved speciation.	1 Extracts and resins are operational measures that lump different association/ dissociation processes. Pore water concentrations are the outcome of several processes.
In vitro tests to mimic human intake for both organics and inorganics	2 Can use field soils and sediments, but must remove from field for test.	2 Extract the solid phase with simulated physiological fluid.	1 Operational measure that lumps multiple processes.
Extraction and other tests of soils and sediments for organic contaminants			
Fluid-phase extractions • Mild solvents • SWE • Supercritical CO_2 extraction • PTD	2 Can extract field sediments, but must remove from field for test.	2 Extracts mimic solid phase exchange reactions.	1 Operational measure that lumps multiple processes.
Solid phase and membrane-based extractions • Tenax • C-18 • SPME • SPMD • DGT	2–3 Can use field soils and sediments, but must remove from field for test. May be able to use SPME, SPMD, DGT in situ.	3 Directly applicable to the solid phase or slurry.	1 Operational measure that lumps multiple processes.
Other desorption tests • Gas purge • Desorption kinetics and activation energy	2 Can use field samples, but difficult to sustain in field setting.	3 Directly applicable to the solid phase or slurry.	2 Single vs. lumped processes can be decoupled by careful experimental design and working with component materials.

Immediately Relevant to Entry into Living Cell	Ability to Generalize	Relevance to Regulation	Usefulness as a Research Tool
2 Extracted concentrations are linked to biouptake by correlation. Pore water concentrations are linked by inference that unassociated form is taken up; most useful for plant uptake.	2 The best methods (like DGT) correlate with bioavailability, but there is uncertain reliability of generalizations.	3 Used in some instances as trigger values for soils. Some sediment guidelines use porewater concentrations.	2 Used in research, although relationships are correlative rather than mechanistic.
2 Infers that what can be extracted will be taken up by organism (biomimetic).	1 Site-by-site test. Limited for generalization.	3 Simplicity makes it attractive to regulators.	2 Operational aspects limit use in research.
2 Infers that what can be extracted will be taken up by organism (biomimetic).	1 Reliability of generalizations is unproven.	1 Regulators seldom use such information for soil/sediment criteria; may be useful eventually.	2 Operational aspects limit use in research.
2 Biomimetic but still an inferential link to biouptake.	2 Reliability of generalizations about bioavailability is unproven; work in progress.	1 Regulators seldom use such information for soil/sediment criteria; may be useful eventually.	3 Potential to measure processes important to biouptake (e.g., can get rates of release).
1 Inferential link to biouptake by correlation or mechanistic model.	3 Generalizations possible with careful experimentation on component materials from different sites.	2 Potential to reveal the relationship between aqueous and solid phase concentrations and soil quality criteria.	3 Can lead to greater mechanistic understanding.

continues

TABLE 4-2 Continued

Technique	Application to the Field	Application to Solid Phase	Single vs. Lumped Processes
Normalizations			
Organic and inorganic correlations • Ratios and models • AVS/SEM • EqP	2 Extracted contaminant normalized to *in situ* conditions. Difficult to mimic field setting.	2 Designed to describe associations with the solid phase that are relevant to biouptake (e.g., those that control exchange).	2 Ratioing assumes specific processes are described, and infers that they define biouptake.
Biological approaches to measuring uptake			
Assimilation efficiency	1 Can use natural samples, but requires spiking and loss of *in situ* influences.	2 Direct intake from solid. Allows inferences about natural solids that are ingested.	3 Mechanistic. Determination of single process (biouptake).
Mineralization/ assimilation assays for microorganisms	1 Requires sample removal, and sometimes spiking.	1 Requires contaminant transfer to aqueous phase.	1 Measures the composite effect of several processes.
Bioassays: cell cultures and isolated organs/tissues	2 Can use field soils and sediments, but must remove from field for test.	1–2 Some techniques can use solid phase material while others require extracts.	3 Mechanistic. Determination of single process (biouptake).
Bioassays: whole organism bioaccumulation • Plants • Invertebrates • Fish • Birds • Mammals (all exposure routes)	1–2 Can use field soils and sediments, but must remove from field for test. May have to spike dermal tests.	2 Solid phase materials can be tested directly.	2 Whole organism bioaccumulation integrates influences of several biological processes, but is indicative of biouptake.

Immediately Relevant to Entry into Living Cell	Ability to Generalize	Relevance to Regulation	Usefulness as a Research Tool
2 Inferential link to biouptake or toxicity via correlation. Some tests assume pore water is the only route of intake.	2 Generalizations can be made, but uncertainties add controversy.	2 Accumulation ratios are used (EqP and AVS/SEM) or proposed for use in regulations because of simplicity.	2 Useful for want of better method, but operational simplifications limit use in understanding bioavailability processes.
3 Directly measures biouptake.	2 Can generalize about intake from food types only.	1 Unused. Potential if used with models, but complex.	3 Simple and reliable way to study important bioavailability processes other than just intake. Mainly useful for small animals.
2 Requires intracellular activity in bacterium, so must assume a link between biouptake and degradation.	1 Used for site-specific measures; generalizations difficult to draw.	2 Unused, but perhaps could be standardized.	2 Might shed light on the biouptake step for microorganisms.
3 Directly measures biouptake.	2 When experiments are focused on mechanisms, results can be generalized.	1 Unused. Potential if used with models, but complex.	3 Simple and reliable way to study biouptake *in vitro*.
3 Can be used to directly measure biouptake.	1 Generalization possible only if data are available for a broad array of sites or situations.	3 Used directly in risk assessments.	2 Mostly a tool for empirical measurements, but commonly used as a probe of bioresponse in experimental research.

continues

TABLE 4-2 Continued

Technique	Application to the Field	Application to Solid Phase	Single vs. Lumped Processes
Field survey: whole organism bioaccumulation	3 *In situ* test.	2 Integrates exposure from all influential media, including solid phase.	2 Whole organism bioaccumulation integrates influences of several biological processes, but is indicative of biouptake.
Biological approaches to measuring organismal response and toxicity			
Reporter systems	2 Can use field soils and sediments, but usually must remove from field for test.	1 Usually does not directly assess the sorbed phase, but an extract. *In situ* tests may be available soon.	1 Measures the composite effect of several processes.
Biomarkers	2 Can use field soils and sediments, but usually (not always) must remove from field for test. *In situ* tests with invertebrates possible.	2 Solid phase materials can be tested directly (but generally not *in situ*).	2 Measures the composite effect of several processes, but gene expression techniques can be used to interpret single processes.
Toxicity tests: spiked • Plants • Invertebrates • Fish • Birds • Mammals	1 Simulates exposure.	1 Indirect application to solid phase. Although solids are used in tests, they are not natural samples.	1 Measures the composite effect of several processes.
Toxicity tests: site-specific materials	2–3 Use sediment or soil from nature; *in situ* tests increasingly used.	2 Solid phase materials can be tested directly (some *in situ*), but response integrates exposure from other media as well.	1 Measures the composite effect of several processes.

Immediately Relevant to Entry into Living Cell	Ability to Generalize	Relevance to Regulation	Usefulness as a Research Tool
2 Can be used to directly measure biouptake, but as an integrated response to influential biological and physicochemical processes.	1 Generalization possible only if data are available for a broad array of sites or situations.	3 Concentrations can be used to regulate exposure, but guidance is often limited, especially for ecosystems.	2 Mostly a tool for empirical measurements, but commonly used for research applications.
2–3 (for bacteria) Must assume a link between biouptake and response being measured by the reporter system.	1 Site-specific use is most viable. Ultimately may be able to draw generalizations (e.g., relative availability of chemicals).	2 Standardization and use feasible, and hence the potential for use in regulations.	2 Research may shed light on relative bioavailability to microorganisms under different conditions.
2 Must assume a link between biouptake and biomarker response.	1 Site-specific use is most viable. Ultimately may be able to draw generalizations from experimental studies.	2 Need to evaluate every site; may be extrapolated for some regulations.	2–3 Historically used as a research tool. More recently used as a site-specific tool in the field. Potential for understanding what controls bioavailability at molecular level.
2 Must assume a link between biouptake and toxic response.	2 Generalizations most useful for extreme cases. Uncertain generalizations in natural settings.	3 Simple test commonly used for regulation, although it does not reflect the natural condition.	2 Primary research tool but not mechanistic. Most effective tests attempt to mimic nature.
2 Must assume a link between biouptake and toxic response.	1 Site specific tests have limited generalization. Generalizations are correlative.	3 Used for some regulations. Need to test every site.	2 A primary research tool but not mechanistic. Has potential for site-specific use as survey tool.

continues

TABLE 4-2 Continued

Technique	Application to the Field	Application to Solid Phase	Single vs. Lumped Processes
Microbial community bioassays	3 Tests microbial communities *in situ*.	2 Biological signal associated with solid phase is feasible.	1 Measures the composite effect of several processes.
Ecosystem level mesocosms	1–2 Simulates exposure in nature (with field samples—2 or artificial samples—1).	2 Solid phase materials can be tested directly, but response integrates exposure from other media as well.	1 Measures the composite effect of several processes.
Environmental exposure studies	3 Study conducted in natural setting.	1 Indirect application to the solid phase.	1 Measures the composite effect of several processes.

No one method achieves the highest rating in all columns, and none of these methods fail all criteria. Because all approaches involve tradeoffs, there is not a universal method that meets all needs for characterizing the complex processes that determine bioavailability. For example, some approaches focus on understanding physical desorption of the contaminant from the solid phase but implicitly assume that desorption equals bioavailability (ignoring, for example, a dietary component to bioavailability). A number of tests encompass multiple processes in a single measurement such that isolating individual influences is difficult. Others attempt to isolate individual processes and as a consequence may have endpoints that are marginally relevant to bioavailability. The Table 4-1 and 4-2 entries also suggest how the tools would rank for other parameters of interest. For example, the uncertainty of a method's results can be inferred from its status, the ability to generalize its results, and its relevance to regulations.

An important factor that is not addressed directly in Table 4-2 is the inherent conflict of scale between the methods and processes experienced by an organism. That is, many tools measure outcomes at a scale different from the processes that influence bioavailability. For example, some probes of specific forms determine interactions at the molecular scale or at an individual site, whereas bioavailability in a natural setting will result from integration across a number of sites, not all of

Immediately Relevant to Entry into Living Cell	Ability to Generalize	Relevance to Regulation	Usefulness as a Research Tool
1 Must assume many links between biouptake and ecosystem processes.	1 Site-specific usually. Broad generalizations can be drawn from experiments or correlative studies.	1 Unlikely regulatory use unless "control" site available.	2 Limited potential to elucidate processes, but new methods assess microbial community function and diversity.
1 Must assume many links between biouptake and ecosystem processes.	2 Can broadly extrapolate to field conditions. Uniquely able to generalize about responses across ecosystem-level processes.	1 Could provide valuable information but regulators reject complexity.	3 Very useful research tool if designed carefully. Replication is big challenge.
2 Must assume links between biouptake and other physiological processes.	1 Site-specific nature limits generalization.	3 Used in regulation because human population studied.	2 Limited use as a research tool, but has potential when coupled to exposure models.

which are necessarily similar to that characterized by the probe. More gross techniques, like extraction-based methodologies, have the opposite problem. They determine form from a sample that may encompass much more sediment or soil than the microhabitat-scale at which many organisms experience their environmental milieu.

The purpose of Tables 4-1 and 4-2 is to show that a variety of tools can be applied to the question of contaminant bioavailability and show what processes the tools address. The categories are meant to guide readers toward potentially practical tools for their individual needs, and to compare the varying attributes of different tools. Of course, many of the categories have an element of subjective judgement, and experts may disagree about the details of some entries. Thus, the tables (particularly Table 4-2) are not meant to provide a quantitative scoring system to compare methods, or to provide precise justifications for choosing one approach to studying bioavailability over another. Rather, they are intended to help understand that tradeoffs are always involved in choosing tools to evaluate any bioavailability question, and to provide some general guidance about what the broadest tradeoffs might be.

The following sections describe and evaluate tools and techniques, many of which remain the state of the science for risk assessment. For each method we

explain the technique and why it is useful for measuring an aspect of bioavailability, weigh advantages and disadvantages with regard to evaluating bioavailability processes, and when possible evaluate performance in terms of reproducibility, repeatability, multi-lab calibration, and other factors. Because of the varied status of each tool (see Table 4-1), the evaluations are not equivalent in that not all of the same information is provided for each tool. In addition, some sections focus on an individual technique (e.g., X-ray spectroscopy), while others cover an entire approach (e.g., sediment bioassays). Thus, the details of each discussion, which focus on strengths and weaknesses, necessarily vary throughout. For example, in some cases methodological problems will be highlighted, while for other tools their potential for practical application will be assessed. In all cases, references are provided to direct the reader to further information about any specific test. Finally, to increase the utility and uniqueness of the tables, not all of the information presented in Tables 4-1 and 4-2 are repeated in the following discussions. Points to consider when choosing tests specifically for use in human health and ecological risk assessment, including criteria for validation, are presented at the end of the chapter.

Although it is difficult to encompass all the methods used to evaluate bioavailability processes, some relatively safe generalizations are possible from the discussions that follow.

- Although approaches to measuring bioavailability can be quantitative or qualitative, ultimately those approaches that allow quantitative estimation of bioavailability are the most important.
- Mechanistic approaches (that unambiguously determine the form of a contaminant) have the greatest potential to ultimately result in useful approaches for defining bioavailability processes and narrowing uncertainties. But they are less applicable at present.
- Regulatory and industry interests prefer simplified approaches that are operational (e.g., extractions), that provide shortcuts to estimate mechanistic processes (e.g., equilibrium partitioning), or that estimate bioavailability indirectly via complex responses (e.g., toxicity bioassays). Such approaches have important practical and scientific tradeoffs. Because some of these approaches lack explanatory capability, have narrow applicability, and have uncertain meaning, they should be employed cautiously in the current regulatory environment so as not to increase uncertainty or the degree to which actions seem arbitrary.

TECHNIQUES TO CHARACTERIZE INTERACTIONS AMONG PHASES

Contaminants occur in soils and sediments as a complex mixture of solid-phase chemical compounds associated with particles of varying size and morphology. Contaminant forms include discrete mineral phases, co-precipitated and

sorbed species associated with solid minerals or organic matter, complex compounds or associations among organic moieties, and dissolved species that may be complexed by a variety of organic and inorganic ligands. The occurrence and relative distribution of contaminants among various phases, and the physical relation between the phases and the soil or sediment, will control a contaminant's dissolution properties and its bioavailability. The spatial heterogeneity of these complex mixtures in soil and sediment will be reflected in variable bioavailability of an element at a site.

This section discusses methods that can be used to investigate physicochemical forms of solids and contaminants and interactions among forms and phases as well as methods to obtain information on soil and sediment characteristics, which is often necessary to understand form. The methods include those that investigate both the microscale location and association of contaminants within solid matrices and the nature of the contaminant binding. The purpose of these tools is to provide a better mechanistic understanding of the chemical release portion of bioavailability and for interpreting differences in bioavailability of contaminants residing in different environmental matrices.

Physical and Chemical Characterization of the Solid

Although basic solid parameters are probably not sufficient to understand bioavailability processes, they provide critical ancillary information. In the absence of this information, more direct tests of bioavailability are difficult to interpret, making generalizations to other places, circumstances, or times problematic. Over the long term, knowledge of contaminant behavior in the field, combined with comparable basic characterization data of solids from many study sites, will improve our understanding of the factors that control bioavailability. This section describes physical and chemical analyses that can be useful as a routine part of evaluating bioavailability at contaminated sites.

Basic Characterization of Soil or Sediment

The measurements described below provide important contextual information about the solid matrix in which contaminants occur, and they are inexpensive and routinely conducted. Some of the measurements can be used as screening tools to make simplistic estimates of contaminant availability. Methods for conducting these analyses are described in Page (1965), Sparks (1996), and Meyers (1998).

Organic Carbon and Organic Matter Content. Organic carbon content (measured as f_{oc}) provides a simple index of solid reactivity to hydrophobic organic contaminants and some metals and is, therefore, an indicator of potentially reduced bioavailability. Organic matter content (f_{om}) provides similar information, except that the entire mass of organic matter including hydrogen, oxy-

gen, sulfur, and nitrogen is determined. Organic carbon content can be used to obtain a reasonably accurate estimate of the sorption distribution coefficient for hydrophobic organic compounds in most modern soil or sediment samples that have ≥ 0.5 percent carbon (wt/wt) (Allen-King et al., 2002). However, the estimate may be poor for some subsurface samples (e.g., those with lower carbon contents or when contaminant concentrations are low). A variety of methods are available to determine f_{oc} or f_{om} in a sample. Methods to measure f_{om} typically do not include black carbon, while methods to measure f_{oc} that rely on high temperature combustion (see Heron et al., 1997) will include all forms of non-carbonate carbon, from humic materials through black carbon.

Particle Size. This measurement provides a crude indication of the grain or particle surface area and can be used to estimate soil or sediment permeability and to better understand chemical release from the solid. Particle size provides screening information on the rate and magnitude of contaminant desorption, which can control bioavailability. If the desorption mechanism involves diffusion within a particle or grain (such as shown for lead in Figure 3-9), then smaller particle-size would result in a smaller diffusion distance and more rapid uptake and release rate compared to larger sizes. For example, Ball and Roberts (1991) found that the time needed for perchloroethylene to attain sorption equilibrium with large grains was much greater than for smaller grains from the same sandy aquifer. The difference was attributed to diffusion to sorption sites within the grains or particles. When the mechanism is primarily sorption to external grain surfaces, then surface area (per unit mass), and hence reactivity, are generally greater for smaller compared to larger particles. In this case, particle size measurements provide information directly related to the magnitude (but not the rate) of sorption. It should be noted that the magnitude and rate of contaminant desorption cannot be reliably estimated from particle size alone, because other characteristics of the particle and contaminant are important in controlling behavior. However, information on contaminant concentration and desorption rate *by particle size* may reveal the mechanisms that limit bioavailability on a site-specific basis and provide information that will improve long-term predictions.

Surface Area. Surface area (also frequently termed specific surface area or SSA) provides indirect information on the types of mineral surfaces present and on reactivity, especially for metal ions. Silicate minerals that do not have internal porosity typically have low SSA (≤ ~1 m^2/g), while some clay minerals can have much greater surface areas (> ~100 m^2/g) (Selker et al., 1999). SSA is frequently determined by developing N_2 gas adsorption isotherms using the Brunauer-Emmett-Teller method (Gregg and Sing, 1982). The isotherm shape can be used to interpret the micro- and meso-porosity connected to the external particle surface. Mercury porosimetry is another method that can be used to determine SSA and intragranular porosity, as described extensively by Gregg and Sing (1982).

Finally, ethylene glycol monoethyl ether vapor adsorption is frequently used to evaluate the surface area of clay mineral phases.

pH. Many contaminants, particularly cationic metals, tend to be more mobile (and thus bioavailable) in acidic soils. Furthermore, the low pH characteristic of some contaminated sites can cause dissolution of relatively high surface area iron oxyhydroxide grain coatings that in turn results in release of cationic species, including metal co-contaminants. Sorption of weak acids or bases to solids is also generally pH dependent. Neutral compounds tend to be least affected by system pH.

Cation Exchange Capacity. The cation exchange capacity (CEC) is the total charge excess of cations over anions for a soil. It is generally measured by procedures designed to saturate the exchangeable sites with particular "probe" cations (e.g., Mg^{+2}) under controlled ionic strength and pH conditions and is, therefore, somewhat empirical. The CEC provides information on the reactive surface properties that are particularly relevant to sorption of cationic metals. A larger CEC (within the range typically observed for soils) indicates a relatively high content of high CEC mineral phases, such as for smectite or montmorillonitic clay, and is generally associated with greater cationic metal sorption.

Characterization of Carbonaceous Phases

Characterizing the type, chemical composition, and structure of carbonaceous materials can provide information about the extent of hydrophobic organic compound (HOC) sorption. As discussed in Chapter 3, substantial sorption of HOCs onto the more condensed forms of carbonaceous materials and black carbons (chars, soots, coals, and kerogens) may make them much less bioavailable than those associated with natural organic matter coatings on mineral solids. There are multiple techniques to identify forms of carbonaceous materials, including extraction and separation of fulvic and humic acid fractions, nuclear magnetic resonance spectroscopy, petrographic and elemental analysis and pyrolysis, and infrared absorbance.

Separation of Humic, Fulvic, and Humin Fractions. Humic, fulvic, and humin fractions of soil organic matter (SOM) are operationally defined by acid and base extractions (Swift, 1996). Determining these fractions in a soil sample can lead to a better understanding of contaminant sorption because each fraction has a different affinity for HOCs. For example, Njoroge et al. (1998) demonstrated the heterogeneity of soil organic matter with respect to sorption by determining its fulvic acid component. At this field site, it was shown that sorption could be adequately modeled by assuming that the sorbent was a mixture of two materials with different affinities for the HOCs. Differences in sorption magni-

tude and nonlinearity for humic and humin fractions from a peat soil have also been demonstrated (Chiou et al., 2000).

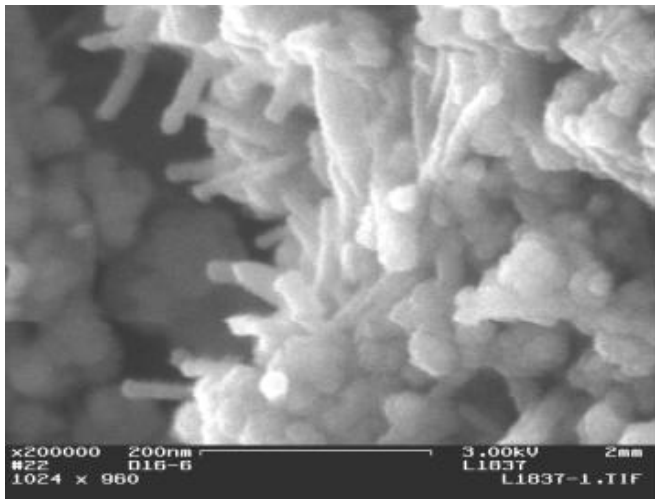
Nuclear Magnetic Resonance. Solid-state ^{13}C nuclear magnetic resonance (NMR) with cross-polarization and magic angle spinning has been used to characterize the relative abundances of different functional groups within soil, and these characteristics have been related to sorption magnitude, desorption rate, and bioavailability. For example, NMR has been used to detect aromatic character (Xing, 1997) and more reduced and condensed organic matter (Lueking et al., 2000), both of which were correlated with greater sorption of polyaromatic hydrocarbons (PAHs). Because the results are qualitative and the technique requires a relatively strong magnet and long times (see Swift, 1996), NMR is useful primarily in research applications.

Petrography and Elemental Analysis. Several techniques to characterize carbonaceous materials have been used extensively as geochemical tools in oil and coal reservoir analysis, including coal petrography, extraction followed by elemental analysis, and pyrolysis techniques. These methods have recently been used to show correlations between HOC sorption and various properties of the carbonaceous material. They are most successful for materials that are relatively rich in carbon and resistant to acid treatment.

Petrographic methods are qualitative to semi-quantitative and are best used to identify the relative proportions of different phases. For example, recent studies have correlated the types of carbonaceous matter in sedimentary rocks or unconsolidated sediments to HOC sorption behavior (Kleineidam et al., 1999; Karapanagioti et al., 2000). Coaly particles had a significant effect on sorption even though they comprised a low proportion of the carbonaceous material.

Elemental analysis has been used to characterize differences in primarily hydrogen (H), oxygen (O), carbon, and nitrogen content, which indicate the degree of condensation and polarity of carbonaceous materials. The carbonaceous material H/O ratio has been correlated to sorption of chlorinated solvents within a series of samples representing a wide range of properties, from fulvic acid to hard coals and shales (Grathwohl, 1990), and for a series of kerogen-containing subsurface sediments (Binger et al., 1999). Because the method is relatively simple, commercially available, and quantitative, it shows promise. However, to date the relationships between the elemental composition of soil carbonaceous matter and sorption magnitude have been tested for only a few combinations of soils/sediments and contaminant compounds.

Infrared Absorbance. Radiation in the infrared (IR) range corresponds to the stretching and bending vibrational frequencies of covalent bonds. Thus, the IR absorbance spectrum (usually measured by Fourier transform IR instruments, FTIR) can provide structural information about an organic molecule. Like NMR,



Scanning electron micrograph of goethite laths developed around a ferrihydrite substrate.

FTIR provides qualitative information regarding complex bonding in natural carbonaceous matter. It has been used primarily to characterize humic substances in soils. Because it responds to bulk sample properties, it is not likely to be well suited to low f_{oc} materials or for discerning subtle differences between samples. FTIR microspectroscopy makes it possible to analyze IR absorbance at small spatial scales, such as the sub-grain scale in soil or sediment. This research grade tool has been used to characterize the chemical properties of surfaces in concert with contaminant analysis to better understand the nature of contaminant binding, as further described in Box 4-1.

Probing Contaminants within the Solid Phase

A variety of spectroscopic techniques are available to evaluate the chemical and mineralogical properties of contaminants associated with soils and sediments. Spectroscopy can also provide information about the solid phase itself and thus can be used to complement the techniques described in the preceding discussion. Although some of these spectroscopic methods are commonplace, most are research grade tools.

BOX 4-1
Complementary Sediment Characterization and Contaminant Distribution Facilitates an Understanding of Bioavailability

Detailed physical and chemical characterization of the solid phase provides information complementary to contaminant concentration and release data regarding mechanisms controlling bioavailability. Recent studies have determined the associations between PAHs and particles of a harbor sediment (Ghosh et al., 2000a; Talley et al., 2002). In these studies, seven different sediment fractions enhanced in particular particle types were obtained by a combination of density and particle-size separations. As shown in Figure 4-1, PAH analysis showed that more than half of the total extracted

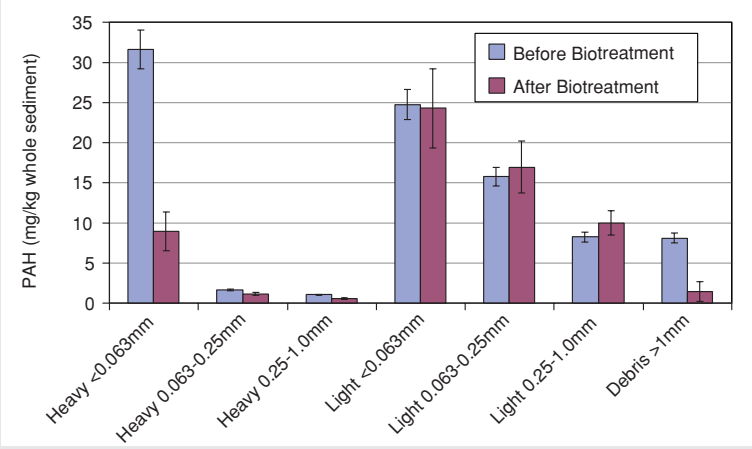


FIGURE 4-1 PAH distribution in sediment size and density fractions before and after bioslurry treatment showing loss of PAHs from the clay–silt fraction (< 0.063 mm) and no change in PAHs from the light coal/wood derived fractions. SOURCE: Reprinted, with permission, from Talley et al. (2002). © (2002) American Chemical Society.

X-ray Diffraction and Scanning Electron Microscopy

X-ray diffraction (XRD) and scanning electron microscopy (SEM), with associated energy dispersive spectroscopy, have been used extensively to characterize solids. X-ray diffraction defines the crystalline structure of the solids, while scanning electron microscopy provides information on particle size and morphology along with elemental composition, all of which are pertinent to evaluating contaminant bioavailability, particularly for metals. Thus, for example, scanning electron microscopy and electron microprobe analyses can establish not only the chemical composition, size, and morphology of individual soil particles but also the distribution of a particular element (for example, lead) within a soil particle. These data can be used to estimate or model the solubility, and hence the bioavailability, of the mineral assemblage in a particular soil. Indeed, analysis of

PAH mass was associated with the "light" (low-density) sediment fractions, although these fractions comprised less than 5 percent of the total sediment by weight. Approximately one third of the total PAH mass was associated with the "heavy" clay–silt size (< 0.063 mm mineral) fraction, and about one tenth was associated with the coarse material (>1 mm). Hence, PAH concentrations on light particles were approximately two orders of magnitude greater than on the heavy particles.

Characterization of the particles provided complementary insights into the nature of PAH binding. The particles in the light fractions were identified by petrographic analysis as primarily coal and coal-derived, and were thought to originate from historic coal shipping and processing operations in the harbor. The light fractions also contained particles of wood and vegetative debris. The more dense fractions were composed primarily of silicate minerals. An FTIR analysis of the heavy clay–silt size fraction revealed that its associated organic matter is more polar than the carbonaceous matter in the light fractions. Consistent with other work (Grathwohl, 1990; Karapanagioti et al., 2000), these results demonstrate that the sorption capacity for HOCs of more condensed coal-derived carbonaceous matter is much greater than the sorption capacity of the more polar organic matter coating silicate grains.

Based on these results and the results of sequential Tenax extractions, the authors posited that PAHs sorbed to the coal-derived materials were more strongly bound than PAHs sorbed to the heavy clay–silt size fraction, and so less bioavailable. To test the hypothesis, the authors subjected the sediment to bioslurry treatment followed by PAH analysis by density and particle size separation. The findings supported the hypothesis (Figure 4-1, dark bars). The total PAH concentration in the heavy clay–silt size fraction diminished by about 75 percent with bioslurry treatment, while there were no significant changes in the total PAH concentrations in any of the three fractions dominated by coal-derived material. This study demonstrates that using techniques to determine the composition of the solid can provide information complementary to the identification of contaminant associations and provide insights on the mechanisms controlling bioavailability.

lead-bearing soils has indicated that the chemical forms and sizes of lead-bearing particles control the oral bioavailability of lead (Ruby et al., 1999), and the same appears to be true for arsenic in soils. U.S. Environmental Protection Agency (EPA) Region 8 has participated in the development of a protocol for site-specific assessment of lead and arsenic mineralogy in soil using electron microprobe analysis (CDM, 1994). However, given the complexity of lead and arsenic associations within soil solids, and the multitude of reactions that may lead to their dissociation, data regarding contaminant phases and size alone have not been deemed adequate to estimate bioavailability. X-ray diffraction has two primary limitations: (1) only crystalline solids are detected and (2) detection requires greater than 1 percent of the specific phase. The drawback with scanning electron microscopy is simply that particle morphology and shape do not translate directly into a relationship with bioavailability.

X-ray Absorption Spectroscopy

X-ray absorption spectroscopy (XAS) has recently proven to be a powerful means for obtaining the speciation and structure of elements (such as metals) present in complex media. It has a number of advantages for studying natural materials that include element specificity, the ability to probe local chemical and structural states of an element, and the ability to analyze materials *in situ* (meaning that a natural water, soil, or sediment sample can be placed directly in the spectrometer without further alteration). XAS probes the local chemistry and structure of a single element throughout a sample, revealing a "view" of the element's electronic structure and the atoms that coordinate it, as illustrated in Figure 4-2. The oxidation state, types of nearest neighbors, coordination number, bond distances, and orbital symmetries of the x-ray-absorbing element can be accurately determined in an array of media (Eisenberger and Lengeler, 1980). XAS is not useful for detecting trace quantities of a contaminant and is not ideal for most organic contaminants. It is, however, extremely useful for identifying the stability of metal contaminants residing in the solid phase of soils and sediments at part per million levels. Few techniques provide greater information on the chemical environment of a metal within natural materials.

X-ray absorption spectroscopy can be broken into two main subsets—X-ray absorption near edge structure (XANES) and extended X-ray absorption fine

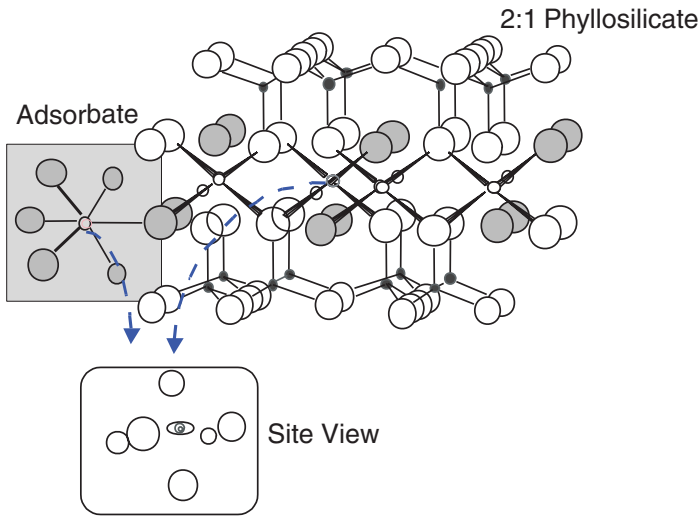


FIGURE 4-2 An illustration of the hypothetical view obtained by X-ray absorption spectroscopy. The eye in the "site view" represents the atom around which the image is centered, which can be either an adsorbate or a mineral atom. The image reveals the configuration of that atom's nearest neighbors.

structure (EXAFS) spectroscopies. XANES spectroscopy provides an opportunity to ascertain the oxidation state or speciation of elements in soils or sediments—until recently a near impossible task. To date, the oxidation states of uranium, manganese, chromium, iron, selenium and other elements have been elucidated with XANES spectroscopy (see Table 4-3). EXAFS spectroscopy, in contrast, probes the local structure of an element within solids, providing detailed information on its bonding environment.

To date, most studies utilizing EXAFS spectroscopy have been conducted on rather simple, homogeneous systems, such as to elucidate the structure of ions on surfaces common to soils or sediments. This information is essential for determining the stability of a sorbate, which dictates the potential for desorption. Furthermore, such information is needed to develop accurate mechanistic models that can predict the fate of metal ions. Whether a metal ion binds electrostatically or chemically can be discerned with EXAFS. Electrostatic retention was observed for lead on corundum (Bargar et al., 1996), while chemical complexes of numerous ions have been noted on many surfaces (for example, Scheidegger et al., 1997). In the case of chemical binding, the coordination environment (i.e., the interatomic distances and coordination number) of the surface complex can be obtained.

To provide the intensity necessary for performing XAS measurements in a reasonable time period and in solvated systems, high intensity X-ray sources—

TABLE 4-3 Selected XAS Studies of Metal Contaminants within Soils and Sediments

Subject	Reference
Selenite and selenate on goethite	Hayes et al. (1987)
Np(V) retention on goethite	Combes et al. (1992)
Cr oxidation states on ferrous hydroxides	Bidoglio et al. (1993)
Speciation of U in soils and sediments	Bertsch et al. (1994)
S speciation in marine sediments	Vairavamurthy et al. (1994)
Se speciation in sediments	Tokunaga et al. (1996, 1998)
Pb complexes and α-alumina	Bargar et al. (1996)
Arsenate sorption on ferrihydrite	Waychunas et al. (1996)
U in soils	Duff et al. (1997)
Mn forms in lake waters and sediments	Friedl et al. (1997)
Precipitation of selenocyanate in water	Manceau and Gallup (1997)
Ni complexes and precipitates on phyllosilicates	Scheidegger et al. (1997)
Cr in soils	Szulczewski et al. (1997)
Cu and Pb on soil humic substances	Xia et al. (1997)
Pb on goethite and alumina	Bargar et al. (1998)
Zn, Cd, Pb in river sediments	O'Day et al. (1998)
Zn and Pb in Penicillium	Sarret et al. (1998)
Cr in soil columns	Jardine et al. (1999)
Pb in mine tailing	Ostergren et al. (1999)
Mn in automobile exhaust particulates	Ressler et al. (2000)

generally found only at synchrotron facilities—are necessary. High intensity synchrotron X-ray sources permit *in situ* investigations of most elements (those heavier than boron). Facilities are being developed to analyze lighter elements of biological significance, such as carbon and nitrogen in thin films of water. Because synchrotron X-ray sources are required, it is unlikely that this technique will be a mainstream analytical tool but will rather serve as a means for describing high priority samples or for calibrating more accessible methods. Box 4-2 describes the use of XAS in conjunction with other tests to help determine bioavailability of metals in sediment.

BOX 4-2
X-ray Absorption Spectroscopy Provides Molecular Understanding of Metal Bioavailability in Sediment

O'Day et al. (2000) assessed metal speciation and bioavailability for contaminated estuarine sediments obtained from the East Outfall Site of the Seaplane Lagoon, at the former Naval Air Station Alameda, located on an island in San Francisco Bay. The researchers assessed a measure of bioavailability proposed by EPA for five metals (cadmium, copper, lead, nickel, and zinc) based on comparison of simultaneously extracted metals (SEM) to acid volatile sulfide (AVS) (see Chapter 2). If $\sum$SEM/AVS > 1, there is potential bioavailability because of insufficient FeS(s) to precipitate the five toxic metals. Toxicity of lagoon sediment to sand dollar embryos and adult amphipods was compared to SEM and AVS measurements and to the speciation and local molecular bonding of metals in sediment as determined by synchrotron radiation X-ray absorption spectroscopy.

The results showed that assumptions about $\sum$SEM/AVS were not valid for this study. Of six metals studied, only cadmium was present in sediment exclusively as a sulfide phase; chromium and lead were coordinated with oxygen. Toxicity tests with amphipods and invertebrate embryos also did not support $\sum$SEM/AVS predictions. In surface sediments, this ratio was between 2.7 and 5.25, yet the sediment was nontoxic, while sediments from 30-cm depth gave 100 percent toxicity despite $\sum$SEM/AVS = 0.54. Toxicity may have been due to either high ammonia or low dissolved oxygen. There was no evidence that FeS(s) was the primary contributor to AVS, as assumed in the AVS method.

XAS showed that the metal contaminants were present in reduced sediment as both sulfide and oxide solid phases. Thus, the assumption that metal and iron monosulfides control the partitioning of toxic metals was not substantiated (except for cadmium and to some extent for zinc). For chromium and lead, and possibly for copper and nickel, pore-water concentrations were dependent on sorption and precipitation processes associated with clays, carbonates, and/or oxyhydroxide minerals.

This study demonstrated the potential for using multiple bioavailability tests to gain mechanistic understanding of bioavailability processes. The toxicity tests and X-ray absorption spectroscopy did not fully support $\sum$SEM/AVS predictions, which should warn against using this ratio to infer mechanisms. Spectroscopic techniques can verify contaminant speciation and thus substantiate proposed standard sediment tests to provide a molecular basis for interpretation and extrapolation.

Laser Desorption and Laser Ionization Mass Spectrometry

In general, spectroscopic assessments of solids provide information on the functional group structure of organic material and the associations of atoms in an organic matrix. However, to date there is a lack of methods that provide direct identification of *organic* contaminant molecules and their specific locations in soils or sediments. A new technique—microprobe laser desorption/laser ionization mass spectrometry (μL^2MS)—offers the opportunity to determine where exactly on solid surfaces organic contaminants reside. μL^2MS involves desorption of constituent molecules on a particle using a pulsed IR laser beam followed by selective ionization of the desorbed molecules with a pulsed, tunable ultraviolet laser. The resulting ions are then extracted into a reflectron time-of-flight mass spectrometer. The PAH detection limit of the μL^2MS instrument is estimated to be in the sub-attomole range. Depth of penetration of the desorption laser is approximately 0.5–1.0 microns based on test results with PAH embedded in thin resin sections. Currently the resolution of the instrument is a circular spot 40 microns in diameter with the potential to be much smaller, in the range of 10 microns.

This method has been used to measure PAHs on field soils and sediments to determine their relative distribution and locations (Gillette et al., 1999). Along with particle sectioning procedures, it can assess the precise distribution of contaminants within sectioned particles (Ghosh et al., 2000b). Such information allows better understanding of microscale sorption mechanisms and can be used with other measurements to assess how organic contaminant locations and sorbent interactions affect bioavailability. This instrument is a unique research tool and is not available for regular screening of environmental samples.

Secondary Ion Mass Spectrometry

Direct surface analysis of environmental matrices for organic and inorganic contaminants is possible using secondary ion mass spectrometry (SIMS) methods. SIMS works by bombarding a specimen with either an ion or molecular beam; surface layers are then "bumped" off the surface and their speciation is determined by mass spectrometry.

For inorganic analysis, SIMS has principally been used to determine the distribution of elements on surfaces of soils or sediments (e.g., Eick and Fendorf, 1998; O'Day et al., 2000). SIMS has also shown utility for investigating organic contaminants when a molecular beam is used. For example, Ingram et al. (1997) used SIMS to analyze 16 pesticide residues on the surfaces of soil, leaves, grass, and stainless steel. Typical spot sizes for SIMS analysis are 3 to 6 mm^2, and minimum detection limits range from 0.03 monolayers (2100 ppm) for tributyl phosphate to 0.005 monolayers (6 ppm) for the pesticide paraquat (Ingram et al., 1996, 1997).

One of the greatest benefits of SIMS is the low detection limit, at least relative to other solid-phase techniques. Generally one can detect species in the range of 10^{-5} to 10^{-6} mg contaminant per kg of solid. However, samples must be subjected to a high vacuum environment. Due to the relatively large spot size, SIMS may not be widely applicable to determine sorption mechanisms, but may be a valuable technique to determine surface concentrations on different environmental matrices.

Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) gives information about the number and nature of the immediate chemical environment of each type of a target atom, as described earlier in this section. In addition to characterizing organic matter itself, NMR has been used to investigate the binding of organic and inorganic contaminants to soils and sediments. Weissmahr et al. (1997) studied the specific sorption of nitroaromatic compounds including trinitrotoluene, nitrobenzenes, and herbicides onto clay minerals with NMR. ^{15}N-NMR studies also provided direct evidence of covalent binding of aromatic amines to humic substances (Thorn et al., 1996). Solid-state ^{15}N-NMR studies of humic acids extracted from ^{15}N-2,4,6-trinitrotoluene show that the explosive is reduced to aromatic amines, and some of the products are covalently bound to the natural soil organic matter (Achtnich et al., 1999; Knicker et al., 1999). Each of these cases is notable because covalently bound contaminant residues may not be bioavailable. Interactions between PAH molecules and aromatic structures within coals have also been studied using NMR techniques (Sakurovs, 1998). NMR has been used to better understand the binding of inorganic contaminants such as cadmium (Sharps et al., 1993; Otto et al., 2001), aluminum (Casey et al., 1998) and vanadium (Lu et al., 1998) to soils and sediments.

The greatest limitation of NMR is that the nuclei of the target contaminant must have unpaired spin-states to be active. Additionally, the material must have limited quantities of interfering species; iron is a noted problem with NMR and restricts its use on natural materials. Commonly studied nuclei of environmental interest include hydrogen, carbon, cesium, phosphorus, fluorine, and aluminum.

Electron Paramagnetic Resonance

Electron paramagnetic resonance (EPR) spectroscopy has been used extensively for the study of selected elements and molecules. In contrast to NMR, EPR probes the chemical nature of a species through electron spin interactions. For an EPR signal to be produced, the species must have an unpaired electron spin state. By definition, all free radicals fulfill this requirement, as do inorganic contaminants such as Mn(II), Cu(II), and Cr(III). EPR has been used to decipher the chemical state of elements such as manganese and copper within soils and sedi-

ments (McBride, 1982; McBride et al., 1984; Bleam and McBride, 1986). In fact, EPR provided some of the first details on the chemical interactions of transition element contaminants with clay minerals. More recently, EPR has been employed to provide direct information about the molecular-scale environment of xenobiotics in natural porous media (Dumestre et al., 2000). A virtue of EPR spectroscopy is that samples are easily prepared; soil or sediment suspensions can be placed directly in an EPR glass tube and inserted into the spectrometer. The main drawback is that studies are restricted to EPR-active compounds, as mentioned above. Also, a number of interfering species may reside within natural material.

X-ray Photoelectron Spectroscopy

X-ray photoelectron spectroscopy involves bombarding a solid with x-rays of fixed energy and then resolving the kinetic energy of the ejected photoelectrons to provide information on the solid's oxidation state and bonding (chemical) environment. XPS is useful only for solids (and adsorbed elements) and has been applied to soils and sediments for at least 30 years, albeit not routinely. Because XPS is rich in information and useful for elements that may reside in different oxidation states, it has been used extensively to characterize surfaces of sulfur and manganese solids (Junta-Rosso and Hochella, 1994; Nesbitt et al., 1998a,b) and for defining the chemical state of arsenic on the surface of soils and sediments (Soma et al., 1994). Unfortunately, there are two distinct drawbacks of XPS. The first is the need for a high vacuum environment, which may potentially distort an environmental sample. The second (and more problematic) is the poor detection limit. A specific element of interest generally needs to be present at concentrations greater than 1 percent of the solid phase.

Summary

Mechanistic understanding of physicochemical phenomena controlling bioavailability processes requires knowledge of the geochemical compartments that contain the contaminant, the forms of the contaminant, and interactions of the contaminant within the compartment. New instruments are helping to develop this understanding. For example, NMR and microscale surface mass spectrometric and microscale infrared spectroscopic methods are capable of describing the occurrence and role of black carbon that may serve as an especially strong sorbent for organic contaminants. X-ray absorption near-edge structure and X-ray absorption fine structure spectroscopy can discern the distribution and bonding of metals in solids. Thus, new spectrometric and spectroscopic methods can identify the locations of specific organic compounds in natural materials, while X-ray absorption spectroscopy can provide data on element mineralogy, which is useful in modeling the solubility of mineral assemblages. Owing to the sophisticated,

specific nature of the instruments needed to address these questions, most of these methods will remain research tools. However, detailed examination of selected samples advances mechanistic understanding and thereby furthers the development of validated conceptual models for describing the chemical and kinetic factors controlling contaminant release, transport, and exposure.

PHYSICAL/CHEMICAL EXTRACTION TECHNIQUES FOR MEASURING BIOAVAILABILITY

A wide variety of extraction tests have been proposed for estimating the bioavailability of organic and inorganic compounds to humans and ecological receptors. The tests involve chemical extraction for metal contaminants and extraction using organic solvents or solid phase adsorbents for organic contaminants. These techniques attempt to provide a site-specific measure of the bioavailable fraction of a contaminant as opposed to the total extractable contaminant based on a rigorous extraction procedure, and they are meant to be simple and reliable. For human exposures, these tests have generally been physiologically based (i.e., relying on knowledge of the mechanism by which the chemical would become solubilized and available for absorption). Extraction tests are generally not considered valid until they have been shown to correlate with an inherently biological measure of bioavailability. The fact that many have not yet been validated reflects the difficulty and expense of measuring the bioavailability of xenobiotics in humans, ecological receptors, or an appropriate surrogate.

Extraction Tests for Inorganic Contaminants in Soils

Extraction tests for inorganics in soils have long been used, particularly for agricultural applications. Thus, most of the tests discussed below were initially developed to mimic plant uptake of metals so that plant tissue analysis would not be needed to determine a soil's ability to provide nutrients. These tests were designed to be easily reproducible, rapid, and relatively inexpensive (O'Conner, 1988). Soil tests were initially developed to predict nutrient deficiencies in soil, and they were calibrated with plant response across different plant species and soil types. In general, it has been possible to determine critical extract levels for certain elements and crops within soil series, but not across all soil series (e.g., Cox, 1968; Lindsay and Norvell, 1978). Extraction methods will undoubtedly need to vary by soil type.

Because the vast majority of extractions were developed to predict metal deficiencies, they tend to be fairly aggressive in order to mimic plant behavior. Traditional extracts, which vary with soil type, generally contain organic chelates and/or acids to solubilize labile pools of soil nutrients. For example, to test for phytoavailable zinc, diethylenetriaminepentacetic acid (DTPA or DTPA-AB) is used as an extract in neutral to calcareous soils, the Mehlich-I or III method is

used in acidic southeastern soils, and the dilute hydrochloric acid method is used for neutral and acidic soils in the north central United States (Reed and Martens, 1996). Predicting plant uptake of elements that are present at potentially phytotoxic concentrations requires a different approach, because plants are generally not aggressively manipulating the rhizosphere to solubilize these elements. Several passive extracts have been developed to predict plant behavior under these conditions. Figure 4-3 shows a conceptual diagram of four extraction strategies for inorganic compounds, examples of which are given below.

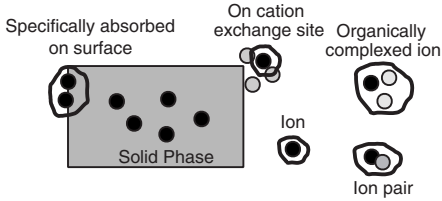
Passive Approaches

Because plant uptake of metals from soils occurs only via soil solution, measurement of contaminant concentration in soil solution gives an instantaneous view of the bioavailable fraction of the contaminant. Passive approaches, including passive extractions, pore water measurements, and some exchange resins, evaluate the concentration of contaminants that are present in soil solution or are readily soluble (that is, the portion held electrostatically on soil exchange sites). Metals in soil solution will be present as hydrated ions, ion pairs, chelated complexes, and complexed on colloidal material (Helmke, 1999). The most specific approach is to measure the concentration of free ions in solution. Indeed, there are indications that only the free ionic species of an element in soil solution is accessible to plants (Parker et al., 1995). Although there are cases demonstrating the uptake of metals present as ion pairs or chelated complexes, this is likely to be much less significant than uptake of free ions (Bell et al., 1991; Smolders and McLaughlin, 1996). Because these approaches do not involve altering the solid phase, they tend to be useful across a wide range of soil series.

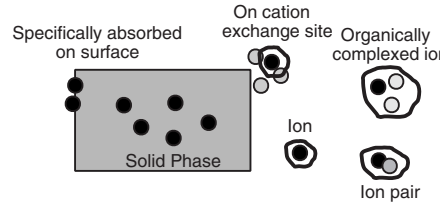
Passive Extractions. Of the passive extracts, water and neutral salt extracts are the most widely used, simplest, and best correlated with plant uptake. A range of neutral salt extracts have been used including $Ca(NO_3)_2$, $CaCl_2$, $SrNO_3$, $NaNO_3$, NH_4NO_3, and $MgCl_2$ (McLaughlin et al., 2000). The technique involves collecting soils, adding either water or a neutral salt solution to the soil, shaking, and filtering. The filtrate is generally taken to be representative of the soil solution and readily soluble fraction, and unless otherwise specified, is analyzed via either atomic adsorption or inductively coupled plasma spectroscopy.

Although these extracts are very straightforward, there are some minor complications. For example, when water is used as the extractant, the normal salt concentrations and ionic strength of the soil solution will be diluted, and this will effect the partitioning of metals between exchange sites and solution. In a similar vein, the use of a neutral salt extract may affect changes in metal partitioning that would not otherwise occur under natural conditions—the extent of which varies depending on the salt chosen.

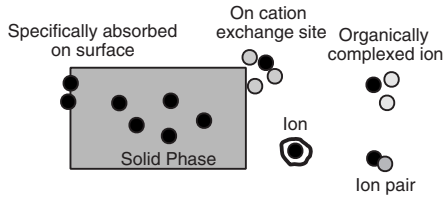
In spite of these complications, studies have generally shown that neutral salt



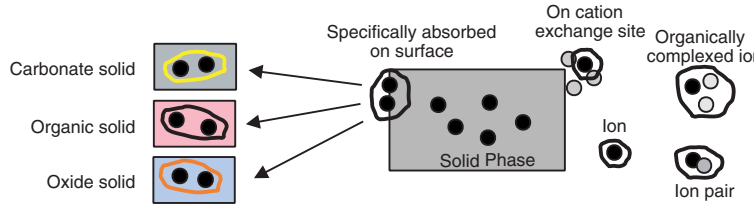
A. Soluble and potentially soluble metals--conventional extracts, exchangeable resins



B. Soluble and exchangeable metals--passive extracts



C. Free ions--pore water measurements



D. Different solution and solid phases--sequential extracts

FIGURE 4-3 Different strategies for extraction tests to remove inorganics from soil and sediment. Conventional extracts and exchangeable resins (A) attempt to quantify all metals that are soluble or have the potential to be available. Less aggressive extracts (B) such as dilute salt extracts are directed towards the soluble or exchangeable fraction. Methods have also been developed to measure the concentration of ionic species in solution (C). Finally, sequential extraction methods (D) attempt to differentiate between solution and different forms of precipitated ions in soils and sediments.

extracts are more predictive of plant metal concentration than total metals in contaminated soils (Symeonides and McRae, 1977; Hani, 1996; Zhang et al., 2001). Lebourg et al. (1996) reviewed 20 years of research comparing soil extracts to plant uptake of cadmium, chromium, copper, lead, nickel, and zinc and concluded that unbuffered salt solutions were the most appropriate way to estimate the transfer potential of these elements from soil to plant, as well as to define guide values for risk assessment. Sauerbeck and Styperek (1984) reported r^2 values between 0.66 and 0.8 for the correlation between $CaCl_2$-extractable soil cadmium and plant cadmium for five crops grown in pot studies using three different soils. Correlation of plant uptake with total soil cadmium was much lower (between 0.01 and 0.22). These extracts show the best correlation over a wide gradient of contamination. They are much less effective at low contamination levels when soil solution is not the primary contaminant source for plants.

Passive extracts can also be used to predict metal bioavailability to other soil organisms. Janssen et al. (1997) found that earthworm bioaccumulation of arsenic, cadmium, copper, and lead was correlated with metal in $CaCl_2$ extracts from contaminated soils (although the correlations were not particularly strong—$r^2 = 0.39$ for $CaCl_2$-extractable arsenic, compared to a correlation of earthworm bioaccumulation with total arsenic concentration of 0.27). Conder et al. (2001) similarly reported that reductions in $Ca(NO_3)_2$-extractable zinc in a smelter contaminated soil were correlated with reductions in earthworm mortality.

Pore Water Measurements. Where uptake of the free metal ion from the aqueous phase is the dominant exposure pathway (as it is for metal uptake into plants—see Chapter 3), pore water measurements can be valuable. Soil solution can be directly measured by centrifuging moist soil or through the use of soil solution samplers. Centrifuging soils requires large volumes of soil to generate sufficient solution for analysis, and thus is not practical for many applications. Soil solution samplers have been recently developed and show promise. These are robust enough for use in pot studies and have also been used in field situations with excellent correlation to plant tissue concentration, although their detection limits can be poor (Doberman et al., 1994; Knight et al., 1998; Farley and Fitter, 1999; Zhang et al., 2001).

Ion specific electrodes are straightforward to use and are relatively rapid and inexpensive. However, for many elements, the electrodes are prone to interference and do not have sufficient sensitivity to be useful for environmental samples. At the present time, copper is the only element that has a sufficiently low detection limit in combination with a lack of interference to be viable for environmental samples. Solution activities of Cu^{2+} have been measured with a copper electrode and compared to plant uptake for three plant species (Sauvé et al., 1996).

Anodic stripping voltammetry (ASV) has been used to operationally define free ion concentrations for a range of elements, including copper, cadmium, lead, nickel, selenium, and zinc, with detection limits of approximately 10^{-9}–10^{-10} M

Plant growth response, as measured in these pot studies, is often used to validate extractions tests for inorganics in soil.

(Shuman, 1996; McBride, 1998; Sauvé et al., 1998). The assumption underlying this method is that only the ionic species from easily disassociated ion pairs or very weak organic complexes can be concentrated on the electrode. ASV has been used to correlate the free ion concentration of cadmium, copper, lead, and zinc in soil solution to a range of soil parameters, including pH, total organic carbon, and total metal (del Castilho et al., 1993; Sauvé et al., 1997, 1998, 2000). Unfortunately, attempts to correlate ASV results to plant uptake are absent from the literature. Validation with plant uptake in field studies will be required before the value of this method can be assessed.

Exchange Resins. Exchange resins have been used extensively to quantify free ion activities, solution fractions, and labile pool concentrations of metals in soils. These different fractions are assessed by varying the particular resin used, the ratio of resin to soil solution, and the equilibrium time. The basic procedure involves circulating soil solution, or soil solution extracted with water or a dilute salt, through an acceptor solution that contains a resin that has been impregnated with a particular cation. The amount of metal exchanged onto the resin is then operationally defined as corresponding to a portion of the total present in soil solution. For detailed discussion of individual tests see Cox et al. (1984), Fitch and Helmke (1989), Jing and Logan (1991), del Castilho et al. (1993), Lee and Zhang (1993), and Holm et al. (1995).

Currently, there is no standardized technique for measuring the free ion in soil solution using resins, making interpretation problematic (Skogely and Doberman, 1996). In addition, most studies use different techniques to measure labile concentrations (e.g., different soil:solution ratios, different equilibrium times, and different types of resins). In some cases, extraction tests using exchange

resins have been shown to correlate well with plant uptake, as in the case of cadmium (Jing and Logan, 1991; Lee and Zheng, 1993). This technique has the potential to be robust across a range of soil series. With standardization, exchange resins could become a widely applicable tool to measure bioavailability.

One concern with passive extracts and pore water measurements is that the measured information (the instantaneously labile pool of contaminants) may not be sufficient to evaluate the potential for contaminants to become more available with time. Exchange resins have the potential to measure both the solution *and* potentially soluble fraction of total soil metals. Strong resins can be used to remove metals from soil solution, after which metals in the solid phase will replenish the solution concentration. If the time course of this replenishment can be quantified, it may indicate the fraction of total metal that will become bioavailable over time. Thus, such resins could be used to indicate both the immediate and potential bioavailability of a contaminant. A technique recently developed for this purpose involves the use of a diffusive gradient in thin films (DGT) (Zhang et al., 1998; Hooda et al., 1999). As discussed in Box 4-3, DGT has the potential to serve as a single resin type for a wide range of contaminants and soils. A new version of the resin technique, DGT was initially developed for use in sediments and water and has only recently been used for soils (Davison and Zhang, 1994; Zhang and Davison, 1995; Hooda et al., 1999). It has not been robustly tested, standard protocols have not been developed for its use, and it has not been validated across different levels of contamination or different soil series.

Conventional Extractions

More aggressive extractions have also been used to define the bioavailable fraction of total metals in contaminated soils. These extractions, including DTPA, Mehlich I, II and III, 0.1 M HCl, and EDTA (disodium ethylenediaminetetraacetate), were developed to predict nutrient deficiencies and so alter the solid phase of the soil as a plant might (Reed and Martens, 1996). They are not robust across soil series. These extractions correlate much more poorly with plant tissue concentrations than do soil solution-based extracts (e.g., Taylor et al., 1992; Brown et al., 1994; Pichtel and Salt, 1998), and thus are not recommended where potential contaminant toxicity is the primary concern.

Sequential Extractions

Passive extracts measure only the instantaneous bioavailable fraction of the contaminant and provide no information on the solid-bound contaminant. Thus, a number of sequential extracts have been developed to quantify the distribution of metals in various solid phases for both soils and sediments (Tessier et al., 1979; Emmerich et al., 1982; Quevauviller et al., 1993; Berti and Cunningham, 1997). Each successive treatment is more drastic in chemical action, or of a different

BOX 4-3
An Exchange Resin Technique for Measuring Metal Bioavailability in Soils: DGT

DGT devices measure metal bioavailability by being placed directly on the surface of moist soil. The device has a filter that permits diffusion of ions through two gels, the second of which has a resin that absorbs ions, setting up a diffusion gradient. Thus, the resin is separated from the soil by an ion permeable membrane. By monitoring both changes in solution concentration and the amount of metal adsorbed on the resin, metal distribution and flux for particular soils can be evaluated. The method assumes that changes in solution concentration (C_{soln}) are resupplied from the labile particulate phase (C_{LP}) at a resupply rate defined by the constant (k_1) for a particular soil and contaminant (Zhang et al., 2001).

$$DGT \leftarrow C_{soln} \underset{k_1}{\overset{k_{-1}}{\rightleftarrows}} C_{LP}$$

Zhang et al. (2001) assessed the phytoavailable fraction of copper in 29 naturally copper-contaminated soils using DGT and other tools. Copper uptake by *Lepidium heterophyllum* Benth. (pepperwort) was compared to (1) free Cu^{2+} in soil solution measured by an ion specific electrode, (2) total copper in soil solution collected with a soil moisture sampler, (3) EDTA-extractable copper, and (4) the effective copper concentration (Cu_E) as measured using DGT (Figure 4-4). Copper measured by DGT was most closely correlated to plant uptake ($r^2 = 0.98$), with total copper in the soil solution being the next best indicator ($r^2 = 0.85$) (Davison et al., 2000). The performance of the DGT resin was superior to the others, particularly at the low end of copper uptake by plants.

DGT is currently under development. Hooda et al. (1999) showed differences in available metal measured based on soil moisture, incubation time, and thin film thickness. While metal flux into the resin was linear in a soil:water slurry, it was not linear in a saturated soil. In addition, trace metal fluxes across different soil moisture concentra-

nature, than the previous one, such that each solid phase is defined as having different potential bioavailability (see Figure 4-3). Metals in soil solution or exchangeable metals are considered the most labile and bioavailable. Organically bound metals and inorganically bound metals are progressively less labile. The residual fraction extracted using strong acids is seen as unavailable (Sposito et al., 1982).

Sequential extraction has been employed for numerous purposes. For example, it was used to assess the differential uptake of metals by plants grown in biosolids-amended soils (Chang et al., 1984a; Sims and Kline, 1991; Berti and Jacobs, 1996; Sloan et al., 1997; Basta and Gradwohl, 2000). In some cases, these studies have been part of the larger goal of evaluating the success of *in situ* soil

tions were not linear and differed by metal ion. The differences shown in this study were for a single soil with constant total metal concentrations. Thus, it is expected that these deviations will become more pronounced when the range of soils examined using DGT increases.

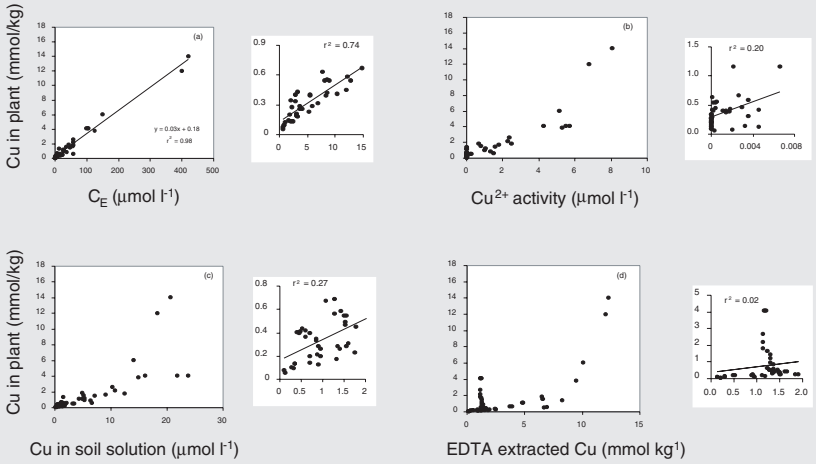


FIGURE 4-4 Plots of [copper] in plant tissue versus C_E measured by (A) DGT (resin), (B) free Cu^{2+} activity, (C) soil solution (passive extraction), and (D) EDTA extracted copper (conventional extraction) for all individual pots. Small graphs show the lower concentration range. SOURCE: Reprinted, with permission, from Zhang et al. (2001). © (2001) American Chemical Society.

amendments to stabilize metals (Berti and Cunningham, 1997). Changes in metal partitioning over time have also been evaluated with sequential extraction. Almas et al. (1999) added cadmium and zinc isotopes to naturally enriched soils, let them incubate for a year, and then used a sequential extraction procedure to evaluate changes in metal distribution. They observed an approximate 50 percent reduction in the mobile fraction with a migration of the isotopes to more inert fractions over time.

Although sequential extracts can reveal changes in how metals are bound to solids, these procedures may be more appropriate for qualitative rather than quantitative determination of metal partitioning. Both soils and sediments consist of heterogeneous layered aggregates; even with an extractant targeted for a spe-

cific phase, it is likely that some phases are leached progressively instead of fully by their selective extractant (Jenne, 1977). Redox-sensitive elements can be gratuitously reduced or oxidized during the procedure, leading to experimental artifacts (Gruebel et al., 1988). In addition, both reprecipitation and readsorption can occur as metals released from heterogeneous phases interact with soluble and insoluble components in the extract (Belzille et al., 1989; Apte and Bately, 1995; Ahnstrom and Parker, 1999; Bunzl et al., 1999). As Nirel and Morel (1990) emphasizes, sequential extraction procedures do not provide actual particulate speciation, and the conditions used during the extraction procedures (strong reagents and fast kinetics) are difficult to extrapolate to naturally occurring processes (weak reagents, slow kinetics).

Despite these concerns, efforts to standardize sequential extractions are under way, as evidenced by the Measurements and Testing Programme of the European Commission (Quevauviller, 1995) and the National Institute for Standards and Testing (Ho and Evans, 1997). Although changes in the operationally defined fractions of metals in soil may provide some indication of changes in bioavailability, at the current time sequential extractions are best used in comparative experiments. The qualitative nature of this information makes its use for development of regulations potentially problematic.

Regulatory Leaching Tests

Simple regulatory leaching tests, such as the Toxicity Characteristic Leaching Procedure (TCLP; EPA Method 1311) or the Synthetic Precipitation Leaching Procedure (SPLP; EPA Method 1312), have been used at times to determine the bioavailability of metals from soil. However, while these tests are useful for evaluating the mobility of contaminants in soils, they have minimal relevance for estimating biological absorption. The TCLP test involves leaching a soil sample in an acetic acid solution at a pH of 2.9 or 5.0 (depending on whether the waste is characterized as alkaline or non-alkaline), and was designed to simulate leaching in a landfill environment. The SPLP test involves leaching in a fluid that is pH 4.2 or 5.0 depending on whether the site in question is east or west of the Mississippi river, respectively, to simulate leaching in rainwater. Because neither of these tests bears a mechanistic resemblance to the processes that would control the uptake of contaminants in soil by ecological or human receptors, it is not reasonable to expect that they would be predictive of bioavailability, nor is their use for this purpose recommended. For example, TCLP tests for arsenic on 13 samples of soil and mine waste compared to the EPA Region 8 young swine model tests (for the same set of substrates) indicated no correlation between these two methods (Rodriquez et al., 1999). However, their use as a first level, commercially available test to evaluate mobility in soils may be appropriate for particular situations.

Extraction Tests for Inorganic Contaminants in Sediments

Extraction tests for inorganic contaminants have a parallel history in soil science and aquatic geochemistry, and most of the tests used for soils are applicable for sediments as well. Thus, for example, pore water measurements in sediments can be used to estimate bioavailability in those cases where the major exposure pathway involves metals in the aqueous phase. An example is tributyltin; dissolved forms of the compound that have leached directly from vessel hull paints are more available that solid-bound forms, such that regulatory thresholds for tributyltin in Puget Sound sediments have been based on correlation between interstitial water and invertebrate tissue concentrations (Michelsen et al., 1998). In other cases, however, more complex tools are required because of the aggregated and complex nature of multi-ligand particulate material with which metals interact. Chemical methods that would selectively extract specific forms of metals once seemed an attractive approach. Thus, batch or more commonly sequential extractions with appropriate reagents were devised for aquatic sediments (Jenne, 1977; Jenne and Luoma, 1977), following the theoretical constructs laid out for soils. As with soils, the goal of sequential extractions of sediments is to leach successive fractions of metal selectively from the sample; that is, at each step to extract a metal completely from a given phase while leaving more or less intact the same metal bound to other phases.

The extractants used fall into the typical classes of chemical behavior, including inert electrolytes, weak acids, reducing agents, complexing agents, and oxidizing agents (Campbell and Tessier, 1989). In general, the outcome for extractions of sediments has been much the same as for soils. A few approaches are somewhat selective, but most are not (see reviews by Campbell and Tessier, 1989; Luoma, 1989). Sequential or batch extractions are probably the least selective for cadmium, copper, and zinc, but may be more effective for establishing forms of mercury (Davis et al., 1997), selenium (Cutter, 1985) or chromium (EPA Method 7195, SW846) because of the unique chemistry of these elements and because analytical methods exist for differentiating oxidation states of these elements once they are extracted. Verifying the specificity of extractions has been difficult because few methods exist to demonstrate metal form in complex natural sediments *other* than extractions (i. e., comparative verification is difficult). X-ray adsorption spectroscopy, which can determine both oxidation state (XANES) and mineral form (EXAFS) of a range of elements, may at some future date be useful in this regard.

Many extractions of sediment purport to remove that fraction of metal that organisms remove from sediments, either by empirically reflecting sediment–water exchangeability or imitating digestive removal. Reviews of the numerous attempts to establish such correlations conclude that no one universal extractant procedure can closely define the availability of all metals (Luoma, 1989; Apte and Bately, 1995), although some successful correlations are found in certain

instances with specific trace elements. For example, extractions may improve understanding of available concentrations of a trace element by excluding the most recalcitrant and unavailable forms (for example with selenium—Schlekat and Luoma, 2000). Mimicry of digestion is also a possible avenue wherein extractants might offer some value in explaining uptake. Recent studies have used extractions with the digestive fluids of invertebrates (the "biomimetic" approach discussed below for humans) to successfully explain bioavailability of at least some metals (Mayer et al., 1996; Chen and Mayer, 1999). Extraction by weak hydrochloric acid seems to greatly improve predicted silver availability to bivalves (Luoma, 1996), although the mechanistic reasons are not clear. Nonetheless, as discussed in Box 1-2, simple correlations between extracted concentrations and bioaccumulation are rare when applied across diverse sediments.

Extraction Tests for Inorganics that Mimic Human Exposure

Extraction tests that are intended to predict the extent of oral bioavailability of inorganic elements in humans have been available for several decades and first appeared in the field of nutrition. In the late 1970s and 1980s, several research groups were developing *in vitro* extraction tests that simulated the function and chemistry of the human gastrointestinal tract to predict the amount of iron in food substances that would be bioavailable upon ingestion (Bezwoda et al., 1978; Miller and Schricker, 1982; Reddy et al., 1988). Several of these groups also attempted to "validate" their *in vitro* extraction tests against iron bioavailability results observed in swine. These tests were the forerunners of the extraction tests currently used to estimate the oral bioavailability of toxic metals in soil. To date, there has been extensive work to develop and validate an *in vitro* extraction test for lead, a moderate amount of work on arsenic and mercury, and a small amount of work on beryllium, cadmium, chromium, and manganese.

These *in vitro* extraction tests simulate dissolution in a fasting gastric environment because lead and cadmium, and most likely other inorganics as well, are more bioavailable under fasting than fed conditions (James et al., 1985; Maddaloni et al., 1998). The gastric phase may be followed by a small intestinal simulation of near neutral pH that contains various enzymes and acids (e.g., pancreatic enzymes and bile acids). (See Oomen et al., 2002, for a recent comparison of five different digestion models.) Obviously, such tests are only capable of simulating dissolution of metals from soil in the gastrointestinal tract (bioaccessibility) and do not simulate the process of absorption across the intestinal epithelium. Therefore, if absorption is the rate-limiting bioavailability process, rather than the rate or extent of dissolution from soil, then these tests will not be capable of predicting oral bioavailability. However, for lead and arsenic in soil, the extent of dissolution in the acidic stomach environment appears to be the determining factor for oral bioavailability, based on comparison to bioavailability

studies for lead in rats (Ruby et al., 1996) and swine (Medlin, 1997) and for arsenic in swine (Rodriguez et al., 1999).

In addition to predicting oral bioavailability, *in vitro* extraction tests have been used to examine the effects of gastrointestinal tract chemistry on the availability and chemistry of metals in soil. For example, the tests described above have been used to examine the effects of pH and gastrointestinal fluid composition on lead and arsenic bioaccessibility, as well as the effect of gastric fluid pH and chemistry on the rate and extent of hexavalent chromium reduction.

In vitro tests are often used to estimate exposure parameters for human health risk assessment, and because they are relatively new, some validation has been conducted to promote regulatory acceptance. This has involved comparison of the extraction test results to those from an *in vivo* model for a set of samples that is large enough (on the order of 10–20) to develop a statistically significant correlation. The cost of generating this amount of *in vivo* data is the primary limitation to the development and validation of this type of assessment tool. To date, only an *in vitro* extraction test for lead has received such validation (against the EPA Region 8 young swine model for lead bioavailability), while an extraction test for arsenic is in the process of validation (against the young swine model and a primate model). At this time, no *in vitro* to *in vivo* comparisons are available for beryllium, cadmium, chromium, or mercury in soil.

Extraction tests using real or synthetic sweat have been used to evaluate the fraction of chromium that might dissolve at the skin surface and become available for dermal absorption. Horowitz and Finley (1993) determined that 0.1 percent of Cr(VI) and 0.3 percent of total chromium in soil samples contaminated with chromite ore processing residue would be extracted by human sweat. Using similar soils (i.e., those containing chromite ore processing residue from the same site), Wainman et al. (1994) concluded that synthetic sweat could reliably be used to estimate dermal exposure to chromium in soil. Given the expense of measuring dermal Cr(VI) absorption using animal studies, or *in vitro* studies using human cadaver skin, it is possible that such extraction studies in real or synthetic sweat will receive further consideration in the future.

Extraction Tests for Organics

Analytical methods for measuring concentrations of organic chemicals in soil or sediment have historically entailed vigorous extraction with low polarity organic solvents to remove all or as much hydrophobic organic contaminant as possible. Common techniques include Soxhlet extraction, XAD extraction, and solid-phase extraction microcolumn. As one would expect, the bioavailability of contaminants in soil and sediments is overestimated by such analytical methods. For example, exposure estimates based on Soxhlet extracts of organic compounds do not accurately reflect the concentrations of compounds available for uptake, as

determined by the number of bacterial mutations in treated soil (Alexander and Alexander, 2000).

Several milder extraction tests have been developed that correlate with bioavailable organic compound concentrations in soil and sediment and may serve as surrogate assays for bioavailability. These chemical tests are being developed for use in site-specific assessment because they are generally less time- and resource-dependent than biological assessments. A limitation of most of the tests described in this section is that they are typically applied *ex situ* and may not be truly site specific because of biases inherent in sample collection and disruption.

Figure 4-5 shows a conceptual diagram of four extraction strategies, examples of which are given below. Extraction tests can be differentiated by (1) the need to make a slurry of the soil or sediment sample versus using the sample directly, (2) use of a semipermeable membrane, and (3) use of a liquid or solid

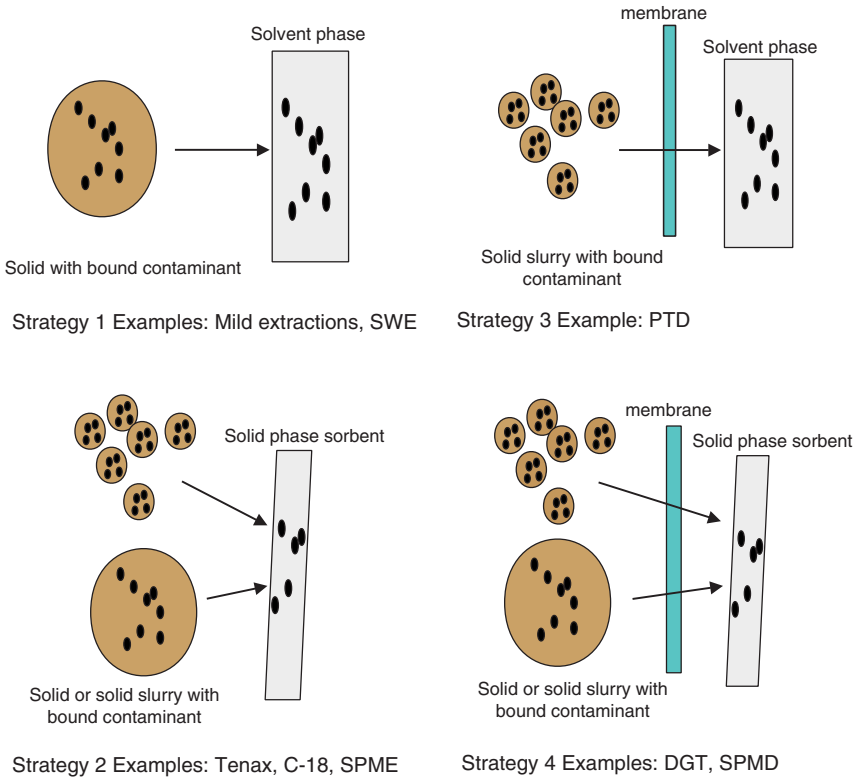


FIGURE 4-5 Four different strategies for extraction tests to remove organics from soil and sediment.

adsorbing phase. First, techniques that require a soil or sediment slurry measure contaminant concentration in the pore water of samples, and thus are an indication of aqueous phase concentrations in the natural environment. They cannot be used *in situ*. A semipermeable membrane allows one to differentiate between contaminants that have desorbed from the soil or sediment to the aqueous phase and those that are still bound to colloids. Techniques that do not use semipermeable membranes tend to measure higher contaminant concentrations than those that do. Finally, the adsorbing phase is typically something that might mimic a biological system (biomimetic). As discussed above for extraction tests for inorganics, techniques using solid-phase adsorbents (e.g., exchange resins) can measure both the instantaneous bioavailable contaminant fraction as well as the fraction potentially available over time (i.e., the rate of desorption).

As with inorganic contaminants, there is no ideal extraction strategy for organic contaminants bound to soils and sediment. Rather, the techniques must be chosen to reflect the conditions present at the site, particularly soil or sediment type and the feeding behavior and uptake mechanisms of the receptors. Ideally, experiments should be conducted within and between different soils and sediments (to determine the effects of the soil or sediment matrix on experimental results) and the tools should be validated by comparison to a bioassay.

Mild Extractions with Various Solvents

As with the passive extractions conducted for inorganics, there are several mild extractions that can be used to determine the available fraction of an organic contaminant in a soil or sediment sample. The techniques generally involve collecting a soil or sediment sample, adding the solvent, shaking and filtering, and analyzing the contaminant in the solvent phase.

Moderately Polar Organic Solvents. Extraction with moderately polar organic solvents, such as butanol, methanol, n-propanol, or ethyl acetate, has been used to estimate the availability of PAHs and a few pesticides in soil, and validation by comparison with biological assays has been achieved. For example, in several studies with freshly added and aged chemicals in soil, a good correlation was observed between the fraction of contaminant extractable using a moderately polar solvent and the fraction taken up by earthworms (Kelsey et al., 1997; Tang and Alexander, 1999). Similar experiments have shown good correlations between extractable fractions and other measures of bioavailability, such as biodegradability or soil mutagenicity assessed by a bacterial genotoxicity assay (Alexander and Alexander, 2000; Liste and Alexander, 2002). Comparisons suggest that the strength of the correlation to bioavailability varies by solvent, at a minimum (e.g., Reid et al., 2000). While these tests have shown promising results for assessing how bioavailability changes with contaminant aging in a particular soil, they demonstrate little consistency among different soils (Chung and

Alexander, 1998). Because these methods are also relatively simple, a better understanding of the attributes of soils that control or limit consistency are needed before such tests could be employed for wide-spread use. Additionally, these methods have not been widely tested on sediments.

Supercritical Fluid Extraction. Supercritical fluid extraction using CO_2 from 40 to 150°C has been developed for sequential extraction of polychlorinated biphenyls (PCBs) associated with field contaminated soils and sediments (Bjorklund et al., 1999). In this method, the sample is placed in a pressure- and temperature-controlled extraction chamber through which supercritical CO_2 is flushed. By increasing the temperature of the supercritical fluid, the solvency power of the extractant is increased in a step-wise fashion (similar to sequential chemical extractions described earlier). Hawthorne and Grabanski (2000) used sequentially stronger supercritical fluid extraction conditions to selectively extract PAHs associated with "fast" (or "rapidly desorbing"), "moderate," "slow," and "very slow" sites on the soil collected before and during one year of field bioremediation of a manufactured gas plant site soil. They found that supercritical fluid extraction under the mildest conditions (120 bar, 50°C) gave good quantitative agreement with removals achieved after one year of bioremediation for PAH compounds ranging from two to six rings. This is a promising method to assess quickly the easily available contaminant fraction in soils (as in Hawthorne et al., 2000a), but it needs to be verified on several other soil and sediment samples.

Subcritical Water Extraction. Subcritical water extraction (SWE) involves varying temperature and pressure to change the polarity of the water solvent. Thus, at low temperature, water extracts polar organics, while at higher temperature, water extracts moderately polar and nonpolar organics. By varying the temperature of extraction, quantitative recoveries of a range of compounds of varying polarities (e.g., PCBs, PAHs, alkylbenzenes, aromatic amines) have been achieved from soils and sediments that compare well to exhaustive solvent extraction methods (e.g., Hageman et al., 1996; Hawthorne et al., 1998, 2000b). In at least one study, temperature was the most influential experimental factor affecting extraction efficiency and kinetics (Krieger et al., 2000). At lower water temperatures (more mild conditions), this method has also shown diminishing contaminant recovery with aging in soil. As such, this relatively simple method shows promise in estimating bioavailability. At this time, there have been no reported correlations of subcritical water extraction results to bioassays.

Extractions with a Solid-Phase Sorbent

Solid phase extraction is a technique that can assess both the rate and extent of desorption of a sorbed organic compound because the solid phase (e.g., an exchange resin) acts as a contaminant sink. These approaches are termed "bio-

mimetic" because they mimic uptake from the solid or pore water directly to the organism.

Slurries with Sorbents. Most widely employed are variants on solid-phase extractions that use soil or sediment slurries (Yeom et al., 1996; Cornelissen et al., 1997a; Gustafson and Dickhut, 1997; Macrae and Hall, 1998; Morrison et al., 2000; Krauss and Wilcke, 2001). In these assays, a strong sorbent for the target compound is intimately mixed with the soil or sediment and water in a batch reactor. An adequate amount of sorbent is provided to ensure that the aqueous phase concentration of the target compound is maintained near zero to ensure a maximum driving force for desorption from the contaminated soil or sediment. Polymeric resins such as XAD-2, XAD-4, and Tenax TA (a 2,6-diphenyl-p-phenylene oxide based polymer) or C-18-coated materials such as Empore™ discs are typical sorbents for extracting HOCs from soil or sediment. After exposure, the sorptive phase is physically removed from the sample, and extracted to determine the total contaminant concentration. Because only the sorptive phase-associated concentrations are measured, this method quantifies the fraction of contaminant that was physically transferred into aqueous phase from the soil or sediment, under the assumption that partitioning to the sorptive phase is rapid. A potential artifact of these measurements is the possible inclusion of colloidal-associated contaminants if the colloids are incompletely separated from the resins during the separation step. The test result can be expressed as a rate (flux or kinetics of contaminant desorption from solid phase), but more commonly it is expressed as a mass (amount of a contaminant that can enter the aqueous phase after a defined incubation time and conditions).

Correlations between the contaminant mass removed by these desorption techniques and other bioavailability assays have been developed. In addition, results from many studies have revealed the existence of fast- and slow-release fractions of contaminant in the soil or sediment (Cornelissen et al., 1997a; Ghosh et al., 1999; Opdyke and Loehr, 1999). For example, strong correlations were observed between rapidly desorbed fractions of DDT, DDE, and DDD (as determined by C-18 extraction) from soils in which pesticides were freshly added or aged and earthworm assimilation (Tang et al., 1999; Morrison et al., 2000). In the study by Tang et al. (1999), worms assimilated 3 to 66 percent of the compounds in test samples with freshly added pesticides or residues persistent in the field for 49 years, which correlated well with the amount of pesticides taken up by C-18 disks. Similarly, the total mass of biodegradable PAH that desorbed during the "fast" phase as determined by Tenax TA extraction closely matched that which was bioavailable as determined by bioremediation via soil-slurry or land-farm treatment (Cornelissen et al., 1997a). Although these correlations suggest the technique can represent bioavailability to some organisms, limited validation has been performed. No validation on the purported correlation to bioconcentration in higher organisms has been reported.

These tests provide more information about the timing of contaminant release from the soil and sediment than mild extraction tests. However, the long time frames involved to obtain the information, particularly for the most hydrophobic compounds studied, can be a disadvantage (although C-18 disks require shorter equilibration periods and simpler extraction procedures). Furthermore, the distinction between the "fast" and "slow" release periods is empirical. Thus, these methods may be most useful for estimation of the contaminant fraction within the soil that is bioavailable within a reasonably short period of time, comparable to the study duration. Finally, these assays cannot be employed *in situ*, but require some type of solid–liquid slurrying.

Solid Phase Microextraction. Solid phase microextraction (SPME) is a relatively recent analytical development that is seeing increased use for aqueous, air, and soil analysis of volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs) (Eisert and Pawliszyn, 1997; Penalver et al., 1999; Alpendurada, 2000). The SPME fibers consist of a sorbent coating (several types are available) on a support. As the fiber (approximately 1-cm long) is exposed to the sample, compounds are sorbed. Following exposure, the entire fiber is desorbed in the injection port of a gas chromatograph for analysis. Thus, SPME does not employ slurries of soil or sediment samples. Because of the small sizes and masses involved in the analysis compared to other procedures, and the now greater variety and selectivity of sorbent coatings, SPME has potential to be used as a biomimetic technique. Headspace solid phase microextraction is a modification that has been proposed as a sample concentration and preparation technique for the analysis of volatile and semi-volatile contaminants in soil (Havenga and Rohwer, 1999; Llompart et al., 1999). Inter-laboratory validation studies to determine the presence of different organic compounds at ppt levels demonstrated use of the technique for quantitative analysis (Alpendurada, 2000).

Solid phase microextraction requires no solvents, and it permits sample transfers and analyses with little modification of chromatographic equipment. Thus, cost can be kept at a minimum. The sample sizes and the extraction times needed to reach equilibrium are small compared to C-18 slurry methods. Moreover, the SPME technique is simple, amenable to automation, and suitable for field and on-site application. However, the method may suffer from the fact that samples are not "cleaned up" as with traditional chromatographic methods, resulting in interference. Thus, it may be useful only for certain chemicals in certain settings. Further, SPME does not give information on concentration but rather on the mass of compound collected over some prescribed interval. This suggests the need for rigorous QA/QC protocols along with proper calibration. There are no published studies that relate the rate or extent of uptake to a SPME fiber directly to a bioassay, although bioaccumulation tests using terrestrial organisms (i.e., enchytraeids and earthworms) are now being conducted. Initial results suggest that SPME fibers may be appropriate for measuring bioavailability of chemicals

with a log K_{ow} up to 6.0 in soil (De Maagd and Staeb, 2002; Heslie et al., 2002; van der Wal et al., 2002).

Aqueous or Solvent Extractions using a Membrane

Polyethylene Tube Dialysis. An *ex situ* method that separates the contaminant sink from the solid–liquid matrix is polyethylene tube dialysis (PTD), wherein the slurry is placed inside a polyethylene tubing suspended in a strong organic extractant (e.g., pentane) and tumbled for 24 hours (Macrae and Hall, 1998). After exposure, the solvent is removed, concentrated, cleaned and analyzed by gas chromatography. Because contaminants associated with soils or sediments cannot pass through the tubing associated with the technique, only dissolved forms of the chemical are measured. This is an important concern for accurately measuring very hydrophobic compounds (certain pesticides, PAHs, and PCBs) which are sorbed strongly onto colloids.

Because less material is extracted than in Tenax TA methods, the detection limit of PTD for PAHs is approximately an order of magnitude greater (Macrae and Hall, 1998). Limited direct comparison of membrane-less and membrane-containing extraction assays has indicated that the former, expectedly, results in larger contaminant recoveries (Macrae and Hall, 1998). Nonetheless, there are situations where membrane-containing extraction assays may extract more contamination than membrane-less tests. For example, the availability of PAHs in marine sediments was greater when measured using polyethylene tube dialysis than Tenax TA methods or a semipermeable membrane device (SPMD, described below) (Macrae and Hall, 1998). The higher availability measurements from PTD were because some of the pentane used in the extraction passed through the tubing and acted as a co-solvent, facilitating desorption of the PAH from the sediment.

Polyethylene tube dialysis is useful when only small samples are available. This is a relatively inexpensive tool for initial evaluation or screening of contaminated soils and sediments. However, the technique imposes conditions that are hardly reflective of conditions *in situ*. Of the Tenax, SPMD, and PTD methods, comparison has shown that the Tenax extraction method was the least expensive, recovered the larger molecular weight compounds more efficiently, and worked well for heterogeneous environmental matrices (Macrae and Hall, 1998). There are no studies that compare extractability by PTD directly to a bioassay.

Membrane-Based Desorption Tests using a Solid-Phase Sorbent

Of slightly increased complexity are desorption assays wherein the solid sorptive phase sink is separated from the solid–liquid phase extractant by another phase (usually some kind of membrane). (The DGT technique described earlier for extracting inorganics from soil and sediment is an example.) Although such

techniques can be used with either a moist solid sample or a solid slurry, in practice most techniques have been developed for application to moist soil samples and sediments. Because of the need to cross a membrane, only truly soluble (i.e., non-colloid-associated) contaminants are measured in these assays.

Semipermeable Membrane Devices. SPMDs are patented devices used to measure the bioavailability of hydrophobic contaminants in the aquatic environment (Huckins et al., 1990, 1993, 1996; Lebo et al., 1992). The device is made from nonporous polyethylene tubing coated with a thinly spread layer of triolein. Hydrophobic contaminants become concentrated in the lipid relative to the water phase according to their respective partitioning coefficient as they would in organismal lipids or tissues. This method allows exposures to be determined without having to account for the variation between individual test organisms or the metabolism or depuration rates in organisms.

Semipermeable membrane devices may be employed in the field at the test site where affected organisms have been located. PAHs with up to five rings have been successfully recovered from marine sediment using this approach (Macrae and Hall, 1998). However, other methods such as the Tenax TA extraction gave higher recoveries for five- and six-member PAHs (Macrae and Hall, 1998). This was attributed to a slow transfer rate of large PAHs across the polyethylene tubing in the SPMD. For lower molecular weight compounds, SPMD was found to be superior to Tenax TA but more expensive.

As with other techniques using a solid-phase-supported sorbent, SPMD should be useful for estimating the fraction of contaminants in soil or sediment samples that will be bioavailable in a reasonable amount of time. However, sorption kinetics to these phases are relatively slow so that weeks may be required before pseudo-equilibrium is reached. Relatively large sample volumes may be necessary to obtain a sufficient level of analytical sensitivity.

The most important limitation of SPMD (or any solid-phase technique) is its dependence on partitioning alone to simulate biological exposure. Partitioning has a first order influence, but as discussed in Chapter 2 has often been shown to not be fully predictive of bioavailability to many organisms.

Both SPMD and DGT can be employed *in situ*, and concentration measures with both techniques were in good agreement with actual aqueous concentrations (Macrae and Hall, 1998; Zhang et al., 1998). With appropriate mathematical modeling, results from DGT or SPMD permit an estimation of the contaminant flux (Zhang et al., 1995, 1998). To date, there are no studies that compare extractability of organics by SPMD or DGT directly to results from a bioassay.

Other Extraction Techniques

Gas Purge. Gas purging of soil or sediment is one of the early techniques to measure the availability and desorption kinetics of semi-volatile organic com-

pounds from soils and sediments (Larsson, 1983; Hassett and Milicic, 1985; Karickhoff and Morris, 1985; Larsson, 1985; Gong et al., 1998). In this method, clean air is purged through a soil or sediment slurry in water, and the off gas is passed through organic traps that are sampled at specific intervals. Data from these tests show the mass fraction of organic compound released versus time. The fractional mass released may plateau with time, and from this it is possible to infer what fraction of the organic contaminant is relatively available.

The main advantage of the gas purge is that the method avoids a solid-separation step and thus eliminates any analytical bias in measuring aqueous concentrations due to colloids that may be present in aqueous samples. Although this method ensures measurement of released organic compound, quantification of mass transfer processes or extrapolation to *in situ* conditions is difficult because both solid–liquid and gas–liquid transfers are involved. As an example of this tool's use, Wu and Gschwend (1986) used data from gas purge of chlorobenzenes from a solid slurry to model rate of release and particle size effects.

Enthalpy and Activation Energy of Desorption. The effects of temperature on sorption can provide information about sorption mechanisms and help explain why an organic compound is more or less tightly bound. The effects of temperature may be explored under isothermal or nonisothermal conditions. Desorption rate tests conducted at different temperatures yield kinetic rate constants from which the temperature dependence of the rate constant may be described by an activation energy (Cornelissen et al., 1997b; Ghosh et al., 1999). High activation energies would be associated with slow release, as in activated diffusion. Activation energies may also be determined by assessment of compound release under continuously increasing temperature conditions, as in automated thermal program desorption techniques (Ghosh et al., 2001). These measurements allow inferences about factors controlling the rate of release and the solid's geochemistry. For example, in sediment significant differences were noted in activation energies for PAH release from clay–silt (37-41 kJ/mol) versus coal-derived particles (115-139 kJ/mol) (Ghosh et al., 2001). High PAH desorption activation energies from coal-derived particles were associated with low lability compared to PAHs on clays and silt. The relationships among rate of release, activation energy, the solid's geochemistry, and the compound's lability were linked in one study for PAHs in sediment and shown to correlate with biodegradability and bioaccumulation (Talley et al., 2002). Such tests are still largely in the development phase. Box 4-4 describes how desorption tests at different temperatures provide some insight on the nature of organic compound interaction with solid sorbent.

Extraction Tests for Organics that Mimic Human Exposure

In vitro extractions for predicting the oral bioavailability of hydrophobic organic compounds in soil to humans also exist, although they are less well

BOX 4-4
Temperature Desorption Techniques

According to the van't Hoff relationship, increasing temperatures result in decreasing equilibrium distribution coefficients for an exothermic process. For heterogeneous surfaces containing sites with different energies, sorption to higher energy sites is often characterized by larger negative enthalpies (or greater heat of sorption). This means that an increase in temperature decreases the amount sorbed, and more so at lower sorption values (Werth and Reinhard, 1997a). Thus, if the heat of sorption is positive at low sorption values and decreases with increasing sorption values, then different temperature isotherms converge with increasing sorption values. Werth and Reinhard (1997a) invoked these considerations to infer whether trichloroethylene (TCE) (1) partitions into natural organic matter or sorbs on water-wet mineral surfaces, which would show small changes with temperature, or (2) adsorbs in hydrophobic micropores that would show very large heats of sorption and significant differences at different temperatures. They demonstrated that temperature did not significantly affect sorption onto sand, aquifer sediment, or soil, suggesting that sorption onto mineral surfaces or partitioning into organic matter controlled equilibrium. However, for a clay and silt a large heat of sorption was calculated for low equilibrium concentrations, indicating that sorption in this region was occurring in micropores. In follow-on work, the authors concluded that the fraction of the TCE mass contributing to slow desorption was attributed to activated diffusion in micropores (Werth and Reinhard, 1997b). Young and Weber (1997), using supercritical fluid techniques to examine desorption, concluded that relatively weak nonspecific forces governed the binding of phenanthrene to three soils.

developed than those for metals. The mechanism by which organic compounds become bioaccessible from soil appears to be primarily a matter of lipid chemistry in the gastrointestinal tract rather than simply dissolution in the acidic gastric environment. Thus, *in vitro* extraction tests for organics in soil use the framework established for *in vitro* extractions for metals with additional chemical components to simulate the lipid and protein chemistry of the gastrointestinal tract.

In the human gastrointestinal system, ingested lipids are hydrolyzed into absorbable forms (fatty acids and monoglycerols) by gastric and pancreatic lipases (Hernell et al., 1990). These fatty acids combine with bile salts to form mixed micelles—a core of hydrophobic lipids surrounded by a shell of lipoproteins. In the small intestine, bile salts form the outer layer of these micelles, which can traverse the mucine layer adjacent to the intestinal wall and then be absorbed across the intestinal epithelium. It is believed that these bile salt micelles in the small intestine provide a lipid sink into which HOCs can partition, and that the HOCs are then absorbed across the intestinal mucosa along with the micelle (Hack and Selenka, 1996; Guha et al., 1998; Holman, 2000; Oomen et al., 2000a). For this reason, bioaccessibility tests for HOCs in soil have all included

some form of bile salt micelle. Different lipid sources have been used to form bile salt micelles, including powdered whole milk (Hack and Selenka, 1996; Wittsiepe et al., 2001), a mixture of oleic acid, monoolein, diolein, and lecithin (Holman, 2000), and oleic acid alone (Oomen, 2000b; Ruby et al., 2002). Various HOCs also appear to partition into protein phases during simulated human digestion (Hack and Selenka, 1996; Oomen et al., 2000a). Therefore, a representative protein such as bovine serum albumin has been added to some extraction systems. Finally, mucin (a viscous mixture of glycoproteins and enzymes present in the mammalian stomach and intestines) has been used in several *in vitro* extraction systems because it has been observed to increase the fraction of HOCs liberated from soil (Hack and Selenka, 1996).

To date, *in vitro* extraction systems have been applied to PCBs (Hack and Selenka, 1996; Oomen et al., 2000a), PAHs (Hack and Selenka, 1996; Holman, 2000), polychlorinated dibenzodioxins/furans (Rotard et al., 1995; Wittsiepe et al., 2001, Ruby et al., 2002), and lindane (Oomen et al., 2000a) in soil and solid wastes. Results from these studies indicate that the fraction of different HOCs extracted is variable, and depends greatly on the composition of the test fluid. For example, for both PCBs and PAHs, extractability from soil is relatively low when only the gastric phase of the extraction is employed, but it increases dramatically when the small intestinal phase of the extraction is added (Hack and Selenka, 1996). For all of these HOCs, the inclusion of bile salts and a lipid source to the extraction test greatly increased the fraction of the HOC liberated from the soil or waste. In general, the more thorough extraction tests gave bioaccessibility results of 20 to 60 percent for PCBs, PAHs, and polychlorinated dibenzodioxins/furans from soils and solid wastes. Because there have been no published comparisons between these *in vitro* data and those from *in vivo* studies for the same soil substrates, the predictive value of these *in vitro* tests is unknown. The lack of *in vivo* studies for HOCs in soil is not surprising given their cost and difficulty. Substantial effort will be required to validate these *in vitro* extraction tests for HOCs in soil.

At this time, no work has been reported on the development of extraction tests to estimate the dermal bioavailability of organic compounds in soil or sediment.

Normalization Techniques

Normalization of extracted contaminant concentrations with measured soil or sediment characteristics is a long-standing approach that has been used to move beyond the simple assumptions of extractions and to incorporate chemical and biological complexities. In this approach, contaminant concentrations in sediments or soils are arithmetically "corrected" via geochemical or biological factors thought to influence bioavailability. Many normalizations were developed from observing correlations between factors during field studies and as such do

not have a complete mechanistic underpinning. Others are based on a more theoretical construct.

Soil scientists have long combined results from extractions with correlative normalizations to empirically develop predictive equations for metal availability to plants (Pickering, 1981). For aquatic sediments, Luoma and Davis (1983) suggested that contaminants generally associate more completely with a geochemical component of the sediments as the number of binding sites of that component increases in the sediment. If that component–contaminant association reduces bioavailability, then higher component concentrations (e.g., iron oxide) result in reduced bioavailability. Some impressive empirical correlations with bioavailability have been obtained using this general approach. For example, correlation was demonstrated between lead bioaccumulated by bivalves and lead:iron ratios in oxidized surface sediments across 17 English estuaries (Luoma and Bryan, 1978). Tessier et al. (1984) showed a similar relationship for a variety of Quebec lakes. Other successful normalizations were found for arsenic:iron ratios in sediments and arsenic uptake by bivalves in English estuaries (Langston, 1980) and mercury:carbon ratios in a variety of environments (Langston, 1982; Breteler et al., 1981).

The studies cited above included broad concentration gradients, careful biological sampling, and complex geochemical conditions across the data set (i.e., they were not the result of co-variances or simple conditions). Nevertheless, this approach has not been extensively employed in recent years. One reason is that the approach is not mechanistic, although mechanistic explanations have been offered (Luoma, 1989). In addition, there is a lack of models or direct analytical techniques to relate metal form to the normalized concentration. Indeed, the quantitative relationship between bioavailability and normalized concentration is unique for each metal–species–environment combination, making development of generalized models difficult. Finally, factors affecting bioavailability are more complex than accounted for by simple normalization for many metals or animal species (e.g., Luoma and Bryan, 1982; Amyot et al., 1994).

Normalization techniques also were developed as shortcuts or surrogate measures to represent expected outcomes of a mechanistic theory like equilibrium partitioning (DiToro et al., 1991). Equilibrium partitioning (EqP) theory uses hydrophobicity and normalization to organic carbon in sediment to predict porewater contaminant concentrations, assuming bioavailability is controlled by pore waters alone (DiToro et al., 1991). Normalization to lipid content of the organism(s) can also improve such relationships (e.g., Lake et al., 1996). Bioassays that manipulated organic carbon in sediments provided the experimental substantiation of the theory (Swartz et al., 1990). But field studies have begun to indicate that bioaccumulation of organic chemicals is not necessarily predictable from EqP alone (Pereira et al., 1988; Swackhamer and Hites, 1988). Dissolved organic matter (DOC) is one factor important in deviations from EqP-predicted distributions of organic chemicals in soil and sediment (DeWitt et al., 1992;

Suedel et al., 1993). Complexities affecting the form of the chemical, including the age of the association, also cause deviations (Meador et al., 1995). Apparently, this approach is most valid if the biological processes involved are relatively simple. In complex food webs, though, additional considerations are necessary to predict bioavailability to upper trophic level animals (Kidd et al., 1995).

Perhaps the best-known normalization technique for defining metal bioavailability is based on redox condition for metals and EqP theory. As discussed in Chapter 2, DiToro et al. (1990, 1991) suggested that normalizing metal concentration in sediment by acid volatile sulfide (AVS) might explain metal bioavailability from sediments. This approach was initially tested using bioassays and manipulations of natural sediments. Recent analyses of the early approaches suggest co-variance among total metal concentration and AVS effects might confound some of those tests (Lee et al., 2000). Additional limitations of this technique have been noted in Chapter 2 and Box 4-2. The AVS normalization technique has not been tested using a field correlative approach—that is, correlating AVS measurements from field samples with bioaccumulation of chemicals in organisms residing at the field site.

Analysis of Extraction Techniques

Operational extractions, empirically and theoretically developed normalizations, and models like equilibrium partitioning have each provided some of the ingredients needed to explain when, where, and how contaminants in soils and sediments become bioavailable. It is important when using these extractions to understand their strengths and limitations, many of which have been touched upon in this section.

Perhaps most importantly, extractions should be verified by comparing the chemical predictions with responses in biological indicators. There is an extensive body of data comparing certain extracts with plant uptake, with substantial observed correlation over a large gradient of contaminant concentrations and soil types. A much smaller body of information exists for other biological endpoints (with earthworm bioaccumulation tests being a commonly used bioassay). Several different extraction procedures have shown correlations with metal bioaccumulation in experimental organisms (Luoma and Jenne, 1977; Pickering, 1981; Fisher and Telyssie, 1986). However, not all of the validation efforts reported to date have been successful, calling into question the reliance on extraction procedures to measure bioavailability. Certainly no one universal extraction procedure has been shown to consistently correlate with tissue concentrations in plants or animals across complicated environmental conditions.

The greatest validation with bioassays has come for those extractions designed for specific metals or chemicals and specific endpoints. For example, extraction of silver from oxidized sediments with 0.5–1 N HCl appears to consistently improve correlation of sediment-bound silver with bioaccumulated silver

(Luoma et al., 1991). When such extracts are used in situations for which they were not developed, they are generally unreliable. For example, one of the extractants employed most widely and successfully in studies of plant-available copper from calcareous soils is 0.004 M DTPA (Lindsay and Norvell, 1978). Correlations between the extraction results and plant uptake have been found to be insignificant among soil series (Pickering, 1981), primarily due to inappropriate application of the test. The use and misuse of the DTPA test for plant-available nutrients and contaminants are described in Box 4-5.

Most extractions account for contaminant release from the solid surface to pore water. Thus, they are most successful (i.e., predictive) when biological uptake is dominated by a pore-water pathway (e.g., plant uptake of metals). Extractions cannot account for other, more complicated uptake mechanisms that control an organism's overall dose (Landrum et al., 1992; Luoma et al., 1992; Luoma and Fisher, 1995). Contaminants are distributed among solution, suspended particles, sediments, pore waters, and specific (living and non-living) food sources within all of these. Each species' exposure to those contaminants is determined by how the species "samples" this complex milieu, and by the accessibility of pollutants within each compartment of the milieu. Digestion is a flexible, adaptive, multi-faceted living process that differs among species and can change within a single species with life history or environmental conditions. Thus, to the extent that dietary uptake of a contaminant is important, simulation of that process by single chemical extraction will be close to impossible. This limitation also extends to extraction tests that use a solid-phase absorbent meant to simulate biological exposure via a first-order partitioning mechanism. In addition to dietary uptake, organisms differ in the rate at which they pass water across their gills, in the ways they are exposed to soil and sediment, pore waters and surface waters, as well as in trophic relationships. These factors have important implications for bioavailability that solid-phase extractants cannot simulate.

Extraction procedures do not (with a few exceptions) remove metals or organic compounds from specific components of soils and sediments, nor can they explain the type or character of the sorbent phase to which an organic sorbate may be sequestered. Thus, they are operational, not mechanistic, methods for estimating contaminant availability. Several obstacles preclude the development of extractions and other tools that can directly determine critical forms of contaminants at the proper scales, concentrations, and conditions. Metal ion activities in solution, for instance, are difficult to determine in pore waters due to problems with detection limits at small scale. This limitation has lead to sediments and pore waters being characterized geochemically on scales much broader than the microhabitat scales experienced by benthic or other organisms (Luoma and Ho, 1993).

Extraction approaches and the use of normalizations coupled with extractions have a mixed history of success (with more success in soil systems than sediment systems). Many prove limited in their abilities to consistently predict

BOX 4-5
Misuse of DTPA Soil Test

The DTPA soil extraction was designed to predict micronutrient deficiencies in neutral to calcareous soils (Lindsay and Norvell, 1978). When used appropriately, the test is effective, reproducible, and accurate. In calcareous soils, micronutrients such as zinc, iron, manganese, and copper will generally be sparingly soluble, and nutrient deficiencies are common. As a result, plants may aggressively manipulate the rhizosphere to access required but insoluble micronutrients. Graminaceous plants (grass species) secrete phytosiderophores into the soil solution. These compounds chelate iron as well as other cations in solution, thereby reducing the concentration of free metal ions. In response to this change in equilibria, a portion of the cations in the labile solid phase will come into solution and be available for complexation. The transpiration stream will transport the chelated cations to the rhizosphere where they can be absorbed by plants (Marschner, 1995). The DTPA extraction was developed to mimic the behavior of plant roots under these circumstances.

In developing the extract, Lindsay and Norvell tested a range of pH levels, time intervals, and solution molarities to determine which were best correlated with plant behavior (as shown in pot studies using 77 soils from Colorado). Both corn and sorghum were used to calibrate the extraction. Since its development, the DTPA soil extraction is one of the most widely used to predict micronutrient availability in soil systems (Loeppert and Inskeep, 1996). It has also been used for regulatory purposes; the Wyoming Department of Environmental Quality has set a suitable soil selenium level as < 0.1 mg kg^{-1} as measured by the ammonium bicarbonate-DTPA extract (WDEQ-LQD, 1984).

Because of this success, the DTPA extract has been used to assess micronutrient availability under a range of soil conditions as well as for a range of elements (e.g., Amacher, 1996; Li and Shuman, 1997). Outside of its intended use, however, results from the extract often do not correlate with plant uptake data (e.g., Bidwell and Dowdy, 1987; O'Connor, 1988; Kuo, 1990; Miner et al., 1997). This can be the result of many factors. The soil test was developed for neutral soils with a potential for deficiencies and to mimic the behavior of graminaceous species. When it is used to predict uptake by other species, the potential for poor correlation with plant data increases. When the extract is used for soils with different properties, results may also not relate to plant uptake.

Using the DTPA test to measure the plant available fraction of contaminants in disturbed soils may be inappropriate for two reasons: the extract may be overly aggressive in relation to plant behavior in these environments, and the chelate may become saturated and not truly measure the full extractable pool. Of these, the first is the most significant. In one study where the soil:solution ratio was corrected to compensate for high solution metal concentrations, there was no relationship between plant uptake and DTPA extractable metals (Li et al., 2000). Dilute salt extractable metals were a much better predictor of plant uptake. Similarly, Brown et al. (1994) found that dilute salt or water extractable metals were a much more accurate measure of plant available metals than DTPA in smelter-contaminated soils. Less aggressive extracts appear to be more appropriate for cases where excess, rather than deficient, concentrations of metals are expected.

bioavailability processes when applied across a wide range of conditions. The best that can usually be hoped for is, for example, prediction of 50 percent or so of the variance in bioaccumulation in the field (see Box 1-2). Given the heterogeneity of soils and sediments and multitude of exposure pathways present to a given organism, it is unrealistic to expect extraction tools to fully account for such variability. Rather, such tests should be viewed as qualitative measures of reactivity that may be useful as screening tools. Given the cost of bioassays, the use of extractions is likely to increase in the human health risk assessment arena, particularly for metals where some *in vitro* extraction tests have been validated.

BIOLOGICALLY BASED TECHNIQUES FOR MEASURING BIOAVAILABILITY

The role of physical and chemical processes is well recognized in bioavailability discussions, but biological processes also play important roles. Biological techniques are employed to study influential biological processes themselves, and as probes to study physical and chemical processes. In a controlled experiment, almost any technique that measures a biological response to contaminant exposure is suitable. However, interpreting the results from such experiments is not always straightforward. This is because biological processes other than the one under investigation can confound the results, making generalizations among experiments or about natural settings a challenge.

Tests that measure biological responses at levels of organization closest to contaminant transport across the membrane, of which assimilation efficiency is perhaps the best example, are easy to interpret from a mechanistic standpoint compared to responses that take place at more complex levels of organization (see Figure 4-6). Gross rates of contaminant biouptake (across the gills or the gut) provide a direct and unambiguous evaluation of bioavailability process D. Whole organism bioaccumulation tests are more complicated in that they reflect not just movement across the membrane, but also how the organism encounters its environment and species-specific internal processing mechanisms like digestion. However, depending on the length of the exposure and the organism under study, these internal processes may be minimized. The "uptake bioassays" discussed in this chapter include those that measure the initial biouptake of a contaminant across a biological membrane (bioavailability process D) as well as longer-term bioaccumulation tests.

Other tests that measure more complicated biological responses or groups of processes reveal less about uptake and accumulation but are valuable for studying toxic effects. For example, biochemical responses to exposure at the cellular level can be measured with biomarkers such as P450. While P450 levels might be unambiguously related to contaminant transport across a biological membrane if all else is controlled, in a natural setting elevated P450 can result from exposure

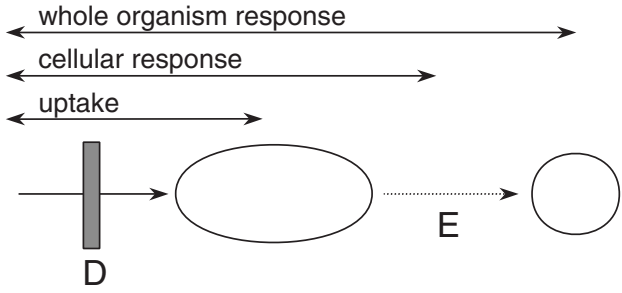


FIGURE 4-6 Biologically based tests measure responses at different levels of organization.

to any of several possible stressors. Toxicity tests (acute and sublethal) are widely used both in the lab and *in situ* to evaluate bioavailability because they are practical, they depict responses of high relevance, and they are particularly useful for helping to understand the effect of contaminant mixtures. Because the number of potentially confounding factors grows beyond those relevant to whole organism bioaccumulation (e.g., detoxification of chemicals can differ widely among species or even among environments), toxicity tests are not optimal mechanistic indicators of bioavailability processes (as defined in Chapter 1).

The section begins with tests that measure uptake (synonymous with absorption) only, including tests used for human health risk assessment purposes and those used primarily for ecological receptors. Determining absorption of chemicals in the digestive tract is one way to evaluate process D in Figure 1-1 (although as noted in Chapter 1, human health bioassays measure absorption into *systemic* circulation, which includes some E bioavailability processes). Absorption itself can be the subject of study, or absorption efficiency can be used as a probe to test site-specific soil or sediment properties or draw generalizations about effects of bioavailability processes A through C. Methodologies for determining absorption exist for both the mammalian model (using cell cultures, organ studies, and feeding studies of whole animals) and for lower-order organisms (using assimilation efficiency). After assimilation efficiency, the tests described encompass additional processes, including cellular responses and toxic effects within an organism. They are organized by scale, starting with molecular approaches, organismal approaches, and finally ecosystem-level approaches. Within the discussion of organismal approaches, uptake and effects-based tests are discussed for each organism type. The conditions present during these studies can vary widely, from laboratory tests using synthetic or field samples, to *in situ* bioassays with caged animals, to field studies with only natural elements.

Adsorption by Mammals: Human Health Bioassays

The tools discussed in this section are used primarily to gain insight on the bioavailability of chemicals in humans. They are focused on the bioavailability processes related to absorption of chemicals via direct ingestion and dermal contact. Although many of these techniques have been developed in the context of drug bioavailability, they hold potential for measuring the bioavailability of environmental contaminants from soils or sediments, but have not yet been used for this purpose.

The best source of information on bioavailability of environmental contaminants to humans would come from studies of humans, but there are very few of these studies. Experimental and ethical constraints dealing with the kinds of dosing and sampling that are possible with human subjects greatly limit clinical research on bioavailability of environmental contaminants. Consequently, most of the bioavailability information used in human health risk assessments must come from laboratory animals serving as surrogates (including pigs, rats, mice, monkeys, rabbits, and dogs) or from experimental model systems. The discussion begins with the simplest *in vitro* models, progressing to more complex mimics of human physiology, and finishing with approaches for deriving information from humans.

Cell Culture Studies

The simplest biological systems to study absorption (specifically bioavailability process D in Figure 1-1) in humans are cell cultures.

Gastrointestinal Absorption. The most established example of a cell culture system to understand absorption processes is the Caco-2 cell, which is a human colon adenocarcinoma cell line. In culture, Caco-2 cells differentiate to form a monolayer with brush borders resembling the apical surface of enterocytes (Pinto et al., 1983). They have been used extensively to study nutrient uptake from food and to explore intestinal absorption of drugs. They have not been used to any great extent to study intestinal absorption of soil or sediment contaminants for a number of reasons as discussed below, although theoretically there is no reason why they could not be adapted to do so.

During experiments using Caco-2 cells, the cells are allowed to form monolayers, substances of interest are added to the cell culture, and the rate of uptake into the Caco-2 cells is measured. The advantages of cell cultures are that they are easily manipulated and are well suited to the study of membrane transport mechanisms and of interactions among substances that affect substance uptake into enterocytes. Caco-2 cells have, for example, provided valuable information on the effect of dietary composition on iron absorption (Glahn et al., 1998). The disadvantage of Caco-2 cells is that they can provide information on only one

facet of bioavailability—absorption in the enterocyte. Absorption that occurs elsewhere, such as the gastric mucosa, and bioavailability processes occurring before and after enterocyte uptake are not addressed. In addition, absorption may not reflect the amount of chemical reaching the systemic circulation for chemicals that undergo presystemic elimination (see Chapter 1).

Cell culture methods have undergone rudimentary validation. Au and Reddy (2000) reported excellent correlation between iron uptake ratios in human subjects and Caco-2 cells. Studies comparing uptake in Caco-2 cells with permeability[1] in the jejunum of the small intestine measured in humans found reasonably good agreement for rapidly absorbed chemicals, but up to 1,000-fold lower permeability in Caco-2 cells than in humans for poorly absorbed chemicals (Lennernas, 1998). These observations indicate that Caco-2 cells may have limited value as quantitative indicators of absorption. Caco-2 cells have been used primarily as a research tool, and formal studies of reproducibility and inter-laboratory consistency have not been conducted.

Dermal Absorption. For dermal absorption, a number of commercial "human skin equivalent" products have been developed. These are based on stratified epithelial cells in culture with an architecture resembling normal human epidermis (Ponec et al., 2000). Examples include Epiderm, Episkin, Skin2, and SkinEthic. These tools have been used primarily in developing *in vitro* tests for skin irritancy and, as part of their development, tests of intra- and interbatch consistency of morphology (e.g., Boelsma et al., 2000) and intra- and inter-laboratory irritation tests have been conducted (e.g., Fentem et al., 2001). The possibility of using these skin tests for examining dermal absorption of chemicals has only recently been explored (Zghoul et al., 2001). The potential advantages of using "skin equivalent" cultures to study absorption of chemicals include lower cost than *in vivo* studies and the ability to avoid use of human and animal subjects. However, there are virtually no data available at present with which to judge the predictive capabilities of this tool.

Isolated Tissues and Organs

Gastrointestinal Absorption. Gastrointestinal absorption has been measured in isolated segments of the gastrointestinal tract of laboratory animals. There are two basic approaches. One involves excision of a segment of the gut and perfusion with a specialized apparatus, such as when a rat jejunal segment is mounted in an Ussing Chamber. Uptake by passive diffusion or transport can be readily measured. Rank order of permeabilities in this system among several

[1]Permeability as used here refers to the rate of movement of a substance across an absorptive surface such as the intestinal lining or cell membranes. It offers a useful basis for comparing relative absorption in different models and systems.

drugs was observed to be similar to that in jejunal permeability studies in human volunteers, although the permeability rates from the rat jejunum segment were lower, particularly for transported substances (Lennernas, 1998). Lower permeabilities may result from the absence of blood flow in the excised segment, which could lead to lower concentration gradients across the jejunum and perhaps deficiencies in cofactors needed for optimum function of transporters.

The second approach is to surgically isolate, but not remove, a segment of gut. The procedure is performed with the laboratory animal under anesthesia. Substances to be measured are introduced into the lumen of the surgically isolated segment. In order to measure the absorption rate, the vasculature serving the gut segment may be sampled. Alternatively, the vasculature may be cannulated and perfused with an artificial medium that is periodically sampled. The rat is often used, but other animals including catfish have been employed (Kleinow et al., 1998). This method has the same advantages as the excised segment model described above but it more closely approximates the intact gut. In a study of rat jejunum *in situ*, permeabilities were higher than measurements in excised segments (as above), but still less than observations in human subjects (Lennernas, 1998).

Both isolated gut models have the advantage of measuring absorption in gut tissue that is morphologically intact. Absorption processes affecting movement of the chemical from the gut lumen to the serosal side of the gut tissue are measured, offering a greater integration of events than is possible with cells in culture. Also, xenobiotic metabolism enzymes in intestinal epithelium remain active for some period of time, permitting an examination of the nature and extent of biotransformation that occurs during absorption. Ease of manipulation of these model systems makes them very useful research tools. As with other *in vitro* tools, they afford the ability to measure large numbers of samples or combinations of chemicals in a short period of time. However, the validation studies performed to date suggest that these models probably underestimate absorption rates in the intact human gut, at least for many chemicals. Further, as discussed above, absorption rate across the gut may not be the most important determinant of bioavailability, particularly for chemicals that undergo presystemic elimination in the liver. Therefore, the absorption rates may not correlate with absolute bioavailability *in vivo* (although this is not a concern when conducting relative bioavailability studies). There are no examples of this system being used to measure uptake from contaminated soil or sediment.

Dermal Absorption. The use of skin tissue from humans or animals to study dermal absorption *in vitro* is very common. The basic design consists of either human or animal skin placed in a chamber, with the skin dividing the chamber into donor and receptor compartments. The dermal dose is place on the donor side, and fluid in the receptor compartment is tested over time for the appearance of the chemical. The receptor fluid, which is usually saline or an aqueous buffer,

may remain in the chamber throughout the experiment (static design), or receptor fluid may be circulated through the chamber (flow-through design). Results may be expressed as a rate of chemical movement across the skin barrier or in terms of a percent of the amount of chemical applied on the donor side reaching the receptor side over a specified period of time.

In vitro skin tissue offers a more convenient means by which to study the dermal bioavailability of chemicals than *in vivo* tools. Properly excised skin can maintain the anatomical barrier of the stratum corneum. There is evidence that fresh skin tissue may also retain, at least temporarily, xenobiotic metabolism activities of skin *in situ*, allowing the opportunity to study metabolism of chemicals during the dermal route. Studies comparing dermal absorption of chemicals in human subjects with results from human skin *in vitro* have generally found good agreement. For example, Bronaugh and Franz (1986) found that dermal absorption of benzoic acid, caffeine, and testosterone applied to the skin of human volunteers was comparable to absorption *in vitro* through human skin, both in terms of percent dose absorbed per hour and total percent absorbed.

There is considerable interest in using skin from species that could act as surrogates for humans. The monkey and pig as well as the hairless mouse have been used, although there are examples of chemicals for which concordance was poor. Perhaps the most striking example of this is paraquat, with a permeability in the guinea pig and mouse 268- and 1,461-times that in human skin, respectively (Bronaugh and Collier, 1993).

There have been a limited number of *in vitro* studies conducted with contaminants bound to soil or sediment. Wester and Maibach (1998) measured the percutaneous absorption of DDT, benzo(a)pyrene, chlordane, and pentachlorophenol from soils after application to *in vitro* skin tissue. Both the chemical concentration in the receptor fluid and that remaining in the skin at the end of the experiment were lower when the soil-bound chemical was applied vs. the pure chemical. Similarly, the concentration of PCBs (Aroclors 1242 and 1254) in receptor fluid and skin was diminished when administered in soil as compared with a mineral oil vehicle, and absorption of arsenic, cadmium, and mercury from soils was less than from water. These studies can provide quantitative information on the extent of absorption from soils useful in human health risk assessment. There are a number of factors that must be considered in applying this information. Percutaneous absorption of a chemical from soil, both *in vitro* and *in vivo*, may not be linear over time. This raises questions about how the extent of absorption observed *in vitro* over one period of time should be applied to environmental exposures that may occur over a different period of time. Also, there is some uncertainty as to how the dose retained in the skin during *in vitro* studies should be regarded. In the *in vitro* studies of dermal absorption described above, the percent of dose remaining in the skin at the end of the experiment was greater than the percent of dose in the receptor fluid for most of the chemicals studied, both organics and metals. It is not clear if the percent remaining in the skin will

ultimately reach the systemic circulation *in situ* (as some may be lost through skin exfoliation). If so, it should be considered as contributing toward systemic bioavailability. Greater information regarding the fate of metals absorbed through the skin is needed to resolve this. Finally, studies of the reproducibility and repeatability of *in vitro* skin absorption studies have not been conducted in the context of assessing the bioavailability of chemicals from soils or sediments.

Whole Animal Approaches

Several approaches are available for measuring both oral and dermal bioavailability in laboratory animals. These tests are sometimes used to validate the physical and chemical tools discussed earlier, or to provide complementary evidence about bioavailability processes in a system. The best approach for a particular situation depends upon the objective (i.e., whether measurement of absolute or relative bioavailability is sought—see Chapter 2), the toxicokinetics of the chemical (e.g., rate and major pathway(s) of excretion), analytical capabilities, and time and financial constraints on the study.

Gastrointestinal Absorption: Blood or Plasma Measurements. Chemicals absorbed and reaching the systemic circulation can be measured in blood or plasma. The systemically absorbed dose is usually determined from the concentrations in blood or plasma over time after a measured dose of the chemical is administered to the animal. For this technique to be effective, the time frame of measurement must cover all of the absorption and most of the elimination of the chemical from blood. Blood or plasma concentrations are plotted against time, and the area under the concentration versus time profile (AUC) is calculated.

In order to determine absolute oral bioavailability, the AUC following oral administration (AUC_{oral}) is compared with the AUC after intravenous administration (AUC_{iv}), the latter representing the AUC expected if the entire oral dose reaches the systemic circulation. The equation below represents the calculation of absolute bioavailability ($F_{absolute}$) based on a single oral dose:

$$F_{absolute} = \frac{AUC_{oral} \times D_{iv}}{AUC_{iv} \times D_{oral}}$$

Notice that the equation includes terms for the oral and intravenous dose (D_{oral} and D_{iv}). This allows the AUCs to be corrected for dose if different doses are used for the two routes. This might be required, for example, if the intravenous dose is limited by poor aqueous solubility of the chemical or pronounced acute toxicity. The use of different doses assumes that the AUC is directly proportional to dose (i.e., linear pharmacokinetics), at least within the range of the doses being compared. This may not always be the case, particularly if the chemical is subject to

saturable absorption or metabolic processes. If the pharmacokinetics are not linear, the use of different doses can result in substantial error in measurement of bioavailability. This method also assumes that the clearance of the chemical is the same following oral and intravenous administration, which for most chemicals is not an unreasonable assumption.

An analogous approach can be used to assess relative bioavailability. In this case, bioavailability under differing sets of conditions (e.g., oral bioavailability of a chemical from a soil matrix versus from water) can be obtained from the ratio of the their AUCs, with one designated as the reference for comparison ("condition A", in the equation below).

$$F_{relative} = \frac{AUC_{(condition\ B)} \times D_{condition\ A}}{AUC_{(condition\ A)} \times D_{condition\ B}}$$

As with the measurement of absolute bioavailability, doses of different size can be used, but only if they are in the linear pharmacokinetic range.

In addition to providing information on the extent of absorption of a chemical, blood or plasma data provide the best information on the rate of absorption. Although the method can theoretically be applied to virtually any chemical, this approach is best suited for chemicals eliminated from blood in a matter of hours to a few days. Also, reliable AUC measurements require several blood or plasma samples with chemical concentrations that are measurable. Animal subjects must be large enough to provide the number of samples and blood volume dictated by the experimental design and the sensitivity of available analytical methods. This limits the utility of small animals for these studies, and often makes the testing of environmentally relevant doses of chemicals difficult.

Gastrointestinal Absorption: Urine Measurements. Many chemicals are excreted extensively in urine following their absorption, and analysis of the urine can provide an indication of absorbed dose. Typically, the animal subject is given a measured dose of the chemical, and urine is collected over time. The appropriate urine collection period depends on the elimination rate of the chemical but is usually extended until the chemical reaches undetectable or background concentration in urine. Based on the concentration of chemical in urine samples and their volumes, the cumulative amount excreted is calculated.

The absolute oral bioavailability of a chemical can be calculated from the amount excreted following an oral dose ($A_{urine(oral)}$ in the equation below) divided by the amount excreted after an intravenous dose ($A_{urine(iv)}$). Analogous to the approach using blood or plasma data, the intravenous dose is intended to represent the amount excreted in urine if the entire oral dose is absorbed. If doses of different sizes are used, the excreted amounts can be corrected for dose, if it is known or can be assumed that the amounts excreted are linearly related to dose.

$$F_{absolute} = \frac{A_{urine(oral)} \times D_{iv}}{A_{urine(iv)} \times D_{oral}}$$

Sometimes, urinary excretion data are used to draw inferences on absolute bioavailability without benefit of a comparison with an intravenous dose. The amount excreted in urine provides an indication of absorbed dose only if other routes of excretion (e.g., biliary, pulmonary) are negligible and elimination of the dose of chemical is complete. Because these conditions are rarely satisfied fully, bioavailability is usually underestimated by this method. Urinary excretion data can also be used to assess relative bioavailability by comparing the excreted amount under two different dosing conditions (see equation below).

$$F_{relative} = \frac{A_{urine(condition\ B)} \times D_{condition\ A}}{A_{urine(condition\ A)} \times D_{condition\ B}}$$

This technique is less invasive than blood or plasma measurements and can provide reliable bioavailability measurements for chemicals excreted primarily in urine. This approach should not be used if urinary excretion accounts for less than 20 percent of the dose. Also, accurate measurement of bioavailability requires complete urine collection, not just discrete urine samples, which may be difficult in some circumstances.

Gastrointestinal Absorption: Fecal Measurements. Fecal excretion represents the inverse of oral bioavailability. A chemical that is not absorbed following oral exposure will ultimately be excreted in feces. Therefore, measurement of fecal concentration can be used as an indication of the extent of absorption. Measurement of oral bioavailability involves collection of feces following single or multiple doses of the chemical. The collection interval must be sufficiently long to accommodate the gastrointestinal transit of the dose. Also, some chemicals do not reach the systemic circulation, but are instead excreted in the feces as the epithelial lining is sloughed into the lumen of the gastrointestinal tract. The collection of the unabsorbed dose must take into consideration the time course for these events.

Absolute oral bioavailability can be estimated by comparing fecal excretion of the chemical following both oral and intravenous doses. The intravenous dose is important because it provides information on the extent of biliary excretion of the chemical and diffusion of the chemical from systemic circulation into the gut. Both contribute to chemical in the feces, but represent absorbed, rather than unabsorbed, chemical. Some investigators have suggested that an intraperitoneal dose of the chemical (obviously relevant for animal studies, but not humans) can be used for the same purpose. The amount of chemical excreted in the feces after an oral dose ($A_{feces(oral)}$ in the equation below), corrected for these confounding

inputs ($A_{feces(iv)}$), can then be compared with the dose to obtain an estimate of oral bioavailability.

$$F_{absolute} = 1 - \left(\frac{A_{feces(oral)} \times D_{feces(iv)}}{D} \right)$$

If biliary excretion is known or assumed to be negligible, then fecal excretion data from oral dosing alone can be used to approximate the oral bioavailability. However, to the extent that this assumption is in error, the approximation will underestimate the actual bioavailability. It is also important to recognize that this method estimates absorption into the portal circulation, which is not necessarily equivalent to systemic absorption. For chemicals with substantial hepatic first-pass metabolism that detoxifies them in the liver, fecal excretion will overestimate systemic bioavailability. If the extent of pre-systemic elimination by the liver is known or can be estimated, this can be used to correct the apparent oral bioavailability based on fecal excretion to reflect systemic bioavailability.

This approach is generally less invasive than methods based on blood or plasma but requires quantitative collection of feces. For chemicals that are extensively absorbed, have substantial pre-systemic elimination by the liver, or prominent excretion in bile, fecal excretion data may not be a reliable bioavailability tool.

Gastrointestinal Absorption: Tissue Measurements. Tissue concentrations may be used in combination with measurements of excreta to assess absorbed chemicals using a mass-balance approach. Mass-balance approaches require measuring the chemical in various tissues in the body to determine the total internal dose. Unabsorbed dose and the amount of dose excreted are also measured, such that the entire dose can be accounted for. From these measurements, the amount absorbed can be calculated. Measurement of absolute oral bioavailability can be accomplished without the need for a comparison intravenous dose, but the mass-balance approach is analytically intensive and obviously unsuitable for measurements in humans.

Alternatively, tissue concentrations alone can be used in some situations to assess oral bioavailability. This approach assumes that the concentration of chemical in tissues is directly proportional to the absorbed dose. It is best suited to measurement of relative bioavailability, and is similar to the feeding tests described later for birds and mammals. Animal subjects may be administered the chemical in one or multiple doses. At specified times, animals are euthanized, and the concentration in one or more tissues is measured. Relative bioavailability is determined from the ratio of the tissue concentrations between the different types of oral doses ($C_{tissue(condition\ A)}$ and $C_{tissue(condition\ B)}$ in the equation below). If the oral doses compared are of different size, the tissue concentrations can be

corrected for dose, provided that the relationship between dose and tissue concentration is linear.

$$F_{relative} = \frac{C_{tissue(condition\ B)} \times D_{condition\ A}}{C_{tissue(condition\ A)} \times D_{condition\ B}}$$

The tissue(s) selected for analysis may represent a target organ for toxicity or, more commonly, a tissue to which the chemical preferentially distributes. This facilitates accurate measurement of concentration, particularly in studies using small animals where the size of the tissue sample available for analysis may be limited.

Tissue ratios offer the advantage of an internal measurement of systemic bioavailability, and they may be more suitable than blood or plasma measurements for chemicals with protracted elimination phases. However, they provide little or no information on absorption rate, and the tissue(s) to be measured and the timing of measurements must be carefully considered to avoid misleading results. Also, use of this approach requires the assumption that distribution and clearance of the chemical are equivalent under the two dosing conditions.

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The principal advantage of whole-animal oral absorption studies is that they measure bioavailability in its most clinically relevant form, that is, the absorption of chemicals from the gastrointestinal tract and into the systemic circulation. This integrates all of the relevant biological components related to systemic absorption, including presystemic elimination if present. By using the animals as surrogates for humans, these studies avoid the experimental and ethical problems associated with the use of human subjects. Currently, certain *in vivo* bioavailability studies conducted with an appropriate species are considered the "gold standard" for developing bioavailability information suitable for use in quantitative human health risk assessments, and they are often used to validate other bioavailability tools. For example, the young swine model for lead bioavailability has been used to validate *in vitro* extraction tests. The principal disadvantages of whole animal bioavailability studies are their expense and the time required to conduct them.

The assumption that certain species serve as valid models for human absorption comes primarily from studies in the pharmaceutical industry rather than direct animal-to-human comparisons for environmental contaminants. As discussed below in the section on clinical studies, there are almost no definitive data on the absorption of environmental contaminants in human subjects to serve as the basis for comparison. However, the extensive use of animal models in preclinical drug development for a variety of different kinds of chemicals offers some assurance that data derived in appropriate animal subjects is relevant to humans.

Dermal Absorption. In theory, the same approaches used to assess oral bioavailability can be used to test bioavailability for the dermal route (except, of course, measurement of fecal excretion). The principal difficulty in applying these methods to dermal bioavailability involves analytical sensitivity. Simply put, the doses absorbed through the skin in typical dermal uptake experiments are often too small to measure in blood, urine, or tissues. In order to maximize sensitivity of measurement, many dermal absorption studies use radiolabeled chemicals. This has been employed successfully in the measurement of dermal absorption of chemicals in soils. However, the use of radiolabeled compounds precludes testing bioavailability of a chemical in soil samples other than those prepared in the laboratory. As discussed elsewhere in this report, such soils may or may not reflect bioavailability of contaminated soils found in the environment.

To assess dermal bioavailability, a measured dose is placed on the skin. For experiments involving laboratory animals, the skin is shaved unless the animal is hairless (e.g., the nude mouse). A measured dose is applied to the skin, either in a liquid vehicle or solid matrix. The dose is left in place for a prescribed period (often 24 hours), and the amount absorbed is assessed in a variety of ways (that can include measurement of blood, urine, or tissue concentrations). Interpretation of results is analogous to that described above for oral bioavailability studies.

Another approach is to estimate absorption by measuring disappearance of the dose from the skin surface. After the exposure period, the applied dermal dose is removed and measured. Removal can consist of simply washing the skin and collecting the wash and rinse solutions for measurement, or may be more aggressive in the form of tape stripping. Strips of cellophane tape are successively applied to the skin in the dose area and removed, taking with them cells of the stratum corneum containing unabsorbed chemical. This may be repeated 20 or 30 times, and the amount of chemical on the tape strippings is then determined. If the chemical is radiolabeled, the amount remaining on the skin may be determined by placing a detector over the skin area and quantitating remaining radioactivity. Regardless of the procedure used to measure dose remaining on the skin, it is assumed that the amount of applied dose not recovered was absorbed.

The choice of where to apply the dermal dose is an important consideration. Generally, the dose is placed on an area that is most convenient for the investigator and offers the least potential for interference from the animal (e.g., from scratching or licking). In animals as in humans, the dermal permeability can vary with location on the body (see Chapter 3) and the results obtained from one area of placement may not be representative of dermal permeability elsewhere. Also, the choice of animal model is important. As discussed earlier under *in vitro* methods, the monkey and pig appear to be the best models for human dermal absorption, but are more expensive and can be more difficult to handle than smaller animals such as rats and mice. The disadvantage to using rats and mice is that their dermal permeability is usually much greater than human skin, and results obtained may therefore overpredict dermal absorption. One approach to

overcome this has been the use of a skin flap model in which human skin is grafted to a suitable animal host such as the nude mouse. The grafted skin maintains the morphological features of human skin, and the model can be used for up to six months.

The dermal absorption of different radiolabeled contaminants mixed with soil has been measured in rhesus monkeys (Wester and Maibach, 1998). Compared with delivery in acetone, dermal absorption of chlordane and pentachlorophenol was slightly reduced. More significant reductions were observed for DDT and benzo(a)pyrene. A reduction of more than 30 percent was observed for Aroclor 1242 when administered in soil compared to acetone, but essentially the same extent of absorption was observed for Aroclor 1254. A 30 percent reduction was consistently observed for both Aroclors in soil when compared with application of the doses in mineral oil.

The advantages and disadvantages of the use of animal surrogates for studying dermal absorption are the same as those described above for gastrointestinal absorption. However, the need to use radiolabeled compounds in most dermal studies in order to achieve adequate measurement sensitivity limits such studies to non-human subjects.

Clinical Studies

Oral, dermal, and even inhalation bioavailability studies are regularly conducted on humans by pharmaceutical researchers in the context of drug development. The employed methods parallel those described above in the section on whole animal studies. The conceptual approaches and techniques are the same, except of course that tissue sampling is precluded. Some specialized procedures, such as the technique for isolated segment permeability studies in the gut (Lennernas, 1998) can be fairly invasive.

In contrast, there has been almost no clinical study of the bioavailability of environmental contaminants in soils and sediments. There are several impediments to this type of research. It may be difficult to convince potential subjects and Institutional Review Boards that it is appropriate to intentionally expose healthy humans to environmental contaminants, even if assurances can be provided that the doses will be well below those associated with adverse health effects. Another impediment is analytical sensitivity. Bioavailability studies in pharmaceutical research can be performed with substantial doses of the chemical because the objective is to determine absorption under conditions of clinical use where effects from the drug are expected. For environmental contaminants, the doses must very low to avoid any possibility of effects. This means that the analytical methods must be able to reliably measure very low concentrations. There also must be some means to distinguish low administered concentrations from “background” levels in the body resulting from the subjects’ incidental environmental exposure to the chemical. One approach might be to use

radiolabeled chemical, but there are potential risks to the subject from exposure to radioactive material, and this would restrict the soils and sediments tested to those created in the laboratory rather than samples from contaminated sites.

One study of lead bioavailability from soil has taken advantage of the presence of naturally occurring stable isotopes of lead to avoid these problems (Maddaloni et al., 1998; see Box 4-6). In measuring the stable isotopes by mass spectrometry, adequate sensitivity could be achieved to follow gastrointestinal absorption of relatively small doses of lead in soil (approximately 200 μg). Also, using differences in stable isotope ratios that exist naturally, they were able to identify lead in the blood originating from the soil sample as opposed to lead from other sources. Unfortunately, the unique circumstances for lead (i.e., the existence of naturally occurring stable isotopes in different ratios in soils and individuals from different areas) make this approach difficult to reproduce for other chemicals.

Assimilation Efficiency

The equivalent of determining absorption in humans is also done in invertebrates and fish. A tool that quantitatively integrates processes in the gut that affect uptake is *assimilation* or *absorption efficiency*. Assimilation efficiency is defined as the fraction of contaminant absorbed by the gut (measured either *in vitro* for larger animals or in the whole organism for smaller animals) relative to the amount ingested (per gm weight food, per gm organism, per day). It is a direct measurement of biouptake in that it determines how much of the ingested contaminant is transported across a biological membrane. When assimilation efficiency is combined with feeding rate and concentration in the ingested material, the final concentration of a contaminant taken up can be modeled (Luoma et al., 1992; Wang et al., 1996a; Luoma and Fisher, 1997).

Assimilation efficiency is most applicable to benthos and water column dwelling organisms that ingest bed sediments or suspended materials that move in and out of sediments. Although the importance of this pathway to overall exposure has been somewhat controversial, a body of work supports the view that diet is consistently responsible for half or more of contaminant uptake by most organisms that ingest sediments, provided the experimental conditions are typical of nature (Landrum et al., 1992; Kidd et al., 1995; Reinfelder et al., 1998).

Because assimilation efficiency is a direct measurement of a biological mechanism (Luoma and Fisher, 1997), it is unambiguously comparable among species, contaminants, and environmental conditions. Thus, comparing assimilation efficiencies by a deposit feeder among sediments of different character is a way to determine how those sediment characteristics affect bioavailability. Assimilation efficiency is especially valuable for invertebrates because it is relatively simple to use, such that experiments can be conducted quickly.

BOX 4-6
Measurement of the Bioavailability of Lead from Soil in Humans

Maddaloni et al. (1998) utilized the stable isotopes of lead that exist naturally to measure absolute bioavailability of lead from soils in humans. Lead (Pb) has four stable isotopes—^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb. Three of these isotopes (^{206}Pb, ^{207}Pb, and ^{208}Pb) are produced continually by radioactive decay, and consequently the ratio of these isotopes varies from location to location with the geologic age of the lead deposit. Given the geographic differences in lead isotope ratios in soils, it is not surprising that lead isotope ratios in human blood can also vary with location. If individuals ingest soils with a very different lead isotope ratio than the one that exists in their body, the change in ratio after ingestion can be used to estimate the amount of lead absorbed from the soil.

This study sought information on lead bioavailability from the soil at the Bunker Hill, Idaho Superfund site. The ratio of ^{206}Pb/^{207}Pb in this soil was 1.057. Twelve adult volunteers were selected from the New York area, all with a ^{206}Pb/^{207}Pb ratio > 1.190. The subjects were divided into two groups. One group received a soil dose (250 μg Pb per 70 kg body weight) after an overnight fast, while the second group received the same dose immediately after a standardized, high-fat breakfast. Blood and urine samples were collected from each subject over a 30-hour period after the dose. After the soil dose, blood lead concentrations increased while the ^{206}Pb/^{207}Pb ratio decreased. From the change in blood concentration and isotope ratio, the percent of the lead dose in the blood compartment of the subjects could be calculated. Among fasted subjects, this averaged 14.4 ± 4.5 percent of the administered dose. Data from a previous study indicated that at 24 hours after an intravenous ^{203}Pb dose to human volunteers, 55 percent of the dose remained in the blood compartment. This indicated that the average absolute bioavailability of lead from the soil samples was 26.2 ± 8.1 percent (14.4 ÷ 0.55 = 26.2). For non-fasted subjects, the absolute bioavailability was much lower, averaging only 2.5 percent. Figure 4-7 shows how the isotope dilution technique was able to distinguish blood concentrations resulting from the soil lead dose from total blood lead concentrations, as well as differences between fasted (A) and fed (B) subjects.

Until the last ten years, assimilation efficiencies were poorly known for aquatic organisms, although the concept has long been employed to study contaminant uptake in higher animals. Decho and Luoma (1994) and Wang et al. (1996a) first showed that repeatable assimilation efficiencies can be determined for sediment-bound contaminants under a variety of conditions and for a variety of species. There are few impediments to determining assimilation efficiency in any species that ingests sediments if the animal can be fed in the laboratory. The traditional approach in mammalian physiology, nutritional physiology, and more recently with invertebrates is to use radionuclides in pulse-chase experiments (Reinfelder and Fisher, 1991; Decho and Luoma, 1991, 1994; Luoma et al., 1992). Particles can be fed directly to the experimental animals or in any feasible matrix or configuration. Experimental conditions can be set up to eliminate the confounding influence of pore water contamination or desorbed contaminant and thus isolate the contribution of ingested material to overall bioavailability. *In*

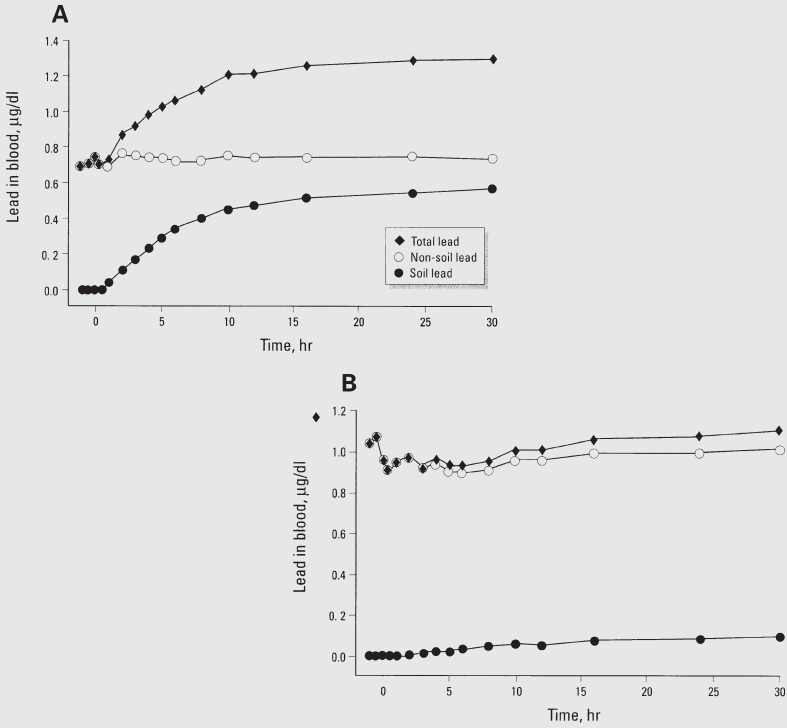


FIGURE 4-7 Change in blood lead concentrations over time after receiving a dose of lead in soil for both fasted (A) and fed (B) subjects. Reprinted, with permission, from Maddaloni et al. (1998). © (1998) Environmental Health Perspectives.

vitro studies with excised gut are possible, especially with larger organisms; *in vivo* studies with whole organisms are used most frequently with invertebrates.

Recent studies show that assimilation efficiencies can vary widely among food types and among species, and some unexpected results have emerged. For example, bivalves appear able to absorb a substantial fraction (20 percent or greater) of what might be considered recalcitrant forms of trace elements from sediments. Both a deposit feeding bivalve *Macoma balthica* and the mussel *Mytilus edulis* absorbed 15–30 percent of sulfide-associated cadmium and silver from ingested particles (Lee et al., 2000). As discussed in Chapter 3, the form or source of the contaminant in the sediment or suspended material that deposit-feeding or detritus-feeding animals ingest also has a strong influence on assimilation efficiency.

A limitation to using assimilation efficiency to measure bioavailability is its dependence on radioisotopes. Exchange between the radioisotope and the stable

Laboratory set-up for measuring assimilation efficiency of clams.

chemical and whether the isotope fully reflects the chemical forms present in the sediment are critical considerations. (The same limitation applies to any spiking experiment.) High specific activity radioisotopes are sometimes difficult to obtain for some contaminants, and handling radioisotopes requires special precautions. Stable isotopes might eventually replace radioisotopes but will not have the

advantages of using gamma isotopes (for which non-destructive determinations are possible).

Models of exposure and bioaccumulation can incorporate assimilation efficiencies, such that the potential uncertainty associated with different sediment types can be determined. This may be especially useful in forecasting potential bioaccumulation under different conditions. (If applied to a specific field setting, however, the assimilation efficiency–model approach would still involve characterizing the sediment character in that setting.) As discussed later in this chapter, Dynamic Bioaccumulation Models (DYMBAM) use assimilation efficiency and seem to provide good estimates of uptake when compared to field results. These models may be an attractive next step (beyond the more empirical methods used to forecast bioaccumulation from sediments like BSAF) for quantifying the implications of considering bioavailability processes (Luoma and Presser, 2001).

Molecular Approaches

The remaining tests in this section have endpoints beyond initial biouptake and include molecular, cellular, and organismal responses to exposure to contamination. Because the success of bioavailability screening may hinge on the speed of the determination and on the reliability of the results, molecular tests are expected to have a significant impact on bioavailability assessment in the future. It is envisioned that bioavailability assessment protocols will require sufficient throughput capacity to be able to handle a relatively high number of samples and, hence, will be of relatively low fidelity. Moreover, such assays also will need to be economical to conduct. Molecular tools hold promise for providing such rapid and accurate assays of bioavailability that are modest in terms of expense (Dodi et al., 1999; Pennie, 2000). Indeed, the past decade has seen an explosion in the number and variety of techniques available for molecular analysis of exposure and toxicity and thus bioavailability, including molecular reporter systems and molecular biomarkers.

Molecular Reporter Systems using Bacteria

Whole cell bioreporters measure the intracellular response of a microorganism (typically bacteria) to an extracellular chemical or physical signal, and as such may provide an indirect measure of contaminant concentration and bioavailability. The attractiveness of a bioreporter system derives from the ease with which the signal, typically bioluminescence (light emission), can be measured. Further, because the response for many chemical-specific bioreporters is contingent on the contaminant molecule passing the microorganisms' membranes, these assays may be relevant to other ecological receptors.

Most bioreporters are based on a variant DNA construct that is inserted into a bacterial strain, which will then be put in contact with a contaminated sample.

The DNA construct links genes that respond to the presence of a contaminant with a "reporter" gene whose output can be easily detected. The *luxCDAB* or *luxAB* cassette, a set of genes derived from the marine eubacterium *Vibrio fisheri* that results in luminescence, is frequently used (Meighen, 1991). Other commonly used reporter genes include *luc*, which encodes firefly luciferase; *lacZ*, which encodes β-galactosidase; and *inaZ*, which encodes the ice nucleation protein (Loper and Lindow, 1994). After an appropriate incubation time between the organism and the sample, the signal is recorded with a luminometer or other device (Reid et al., 1998; McGrath et al., 1999; Shaw et al., 2000). A very recent reporter gene that will see increasing use in biosensor development because of its ease of detection and minimal metabolic cost to the host cells encodes for the green fluorescent protein (GFP), originally isolated from the jellyfish *Aequorea victoria* (Tsien, 1998; Cha et al., 1999; Hansen and Sørensen, 2000; Joyner and Lindow, 2000; Stiner and Halvorson, 2002).

Depending on the DNA construct, bioreporters can have either narrow or intentionally broad contaminant specificity. In the former case, the reporter is designed to respond to a sole organic or inorganic chemical species such that response to a secondary species is considered undesirable. Most of these reporter systems are based on promoters and transcriptional regulatory proteins that respond to specific chemical species (e.g., Hg(II), arsenic, antimony, zinc, copper, cadmium, lead, toluene, and naphthalene) (Selifonova et al., 1993; Heitzer et al., 1992, 1994; Taurianen et al., 1998; Stiner and Halverson, 2002). For example, some bioreporters have been designed such that bioluminescence is switched on in the presence of the target contaminant and measured quantitatively. Insertion of the *lux* reporter system into *P. fluorescens* HK44 was reported to successfully provide real-time data of naphthalene bioavailability, degradative activity, and optimal degradative conditions (Heitzer et al., 1992).

If broader contaminant specificity is desired, the reporter is designed to capture the physiological response against a wide range of contaminants. These DNA constructs are either based on the use of constitutive promoter elements where luminescence can be seen as an overall measure of cellular activity (Rattray et al., 1990; Chaudri et al., 2000), or on promoter elements that are expressed systemically in response to cellular stress, e.g., the promoter of *recN* (van der Lelie et al., 1997) and various heat shock stress protein promoter elements (Cha et al., 1999). The original Microtox™ bioassay belongs to the latter group, because it measures the luminescent response of the marine *Vibrio fisheri* containing *luxCDAB* under control of its wild-type regulation (Meighen, 1991). Hence, toxicity or inhibition to the microorganism is inferred from a reduction in luminescence intensity against a control treatment (ASTM method D-5660-95). In this case, reduction in luminescent response is believed to serve as a general indicator of both inorganic and organic toxicant stress. GFP-based systems under control of constitutive promoters can be employed similarly to measure generic cytotoxic effects (Rabbow et al., 2002).

Although bioreporters have the potential of providing real-time data of compound availability, the short lifetime of the bacterial cells is not always optimal for developing biosensors capable of long-term, online monitoring of vapor and aqueous phase contaminants. Another key challenge for biosensors is their limited sensitivity, which is in the 1 nM range for some reported metals, toluene, and naphthalene (Selifonova et al., 1993; Taurianen et al., 1998; Stiner and Halverson, 2002), but may be orders of magnitude higher for others (Willardson et al., 1998). In addition, bioreporters can have limited genetic stability (Heitzer et al., 1992, 1994; Ripp et al., 2000), their results can be confounded by the effect of nonspecificity (many metal-specific and arene-specific sensors suffer from this), there is substantial background response, and there can be strong matrix effects on signal response (Selifonova et al., 1993; Neilson et al., 1999). In general, luminescence- and fluorescence-based biosensors are an inexpensive and rapid technique that may become useful for evaluating the bioavailability of both organics and metals in the soil.

Most bioreporter systems rely on intimate contact between the reporter strain and the environmental matrix. Thus the tests typically employ soil or sediment extracts within which the bioreporting organism is suspended (although pore water that is reflective of *in situ* conditions might be employed) (e.g., Willardson et al., 1998; Rasmussen et al., 2000). A biosensor system that relies on a very small volume of extractant has been developed for assessing heavy metal toxicity of soils, sediments, and sludge (Bitton et al., 1996; Boularbeh et al., 1996). In the future, the use of fiber-optic devices may permit application *in situ* (Heitzer et al., 1994). Little validation of molecular bioreporter systems has been performed, except for the heavy metal biosensors for which the response was reasonably well correlated with phytotoxic (bean and tomato) and zootoxic (*Eisenia fetida*) end points across several different soils (Corbisier, 1999).

Biomarkers

Biomarkers represent responses of living organisms that may indicate exposure to contaminants, predict harm, or themselves be harmful effects (Timbrell, 1998). A biomarker is a biochemical, physiological, or morphological response (usually on a molecular level), but not a population or ecosystem bioindicator (Stegeman et al., 1992). At minimum, a biomarker response discloses that a contaminant (1) is present in the environment, (2) is available to the organism, and (3) has reached the affected tissue or organ in sufficient amounts for a period of time long enough to produce an observed response (Depledge et al., 1993). An impetus for developing biomarker techniques for measuring contaminant bioavailability is that the measurement of parent toxicants or metabolites in biological samples (e.g., urine or blood) is currently limited to about 100 chemicals or classes of related compounds. Less than half of these can be quantitatively related to exposure.

Biomarkers have been categorized as biomarkers of exposure or effect. A biomarker of exposure indicates the presence of a xenobiotic substance or its metabolite(s) or is the product of an interaction between a xenobiotic agent and some target molecule or cell (such as the formation of a macromolecular adduct) (DeCaprio, 1997). Biomarkers of exposure are mainly useful in establishing contaminant dose in both ecological as well as human studies where they provide information about long-term exposure to carcinogens (Waterfield and Timbrell, 2000). Generally, these biomarkers reflect recent exposure, although the half-life of the contaminant must be taken into account. Biomarkers of exposure are the most convenient to determine. For example a contaminant or its metabolites often can be quantified from samples of blood, breast milk, feces, or urine, as well as tissues obtained through biopsy or necropsy (Fossi et al., 1994). While macromolecular adducts do provide some degree of specificity and sensitivity, they are expensive to evaluate and not always quantitatively related to exposure.

Biomarkers of effect indirectly indicate exposure and are defined as any measurable biochemical, physiological, or other alteration within an organism that can be recognized as an established or potential health impairment or disease (Hugget et al., 1992). This includes induction of proteins (e.g., metallothioneins and heat-shock proteins). Markers that are the result of pathological damage can be considered separately from markers that indicate a metabolic lesion. Clinical or behavioral observations can also be considered a separate type of biomarker. Biomarkers of effect vary markedly in their specificity, sensitivity, usefulness, and feasibility. A consideration for the well-documented biomarkers (e.g., metallothioneins and stress proteins) is that they are "general" responses induced by exposure to a variety of compounds. This can be an advantage in situations where the total biological response, for example from a mixture of contaminants, is the preferred endpoint.

The use of biomarkers to further mechanistic understanding is made difficult by the fact that biomarkers indicate the cumulative effects of chemical interactions and reflect a temporal and spatial integration of exposures. Ideally, a suite of biomarkers would be needed to observe different classes of chemicals. Biomarkers could be used as early warnings to detect exposure shortly after it has occurred. However, their use over long periods of time may be hard to interpret unless more is known about the duration of the actual response. The specificity of biomarkers decreases with an increasing level of organization such that molecular biomarkers are more specific than organ- or organism-level ones. Finally, it can be hard to relate lab results to the field because of interspecies differences and ecological impacts. As discussed later, gene expression technology holds great promise in complementing more "general" biomarkers to further mechanistic understanding. [Interestingly, even though data are being assembled on gene expression during exposure to specific contaminants (see following section), the number of genes whose activation has been linked to a biomarker is minor. Ideally, activation of a gene or gene cluster would signal a specific response related to the appearance of a biomarker.]

Four prominent examples of molecular biomarkers with potential applicability to bioavailability processes are stress proteins (see Box 4-7), DNA damage, metallothioneins, and cytochrome P450 activity. The more common methods for determining DNA damage are (1) direct measurements of DNA structural damage, (2) assessment of DNA repair, or (3) determination of mutations present (Shugart et al., 1992). Metallothioneins are a class of small proteins that are rich in cysteine, capable of binding metal ions, and inducible by cadmium, copper, mercury, zinc, cobalt, bismuth, nickel, and silver ions (Waterfield, 2000). The P450 cytochromes are a class of hemoproteins present in a wide variety of organisms and in all tissues in mammals, especially the liver. They are inducible by a variety of organic chemicals (De Caprio, 2000). Although several sensitive assays have been developed for quantifying cytochrome P450 induction (Saint-Denis et al., 1999), the appropriate assay conditions and specificity of response must be ascertained for each species.

BOX 4-7
Heat Shock Proteins as Biomarkers

One of the best-studied biomarkers of exposure is production of stress proteins (Bierkens, 2000). A wide variety of organisms from bacteria to humans produce proteins that provide some protection from cellular damage (Hightower et al., 1985; Hightower, 1993; Morimoto et al., 1995a,b; Hartl, 1996). These proteins, initially described in fruit fly cells during exposures to high temperature (Ritossa, 1962), are termed "heat shock proteins" (*hsp*). Since the initial discovery, a range of environmental stresses has been shown to induce heat shock proteins; thus, the term "stress protein" consequently has been coined. Environmental contaminants that can induce these proteins include both trace metals (Sanders et al., 1991; Bauman et al., 1993; Williams et al., 1996) and organic compounds (Sanders, 1990).

There are several families of heat shock proteins classified by molecular weight: Hsp90, Hsp70, chaperonin, and those of low molecular weights. An increase in the total specific activity of Hsp70 within an organism can be used as a nonspecific indicator of stress, exposure, and potentially bioavailability. Nadeau et al. (2001) demonstrated that stress-induced Hsp70 could be used to monitor exposure of the earthworm species *Lumbricus terrestris* to various soil contaminants. The midgut and intestinal tissues of *L. terrestris* revealed expression of an inducible member of the Hsp70 family after heat shock treatment *in vitro* (positive control) and after exposure to different toxicants in artificial soil. Short-term (24–72 hours) and long-term (14–16 days) exposure to chloroacetamide and pentachlorophenol as well as heavy metals (Pb^{+2}, Gd^{+2}, Cu^{+2}, and Hg^{+2}) in soil induced Hsp70 in the earthworms' midgut and intestinal tissues. This biomarker appears to be sensitive with a good level of reproducibility despite some individual variations. The use of non-exposed animals transposed into contaminated environments should be highly relevant to bioavailability studies. Stress proteins do have some selectivity (Ait-Aissa et al., 2000), as not all contaminants induce a stress response. However, among those contaminants that do induce expression of Hsp70, the potency of induction was related to the octanol–water partition coefficient.

Fouchecourt et al. (1999) and Koganti et al. (1998) evaluated the bioavailability of PAHs from ingested soil via the measurement of several different exposure biomarkers in target organisms, including whole body or organ burden of the toxicant, measurement of cytochrome P450-dependent monooxygenase activities, urine levels, and chemical:DNA adduct levels in lungs. The gastrointestinal absorption and systemic bioavailability of PAHs was determined for soil containing complex organic mixtures. The results of the biomarker assay were compared to PAH bioavailability as measured with soil and organic extract of each soil (Soxhlet) to give a relative bioavailability value for each soil type. In another study (Fouchecourt et al., 1998), rats maintained on a litter of PCB-polluted soil were used to assess bioavailability. PCB burdens and activities of microsomal liver and lung cytochrome P450 monooxygenases were the biomarkers assessed. A near dose-response relationship was found between concentrations of PCB in the litter and activity of the monooxygenase EROD in both the liver and lungs. This suggests that EROD activity measurements in both liver and lung of rats maintained on a litter of PCB-polluted soil can be used to assess the bioavailability of PCBs to mammals.

Gene Expression Techniques. Like other biomarkers, gene expression techniques quantify a molecular response to contaminant exposure. In this case, the response is an alteration in gene expression at the level of transcription, detected by making real-time measurements of particular messenger RNAs. The underlying scientific basis is that an organism's contaminant exposure is manifested in (among other things) creation of unique mRNAs that direct protein manufacture and other cellular responses. Such genomic biomarkers have the potential of acting as a toxicant-specific or at least toxicant class-specific "fingerprint" of chemical bioavailability.

Specific techniques can be divided into two types: high fidelity, low-throughput techniques and low fidelity, high-throughput techniques. Microarrays, subtractive hybridization, and serial analysis of gene expression (SAGE) are high-fidelity assays in that they generate considerable information about an organism's genetic response to exposure. However, because of the amount of information generated, as well as the number of steps involved, the amount of mRNA required, and the costs, these techniques cannot currently be used for high-throughput screening. More rapid flow-through DNA hybridization array or "genosensor" systems are being developed (Fredrickson et al., 2001) that hold great promise in the area of soil and sediment bioavailability. All these techniques are described in detail in Box 4-8.

Microarrays have generated considerable interest in toxicology and thus indirectly for use in studying bioavailability. Using microarray techniques, it is possible to develop a sensitive and inclusive snapshot of the responses of cells, tissues, and organisms to a contaminant without the time requirements, labor, or subjectivity of more traditional analyses. Validating these techniques, and in-

creasing their practicality for specifically assessing contaminant bioavailability from soils and sediments, should occur in the near future.

Organismal Approaches

A variety of bioassays at the level of individual organisms can be used both to assess bioavailability of contaminants in soils and sediment and to validate the physical and chemical tests discussed earlier in this chapter. Bioassays generally can be divided into two basic categories. First, uptake tests directly reflect how much of a contaminant is in the tissue of an organism. Such tests are commonly conducted in plants, invertebrates, and fish, and sometimes in birds and mammals when these organisms are an ecological receptor of concern. For obvious reasons, uptake tests are generally not feasible in microorganisms. Uptake tests generally do not take into account whether the compound of interest is transformed within the body of the organism. The most common measurement is simply the concentration of the compound in the tissue of interest, and when this is measured after a prolonged exposure, the test is referred to as a bioaccumulation test. The second major type of bioassay is a toxicity test to determine what concentration of a compound brings about some toxic effect, such as suppressed growth or death. Following a brief discussion of microbial mineralization assays, this section is organized by organism, with both uptake and toxicity tests discussed for each.

Mineralization and Assimilation Assays for Microorganisms

Microbial mineralization (and concomitant CO_2 evolution) has been applied to assess bioremediation potential of soil-bound HOCs and hence their bioavailability to microorganisms. In this assay, the initial mineralization rate and extent in soil-slurry experiments supplemented with a HOC mineralizing strain are compared with soil-free controls to estimate bioavailability reduction (Guerin and Boyd, 1997; Feng et al., 2000). Such measures for estimating qualitative trends in contaminant availability to microorganisms have long been employed by Alexander and coworkers. Employing ^{14}C-labeled compounds, reduction in degree of mineralization of PAHs and other compounds was linked to bioavailability reduction (e.g., Chung and Alexander, 1998, 1999; Hatzinger and Alexander, 1998; Tang et al., 1999; White et al., 1997, 1999).

Because the method relies on the use of ^{14}C-labeled marker compounds, it is not suitable to measuring mineralization of the relevant aged contaminants, nor can it be conducted in soils or sediments *in situ*. Further, different bacterial strains may yield different results, suggesting that strains have varying abilities to degrade solid phase-associated compounds (Guerin and Boyd, 1992; Friedrich et al., 2000; Grosser et al., 2000).

A wholly empirical but promising technique that estimates the microbially oxidizable fraction of soil- and sediment-bound hydrophobic organic compounds

BOX 4-8
The Pros and Cons of Gene Expression Techniques

The basis of gene expression techniques is that an organism's contaminant exposure is manifested in the creation of unique messenger RNAs. Thus, most of these techniques revolve around detection of specific mRNA species through the use of hybridization techniques.

Membrane-based microarray assays (the forerunner of current hybridization microarrays) employ membrane filters onto which are adsorbed thousands of cDNA sequences related to various aspects of cell regulation. (cDNA is complementary DNA formed using messenger RNA as a template and the enzyme reverse transcriptase.) Such membranes are available from several commercial sources at reasonable cost (Cheung et al., 1999). mRNA from two different cells (test and control) thought to respond to contaminant exposure is then hybridized onto the two filters. In this way, the exact genes that are being turned on in response to the exposure can be identified. Glass slide-based microarray technology is an even faster and more efficient microarray setup, for a variety of technical reasons. Subtractive hybridization is another high-throughput technique that allows for the isolation and cloning of mRNA unique to an exposed population.

Pennie et al. (2001) have shown that cDNA microarrays allow comprehensive coverage of genes associated with entire pathways (such as oxidative stress, signal transduction, and stress response). Tully et al. (2000) used the CAT-Tox(L) assay system (which utilizes human liver carcinoma cells) to examine patterns of gene expression to several heavy metals. Similarly, Liu et al. (2001) used the Atlas Mouse Stress/Toxicology array to observe alteration of gene expression related to stress, DNA damage, and metabolism in mice following acute arsenic treatments.

As an example, DNA arrays containing 148 genes for xenobiotic metabolizing enzymes, DNA repair enzymes, heat shock proteins, cytokines, and housekeeping genes were used to examine gene expression patterns in the livers of mice in response to exposure to cadmium chloride ($CdCl_2$), benzo(a)pyrene (BaP), and TCE (Bartosiewicz et al., 2001a). Each toxicant was found to produce a unique pattern of gene induction or "fingerprint." Exposure to $CdCl_2$ resulted in marked up-regulation of metallothionine I and II, several of the heat shock-stress response proteins, and early response genes. In contrast, exposure to BaP lead to up-regulation of only metabolizing enzymes Cyp1a1 and Cyp1a2 genes and produced no significant increases in any of the stress response genes or the DNA repair genes present on the array. Exposure to TCE was shown to induce gene expression of the heat shock proteins Hsp 25 and 86 as well as Cyp2a.

entails gentle persulfate ($S_2O_8^{2-}$) oxidation (Cuypers et al., 2000). This method assumes the soil organic matter oxidized under the applied conditions is the primary source of readily available HOC. Thermal gravimetry analysis confirmed that oxidation indeed removed the 250–350°C labile organic matter phase (Bierkens et al., 1998). Results from this assay for soil- and sediment-bound PAHs correlated well with batch biological oxidation after 21 days and Tenax TA extraction after 264 days. Hence, this assay may correlate with prokaryal intracellular availability. However, it should be note that this method is not itself a mineralization assay but rather a physicochemical test that is biomimetic.

Bartosiewicz et al. (2001b) expanded on this study by looking at both liver and kidney of mice exposed to five classes of chemicals (PAHs, DNA alkylators, peroxisome proliferators, heavy metals, and oxidative stressors). Each toxicant group gave a similar pattern of gene expression in the liver and kidney, which was dissimilar from that of the other four toxicant groups, with both time and dose being important to class differentiation.

Using *in vitro* techniques, Waring et al. (2001a) investigated whether chemicals with similar mechanisms of toxic action produced similar changes in gene expression. They treated rat hepatocytes with 15 known hepatoxins (carbon tetrachloride, allyl alcohol, aroclor 1254, methotrexate, diquat, carbamazepine, methapyrilene, arsenic, diethylnitrosamine, monocrotaline, dimethyl-formamide, amiodarone, indomethacin, etoposide, and 3-methylcholanthrene) and used microarray technology to characterize alterations in gene expression. Results revealed that gene expressional profiles for toxicants with similar toxic mechanisms formed clusters, suggesting a similar effect on transcription. However, each toxicant produced a unique fingerprint. Along the same lines, Waring et al. (2001b) showed that gene expression changes caused by an agent *in vitro* reflected those produced *in vivo*.

These findings suggest that microarray analysis with a focused set of genes might be capable of discriminating exposure to, and thus bioavailability of, different toxicants.

Other potentially useful gene expression tools include SAGE analysis (Velculescu et al., 1995; Bertelsen and Velculescu, 1998) and differential display (Liang and Pardee, 1992). SAGE quantifies the level of RNA in each individual cell population. With differential display, mRNA samples from several samples can be analyzed at the same time, which is not possible with other techniques. Both SAGE analysis and differential display have the ability to identify previously unknown genes that may be expressed upon exposure. Several researchers have used differential display to identify genes expressed with exposure to certain toxicants (Wang et al., 1996b; Selmin et al., 1996; Kegelmeyer et al., 1997; Donat and Able, 1998; Muhlenkamp and Gill, 1998; Roman and Peterson, 1998; Rodi et al., 1999).

Finally, real-time polymerase chain reaction (Higuchi et al., 1993), scintillation proximity (Harris et al., 1996), and branched-DNA (Waring and Ulrich, 2000) are molecular techniques used to follow the responses in one or a few genes that could be constructed as high-throughput, albeit low-fidelity, screens.

Plant Bioassays

Plant bioassays can be used to measure bioavailability processes for a range of organic and inorganic compounds in soils. Two types of results can be generated. First, plant tissue can be analyzed to determine if the contaminants of concern are present at elevated or potentially toxic levels. It is relatively straightforward to analyze plant tissue for concentrations of toxic inorganic contaminants. The second approach is to measure of the growth and vigor of the plant. If the plant can grow in the presence of a contaminant, then it is possible to conclude that the contaminant is not present in phytotoxic concentrations. For both

types of assays, the results can be used either to determine the bioavailability of contaminants to plants and to organisms that consume the plants, or to estimate bioavailability of the contaminants to other organisms (assuming a correlation between plant and animal uptake can be shown).

This type of testing has been routinely done in agriculture for decades, and has been used to validate many of the extraction tests discussed earlier in the chapter (Leschber et al., 1984; O'Conner, 1988). For example, growth tests are commonly used to better understand the bioavailability of herbicides, and tests that measure plant tissue concentrations are routinely conducted to evaluate plant nutrient status. Tests have most often focused on identifying plant deficiencies of particular elements but are easily adapted to evaluate toxicities (Gettier et al., 1985). Plant uptake has been used to evaluate the effect of soil contamination as well as the ability of *in situ* treatments to reduce those effects (Pierzynski and Schwab, 1993; Chaney and Ryan, 1994; EPA, 1995; Laperche et al., 1997). When used appropriately, plant tissue analysis can provide a quantitative assessment of bioavailability process D in Figure 1-1.

Appropriate methods for plant sampling and analysis have been outlined for a range of agronomic crops (see Westerman, 1990; Rayment and Higginson, 1992; Kalra, 1998). Field studies are generally conducted for a minimum of two growing seasons, while controlled environment studies often involve multiple harvests to mimic changes over time. Depending on the goals, typical measured responses include visual symptoms of toxicity and deficiency or simply aboveground biomass. Analysis of plant tissue for total elements from samples collected at sites of concern is generally inexpensive with a turn around time of one to two weeks. Bioassays using plants in field studies or in controlled environment studies are considerably more expensive as well as time consuming. There are experimental artifacts in metal uptake results obtained in plants grown in pot versus field studies (deVries and Tiller, 1978) in that plants grown in pots with access to a limited volume of soil will have increased metal concentrations when compared to field studies.

Appropriate use of plant bioassay data must take into account characteristics of the plant species tested, the experimental conditions, the role of the contaminant of concern in plant nutrition, and how the contaminant of concern may have interacted with necessary plant nutrients to cause imbalances. For example, metals spiked to soil as salts are generally much more phytoavailable than comparable concentrations added in municipal biosolids or present in historically contaminated sites (Brown et al., 1998). Pot studies using metal salts may greatly overestimate uptake into plant tissue *in situ* (Logan and Chaney, 1983; Page et al., 1987; Sauerbeck, 1991). It is also important to understand the mechanism by which the contaminant is most likely to cause negative health effects. In certain cases, contaminants may not be phytotoxic, but they may be accumulated in sufficient concentrations in edible plant tissue to cause negative health effects to consumer populations.

Metals. The utility of plant bioassays for measuring bioavailability of metals is dependent on the particular metal, its route of uptake, and its potential mode of toxicity. For example, zinc, nickel, copper, and manganese toxicities have been reported for plants growing under field conditions. For these elements, the potential toxicity to plants must be considered in any evaluation of bioavailability. For other elements, their concentration in plants does not vary significantly even with changes in soil concentration that span orders of magnitude. For even other elements, consumption of enriched forages is the primary pathway through which these elements can enter the food chain and cause harm. Here, plant tissue concentration is a viable means of measuring bioavailability to higher organisms, even though plant yields may not be impacted. Specific examples of each of these cases follow.

Cadmium, lead, arsenic, chromium, and cobalt are not generally phytotoxic, even in cases of severe soil contamination in the field. Furthermore, lead, arsenic, chromium and cobalt are generally not taken up by plants in readily measurable quantities (Xu and Thornton, 1985; Chaney and Ryan, 1994; McGrath, 1995; Chaney et al., 2000). When these four metals have been found to be toxic to plants, uptake was generally confined to root tissues; thus, measurements of plant shoot concentrations are not useful. For lead, arsenic, chromium and cobalt, plants are not the most sensitive species, and consumption of contaminated plant material is not a relevant exposure pathway for higher organisms.

For other elements, consumption of foodstuffs with elevated metal concentrations can be an important exposure pathway, although the metals are not toxic to plants themselves. For example, consumption of plants containing elevated concentrations of cadmium has resulted in human fatalities (Kobayashi, 1978). Although plant concentrations alone are not sufficient to determine if consumption of cadmium-enriched foodstuffs will result in negative human health effects, they are an important indicator of bioavailability in a soil system (Chaney et al., 1999). In the case of selenium and molybdenum, uptake into the edible portion of plant tissues is generally not sufficient to cause plant toxicities but has lead to toxicities of animals consuming enriched plant tissue (Foy et al., 1978; Bingham et al., 1986; McGrath, 1995). Thus, for cadmium, selenium, and molybdenum, measuring plant uptake from soil is a means to evaluate their bioavailability to higher organisms. It should be remembered when sampling plants as part of ecological risk assessment that wildlife species may feed on different plant parts, which may accumulate contaminants to different degrees.

Plants are considerably more sensitive than other organisms to manganese and particularly zinc. Indeed, phytotoxicity of zinc is one of the primary concerns of excess zinc in soils. It is not surprising, then, that plant uptake of zinc has been identified by EPA as the controlling pathway for setting maximum permissible zinc concentrations for biosolids applied to land (Chaney et al., 2001). Because zinc will kill plants at concentrations lower than those generally associated with negative health effects in animals, plant phytotoxicity effectively prevents trans-

fer of soil zinc through the food chain (Chaney and Ryan, 1994). Plant zinc concentrations are also effectively used to measure changes in bioavailability as a function of soil treatment with different amendments, such that reduction of plant zinc following amendment is accepted as evidence of the reduced bioavailability of the metal (Basta and Sloan, 1999; Brown et al., 2000).

It is important to understand that there is not a single metal concentration that is associated with growth suppression and phytotoxicity across all plant species. For example, concentrations of zinc in plant tissue associated with phytotoxicity vary greatly both within and across species. Twenty varieties of soybean (*Glycine max* L.) grown on the same high zinc soil, were found to have different uptake as well as yield response (White et al., 1979). Four barley cultivars (*Hordeum vulgare* L.) grown under identical conditions had plant zinc concentrations ranging from 52 to 126 mg kg^{-1} (Chang et al., 1984b). Values for toxic concentrations have been reported to range from 200 mg kg^{-1} (Bingham et al., 1986) to 500–1500 mg kg^{-1} (Chaney et al., 2000). For metal concentrations to be effectively used as a measure of bioavailability, it is important that the threshold values of the plant tested are well understood. In addition, toxicities of certain elements are associated with deficiencies of others. For example, zinc, copper, and nickel toxicities can be associated with iron deficiencies (Bingham et al., 1986), while lead and zinc toxicities can also be related to phosphorus deficiencies (Laperche et al., 1997; Brown et al., 1999, 2000). Behavior of plant species in response to nutrient deficiencies varies, and this response can affect the uptake of potentially toxic elements (Marshner, 1998).

Organics. Use of plant bioassays to assess the bioavailability of organics in soils to plants and higher animals is not well documented. Plant germination and growth tests are routinely used to evaluate the efficacy of herbicides in soils, but these organics were developed specifically to be available to certain classes of plants. Plant assays have been used to measure the uptake and toxicity of explosives (Anderson et al., 1999; Gong et al., 1999a,b; Krishan et al., 2000). But for the majority of organic contaminants, use of plant assays is limited and generally not well accepted because plant uptake of such compounds tends to be minimal (perhaps because they are not required for growth) (Chaney et al., 2001). Organics must be present in the transpirational stream to be taken up by plants, which is unlikely for compounds of limited solubility. Hydrophilic compounds cannot easily pass through the lipid portion of the root's plasma membrane (Marschner, 1995; Burken and Schnoor, 1998), while more hydrophobic compounds can penetrate the membrane but are prohibited from entering the xylem and being translocated to shoot tissue. These compounds are generally bound to the mucigel at the root surface or to the lipid membranes of the root cell walls.

Measurement of organic compounds in plant tissue also presents analytical difficulties. Radiolabeled compounds are often used (Burken and Schnoor, 1997), such that pure compounds as well as metabolites can be detected in plant tissue.

It is not always clear if compounds are metabolized once they have entered the plant tissue, although axenic plants and cultures have been used in an attempt to clarify this process (Hughes et al., 1996; Newman et al., 1997). While these studies have shown that certain species are capable of metabolizing compounds internally, the possible contributions of rhizosphere and endophytic microorganisms must also be emphasized and has generally not been assessed (Banks et al., 1999; Siciliano et al., 2001). Because of these factors, plant bioassays of organic compounds are currently not viable tools for understanding bioavailability processes.

Invertebrate Bioassays

Soil Bioassays. A variety of tests are available to determine the uptake of organic compounds and metals and their toxicity to soil invertebrates (Løkke and van Gestel, 1998). These tests can be conducted with site-specific soils, or spiking studies can be used to predict the potential accumulation from soils that do not currently contain the contaminants of interest. The measured endpoints of soil bioassays include tissue accumulation, altered growth and reproduction, and mortality. Thus, such studies can be either direct measures of biouptake (bioavailability process D) or more indirect measures (that capture bioavailability processes D and E).

Invertebrate tests have been developed for representatives of the major terrestrial groups including protozoa, nematodes (*Caenorhabditis*), annelids (earthworms), crustaceans (isopods), and various insects (Bierkens et al., 1998; Løkke and van Gestel, 1998). However, studies have focused mainly on earthworms and the springtail (collembolan) *Folsomia candida* (Cortet et al., 1999). The terrestrial oligochaetes such as *Lumbricus terrestris*, *Eisenia foetida*, or *E. andrei* typically live burrowing through the organic rich upper third of a meter of soils. Moreover, they are well studied, easy to rear, reproduce rapidly, and are an important part of most soil ecosystems, thus making them an excellent candidate for soil bioavailability studies.

Several tests have been devised to assess the effects of contaminants on earthworms, including bioaccumulation, survival (OECD, 1984), reproduction and growth (van Gestel et al., 1989; Gibbs et al., 1996; ISO, 1996; Holmstrup, 2000), avoidance (Yeardley et al., 1996), as well as a range of immunological (Chen et al., 1991) and biochemical (Arnaud et al., 2000) parameters. In the growth and reproduction studies, adult earthworms are weighed prior to being incubated in food-supplemented test soils for varied periods of time (21–56 days). At the end of exposure the worms are collected and reweighed. In addition, the soil is washed through 2- and 1-mm sieves, and the cocoons collected and counted. Such tests have been applied to contaminated soils (Robidoux et al., 2000; van Gestel et al., 2001). The *E. foetida* 14-day artificial soil test (OECD, 1984) has been adapted to *L. terrestris* and used in assessing mortality, morbidity, and

whole worm burden of contaminated soils (Callahan, 1991; Chang et al., 1997; Charrois et al., 2001). While typically there is increasing sensitivity in going from survivability to biochemical indicators, there is also increased individual variability, with responses often being attributed to non-toxicological events (e.g., handling) (Arnaud et al., 2000).

The springtail (*F. candida*) assay is a 28-day soil assay that evaluates survival, growth, and reproduction (ISO, 1999). Growth is determined by comparing pre- and post-exposure weights. Survival and reproduction are determined by "floating" the springtails out of the soil after exposures. An image of the floating specimens is captured, and from this image the numbers of adults and juveniles can be determined. This assay, while more labor intensive, has been applied to contaminated soils (van Gestel et al., 2001).

Nematodes, especially *C. elegans*, are good biomonitors of soils (Donkin and Dusenbery, 1993). While mortality and to a lesser extent development are the common endpoints with this species (Peredney and Williams, 2000), more recent work has focused on the development of transgenic strains with heat shock proteins (Stringham and Candido, 1994) or metallothioneins (Cioci et al., 2000) linked to the β-galactosidase (*lac-Z*) reporter gene. As noted by Drobne (1997), a variety of endpoints has been developed for terrestrial isopods (e.g., woodlice).

There are a number of factors to consider before deciding whether to use *in situ* or laboratory soil invertebrate bioassays. A major advantage offered by laboratory measurements of invertebrate bioaccumulation is the greater control that the investigator can exert over the test. For example, a field-collected soil can be mixed in the laboratory to help reduce variability in concentrations (if such variability is not the focus of the investigation) or create soils with a desired contaminant concentration. Potential disadvantages of laboratory tests are numerous, and mainly concern the representativeness of the test results to field conditions. Laboratory-maintained cultures of invertebrates may differ from indigenous biota in their ability to tolerate or accumulate contaminants, with evidence that some field populations develop resistance and can accumulate toxins to a greater degree than laboratory counterparts (Ron Chekai, personal communication, 2002). A related issue concerns whether lab experiments are conducted long enough for conditions to reach steady state. It appears that in some cases exposure duration on the order of a few weeks to a month is adequate (Edwards and Jeffs, 1973; Stafford and Edwards, 1985). An important consideration with laboratory tests is the extent to which whole, "undiluted" field soils are used rather than field soils diluted with a reference soil of similar physical characteristics (usually collected from or near the site of contamination) or a standard laboratory reference soil. While such dilution provides a greater degree of control, the modifications produced must be taken into account (e.g., mixed soil is more analogous to an emulsion of particles with higher and lower contaminant concentrations rather than a homogenous mass of particles of intermediate contaminant concentration).

Box 4-9 describes the use of bioaccumulation measurements in earthworms to determine the soil or sediment availability ratio, which is a crude measurement of the relative bioavailability of fresh versus aged contaminants in soil.

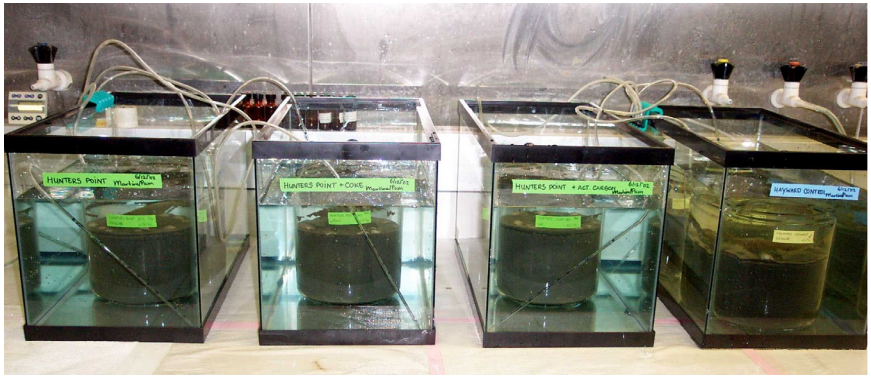
Sediment Bioassays. Both laboratory and field studies of contaminant bioavailability from sediments are conducted with benthic invertebrates. This information can generally be obtained rapidly and applied directly to predictions of direct exposure or food chain bioaccumulation. A range of tests is available to directly determine the uptake and accumulation of organic compounds and metals and their toxicity to invertebrates, in this case aquatic organisms (Giesy and Hoke, 1989; Burton, 1991). These tests can be conducted with historically contaminated field sediments or field or clean sediments that have been spiked to contain the contaminants of interest. Like the soil invertebrate bioassays, the measured endpoints of sediment bioassays include tissue accumulation as well as functional responses such as altered growth and reproduction and mortality.

BOX 4-9
Soil or Sediment Availability Ratios

The soil or sediment availability ratio (SARA) method uses organisms to estimate the relative bioavailability of aged versus unaged organic and inorganic chemicals in soil or sediment (Sijm et al., 2000). This is accomplished by exposing organisms to soil or sediment with freshly added chemicals and to soil or sediment with aged chemicals, both at similar total concentrations. The resulting ratio of the concentrations in the organism gives the relative bioavailability. The most frequently used biological system for SARA involves earthworms, particularly *Eisenia foetida* (Haque and Ebing, 1988; Van Gestel and Ma, 1988; Van Gestel and van Dis, 1988; Belfroid et al., 1995; Kelsey and Alexander, 1997; Chung and Alexander, 1999; Morrison et al., 2000).

Using SARA, Belfroid et al. (1995) measured the relative bioavailability of hydrophobic compounds present for more than 20 years in a field-contaminated soil. They demonstrated that the aged compounds had a bioavailability of < 3 percent relative to the same chemical freshly added to soil samples. This method has also been used to show that select PAHs aged for only three months in sediment had similar bioavailability as freshly spiked sediment (Kraaij et al., 1998). This method has substantial potential for the indirect evaluation of bioavailability of chemicals that have been present in the soil or sediment for an extended period of time and on how contaminant association with the solid phase changes over time. It should be recognized, however, that SARA relies on the comparison of organismal concentrations of contaminant from two different scenarios, for which several bioavailability processes may be different. Hence, the inferred "bioavailability" number should be considered a lumped estimate of several bioavailability processes rather than measurement of an individual process like solid partitioning or gastrointestinal absorption.

Validation of the technique has not occurred, probably due in part to the difficulties in comparing results from different tests.



Set-up for measuring bioaccumulation and toxicity of contaminated sediments to invertebrates using material from Hunter's Point, CA.

Uptake bioassays directly determine the biologically available fraction of compounds associated with sediment (EPA, 2000a,b), that is, they measure the potential for chemicals to partition into benthic biota or other aquatic organisms. These tests are often used to calculate Biotic Sediment Accumulation Factors (BSAF), discussed in Chapter 2. Such bioassays are most appropriate when the contaminants are not toxic to benthic invertebrates but can be bioaccumulated and biomagnified up food chains or through food webs to the point where they may be chronically toxic to higher-order organisms. This is the case for a range of organic compounds such as PCBs and chlorinated dioxins. Benthic invertebrates are tolerant of exposure to these compounds because such invertebrates do not contain the aromatic hydrocarbon receptor through which the critical toxic effects are mediated.

Uptake bioassays can be conducted under either field or laboratory conditions and on field-collected sediments or on spiked sediments (Giesy and Hoke, 1990). Field studies with indigenous invertebrates and *in situ* studies where caged organisms of a known history are placed in the field are more realistic measures of the biologically available fraction than laboratory studies (Salazar et al., 1995; Chappie and Burton, 2000; Burton et al., 2000). *In situ* bioassays with benthic invertebrates are particularly relevant to understanding contaminant bioavailability, because these organisms often feed on sediments or sediment-associated meiofauna or algae. Thus, contaminants in their tissues reach steady-state concentrations more quickly than for higher-order organisms, such that studies can be conducted for shorter duration. It is important to determine if the confined animals have sufficient natural food items available to sustain them (likely for most sediment-dwelling organisms); if they do not, an accurate measure of uptake and accumulation of sediment-bound chemicals is not obtained. In addition, when *in situ* tests are used, it is critical to have reached steady-state tissue con-

centrations of the contaminant of concern, or at least know what proportion of steady state has been obtained.

The greatest limitation of laboratory bioassays using spiked sediment is the questionable ability of the experimental conditions to mimic field conditions. For a variety of reasons (see Chapter 3), chemicals bound in sediments can take a long time to reach a steady state—a situation that may take months or years to simulate using laboratory-spiked sediments. In addition, adding exogenous chemicals to sediments will disrupt the dynamic equilibrium of other chemicals in the sediment and can result in estimates of accumulation that are different from what would be observed for an undisturbed sediment. Similarly, when laboratory studies are conducted with sediments collected from the field, the manner in which sediments are handled can affect the bioavailability of organic compounds and metals in the sediment (EPA, 2001).

Uptake and bioaccumulation of contaminants from sediments has been studied in a range of test designs with a number of species including invertebrates from both freshwater and saltwater environments (EPA, 1994, 2000c). Some of the most useful protocols for the study of benthic invertebrates are for worms, benthic insects, and mollusks such as clams (EPA, 1987a,b). One limitation of both field and laboratory tests that utilize small organisms is obtaining accurate estimates of concentrations and particularly weights or lipid contents for normalization. Also, it is important to be able to separate the material that is actually accumulated into organismal tissue relative to that which is simply adsorbed to the surface. Protocols for conducting both toxicity studies and bioaccumulation studies have been suggested, and some have been adopted by state, provincial, and federal agencies or international bodies (Boese and Lee, 1992). A comprehensive discussion of the theory and issues involved in the standard tests for sediment toxicity testing and determination of bioaccumulation of contaminants from freshwater sediments to benthic invertebrates, as well as specific guidance for test methods, has been provided by EPA and thus is not discussed here (EPA, 1994; Ingersoll et al., 1995; EPA, 2000c). In addition to providing information on the specific tests, EPA (2000c) gives compound- and element-specific bioaccumulation information for a range of species. Specific guidance has been produced jointly by EPA and U.S. Army Corps of Engineers for evaluating dredged materials for both toxicity and uptake and bioaccumulation in invertebrates in marine (USACE and EPA, 1991) and freshwater environments (EPA and USACE, 1998). The strengths and limits of applying such tests to nature have also been discussed in Luoma (1996).

A subset of sediment bioassays are uptake and toxicity tests involving sediment interstitial water (pore water) (Lee, 1978; Giesy et al., 1988, 1990; Ankley et al., 1989; Hoke et al., 1992, 1993). For certain organisms, contaminant concentrations in pore water are more closely related to bioavailability than are concentrations in bulk sediments (Ankley et al., 1992a; Bonnell et al., 1995). Indeed, this is the basis of equilibrium-partitioning methods for predicting bioavailability

of metals and neutral organic compounds (see Chapter 2). Sediment pore water tests are most useful when the duration of the assay is short. Otherwise, the contaminant can become depleted and the tests will underestimate both bioaccumulation and toxicity of bulk sediments (Giesy and Hoke, 1989; Giesy et al., 1990). Obviously, these tests are accurate only for assessing exposure via the pore water pathway.

Another related bioassay is the elutriate test, which was developed to mimic the potential bioavailability (and in some cases toxicity) of contaminants following sediment resuspension in the water column caused by dredging (Palermon and Thackson, 1988; Ludwig, 1989; Bonnet et al., 2000). The test is actually an extraction followed by an uptake or toxicity bioassay on the extracted liquid. Sediments are combined with water in a ratio of approximately 4:1 (Daniels, 1989; Giesy and Hoke, 1990), which leads to fundamental changes in contaminant–sediment binding and hence bioavailability (Harkey et al., 1994). Where compounds are accumulated across membrane surfaces such as gills, this resuspension and the resulting contaminant release into the dissolved phase can increase overall bioaccumulation and toxicity. In cases where primarily particle-bound compounds are taken up (such as with zooplankton), the opposite is generally true (Nalewajko, 1989). Thus, the accuracy of elutriate tests in assessing bioavailability is a function of the compound of interest, the exposure pathway, and the dilution ratio.

Fish Bioassays

Fish have been used in tests of sediment contamination in both field and laboratory studies (Anon, 1978; Ankley et al., 1992b). Accumulation of contaminants from sediments can have direct effects on fish and the predators that eat them, including humans. Thus, fish bioassays can be used in food-chain studies as well as to predict the potential adverse effects these residues could have on the fish themselves. Contaminant concentrations in fish tissues provide an integrated measure of the exposure from all pathways (e.g., ingestion of contaminated water, food, or sediment; dermal contact; and passage across the gills).

In situ studies give the most accurate measurements of bioavailability under field conditions, as long as the study design does not inject biases into the results. Two basic fish bioassays are possible under field conditions. Either wild (feral) fish can be collected at a site, or (because it may be difficult to know if fish are resident or for how long they have been exposed) fish can be caged at a location. There are different measurement endpoints that can be used, including bioaccumulation as measured by tissue concentrations and more functional responses. For example, toxicity—measured by survival or growth—is a standard endpoint. Whether a bioassay that uses accumulation into tissues or a functional measure of response is chosen depends on the particular scenario, including the contaminant of interest. For example, some metals are homeostatically regulated in fish, such

that accumulation of these compounds is a less appropriate measurement of bioavailability. Instead, functional responses that are manifested, for example, on the surface of the gills, might be used.

Field studies of contaminant accumulation and toxicity in fish are complicated by a number of factors that are difficult to control. It is generally impossible to distinguish between different sources of contamination, be it the sediment itself, the water column, and food items, including invertebrates, consumed by the fish. Indeed, the primary route of fish accumulation of many contaminants, especially neutral organic compounds that tend to be persistent in sediments, is through food chain transfers (Jones et al., 2001). Fish are excellent integrators of contamination coming from these multiple sources, but as a consequence fish bioassays in the field are not effective for measuring individual bioavailability processes. Another important logistical limitation to caged fish studies is that unless there is sufficient natural food, the fish need to be fed. If this is the case, the caged fish may not accurately reflect exposure under natural conditions.

Standard protocols have been proposed to determine the accumulation and toxicity of various contaminants under *laboratory conditions*, including metals and organic compounds from both water (EPA, 1975; ASTM, 1980) and sediments (ASTM, 1988, 1994; Ankley et al., 1992b; EPA Region 5, 1994). For such tests that utilize contaminated water only, a direct measurement of biouptake into fish can be made. The ecological relevance of laboratory tests is not always clear because biouptake may not be the rate-limiting step for overall fish accumulation of contaminants from sediment. However, results from such bioassays are valuable as input into simulation models of contaminant accumulation by fish (which attempt to account for uptake from multiple sources of contamination). Laboratory protocols that include sediment should be designed to capture diffusion and disturbance processes in sediments, which may be more important to overall contaminant accumulation than the biouptake process (Magee, 1991; Ankley et al., 1992b).

Mammal and Bird Bioassays

As part of ecological risk assessments, both mammals and birds have been used to monitor for exposure to residues at contaminated terrestrial sites (Phillips and Rainbow, 1993; Talmage and Walton, 1993). Although there are some differences between birds and mammals, there are enough similarities that they can be considered together when discussing the bioassays available for both toxicity and accumulation (Tank et al., 1993). Vertebrates can be exposed to residues in sediments; however, the issue of bioavailability is generally more relevant for their exposure to terrestrial soils (Pankakoski et al., 1994). As with fish, tissue accumulation or toxicity measurements in mammals or birds are integrative measures of exposure. Sentinel animals can be used either in field situations or in closed laboratory systems where soils are brought from the field.

There are several pathways through which vertebrates can be exposed to contaminants in soil, including direct consumption of plants, prey, and soil. However, except for some species of ducks, direct ingestion of soil by birds is rare, such that few if any bird bioassays have been designed to capture that pathway. Rather, tests are designed around the pathway of plant or prey consumption, and contaminants in food are assumed to be 100 percent bioavailable. (This may or may not be true for all organic compounds and metals, but it seems to be true for most neutral organic residues, such as PCBs and DDTs.) For the food consumption pathway, the availability of contaminants in sediments or soils is measured by determining the bioavailable fraction to the prey rather than the predator, using tests described earlier for invertebrates and fish. For example, if midges living in PCB-contaminated sediments will molt and subsequently be eaten by tree swallows, the normal practice is to determine the bioavailable fraction of the PCBs in the sediments to the insects, not to the birds. It is possible to determine the bioavailable fraction to the predator directly, for example by measuring the concentration of the residue in the bird's diet and the fraction remaining in the feces.

In some cases, direct ingestion of soil can be an important exposure pathway for which direct measurements of uptake would be useful. For example, birds that have a crop consume grit in the form of small stones or sand (Solomon et al., 2001). Determining the fraction of the contaminant in soil or sediment that would be biologically available under the conditions in the crop can be approached with standard laboratory feeding studies (Romijn et al., 1995). In the case of birds, the tissue of interest for which the contaminant levels are measured is often the eggs (Keith, 1996).

No specific protocols have been developed to determine the chronic toxicity of soil or sediment contamination to birds. However, the protocols that have been developed to conduct dietary toxicity tests (ASTM, 1999) can be applied.

Field studies are another possibility, using wild, feral, or even domestic mammals (Pankakoski et al., 1994) or birds (Tank et al., 1993; Hothem and Welsh, 1994; Baars et al., 1995; Nabel et al., 1995) to monitor for the exposure expected for wild animals at a specific site. It is difficult to determine the contaminant fraction taken up from soils or sediments by measuring the concentrations of residues in wild mammals and birds, given the various sources of contamination to which they may be exposed and a general inability to control for confounding factors. However, if the site use factor[2] is well known, wild mammals and birds may be helpful to determining potential exposure. Another approach is to release sentinel organisms such as small mammals or birds of a known dietary type at a site (Custer et al., 1996). For gallinaceous, ground-dwelling birds, the domestic chicken is a useful surrogate species (Schuler et al.,

[2]The site use factor is the proportion of time an animal spends in a contaminated area. If the animal is there all the time, the factor is 1.0; if only there half the time it would be 0.5.

1997). Sentinel birds can be placed in cages over the area of interest to determine the degree of accumulation that occurs under relatively natural field conditions. Pinioned birds can be released into larger areas, but recovering the birds can be difficult and predation can be a problem. A third approach is the bird box study, which works well for a wide range of species including blue birds, robins, doves, tree swallows, house wrens, and any species that will build a nest in a nest box (Cain and Bunck, 1983; Bunck et al., 1987; Blus et al., 1993; Bishop et al., 1995; Kemler et al., 2000). Nest boxes can be placed in certain regions to determine the overall exposure that is expected to occur to birds of a particular feeding type. This type of monitoring has been used successfully at a number of contaminated sites (Froese et al., 1998), including the use of kestrels to determine bird exposure to polychlorinated dibenzodioxins in soils (Kemler et al., 2000). Such monitoring obviously cannot account for specific bioavailability processes, because the measured bird contaminant burdens are an integration of multiple processes occurring in the area around their nests. However, if concentrations of the contaminant of interest are also measured in soil, sediment, and other dietary items, then bioconcentration factors, which implicitly include a measure of the bioavailable fraction, can be determined (Froese et al., 1998).

Environmental Health Studies

Environmental health studies are designed to evaluate human childhood exposures to a contaminant in the residential environment. Such studies have been conducted for lead at numerous mining sites and to a lesser extent for arsenic at mining and smelting sites. Although these studies are not intended primarily to evaluate the bioavailability of the contaminant to the exposed population, they can yield this type of data all the same. Given the difficulty of studying absolute and relative contaminant bioavailability in children, environmental health studies offer one of the few mechanisms for obtaining this type of data.

To conduct such a study, a cohort of individuals living within an area with elevated soil concentrations of the element of concern must be recruited. For each individual, all known potential exposure sources within the residential environment are sampled, along with a biomarker(s) for exposure to that element. For example, when conducting such a study for arsenic, sampling would include yard soils (particularly from bare areas), house dust, and tap water as the potential exposure sources. Potential biomarkers include total arsenic, speciated arsenic (i.e., As^{+3}, As^{+5}, monomethylarsonic acid, and dimethylarsinic acid), and creatinine concentrations in urine. Exposure from food would be estimated based on diet, since the extent of this exposure is well characterized. A detailed questionnaire would be administered to identify any behavioral or dietary sources of arsenic exposure. Urinary and fecal arsenic concentrations would be monitored to establish arsenic exposure to each individual. These data would then be used as

input to a human health risk assessment model for the affected community. The risk assessment model requires the relative bioavailability of arsenic from each exposure source to be known or estimated. Since the relative bioavailability of arsenic from soil and house dust, along with soil ingestion rate, are two of the least well characterized variables in the model, combinations of these variables can be tested to establish the best fit to observed exposures. Such an approach was performed for childhood exposures to arsenic in the residential soils at the Anaconda, Montana, National Priorities List site (Cohen et al., 1998). The study yielded plausible estimates for relative arsenic bioavailability in local children. This approach to estimating relative arsenic bioavailability in children contains considerable uncertainty, and, having only been performed once, the reproducibility is unknown. In addition, such a study is sufficiently expensive that it would likely be performed only at the most high profile sites. Finally, the study must be specifically designed to yield estimates of uptake from soil and house dust. Of all the environmental health studies for lead and arsenic conducted to date, only the Anaconda study included the data necessary to evaluate contaminant bioavailability from soil.

Ecosystem Level Tests

Microbial Community Assays

There are no reports that explicitly address contaminant "bioavailability" measurement inferred from a response at the level of whole soil microbial community. However, several community-based assays to measure soil microbial activity exist and could potentially be employed to infer contaminant bioavailability. As microorganisms account for up to 90 percent of the soil biomass and contribute a large proportion of essential soil functions, such as cycling of C and N, examining contaminant effects at the level of the microbial community seems critical. This is supported by the fact that microorganisms are in direct and intimate contact with contaminated soil particles and pore water.

Microbial systems can be investigated at two fundamentally different levels: the level of system function and the level of community structure. System function is most often examined by studying elemental (nitrogen or carbon) cycling. Carbon mineralization can be inferred from CO_2 evolution and measured as a basal level or in response to the addition of specific carbon substrates of interest (Stenstrom et al., 1998; Lin and Brookes, 1999; Gong et al., 2000; Murray et al., 2000; OECD, 2000a). An extension of this method concerns the examination of the community level utilization of different carbon sources (Degens and Harris, 1997), facilitated with tools such as the commercially available Biolog (Garland and Mills, 1991; Rutgers et al., 1998). Measurement of nitrogen cycling is more complicated and requires selective extraction of nitrogen species from soil matrix and subsequent analysis (Johansson et al., 1998; Kandeler et al., 1999; OECD,

2000b). Rapid and inexpensive qualitative assessments of whole system function can be derived from the activity of several key soil enzymes such as dehydrogenases, amylases, phosphatases, arylsulfatases, and cellulases (Kelly and Tate, 1998; Margesin et al., 2000). Unfortunately, the true ecological relevance of these snapshot measurements is not clear, and microbial activity cannot be easily separated from plant root activity.

Direct measurements of community structure—presumably correlated to activity—can be made (Ibekwe and Kennedy 1998; Waldrop et al., 2000). It has long been recognized that cultivation-based techniques to address community structure vastly underestimate community diversity, yielding biased estimates of community structure (Amann et al., 1995). Hence noncultivation-based molecular techniques are typically adopted. Most of those techniques as applied to soil microbial community inspection either target the lipid profiles (Lindahl et al., 1997; MacNaughton et al., 1999; Zelles, 1999) or nucleic acid fraction (Muyzer et al., 1993; Liu et al., 1997; von Witzingerode et al., 1997; Hill et al., 2000) of the community. When targeted at the nucleic acid fraction, analysis can be done with or without enzymatic amplification of the nucleic acid pool. Although vast progress has been made in development of such techniques, their successful implementation awaits standardization and decisive studies on the link between community diversity and contaminant toxicity (and/or bioavailability).

Mesocosm Tests

This chapter has previously discussed field-scale tests of contaminant uptake and accumulation, such as putting caged fish into aquatic systems or putting penned or pinioned birds into a terrestrial area for a known period of time. While conducting such studies at the site of interest is often desirable, it is not always possible. Also, the inability to control or maintain organisms for long periods of time and the inability to control exogenous factors makes interpreting these results difficult. Therefore, semi-field scale tests, referred to as mesocosms or microcosms depending on their size, have been developed to mimic realistic exposure scenarios (Perez et al., 1977) while allowing some control over complicating factors (Brockway et al., 1979; Craft, 1983; Anon, 1984).

Mesocosms are subsets of ecosystems (Giesy and Odum, 1980). They can be bounded natural systems or completely artificial (gnotobiotic) (Graney et al., 1995). Mesocosms are generally used to study population, community, and ecosystem processes, including responses to stressors and chemical toxicants (Davies and Gamble, 1979). This is done to give more realistic exposures and to allow for the study of interactions that occur in these more complex systems (Addison and Holmes, 1995). For example, volatilization, photolysis, and sorption to inorganic and organic matrices may be fairly site-specific and important to bioavailability processes. Of particular importance is the interaction between biota and their environment (Rodgers, 1983).

A wide range of mesocosm types and sizes has been developed to test for the toxic effects and bioaccumulation of compounds (Giesy, 1980; Odum, 1984). For example, intact soil cores have been used in soil leaching studies to estimate the bioavailability of metal ions (Tolle et al., 1985). Mesocosms are also useful for validation of complex models of bioaccumulation (Larsson, 1984; Anderson et al., 1987; Larsson and Sodergren, 1987; Abbott et al., 1995). Mesocosms serve as an intermediate-scale system in both size and complexity, and thus must be large enough to have certain attributes, but not so large that they cannot be studied as experimental units and replicated. Thus, mesocosms allow for potentially more realistic exposure scenarios (and chemical and physical processes) than could be simulated in smaller bench-top studies. Finally, mesocosms can include complex interactions between and among organisms and their abiotic environment to more closely mimic field conditions. Because such tests are complex, they tend to be useful but expensive to conduct. Currently, mesocosms are neither required nor readily accepted as tools to study bioavailability for regulatory purposes.

Summary

This section has discussed dozens of biological tools available for measuring bioavailability to both ecological (microorganisms, plants, and animals) and human receptors. The tools range from those that measure just one process, such as absorption across a membrane (biouptake), to those that measure the integrated effect of multiple processes. There are tradeoffs between such tests, as clarified in Table 4-2. In particular, those tests that directly measure biouptake, such as isolated organ tests or assimilation efficiency, provide unambiguous results about distinct mechanisms, but they may not capture the complexity of the environmental system nor speak to important effects, like mesocosms and toxicity tests can.

Certain biological tests have been used to validate some of the physical and chemical tools discussed earlier, or they have been used to provide complementary evidence about bioavailability processes in a system. For example, assimilation efficiency used in parallel with spectroscopy could reveal the properties of sediments that control bioavailability process A in Figure 1-1. Finally, many of the tools discussed represent the state of the art or require additional research in order to reach their potential, especially molecular tools such as biomarkers and reporter systems.

TOOLS FOR HUMAN HEALTH AND ECOLOGICAL RISK ASSESSMENT

Exposure assessment is central to assessing risks of chemicals in the environment. The tests described in this chapter can be used to incorporate site-specific information into exposure assessment and to improve general knowledge. In order for the results to be acceptable to risk managers and regulators, the

tools used must be well supported technically. This section discusses the tools commonly chosen to provide information about bioavailability processes in site-specific human health and ecological risk assessment. Of course, the choice of a tool or tools is driven by the purpose of the assessment (e.g., to evaluate effects of specific soil or sediment conditions, to improve direct estimates of absorption processes, or to determine how bioavailability processes affect toxicity). Tests that measure uptake and bioaccumulation, are biomimetic (extractions), or measure toxicity directly or in surrogates are deployed on a regular basis for human health or ecological bioavailability assessment. New tools and modeling approaches are also available, and these are discussed as well. Because no tool or approach is universally the best, tradeoffs among tools (as described in Table 4-2) should be considered.

Prior to engaging in any attempt to measure the bioavailability of contaminants from soils or sediments, it is critical to establish an accurate site conceptual model that describes the relevant exposure pathways, the receptors to whom the exposures are occurring, and the environmental conditions under which the exposures are occurring. This is vital because the available tools for assessing bioavailability processes from soil are receptor-, pathway-, and contaminant-specific; bioavailability data for a chemical for one exposure pathway are not necessarily applicable to another exposure pathway. Because the development of a site conceptual model is generally the first step in any human health or ecological risk assessment, this information may already be available for a particular site. The lack of an accurate site conceptual model can lead to (among other problems) measurement of the wrong endpoint or selection of an inappropriate bioavailability tool.

Ideally, the tools chosen should support mechanistic understanding of bioavailability processes and subsequent model development. Only if this is a common goal will bioavailability assessment progress to the point of being used regularly, consistently, and accurately. This focus on processes suggests that a suite of tools is needed to fully assess bioavailability. Tools that collectively cut across different processes are more valuable than having multiple tools for the same process. As an example of why tools should be chosen to better understand fundamental processes, consider the role of soil properties on contaminant retention. As discussed extensively in Chapter 3, the mechanisms by which contaminants are bound to solids are controlled by a number of soil factors (e.g., organic matter content). Measurement of these factors early in the evaluation can guide the selection of other bioavailability tools and help interpret their results. (See Chapter 5 for additional discussion of this aspect of tools selection.)

A third important consideration is that since human health and ecological risk assessments tend to follow specific procedures, in order for bioavailability information to be useful within a risk assessment it must be in an appropriate form. This form may include (1) values or factors that are used in deterministic or equilibrium exposure equations; (2) kinetic models that take into account the time

of exposure and other factors; or (3) direct measures of chemical concentrations or biological responses to exposure.

Regulatory acceptance of the tools used to generate bioavailability information in risk assessment is expected to be influenced by several factors, including the validity of the method (Menzie et al., 2000). Validation variously refers to the performance of a tool or approach in terms of reproducibility, reliability, and multi-lab calibration. More important is validating the application of the approach to bioavailability processes; that is, it must be proven that the approach responds to changes in bioavailability. Ultimately, approaches that allow quantitative estimation of bioavailability should be validated by correlation with a biological response both experimentally and in the field situation where they are most relevant (see Box 4-10 for an example). An appropriate body of experimental and field study work would:

1. clarify where and when a method yields a definitive response;
2. clarify that the tool can be linked to a biological response of a similar magnitude, and that the linkage stands up across a range of conditions in the type of environment that is being managed;
3. test the prediction of bioavailability using different types of experiments and field studies;
4. clarify which types of species-specific biological responses are best predicted by the approach; and
5. include critiques of the best applications and the limits of the approach, especially compared to alternatives.

A method that is well accepted and validated should be given greater weight than one that is new or experimental.

Human Health

Several tools currently exist to evaluate the relative bioavailability of contaminants from soil to humans, all of which are based on exposure through direct contact. The tools include (1) animal studies that compare the absorption of a particular compound from soil relative to its absorption from a soluble salt (for inorganics) or a readily-absorbed vehicle such as oil (for organics); (2) *in vitro* test systems that have been developed to measure solubility or absorption of a chemical under a defined set of exposure conditions; and (3) various indirect techniques that evaluate the chemical forms of inorganics in soil or the manner in which organics are sequestered in soil. To date, this last approach has most often been used with certain metals (arsenic, lead, mercury) in soil; however, this information is generally used to elucidate the mechanisms underlying bioavailability rather than as the sole approach to estimating a bioavailability adjustment factor. Although any of these approaches could potentially be used with sedi-

BOX 4-10
In Vitro Validation Study for Lead

This box describes validation of an *in vitro* extraction test against the EPA Region 8 young swine model for determining the relative bioavailability of lead in soil. This work was a collaboration among a group of academics, consultants, regulators, and industry personnel to validate a simple test method that could predict relative lead bioavailability from soil and could be used for risk assessment.

Initially, the group developed a simplified *in vitro* test, consisting of a well-mixed, 1-hour extraction (37°C) in pH 1.5 HCl that was buffered with glycine (0.4 M). Initial testing indicated that this method correlated well with results from the young swine model. A Standard Operating Procedure (SOP) for the *in vitro* test, and an associated Quality Assurance Project Plan (QAPP), were prepared for the method validation study. The QAPP specified a high frequency of Quality Assurance (QA) sample analysis, including blanks, duplicates, and spikes. Three independent laboratories were selected (National Exposure Research Lab in Las Vegas, Nevada; Bureau of Reclamation, Environmental Research Chemistry Laboratory in Denver, Colorado; and ACZ Laboratories, Inc. in Steamboat Springs, Colorado), and each reviewed the SOP and QAPP. Each laboratory was then sent blind triplicate splits of three samples (nine samples total) and asked to perform the *in vitro* extraction and report the extracted lead concentrations for each of the nine samples. The resultant data were used to evaluate the precision, accuracy, and reproducibility of each laboratory, and to identify any deficiencies so that corrective actions could be instituted. The actual validation study involved submitting blind triplicate splits to each of the three laboratories from the 19 samples that had been tested for relative lead bioavailability in the young swine model. Results from these analysis indicated that the *in vitro* extraction method has good inter- and intra-laboratory reproducibility, and it correlates well with results from the young swine model (*in vitro* to *in vivo* correlation is linear with an $r^2 = 0.93$; Drexler, 1997). The *in vitro* extraction method used in the validation study may be found in Kelley et al. (2002), and the results of the validation study have recently been submitted for publication.

ments, their practical use to date has been limited to soils because this is the medium to which humans are most frequently exposed.

The importance of establishing an accurate site conceptual model during human health risk assessment to guiding the selection of bioavailability tools cannot be overstated. For example, under residential exposure conditions it is generally young children who are the most highly exposed and sensitive population. Their exposures to inorganics in soil are predominantly oral (because of hand-to-mouth activity) and may occur from both soil and house dust (depending on activity patterns and the season). To address such a childhood exposure, it is important to select a tool that measures relative oral bioavailability relevant to children, particularly if they have higher oral absorption rates of the chemical than adults (e.g., as with lead). The young swine model, which was specifically designed to determine the relative oral bioavailability of lead in soil to young

children, is just such a tool. However, were the contaminant of concern an organic compound in soil or house dust, then it is likely that dermal exposures would also be important, and an entirely different assessment methodology might be required. Finally, if it were an adult that was exposed to lead in soil, for example a construction worker exposure scenario, this would be better evaluated using the adult human model for lead uptake from soil.

Table 4-4 provides a summary of the methods that are currently available or are in development for estimating the relative bioavailability of organics and inorganics in soil to humans via oral, dermal, and inhalation exposure routes.

TABLE 4-4 Tools for Estimating the Relative Bioavailability of Soil Compounds to Humans

Exposure Pathway/ Contaminant	Currently Available[a]	In Development[b]
Oral/Inorganics	In vivo: Weanling rat (Pb, Cd) Young swine (Pb, As) Adult primate (As) Adult human (Pb) In vitro[c]: Pb, CN^-	In vivo: Young swine (Cd, Cr) In vitro[c]: As, Hg, Cd, Cr
Oral/Organics	In vivo: Mice (PAHs) Rat (PCBs) Rat, rabbit, and guinea pig (PCDDs/Fs)	In vivo: Rat (DDT) In vitro[c]: PAHs, PCBs, OCPs, PCDDs/Fs
Dermal/Inorganics	In vivo: Monkey (As) Swine (Ni) In vitro[d]: As, Cd, Cr, Hg, Ni	
Dermal/Organics	In vivo: Monkey (PCBs) Mice, rat, guinea pig, and swine (PAHs) In vitro: PAHs, PCBs, OCPs PCDDs/Fs	
Inhalation	None	None

[a]"Currently available" *in vivo* tests are indicated for the contaminants to which they have been applied on a regular basis, while for *in vitro* methods the table indicates contaminants for which tests have received a substantial level of validation, or have been accepted by a regulatory agency.

[b]"In development" indicates methodologies on which development work is actively being conducted.

[c]*In vitro* tests for estimating oral bioavailability are based on extraction in simulated gastrointestinal fluids.

[d]*In vitro* tests for estimating dermal bioavailability are generally based on penetration of a compound through skin (human or animal) in a special test cell.

Given the critical role of the biological system, *in vivo* methods are generally preferred as a technical basis for refining risk assessment for human health. *In vivo* studies, however, require that an acceptable animal model is available or can be developed within the technical, cost, and ethical constraints associated with a particular project. As evident from Table 4-4, the *in vivo* models for estimating the oral relative bioavailability of inorganics (particularly lead and arsenic) in soil have received considerable attention, and therefore are the most fully developed. The *in vitro* tests for these elements, which are based on extraction in simulated gastrointestinal fluids, are also relatively well developed. However, as mentioned in Box 4-10, only the *in vitro* test for lead has been fully validated (against the young swine model).

After oral bioavailability models for specific inorganics, the dermal absorption of hydrophobic organic compounds has received the most attention. Both *in vivo* and *in vitro* methods have been developed, although in this case the *in vitro* tests involve measurement of penetration through actual skin (human or animal) in a special test cell. The dermal absorption of certain metals (Table 4-4) has also received a certain amount of attention, with most of the studies to date having been conducted using *in vitro* rather than *in vivo* methods. This reflects the technical difficulty and expense of designing and conducting studies using animal models. Finally, the oral bioavailability of hydrophobic organic compounds in soil is an area where much work remains to be done. Although animal models have been developed for the major classes of organic compounds, all could benefit from further refinement, and no validated *in vitro* models are available at this time.

To date, no specific test methods, either *in vivo* or *in vitro*, have been developed to measure the pulmonary bioavailability of organics or inorganics from soil. This situation reflects the fact that inhalation exposures from soils are generally small relative to the oral and dermal exposure pathways, except under special circumstances.

Ecological Applications

Ecological risk assessment presents complexities over human health risk assessment because of the potential for contaminants to be differentially accumulated by different organisms and transferred up food chains. The tools that are used must be able to discern the fraction of the contaminant that is available for release, for absorption by critical species, and for further passage into the food web. One way to handle this complexity is to consider tools for measuring bioavailability processes in terms of the exposure pathways to which they apply. Bioavailability tools have been considered, developed, or applied to many of the common ecological pathways in soil, such as soil ingestion, dermal contact, and ingestion of plant matter, as well as for pathways that occur in aquatic environments. Table 4-5 lists those tools available for determining the bioavailability of contaminants in soil and sediment to ecological receptors for all major pathways,

TABLE 4-5 Tools for Estimating the Bioavailability of Compounds in Soil or Sediment to Ecological Receptors

Exposure Pathway	Currently Available	In Development
Soil/Sediment → Invertebrate	• Equilibrium partitioning and AVS methods • Toxicity tests with benthic and soil invertebrates to measure effects • Laboratory or field tests on benthic and soil invertebrates to measure accumulation of chemicals in their tissues	• Use liquid or solid extraction media to simulate uptake and accumulation into invertebrates • Critical Body Residues (CBRs) in invertebrates that are predictive of effects
Soil/Sediment → Plants	• Soil/sediment extraction tests • Toxicity tests with appropriate plant species to measure direct effects on germination and growth • Laboratory or field exposures to contaminated soils/sediments to measure accumulation of chemicals in plants for direct use in food-chain models	• Test aqueous extracts or elutriates of soils in various plant bioassays including algal tests, seed germination, and root elongation • Utilize the Plant Micronucleus test—Tradescantia—to evaluate potential genotoxic effects
Soil/Sediment → Groundwater → Surface Water Biota	• Leaching and desorption tests • Direct measurements of chemicals in groundwater, pore water, or surface water	
Soil/Sediment → Wildlife		• Extraction tests that simulate physiological fluids may be useful for evaluating availability of incidentally ingested soils/sediments • Short-term feeding studies may be useful for evaluating incidental ingestion of soils/sediments • Selected chemical-specific biomarkers
Soil/Sediment → Plant or Invertebrate → Wildlife	• Depending on wildlife diet, measures of bioaccumulation in plants and animal prey can be used and combined with a model	

TABLE 4-5 Continued

Exposure Pathway	Currently Available	In Development
	• Body burden measurements and measures of chemical metabolites can be used as indicators of exposure and of the availability of chemicals in soils/sediments • Selected chemical-specific biomarkers	
Soil/Sediment → [invertebrate or plant] → Wildlife → Predatory birds and mammals	• Depending on wildlife diet, measures of bioaccumulation in plants and animal prey can be used and combined with a model • Body burden measurements and measures of chemical metabolites can be used as indicators of exposure and of the availability of chemicals in soils/sediments • Selected chemical-specific biomarkers	
Soil → Soil Vapor → Burrowing wildlife	• Field measurements of soil gas to estimate exposure • Field measures can be used in soil gas models	

while the discussion below focuses on those pathways that have received the most attention with respect to bioavailability measurements.

Pathway from Soil or Sediment to Invertebrates or Plants

Invertebrates and plants are in direct contact with soils or sediments and therefore subject to chemical exposures at levels that could be toxic either to themselves or to higher trophic levels via food consumption and predation. For this reason, many tools for measuring contaminant uptake and bioaccumulation into plants and invertebrates as well as toxicity tests have been developed. Bioaccumulation test data have been used to create a variety of models of chemical exposure (such as the equilibrium partitioning and empirical methods discussed in Chapter 2 and other more mechanistic models, as described below).

Toxicity tests (described variously throughout the chapter) are frequently applied to evaluate effects of chemicals and chemical mixtures in soils and sedi-

ments to plants and invertebrates. The drawback of laboratory tests is that they may not reflect field conditions. *In situ* tests, on the other hand, generally lack the formal standardization and control available in the laboratory. Although the use of toxicity tests in regulatory programs is well accepted for invertebrates and plants, a number of uncertainties are associated with their application that have fostered debate and further research and development (Luoma, 1996). Most of this uncertainty concerns the selection of test species, test duration, appropriate toxicological endpoints, and extrapolations from simple laboratory conditions to the highly variable field conditions. Because toxicity tests reflect the integration of multiple physical, chemical, and biological processes, they are of limited use for gaining a better mechanistic understanding of bioavailability. However, they provide information that is unique from initial biouptake and bioaccumulation tests and they measure endpoints that are often of greater interest to stakeholders and regulatory agencies.

Bioaccumulation tests on sediment or soil invertebrates and plants that measure tissue concentrations of contaminants following exposure are frequently used to assess bioavailability and to provide input data for such simple empirical models as BSAF. As discussed previously in this chapter, such tools have been developed for the laboratory and the field and are in wide use. The major uncertainty associated with these tools stems from species-specific differences in the degree to which organisms accumulate compounds, which may reflect variability in exposure as well as variability in their anatomy and physiology. Therefore, data developed for a limited number of species may not be directly extrapolated to other species. This uncertainty can be somewhat accounted for by selecting a range of species considered representative of groups of other species (i.e., guilds). These measures of exposure can also be combined with effects information based on critical body residues (CBRs) to estimate toxic effects to target organisms, particularly acute effects (Fitzgerald et al., 1996; Lanno et al., 1997). Indeed, there is much interest in coupling tissue residue measurements with CBRs for bioaccumulative compounds to provide a better measure of risk to organisms than can be achieved solely from chemical measurements in soils or sediments.

One of the most important uses of bioaccumulation data has been to try to develop more sophisticated models of uptake that might take into account soil and sediment properties. Many of these models assume a mechanistic underpinning of equilibrium partitioning between the solid phase, pore water, and tissue. For example, Connell and Markwell (1990) initially proposed an equation to model the distribution of nonpolar organic contaminants in three compartments: soil, soil water, and earthworm tissue. Menzie et al. (1992) later modified the soil-to-earthworm bioaccumulation model to include a variable for soil organic content that yielded predictions that were in agreement with site-specific data. Soil characteristics have also been incorporated into models of heavy metal (cadmium, copper, lead, and zinc) accumulation in radish (*Raphanus sativus L.*), although not all metal distributions were accurately predicted (Davies, 1992).

Sampling sediments in San Fransicisco Bay for clams that bioaccumulate contaminants.

Assuming that contaminants will partition between soil organic matter and organic matter in the root system, Polder et al. (1995) designed a plant uptake model using data for 27 organic compounds, including pesticides and PCBs, and 18 plant species, mostly agriculturally important crops. These models were found to work best when soil organic matter is between 0 and 30 percent. In addition,

accumulation into stem tissues was not well predicted and in some cases only certain root tissues (i.e., root peel but not core) were well represented.

Jager (1998) employed the concept of thermodynamic partitioning between water and lipid phases of tissue to model bioconcentration factors for earthworms. Although tissue concentrations correlated well with aqueous concentrations for chemicals with log K_{ow} values from 2 to 6 ($r^2 = 0.9$), the model overestimated the bioaccumulation factor for earthworms exposed to soil-borne contamination by an average factor of 5.6—a discrepancy explained in part by the differences in the feeding ecology of the various species of earthworm tested.

More complicated models of bioaccumulation were generated by Sample et al. (1998) during a literature survey of 32 studies which examined co-located earthworm and total soil chemical concentrations for nine inorganics (arsenic, cadmium, chromium, copper, manganese, mercury, nickel, lead, and zinc) and two organics (PCBs and tetrachlorodibenzo-p-dioxin). Results from 26 of the studies were used to prepare simple regression models, while the remaining six studies were used to validate predicted values. For PCBs and seven of the nine metallic elements considered (arsenic, cadmium, copper, mercury, manganese, lead, and zinc), the best estimate of tissue concentration in earthworms was given by a natural log-natural log regression with total soil metal concentration. The addition of soil pH data to the regression model did not markedly improve fit, although when soil calcium concentrations were incorporated, a better fit was obtained for cadmium and lead. Tissue concentrations were inaccurately estimated for the transition metals nickel and chromium, by either simple or multiple regression models.

Finally, Saxe et al. (2001) have also suggested a partitioning approach for metals in earthworms that considers both dermal exposure to soil pore water and ingestion of soil particles.

All these examples of simple compartment-type and regression models that incorporate key soil characteristics into the prediction of bioaccumulation are necessarily limited by our lack of knowledge regarding the soil factors most important for influencing bioavailability processes. It may be possible to use such models to bound the reasonable range of bioaccumulation—thereby providing conservative site-specific estimates that are required for screening ecological risk assessment—but additional work is clearly needed in this area.

Pathway from Soil or Sediment to Wildlife via Incidental Ingestion

There are few tools available to measure the bioavailable fraction of chemicals in soils and sediments incidentally ingested by wildlife. Extraction tests that simulate the action of physiological fluids may be useful for evaluating this pathway, although they have not yet been developed for that purpose or tested against actual responses. Some of the more sophisticated methods (e.g., the use of biomarkers in urine or blood, or feeding studies with laboratory animals) are used

only occasionally in site-specific studies, although their results might be generalizable in some circumstances.

Experimental feeding studies with representative species are the most direct way for evaluating bioavailability to wildlife, although they are rarely carried out. Because of variations in the physiology and anatomy of wildlife species, data developed for one species may not apply to another. On the other hand, field studies or surveys have long been useful in identifying bioavailability influences (e.g., Luoma and Bryan, 1978), or how food web relationships influence biotransfer of contaminants that originate from sediments. Use of stable isotopes has proven a relatively inexpensive approach to food web studies (Kidd et al., 1995). However, limitations imposed by logistical complexities and the availability of the proper expertise so far have precluded conducting such work for any but generic purposes.

***

In any pathway where food chain transfer is expected to play a role in exposure, models can be used to predict the exposure expected for each trophic level or individual receptor. One of the most common methods of prediction is the food chain model based on fugacity (and hence on partitioning theory and ultimately thermodynamics) (Ling et al., 1993; Mackay and Paterson, 1991; Nichols et al., 1995). A more mechanistic modeling approach for site-specific exposure assessment for trace elements that takes multiple pathways into account is the Dynamic Multipathway Bioaccumulation Model (DYMBAM) (Wang et al., 1996a; Luoma and Fisher, 1997; Schlekat et al., 2002). This is a relatively new approach similar in principle to generic biokinetic models (Thomann et al., 1995). Yet, DYMBAM is more applicable to specific circumstances because it uses empirically developed physiological rate parameters representative of one or more key native species and environmental data representative of a range of system conditions.

DYMBAM models bioaccumulation as a combination of gross influx and efflux rates, such that key parameters to determine experimentally include influx rates from solution and from food and efflux rates. Influx from solution can be determined with radionuclides in short exposures (e.g., one day) because the goal is to estimate the unidirectional flux. Influx rates from ingestion vary with the food source and so are best determined from the product of assimilation efficiencies (from specific types of food), feeding rate, and concentration. The efflux of inorganic contaminants is characterized by rate constant(s) describing exponential disappearance as function of time (first order isotope-substitution kinetics; Riggs, 1963; Ruzic, 1972; Cutshall, 1974; Luoma et al., 1992). Obtaining the data for such models has been considered onerous in the past (Landrum et al., 1992). However, recent studies show that model behavior can be reasonably constrained (McKim and Nichols, 1994), and manageable methods are available for obtaining species-

specific biological data, especially if whole organism data is the goal (Wang et al., 1996a). If correctly determined, these parameters are directly comparable among species. Thus, in addition to their use in models, these data could lead to better understanding of interspecies differences in bioaccumulation.

DYMBAM is the simplest form of a bioaccumulation model. It lacks, for example, bioenergetic terms or considerations for seasonal gain and loss of lipid that affect both trace element and organic contaminant bioaccumulation (Capuzzo et al., 1989; Cain and Luoma, 1990). Nonetheless, even this simple model approach appears to provide reasonable compatibility with field observations (Luoma, 1976; Luoma et al., 1992; Griscom et al., 2002), although further studies undoubtedly will find ways to improve the model predictions. Data needs for expanding even the simple empirical pathway models are, at present, large. However, as rate constants are defined for common species, and as experiments with different geochemical conditions are related to these mechanistic biological responses, adequate data should become available for site-specific exposure assessments.

One of the goals of developing such models is to help pinpoint those bioavailability tools that should be used in a particular situation. For example, DYMBAM has been used as part of a large framework for modeling selenium fate and transport in the San Francisco Bay (Luoma and Presser, 2001). In addition to DYMBAM predictions of bioaccumulated selenium in marine invertebrates, the framework also incorporates thermodynamic predictions of metal speciation, empirical observations of trophic transfer, and results from toxicity studies. As discussed in Box 4-11, preliminary results suggest that particulate selenium and selenium concentrations in bivalve tissues should be the target of measurement tools.

CONCLUSIONS AND RECOMMENDATIONS

A wide array of approaches can be used to better understand bioavailability processes in soils and sediments. Physical and chemical tests including modern spectroscopic techniques have been developed for determining contaminant form and better understanding contaminant–solid interactions. Simple extraction tests provide operational results about bioavailability. An array of biological approaches are available that vary widely in how they address bioavailability processes. Despite these advances, at the present time, the "tool box" of methods is incomplete. Table 4-1 confirms that few of the tools developed to date are ready for widespread application on any level other than as research tools. The following conclusions and recommendation summarize the future directions that bioavailability tools development should take.

At a given site, a suite of tools is needed to describe bioavailability processes in soils or sediments. No single tool has been developed that can univer-

sally describe or measure bioavailability, and approaches that have attempted this have failed. Thus, a complementary group of tools that characterize different bioavailability processes is a better choice than multiple tools that focus on only one step. Ideally, risk managers should consider processes influencing contaminant concentration, form, or transformation; biological processes affecting uptake; and linkages between internal concentrations and adverse effects in receptors. The complexity of this requirement illustrates the importance of a well-developed site conceptual model and a more comprehensive approach to exposure assessment as compared to a single-value regulatory approach in evaluating contaminant bioavailability. The corollary is that simple tests should be used cautiously. Simplification should only proceed once more mechanistic knowledge has become available, not in lieu of such information.

The suite of bioavailability tools should be comprised of a cluster of standardized and when possible validated protocols (physical, chemical, and biological assays) that are initially applicable at specific sites but with the potential of being general in application. New approaches must continue to be validated via intensive peer review, comparison against actual observations in natural systems or contaminated settings, and pilot testing before they can be applied as unambiguous regulatory tools. Strict validation criteria are necessary to avoid premature or inappropriate application of methods.

There are tradeoffs associated with the use of bioavailability tools such that not all tests for the same bioavailability process are of equal value. Different tests for a single bioavailability process may yield different kinds of information—a fact that must be considered carefully in evaluating the relevance and meaning of various experimental data. In addition, tests that aggregate the effects of multiple processes may have limited relevance to understanding mechanisms (if that is the primary objective), while tests that constrain certain variables for direct elucidation of mechanisms may have less relevance for assessment of overall toxicity.

To avoid misapplying bioavailability tools it is important to understand the environmental setting for which a tool was designed and intended. As illustrated in Box 4-5, some tools have been used in situations for which they were not designed. The long-term success of implementing considerations of bioavailability in the management of environmental hazards depends upon the development of improved models and measurement techniques appropriate to site-specific conditions. Confusion in the regulatory process could result if tools intended for other purposes are misapplied to soil and sediment management.

An intensive effort to develop mechanistic tools and models is critical to future development of bioavailability tools. Many operational tools (e.g., extractions, normalizations, and simple models) have proven ambiguous or shown

BOX 4-11
Forecasting Ecological Effects of Selenium from Discharge of Irrigation Drainage to San Francisco-Bay

Knowledge of many contaminants, including selenium, has grown sufficiently that evaluations of risk can consider the full complexity of processes that lead from inputs to toxicity. A large drainage system has been proposed that would carry selenium-laden irrigation drainage from the farmlands of the San Joaquin Valley and discharge it into San Francisco Bay. Luoma and Presser (2001) introduced an ecosystem-scale modeling approach to forecast potential effects under different load scenarios associated with the irrigation drainage input.

The step-by-step conceptual model is shown schematically and illustratively in Figures 4-8 and 4-9, respectively. At each step, field data, oceanographic principles, and/or simple modeling approaches are used to forecast how selenium is transferred to the next level. Luoma and Presser (2001) forecast environmental concentrations under three different climate (river inflow) conditions. Speciation was inferred from sources and transformations typical of Bay conditions. This then

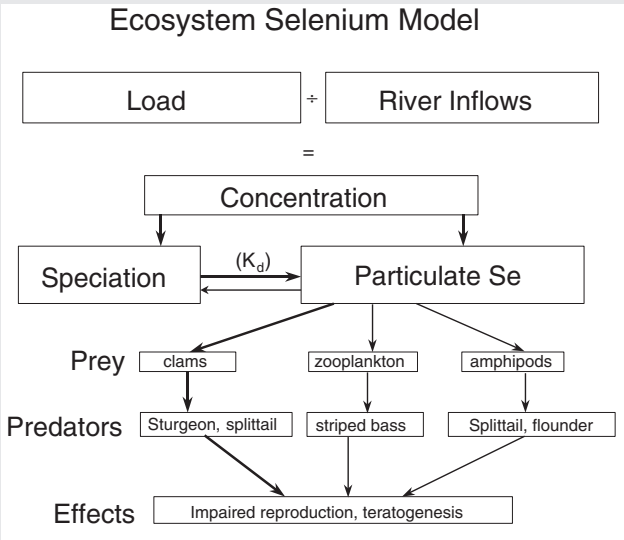


FIGURE 4-8 Schematic ecosystem selenium model.

large uncertainties in their estimates of bioavailability when rigorously tested. Such empirical tests cannot be extrapolated to other sites, nor can they be used with confidence to understand permanence or unforeseen conditions. They are poorly correlated across species and ranges of environmental conditions. Development of a suite of mechanistically based tools is the best way to overcome such limitations.

dictated selenium transformation to particulate form, for which three scenarios were used that encompassed the full range of particle–water distributions (distributions coefficients or K_d) observed in wetlands, rivers, or estuarine conditions. Particulate concentrations were converted to bioaccumulation by invertebrates using DYMBAM model approaches and physiological coefficients developed in laboratory studies (Luoma and Fisher, 1997; Schlekat et al., 2002). Trophic transfer to predators was forecast from empirical relationships in field data from San Francisco Bay. Finally, toxic effects were inferred from previous toxicological studies.

At each step (water, sediment, invertebrate tissues, predators), forecasted concentrations were compared to selenium guidelines suggested in the literature for that media. The modeling revealed that bivalves or particulate selenium would be the most sensitive indicators for monitoring potential changes in selenium effects in the Bay-Delta. Although this full stepwise approach is complex, it confronts uncertainty more directly than traditional methods and it is feasible as a framework for setting site-specific guidelines.

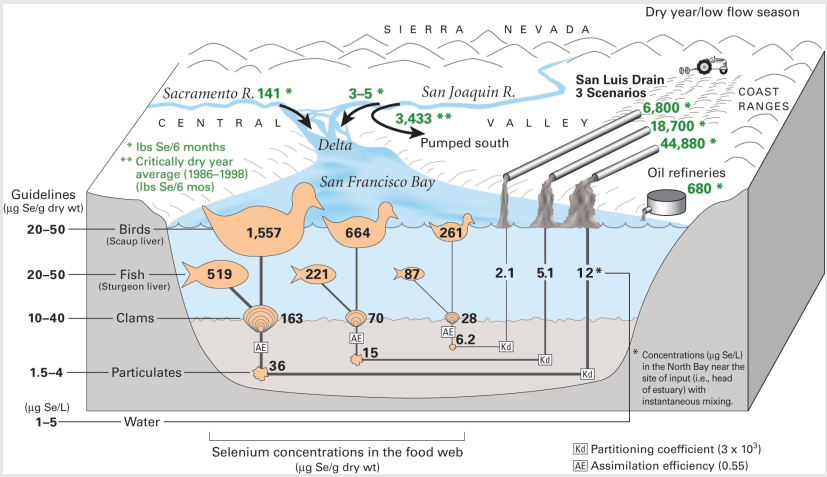


FIGURE 4-9 Illustrative ecosystem selenium model.

Not every bioavailability process must be evaluated and understood in order to get useful measurements for practical application. Rather, measurements can be made that provide information for a specific process or for an integrated group of processes. These might include macro-scale measurements, such as measures of accumulation in organisms, and micro-scale measurements of key processes such as desorption. Micro-scale measurements can be especially

useful for explaining observed variability in the accumulation of a chemical in organisms.

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5

Moving Forward with Bioavailability in Decision-Making

Soils and sediments are the ultimate sink for many persistent organic and inorganic contaminants and have the potential to impact human and environmental health for a long time. Remediation and management of contaminated soils and sediments is often technically difficult and can be very expensive when there are large volumes of contaminated material. To more rationally allocate limited environmental management and remediation resources, there is a need to improve risk assessment by including more explicit consideration of bioavailability processes.

Inadequate scientific understanding has hampered the widespread consideration of bioavailability processes in remedial decision making to date. Uncertainty in the relationship between total contaminant concentrations in soils and sediments and risk has often resulted in a conservative approach to exposure assessment in which the total contaminant present in a particular material is assumed to be available for uptake by possible receptors. Other assumptions (of relative bioavailability being less than 100 percent or about relevant exposure pathways for ecological receptors) may have led to situations where risk was underestimated. All assumptions have important implications with respect to the amount of material that must be treated and to the selection of a technology capable of reaching treatment goals. Explicitly incorporating bioavailability routinely and rigorously into the risk assessment process would offer the possibility of demonstrating in some cases that only a fraction of a contaminant's total mass contained in a soil or sediment actually has the potential to enter potential receptors. In other cases, better understanding of bioavailability processes can lead to

more protective risk estimates, for example by refining a default relative bioavailability factor or identifying an important exposure pathway that was overlooked.

Consideration of bioavailability processes could also be used to improve evaluation of remediation technologies. For example, dredging is a common remediation technology applied to contaminated sediments. In certain cases, natural burial processes have isolated the contamination to the extent that contact between sensitive species and the contaminated matrix is not possible (a situation that can be evaluated through the use of coring studies). Dredging may promote the release of contaminants to the water column, possibly resulting in an increase in mobility and hence bioavailability. In such cases, decision-makers need to consider whether an increase in bioavailability is consistent with the goals of site remediation.

This chapter examines the developments needed in both science and decision-making approaches to promote better consideration of bioavailability processes in remediation and management of contaminated soil and sediment. The chapter examines limitations in our current understanding of bioavailability processes and their implications and what can be done to overcome these limitations. Scenarios in which consideration of bioavailability processes has the greatest potential to impact decision-making are identified, with the hope of focusing science and technology development efforts on these situations. The chapter concludes by recommending specific steps that can be taken to move forward with consideration of bioavailability processes at individual sites, in regulation and decision-making, and in scientific research.

CURRENT LIMITS OF KNOWLEDGE

As demonstrated in Chapter 3, bioavailability of contaminants in soils and sediments to human and ecological receptors is governed by a wide range of physical, chemical, and biological processes. Qualitative and quantitative understanding of some of these processes is substantial, but for other processes there is much to be learned. For example, there is much about contaminant–solid interactions that is only weakly understood. While conceptual models exist for many kinds of contaminant–solid interactions, tools to test these models are often inadequate or nonexistent. As a result, there is significant uncertainty in the models used to describe contaminant–solid interactions and in the parameter values employed in these models. As some description of contaminant–solid interaction will usually be needed for assessment of risk associated with contaminated soils and sediments, the model and parameter uncertainty will transfer directly to the exposure assessment in a risk analysis.

All models and parameters used in exposure assessment have a certain degree of uncertainty associated with them, including those used in bioavailability process considerations. In screening-level assessments for contaminated soils and sediments, this uncertainty is often recognized and dealt with by assuming

that all the contaminant mass is readily available. In practical terms this means that no special adjustments are made to account for bioavailability processes when exposures are estimated. If explicit consideration of bioavailability processes is to become more frequent, the uncertainties inherent in their measurements must be addressed and reduced, if possible.

Some general sources of uncertainty associated with bioavailability processes include:

- a lack of knowledge about how physical, chemical, and biological processes acting at the level of soil and sediment particles influence the binding and release of chemicals;
- variations in soil and sediment characteristics at various spatial scales;
- a lack of knowledge about how biota modify bioavailability of chemicals in soils and sediments that come into contact with external membranes (e.g., skin) or that are taken into the body (e.g., digestive systems), and whether information obtained for one species is representative of another;
- variations in chemical form or properties (e.g., redox state of metals or diffusive rates for organics);
- physical, chemical, or biological changes that might, at some point in the future, change the bioavailability of a chemical.

Given these multiple sources of uncertainty, regulatory agencies have been cautious about moving away from default assumptions concerning bioavailability processes in risk estimates. It is not clear whether there is too much uncertainty associated with bioavailability tools for regulatory agencies to feel comfortable about more explicitly incorporating their results into exposure estimates. Input received by the committee indicates that there is disagreement over this issue. An individual who has a strong precautionary stance might argue against replacing certain default assumptions (e.g., of 100 percent availability) to account for site-specific bioavailability processes. On the other hand, someone who sees large trade-offs among alternatives that hinge on bioavailability considerations would likely support their inclusion in specific situations. Risk assessment practitioners well versed in uncertainty and probabilistic analyses might argue that the uncertainties could be identified and taken into account, thereby providing more complete information to the risk manager.

Explicit incorporation of information on bioavailability processes has occurred in ecological and human health risk assessments for particular types of problems and chemicals where the uncertainty has been relatively low due to extensive testing of certain contaminants and processes. Examples include exposure of humans to lead in soils (oral), and to polychlorinated biphenyls (PCBs) in soils (dermal); leaching of soil contaminants to groundwater; exposure of benthic invertebrates to non-polar organic chemicals (e.g., polyaromatic hydrocarbons or PAHs) in sediments; and site-specific determinations of bioavailability via up-

take studies from soils or sediments to benthic invertebrates, sediment invertebrates, plants, and wildlife (see Table 2-3). Clearly, the inclusion of site-specific bioavailability information has been judged to be important in a number of cases, and uncertainties were addressed at a level appropriate to risk-based decision making.

There have been many other cases, however, in which the level of uncertainty has been judged to be too high for bioavailability measurements to replace default assumptions. A prominent example is the case of the Times Beach, Missouri, Superfund site, where large amounts of dioxin-contaminated soil were excavated and incinerated (see Box 5-1). There was a limited, generic consideration of bioavailability processes in determining the dioxin action levels for soil to be excavated and treated. However, site-specific assessments of bioavailability processes were not used to guide remediation decision-making, at least in part due to uncertainty in the bioavailability process measurements.

WHY THESE LIMITATIONS AND UNCERTAINTIES MATTER

The limitations in our understanding of bioavailability processes and the large uncertainties associated with their measurement have important ramifications for site management. The most obvious is that a lack of knowledge may inadvertently support poor decisions regarding exposure assessment, which has implications for how much contamination should be cleaned up and at what cost. For example, site managers working with incomplete information may be inclined to excavate a contaminated site even if the contaminants are not bioavailable. This could present myriad problems, including increasing the bioavailability of the material and potentially the risk to other receptors, such as wildlife, that were not originally the receptors of concern.

Our lack of understanding of bioavailability processes also has important implications for the remediation of hazardous waste *in situ*. With regard to remedy selection, a large number of treatment and containment technologies rely on biological processes that are partially controlled by bioavailability, such as the transformation reactions of microorganisms. Without a better understanding of bioavailability processes, it is difficult to choose among technologies or to know if they are effective. (Although many might agree with the conceptual model of bioavailability processes outlined in Figure 1-1, there is little consensus on how to identify and quantify the dominant processes relevant for a specific situation.) This is aggravated by the plethora of different bioavailability tools and measurements used, many of which do not actually test a relevant endpoint. Additionally, site managers may not be cognizant of when treatment technologies unintentionally affect bioavailability. Especially for technologies that have yet to be fully tested, like phytoremediation, there may be unanticipated "side effects" that result in undesirable changes in bioavailability to certain receptors. Finally, in the last several years, approaches using simple tests to assess bioavailability at hazardous

BOX 5-1
Times Beach Superfund Site: How Uncertainty Influenced Decision-Making about Bioavailability

The remediation of Times Beach, Missouri, has been one of the largest Superfund projects in the nation after hazardous levels of dioxin were found throughout eight square miles of the small agricultural and residential town in 1982. Waste oil used to spray the roads for dust control in 1972 and 1973 contained dioxin (2,3,7,8 TCDD). After the waste oil application to the roads, animal mortality and human illness were observed. Almost immediately a toxic chemical in the oil treatment was suspected.

The U.S. Environmental Protection Agency (EPA) tested soil samples from the town's unpaved roads and right of ways, revealing dioxin levels ranging from 1 ppb to 127 ppb. The entire Times Beach site is situated within the floodplain of the Meramec River. Shortly after the discovery of dioxin, the Meramec River flooded the city, which spread the contamination. Times Beach was evacuated in February of 1983, and the federal government used $33 million from Superfund to buy the dioxin site and relocate the residents.

The Centers for Disease Control and Prevention (CDC) evaluated the health implications of dioxins in the soil at the site (Kimbrough et al., 1984)—one of the earliest examples of explicitly including bioavailability information in an assessment. CDC investigators noted that "regarding dermal absorption, there is some evidence that TCDD binds to soil and would not be as easily available for absorption." They considered three routes of exposure: dermal contact, incidental ingestion, and inhalation. In their estimates of exposure, Kimbrough et al. (1984) used the available literature values for relative bioavailability—1 percent to estimate dermal uptake and 30 percent to estimate absorption in the digestive system. Bioavailability was not included in the estimate of inhaled dose. Interestingly, in discussing the implications of their assessment for management of the soils at Times Beach, Kimbrough et al. (1984) state: "The precise bioavailability of TCDD from soil is not known. Such bioavailability may vary with the soil type. It has been recently established that TCDD-contaminated soil from Missouri is toxic to guinea pigs and rats, if given orally. It was estimated that the [relative] bioavailability was

waste sites have become popular. Some of these approaches do not seek to better understand underlying bioavailability processes such that their widespread application may become problematic.

Technologies Developed with the Intent to Decrease Bioavailability

A number of treatment technologies have been reported that "decrease bioavailability"—that is, treatment that impedes transfer of a contaminant from the soil or sediment matrix to a living organism. Although institutional controls and containment remedies would theoretically be encompassed by this definition, this discussion focuses on *in situ* treatments that aim to either (1) remove the labile fraction of contaminants (e.g., by microbial or plant mineralization), (2) convert

30–50 percent or more [compared to ingestion of TCDD in corn oil] (McConnell et al., 1984.)"

As a result of the Kimbrough et al. (1984) study, CDC recommended a 1 ppb TCDD action level for residential areas and 20 ppb level for industrial areas. Site-specific assessments of relative bioavailability performed later (Umbreit et al., 1986a, b, 1987, 1988a, b; Shu et al., 1988), which would probably have changed the cleanup goals by a factor of about 2, were not used to guide remedial actions because:

1. There apparently was little communication early in the decision-making process concerning the role that site-specific bioavailability information might have in guiding remediation.
2. Regulatory agencies prefer to err on the side of health protectiveness. Given the uncertainties in the bioavailability information derived from the Umbreit et al. studies, the regulators chose not to apply a bioavailability adjustment in the risk assessment. The Umbreit studies were controversial because the controls used conditions that were dissimilar from the critical toxicity study from which the reference dose for TCDD is derived (Kociba et al., 1978).
3. There was a lack of an accepted framework for incorporating the measurements of site-specific bioavailability processes into risk estimates.

Roads and affected areas at Times Beach containing dioxin levels over 1 ppb were excavated to a depth of four feet of contaminated soil and stored. A 50,000 cubic yard concrete tank with a flood-proof covering was used as a storage facility for the excavated soil, which was subsequently treated via incineration. Contaminated soil from 26 other dioxin sites was also brought to Times Beach to be incinerated—a fifteen-month process resulting in 265,000 tons of waste material. The incinerators ceased operation in June 1997, and the site was declared fully recovered.

the labile fraction to a stable fraction (e.g., by the precipitation of metals), or (3) increase the mass transfer resistance of pollutants (e.g., by modifying the physical structure of the geosorbent). Examples of such technologies include biostabilization (the use of bioremediation to reduce contaminant mobility and toxicity of contaminated soils and sediments); sediment capping (reducing the ability of a bottom dwelling organism to get to the contaminant, and increasing mass transfer distance); vitrification or solidification (decreasing contaminant mobility by vastly increasing mass transfer resistance out of the solid matrix); and chemical alteration (e.g., converting a compound to a low solubility redox state via an amendment).

Biostabilization relies on the microbial degradation of contaminants serving as carbon or energy sources or as electron acceptors. It consists of an initial active

and often engineered bioremediation phase (that may last months) to remove or transform those compounds that are more bioavailable, followed by a passive bioremediation phase (lasting years) to ensure that there is no chemical migration away from the actively treated material. The concept of the second phase is that intrinsic biodegradation rates equal or exceed the rate at which low solubility compounds become available. Box 5-2 discusses the characteristic desorption curves for PAH-contaminated solids, which are a frequent target of biostabilization efforts.

One limitation of biostabilization is that the organic compounds may not meet threshold concentrations needed to drive microbial metabolism. Threshold concentrations of compounds are thought to play a role in energy maintenance and microbial enzyme induction (e.g., Schmidt et al., 1985) and they are experimentally manifested as residual concentrations of pollutants in various biodegradation tests (Bosma et al., 1996; Tros et al., 1996a, b). For a given contaminant, the value of the threshold concentration is determined by the efficiency of microbial metabolism (e.g., the relative values of specific uptake rates versus maintenance coefficients). Thus, thresholds can be affected by external mass transfer limitations, which often occur with aged pollutants in soils and sediments (Bosma et al., 1997). The existence of threshold values may be irrelevant when these values are far below concentrations that present risk. However, when these microbial threshold concentrations are above values deemed to represent a risk, biostabilization may not be a suitable remedial technology.

Other remediation approaches use isolation to reduce bioavailability by employing capping or burial to remove access of a contaminant to the biosphere. In a physically active waterbody, however, capping will not permanently remove contaminants from the bioaccessible or bioavailable location if the sedimentary environment is erosional. To evaluate the potential success of isolation techniques, it is important to take sediment cores and evaluate their sedimentation regimes.

Several technologies to reduce bioavailability of metals in soil, sediment, or other contaminated matrices rely on amending the solid phase to alter the redox or acid–base status of metals or sulfur species (NRC, 1997a). Certain metals (e.g., chromium or uranium) may have highly unavailable (low solubility) species depending on redox conditions, which can be imposed by specific technologies. This has been demonstrated at the Department of Energy (DOE) Hanford Site in Washington, where groundwater hexavalent chromium levels have been reduced from 0.060 mg/L to below detection limits (0.008 mg/L). The zone of reduction was created by injecting reagents that reduce iron naturally present in the aquifer sediments from Fe(III) to surface-bound and structural Fe(II) species, which concomitantly reduces the hexavalent chromium. Other metals may not have such speciation, but they can be precipitated as phosphates or sulfides, and hence the reduction of oxidized sulfur species can reduce their bioavailability (Benner et al., 1999). This strategy is exemplified by the case study presented in Box 5-3,

BOX 5-2
Biostabilization of PAH-laden Soils or Sediments

Biostabilization generally refers to the situation where biological processes alone—intrinsic or stimulated—are deemed sufficient to reduce the risk associated with contaminants in soils and sediments. Although an awkward term, *stabilization* alludes to the fact that the *labile* fractions of the total contaminant are being reduced in size. This remedy has been suggested extensively for soils and sediments contaminated with PAHs, many of which have been documented to undergo microbial mineralization under various redox conditions (Kanaly and Harayama, 2000).

Documenting the success of biostabilization typically requires demonstrating not only a decrease in total contaminant mass but also a decrease in the labile fraction of the contaminant pool between the onset and the end of the examined stabilization period. Popular tests to make these measurements examine the "rate of release" of contaminants using infinite sorption sinks or different extraction solvents (e.g., Cornelissen et al., 1998; Hawthorne and Grabanski, 2000), or they use toxicological endpoints (Loehr and Webster, 1997). Typical results for desorption data are shown in Figure 5-1 for two compounds in contaminated sediment. Detailed studies that directly inspect the soil and sediment phase to determine the stabilization mechanism are rare, and the actual contribution of microbial metabolic activity is only sporadically demonstrated (Ringelberg et al., 2001).

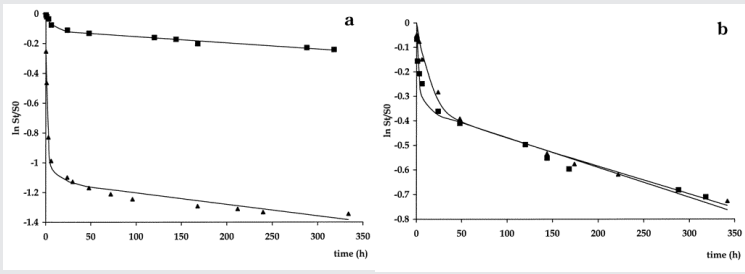

FIGURE 5-1 (A) Desorption of fluoranthene, a compound amenable to microbial degradation, before (triangles) and after (squares) bioremediation. Total fluoranthene concentration dropped from approximately 170 mg/kg to 20 mg/kg over four months of active bioremediation. The shape of the desorption curves are very different before and after bioremediation. The *rapidly desorbing fraction* (obtained from curve fits shown in figure) dropped from 67 percent ± 3 to 10 percent ± 4 after bioremediation. This drop in rapidly desorbing fraction was observed for all the compounds that were biodegraded, suggesting a decrease in their labile fraction, and hence biostabilization. (B) Desorption results for the non-degraded compound benzo(*ghi*)perylene indicating very similar shapes of the desorption curves before and after the bioremediation. SOURCE: Reprinted, with permission, from Cornelissen et al. (1998). © (1998) American Chemical Society.

BOX 5-3
Soil Amendments to Reduce Lead Bioavailability at the Joplin Superfund Site

Joplin, Missouri, was included on the National Priorities List because of soil contamination from the smelting of locally mined lead (Pb) and zinc (Zn) ores. As part of the remedial action undertaken for the site, 2,600 homes in Joplin have had their soil replaced with clean material. In conjunction with this cleanup, a field site was established to test the ability of different *in situ* soil amendments to reduce the bioavailability of soil Pb to children. This project was undertaken by the Inplace Inactivation and Natural Ecosystem Restoration Team (IINERT) of EPA's Remedial Technology Development Forum, whose stated mission was to identify *in situ* technologies that could chemically and physically inactivate hazardous metals in soils by reducing the metal's solubility and bioavailability. This box focuses on human health because of the urban focus of the risk assessment. However, there are adjacent lots at the site where soil amendments are also being tested and where ecological receptors (including plants, herbivores, and insectivores) are the primary receptors of concern.

Background

Several lines of evidence suggested that soil amendments, including different sources of phosphorus, high iron materials, and biosolids compost, might be successful in reducing Pb availability *in situ*. The solubilities of different Pb species are known to vary in relation to the mineral form (Nriagu, 1984). In the presence of phosphorus, lead can form chloropyromorphite, which has a very low K_{sp} ($10^{-84.4}$), such that the compound is likely to be stable under most soil and gastric systems. Thus, amendments that would promote formation of this mineral became the focus of research. Controlled environment studies demonstrated that it was possible to alter the mineral form of Pb in both pure and soil systems (Ma et al., 1993, 1994a, b). Field validation of these technologies was determined to be the appropriate next phase of research, for which hypotheses were developed. The initial phase of research focused on defining an appropriate animal surrogate to measure changes in bioavailability and on determining what extractions or *in vitro* tests can potentially substitute for animal feeding studies. Identifying the mechanisms that are responsible for the observed reduction in bioavailability and the appropriate tools to measure changes in speciation was also a goal.

Animal surrogates and in vitro testing

Initial results from the field site showed that additions of both H_3PO_4 and biosolids compost *in situ* are capable of reducing Pb bioavailability in juvenile swine, and in weanling and adult rats (Casteel et al., 2001; Maddaloni et al., 2001). However, although animal feeding studies have consistently shown reduced lead bioavailability as a function of treatment, the reductions are not consistent across groups or over time after treatment (see Casteel et al., 2001 for details). A second goal of the field study was to determine whether an *in vitro* extraction test could substitute for *in vivo* trials to assess reduction in Pb bioavailability. For the Joplin site soils, the *in vitro* test results at an extraction pH of 2.3 were comparable to the results from the swine studies (Ruby et al., 2001).

Mineral Form

The final goal of the field trial was to determine the mechanisms responsible for the observed reduction in bioavailability. Using X-ray adsorption spectroscopy (XAS) and

comparing field samples to known mineral forms, the formation of chloropyromorphite in the treatments that included phosphorus addition has been confirmed (Scheckel and Yang, 2001). In addition, the portion of total Pb present in this mineral phase increased over time. Mineral forms in the unamended soils have remained constant over time. There also seems to be a relationship between the observed decrease in bioavailability and the presence of pyromorphite [although the correlation is weak—$r^2 = 0.5546$ (Ryan and Berti, 2001)]. For other treatments, results are even less clear. Although compost addition resulted in reduced bioavailability as measured by *in vitro* and *in vivo* (weanling rats) studies, XAS was not able to quantify the formation of a new mineral phase. A shift was observed from carbonate- and S-associated Pb in the control soils to what was identified as adsorbed Pb in the compost amended soils. Clearly, more information will be required before this shift can be accepted as the cause of the observed decrease in bioavailability.

Conclusions

On many levels, the preliminary research at the Joplin field site has been a success. It should be noted that this is the first time that feeding studies on animals have used treated soils. Thus, the methods are clearly a work in progress. All *in vivo* (human, pig, and rat) and *in vitro* studies (data unpublished) have shown that soil amendments are able to reduce the portion of total soil Pb that is bioavailable. In addition, it has been demonstrated that when P is added to the soil, the mineral form of Pb shifts, at least in part, to pyromorphite. This mineral shift appears to weakly correlate with the observed decrease in bioavailability. The stability of this mineral phase also suggests that the observed decrease in bioavailability will persist over time. During the limited sampling time since treatment addition, increasing pyromorphite concentrations have been observed for select treatment.

However, this field site also illustrates some of the complexities involved in the measurement of bioavailability to assess risks posed by Pb in soil. Although all indices used in this study show decreases in bioavailability, they also show considerable variability. At this time, it is not clear if a single, appropriate index can be identified. The initial results from this field site indicate that, while it is possible to reduce the bioavailability of Pb *in situ*, it is not clear how to interpret or utilize these observed reductions in the regulatory arena.

Plots at the Joplin Superfund Site being subjected to soil amendment in order to reduce metal bioavailability to residents.

in which different soil amendments were tested for their ability to reduce the bioavailability of lead in soil to children. Both bioassays (feeding studies) and physicochemical tests (x-ray spectroscopy) were conducted to determine the effectiveness of the soil amendments.

Environmentally Acceptable Endpoints. Of specific relevance in this discussion (particularly for biostabilization) is the increasing popularity of environmentally acceptable endpoints (EAE). The EAE concept is based on the observation that many organic contaminants become less "available" as they age within soil or as the soils undergo treatment, due to changes in the way soils and sediments encapsulate chemicals over time (Alexander, 1995). It has been proposed that this reduced availability should have an impact on cleanup levels and remediation goals and should be incorporated in site-specific risk assessment (Stroo et al., 2000). In some cases, this may involve modifying the default assumptions to reflect bioavailability limitations.

This reduced availability, which has been described for organic contaminants by such mechanisms as sequestration and entrapment, has largely been inferred from the behavior of persistent hydrophobic compounds (mainly PAHs) in the field. After an initially rapid rate of chemical degradation, a period follows with little or no change in chemical concentrations. In the case where the considered chemicals are known to be biodegradable, the lack of continued decline—all other things remaining favorable for microbial activity—suggests that the chemicals themselves have largely become the limiting factor to microbial biodegradation, probably because of reduced availability. It is postulated, but rarely confirmed, that reduced availability to microorganisms relates to reduction in risk posed by the contaminants. Concomitant reductions in toxicity to other more relevant receptors has only occasionally been demonstrated (Salanitro et al., 1997; Olivera et al., 1998).

Although plausible, the lack of availability of contaminants in soils or sediments to resident microorganisms does not suffice to characterize the suite of possible bioavailability processes. As an analogy, consider the fact that exchange of metals from sediments to pore water declines as the metal–sediment association ages (Schlekat et al., 2002). While the risk to water column species may decline with contaminant aging in sediments, there will not necessarily be a change in the risk to species whose food web is connected to ingestion of the sediments themselves. Hence, the evidence on which environmentally acceptable endpoints are based (microbial availability) may be insufficient, unless multiple exposure pathways and multiple receptors are considered. The challenge to all bioavailability assessment is to quantify the relevant bioavailability processes at work in a given situation, which requires an understanding of the importance of all exposure routes and receptors.

Variability in the Tools Used. One of the difficulties inherent with implementing all of these types of remedies is that there is no consensus on the tools or methods that should be employed to measure "bioavailability reduction" in the course of remedial technology selection or on how results from those tests should be incorporated into risk assessment. As a result, the state-of-the-practice consists in applying a battery of assays to the soil or sediment under investigation that all have some relationship (however ill-defined) to contaminant bioavailability. Further, measurements that may approximate only certain bioavailability processes, such as chemical mobility measurements or the water-soluble fraction of a compound, have been employed to infer satisfactory treatment. Again using biostabilization as an example, a recent review of remedies for hydrocarbon-contaminated soils from petroleum refining, wood treating, petrochemical manufacture, and gas and electric utility sites demonstrates the wide variety of surrogate measures of bioavailability utilized. Technical report 25 in Loehr and Webster (1997) measured a reduction in total chemical concentrations as well as in toxicity (via Microtox EC_{50} assays) to assess reductions in bioavailability for petroleum-contaminated soils subject to soil pan and biopile treatability. In a study on bioremediation of soils artificially contaminated with a mixture of chlorophenolic compounds, reduction in the water soluble fraction as well as Microtox-inferred toxicity were used as "bioavailability reduction measures" (Dassapa and Loehr, 1991). In another recent study on soils artificially contaminated with pentachlorophenol (PCP), increased toxicity was measured using the soil bacterium *Bacillus megaterium* as test species, although the aqueous PCP concentration had dropped (McGrath and Singleton, 2000). This finding indicates that transformation products potentially can cause increased biological effects (compared to the parent compound), and that toxicity reduction may be an ambiguous tool for understanding "reduced bioavailability." In yet another set of field-scale bioremediation efforts of unsaturated-zone wood treating site soils, a reduction in Toxicity Characteristic Leaching Procedure evaluations or water-soluble fraction determinations were employed to infer bioavailability reduction with time (technical reports 16, 17, and 22 in Loehr and Webster, 1997).

A recent comprehensive study on a PAH-impacted site applied seven assays to assess the degree of biostabilization and reduction in contaminant mobility that had occurred after various natural and engineered processes (Stroo et al., 2000). These assays were dermal uptake through human cadaver skin over 96 hours, absorption efficiency via 10-day oral uptake in mice, accumulation via 28-day earthworm tests, 14-day exposure earthworm toxicity, Microtox toxicity of soil slurries and aqueous extracts, Synthetic Precipitation Leaching Procedure, and a 119-day desorption test in infinite dilution matrix. Although qualitative consistency among some of the tests was found, quantitatively the results were very different. As recognized by the authors, each of the applied tests had limitations with respect to relevance to real endpoints. Further, all tests reflect a single time point analysis, and the effect of time-varying ecological and geochemical factors

is not typically addressed. The authors concluded that using any specific set of tests to adjust risk-based cleanup criteria would be subjective (although they also suggested development and adoption of a few short-term tests that could be employed in a tiered testing scheme).

More recently, physicochemical-based assays have been applied to infer bioavailability reduction during biostabilization. For example, in PAH-contaminated sediments subject to four months of active bioremediation or two years of land farming, the degree of biostabilization was inferred by comparing the rapidly desorbing PAH fractions, before and after treatment, using the infinite dilution Tenax TA desorption technique (Cornelissen et al., 1998). A similar trend between "extractability or bioavailability" and extent of bioremediation for manufactured gas plant soils was observed when supercritical fluid extraction was used to measure the various fractions of soil-bound PAH (Hawthorne and Grabanski, 2000; Hawthorne et al., 2001). Box 5-4 discusses how multiple complementary tools might be used to address the effectiveness of biostabilization, in this case the humification of trinitrotoluene (TNT), and gain more confidence in the proposed remedial selection.

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There is a general consensus that biostabilization and certain other treatment technologies and natural aging processes might reduce the risk associated with soil and sediment contaminants. However, this has not been conclusively demonstrated in the examples cited above. The types of correlative assays frequently used may aid in short-term decision making for site management. But in the absence of better capabilities to measure bioavailability processes, they must be applied with caution to ensure that appropriate site management decisions are made. In addition, the permanency of treatment technologies that aim to reduce bioavailability has not been addressed, in part because tools to assess bioavailability processes over long time scales and over a range of soil and sediment conditions are not yet developed. Hence, the concept of using EAE-based rather than default cleanup values may have merit, but full acceptance of this concept will be contingent on better understanding and measurement of the constituent bioavailability processes on which it integrally is based.

Technologies Developed with the Intent to Increase Bioavailability

An alternate strategy is one that recognizes that the continued presence of pollutants in soil or sediment will always invoke potential risk. Thus, some technologies attempt to increase pollutant removal or destruction by facilitating bioavailability processes. These technologies increase mass transfer from the sorbed phase via physical means (grinding or mixing to decrease diffusional paths, increasing temperature to increase mass transfer rates) or chemical means

(surfactants, co-solvents, or chelating agents to increase mass transfer by increasing the apparent aqueous solubility of hydrophobic organic compounds, or mediating changes in geosorbent matrix structure). Clearly, such technologies need to be paired with technologies that can capture or destroy the increased flux of pollutant thus generated.

The use of additives to soils or sediments to enhance the extent or rate of desorption has been examined for both inorganic and organic contaminants. Surfactants, of both chemical and microbiological origin, have been applied with varying degrees of success to enhance solubility of hydrophobic organic chemicals (particularly nonaqueous phase liquids). They typically function by micellar solubilization and mobilization of the trapped liquids by lowering the liquid–water interfacial tension (Harwell et al., 1999), leading to an increase in apparent water solubility and solubilization of sorbed contaminants (Kim et al., 2000). The surfactant generally must be present in amounts above its critical micelle concentration. Unfortunately, sorption of the surfactant itself to solids can impede the success of this approach (Dwarakanath et al., 1999; Deshpande et al., 2000). The effectiveness of surfactant use has been widely disparate, with studies demonstrating negative effects, zero effects (Löser et al., 1999), or positive effects on enhancing pollutant availability and subsequent biotransformation (Liu et al., 1995; Tiehm et al., 1997).

For inorganic contaminants, many additives have been used to increase their solubility. For example, chelating agents have been used specifically to enhance the solubility of multivalent cationic species. Technologies based on citrate addition to enhance removal of transition metals and actinides from the solid phase have been developed that rely on the formation of complexes with citric acids (Francis and Dodge, 1998). Recently, it has also been observed that chelating agents may enhance the bioavailability of hydrophobic organic pollutants, presumably by altering the geosorbent matrix, although the exact mechanism has not yet been elucidated (Yang et al., 2001). For example, White and Kottler (2002) found that citrate addition enhanced the plant uptake of weathered 2,2-bis(p-chlorophenyl) 1,1–dichloroethylene (p,p′-DDE) from soil. Nonetheless, without a complete understanding of the bioavailability process and appropriate tools to measure the constituent steps, it is difficult to ascertain with certainty the impact of these bioavailability enhancement techniques on the long-term fate of the contaminants.

Chelating agents have also been used intentionally to promote the uptake of metals and radionuclides into plants from contaminated soils. In particular, EDTA and citric acid can trigger hyperaccumulation in plants (specifically *Brassicaceae*) (Blaylock et al., 1997; Huang et al., 1998; Bricker et al., 2001; Chen and Cutright, 2001). This may be due to the chelator's ability to promote desorption of metals and radionuclides from the solid phase to soil solution. Although the ensuing hyperaccumulation response is very rapid (within 24 hours) (Huang et al., 1998), and several chelating agents are readily biodegradable, this application needs to

BOX 5-4
Humification of TNT via Sequential Anaerobic-Aerobic Soil Slurry Treatment

A technology proposed to reduce the bioavailable fraction of trinitrotoluene (TNT) in contaminated soils relies on the cometabolic reduction of the nitro substituents on the compound. Microbial reduction of TNT occurs readily, leading to nitroso, hydroxylamino, and finally amino derivatives of TNT. The rate and extent to which individual nitro substituents are reduced and the number of nitro substituents reduced per TNT molecule depend partly on the redox status of the environment (Preuß and Rieger 1995; Riefler and Smets, 2000). The nitroso and hydroxylamino functional groups formed as intermediate products during reduction have a high chemical reactivity towards solid-phase constituents. Thus, it is thought that microbial TNT reduction in the presence of the functional sites on soil might lead to biostabilization of the reduced compounds.

Lenke et al. (1998) treated contaminated soil from a former munitions site (176 mg/kg TNT, 45.6 mg/kg ADNT, 2.4 mg/kg 2,4-DANT) as a soil slurry (850 g soil/850 ml mineral medium) subject to an anaerobic fermentative step followed by an aerobic polishing step. No hydroxylaminodinitrotoluenes (HADNT) or triaminotoluenes (TAT) were detected in the slurry supernatants, and no residual methanol extractable compounds were detected after the combined anaerobic–aerobic phase (after approximately 672 hrs). (Methanol extractions are used to release rapidly desorbable fractions.) Also at a technical scale, a sequential anaerobic–aerobic incubation of a TNT- and a DNT (dinitrotoluene)-contaminated soil gave only TNT and DNT as residual extractable compounds at 1.86 and 3.45 mg/kg, respectively, from initial concentrations of 189 and 49.1 mg/kg. None of the reduction products was detected in either aqueous supernatant fractions or in alkaline, base, or methanol extracts of soil, strongly suggesting the formation of irreversibly soil-bound fractions.

Further, no toxicity was detected in aqueous soil eluates after the combined anaerobic–aerobic treatment, according to tests employing a bacterium, *Vibrio fisheri*, an aquatic invertebrate, *Daphnia magna*, or the photosynthetic alga *Scenedesmus subspicatus*. Further, terrestrial tests indicated no earthworm mortality or plant toxicity and acceptable microbial respiratory activities of the soil after treatment. Although these results suggested some type of humification, complementary experiments were necessary to confirm these observations.

To examine stability of the immobilized TNT derivatives and to differentiate between sequestration and covalent binding, samples from the lab-scale experiment that used radioactive TNT were subject to vigorous extraction–derivatization procedures (Achtnich et al., 2000). Very small amounts (1.3 percent to 2.5 percent) of initial radioactivity were extracted after the combined anaerobic–aerobic treatment with methanol. Only with 5.0 M HCl was a significant fraction extracted (8.9 percent). However, chromatographic analysis of the HCl extract showed that all radioactivity remained associated with the humic acid fraction. Silylation, which breaks open the 3-dimensional structure of soil, was able to release 73.1 percent of the initial radioactivity, but chromatographic analysis again indicated that all activity was associated with soil organic matter, and no free TNT metabolites were detected. These speciation analyses clearly supported the notion that TNT derivatives were covalently bound to soil after the two-stage process.

To further understand the observed immobilization of TNT derivatives to the soil, Daun et al. (1998) examined the cometabolic reduction of TNT (0.4 mM) by a glucose fermenting enrichment culture in the presence of individual model soil components: montmorrilonite (3.3 or 10.3% w/v) and humic acid (1% w/v). They observed a very rapid decrease in aqueous phase TNT reduction metabolites, with complete absence of aqueous products after prolonged incubation (340 hrs, 220 hrs) and suggested that HADNT and TAT had undergone strong reactions with the solid phase. Separate experiments confirmed that neither acid nor base hydrolysis could release HADNT and TAT sorbed on montmorillonite and humic acids, suggesting formation of irreversible sorptive interactions.

In a final study (Achtnich et al., 1999), stable isotopes of TNT were employed. $^{15}[N_3]$TNT and [^{14}C] TNT were spiked (4 g/kg) into the same TNT-contaminated soil samples (350 mg/kg) as studied by Lenke et al. (1998), and the sequential anaerobic–aerobic soil-slurry treatment was repeated at the laboratory level. Soil samples, taken at various times throughout the treatment period, were subject to both methanol extractions as well as subsequent fractionations of the soil organic matter (fulvic, humic, humin fractions) to characterize the bound fractions. ^{14}C-based mass balances revealed a vast reduction of the methanol-extractable fraction (from 102 percent at day 1 to 1.1 percent after 83 days), with a gradual increase in the humin-bound fraction (up to 71 percent, after 83 days). Of the humin-bound fraction, only 3.4 percent was HCl-extractable, 44.4 percent could be solubilized in dimethylsulfoxide after silylation (due to humin solubilization), and 23.4 percent remained soil-bound. Importantly, NMR inspections of the humic acid-bound fraction revealed a gradual reduction in the aromatic nitro groups, intermediary accumulation of azoxy functional groups, and accumulation of aromatic amines, tertiary amines, or amides with time, while the NMR spectra of the humin-bound fraction suggested formation of azoxy compounds and imine linkages. Further, the broad NMR line widths of the metabolite spectra provided convincing evidence of strong (covalent) interactions between metabolites and humic acids or humins. Hence, convincing spectroscopic evidence of true soil immobilization of TNT metabolites during reductive transformation of TNT was presented. An illustration of the humification of the TNT derivatives is shown below in Figure 5-2.

In summary, the observations of TNT disappearance (from aqueous phase) during anaerobic cometabolic reductive treatment of TNT-laden soils was confirmed to be in part due to immobilization of TNT reduction products on soil constituents (humification) via the following complementary lines of inspection: (1) aqueous phase monitoring of TNT and all its presumed transformation products, (2) extraction of solid phases with various rigorous extraction procedures, (3) ecotoxicological endpoints, (4) sorption experiments with individual TNT transformation products showing irreversibility, (5) TNT reduction experiments in the presence of model solid components, (6) mass balances employing spiked [UL^{14}C]-TNT, and (7) NMR spectroscopic investigations employing spiked [^{15}N]-TNT.

continues

BOX 5-4
Continued

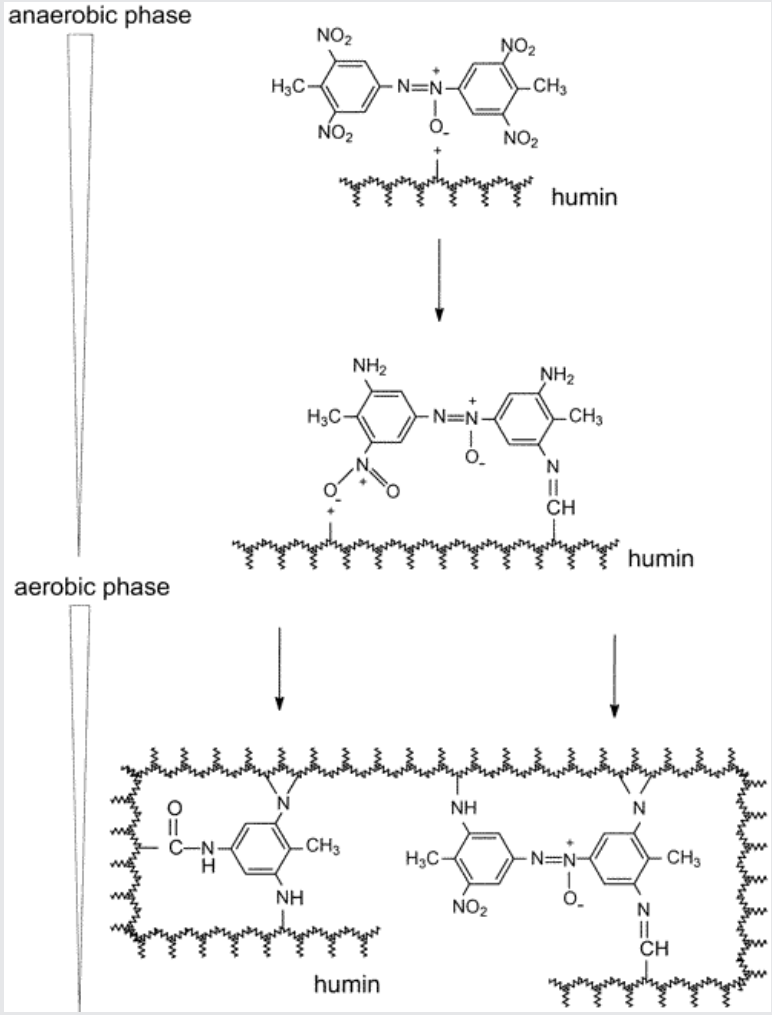

FIGURE 5-2 Proposed pathway of binding of reduced metabolites of TNT to the humin fraction in the anaerobic–aerobic process. SOURCE: Reprinted, with permission, from Achtnich et al. (1999). © (1999) American Chemical Society.

be designed and timed carefully to avoid negative effects on soil microbiota and the unintentional release of contaminants to the underlying groundwater (Grcman et al., 2001; Romkens et al., 2002).

Box 5-5 discusses the second major category of treatment technologies designed to increase contaminant bioavailability—the use of physical mixing and changes in temperature to enhance the biodegradation of hexachlorocyclohexane in soil.

Remediation Strategies with Unintentional Effects on Bioavailability

A number of technologies used for the remediation of contaminated soils or sediments operate through principles of increasing the mobility—and consequently the bioavailability—of contaminants. In some cases, however, technologies that function around principles other than enhancing mobility are also capable of increasing bioavailability, often unintentionally. Although this unintentional effect has been recognized in some cases, it is likely that, in an absence of complete understanding of a technology, such effects might be more common than anticipated.

An example of an unintentional increase in contaminant bioavailability can occur during the dredging of contaminated sediments. In dredging operations there is considerable concern regarding the short- and long-term potential to increase contact between receptors and contaminants after dredging as compared to the levels of exposure that would occur if sediments were not disturbed (NRC, 1997b, 2001a). The objective of the dredging process is to remove sediments from the bed, capture the sediment particles, and then transport the contaminated materials to confined disposal or *ex-situ* remediation processes. The unintentional increase in bioavailability that results may be the outcome of one or more specific processes that occur during or after the dredging is complete. For example, mobilized sediment particles that are subject to transport in the water column may not be adequately captured and have the potential to come into contact with receptors. Certainly efforts to retain a high fraction of the sediment particles are a component of dredging practices, but the small fraction of sediment that escapes is often significant in the analysis of risk at contaminated sites.

Adding to the short-term risk of dredging is the release of contaminants to the water column as bed sediments are brought into contact with overlaying waters. Similar to the concern with sediment transport, any dissolved-phase contaminants are free to move with the flow of water and come into contact with receptors. This mechanism of release may take place only for short periods but can result in the release of contaminants into the aqueous phase at levels considerably higher than was occurring prior to dredging (via diffusing from the sediment bed).

An example of long-term concerns of sediment dredging results from the storage of the materials in confined disposal facilities where redox conditions are

BOX 5-5
Mixing to Enhance Bioavailability as Measured by Biodegradation Rates

It has often been observed that some contaminants are recalcitrant to microbial attack after a certain time, despite favorable environmental conditions (Erickson et al., 1993)—an observation on which biostabilization is premised. In situations where further microbial degradation is desired, it may be possible to manipulate other factors such as physicochemical phenomena and supply of electron donors and acceptors to restart the microbial degradation process (Ramaswami and Luthy, 1997).

The kinetics of mass transfer can control the overall biotransformation rates only if the mass transfer of the substrate or other critical reactant, such as the electron acceptor, is slower than the potential biodegradation rate. The ratio between these two rates is referred to as a Damköhler number; if this value is much greater than unity then physicochemical processes such as desorption, dissolution, or diffusion occur much more slowly than biodegradation, limiting the overall biotransformation rate. If the biodegradation rate is limited by external mass transfer of electron donor or acceptor, then mechanical mixing may enhance the overall rate by increasing contact and the surface area per unit volume. This is illustrated in Figure 5-3 where the biodegradation rate for α-hexaclorocyclohexane [α-HCH] in unmixed soil in the field is practically zero. The rate increases significantly with tilling, and even more so with mixing in a slurry reactor or mixing in a laboratory apparatus. The implication from the data in Figure 5-3 and related desorption tests (Rijnaarts et al., 1990) is that the biodegradation of α-HCH is mass transfer (diffusion or desorption) limited. Thus, activities that can increase mass transfer by reducing the particle size, such as mixing, can enhance biodegradation rates. Temperature has a similar influence in that increasing temperature generally increases mass transfer rates for volatile and semivolatile compounds and thus affects contaminant bioavailability. Indeed, this partially forms the basis of thermal treatment technologies for subsurface contamination.

often different than those in bed sediments. This change in redox conditions has the potential to perturb the partitioning behavior of contaminants associated with the dredge spoils. In particular, those heavy metals that are prone to precipitation under reducing conditions (often present in bed-sediments), but are soluble in aerobic, oxidizing environments (perhaps found in the water column or confined disposal facilities) may become more available and more mobile in a confined disposal facility. The leaching of contaminants from confined disposal represents a long-term concern in sediment management, and may result in greater impacts on the waters near the disposal site than would have occurred in the region of initial contamination without dredging. None of these potential outcomes is desirable or intentional, but all must be considered in the dredging of sediments.

Similar concerns are considered in the excavation of contaminated soils, where particulate matter is prone to atmospheric transport, and volatile contaminants may be lost to the gas phase. In certain cases, the potential for such releases

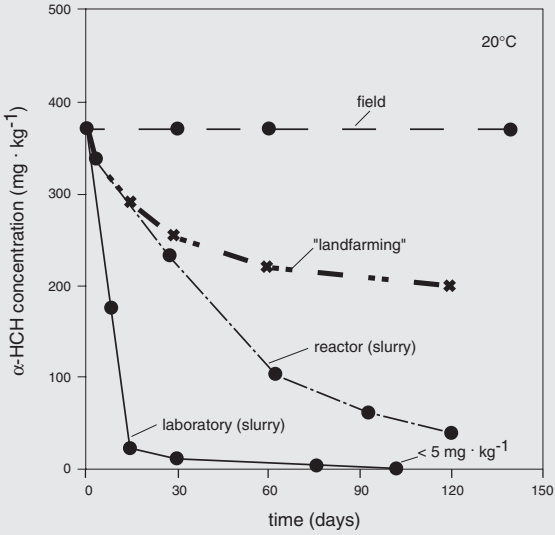

FIGURE 5-3 The microbial biotransformation of hexachlorocyclohexane [α-HCH] in soil systems ranges greatly from the laboratory scale to the field scale. The rate of biotransformation is greatest in laboratory studies and is enhanced by mixing in the field. SOURCE: Courtesy of Alexander Zehnder, EAWAG.

results in the decision not to excavate or dredge, as the resulting exposure to receptors would be greater than that which would occur without intervention.

Examples of how treatment and containment technologies impact bioavailability processes (intentionally or unintentionally) are summarized in Table 5-1.

WHEN WILL CONSIDERATION OF BIOAVAILABILITY PROCESSES MAKE A DIFFERENCE?

Explicit consideration of bioavailability processes in site-specific risk assessment can be technically difficult, time consuming, and costly. As the preceding discussion indicates, uncertainties associated with data and models pertaining to bioavailability processes must be confronted and dealt with. Experience shows that decisions to consider bioavailability processes occur on case- or topic-specific bases. An important dimension for the risk manager to consider is the value

TABLE 5-1 The Effects of Remedial Technologies on Bioavailability

Technology	Primary Effect on Bioavailability	Other Considerations
Bioremediation/ Biostabilization	Mineralizes labile forms or otherwise transforms contaminants into a chemical form that is more resistant to uptake.	May not be effective for contaminants that resist rapid transfer to the aqueous phase; resistant fraction may need to be assessed for possible entry into other life forms.
Phytoremediation	Promotes uptake and transformation of contaminants in plants.	Phytoremediation processes often result in the translocation of inorganic contaminants to tissues that have the potential for consumption by herbivores and direct entry into terrestrial food chains. Hence they might enhance bioavailability at an ecosystem level scale.
Sediment capping	Produces a barrier of "clean" materials to prevent transport of contaminated material to bottom dwelling macrofauna.	May alter the flux of materials into and out of the sediment bed resulting in changes of the biogeochemistry of the contaminated media and subsequently the physicochemical state of the contaminants.
Stabilization/ vitrification	Modifies the soil or sediment matrix to produce a material where the contamination is less prone to transport or biodegradation.	No destruction of contaminant mass occurs such that long-term stability of the solid matrix must be considered.
Redox manipulation	Changes the chemical form of a contaminant to decrease solubility, mobility, and bioavailability.	No destruction of contaminant mass occurs such that long-term stability of the chemical form must be considered. Also, in certain microniches, organisms may be capable of changing redox conditions or chelation processes, reversing the intent of the process.
Surfactant/Co-solvent/Chelatant flushing	Increases the apparent solubility of contaminants and may also increase bioavailability.	Chelates, surfactants, or co-solvents may change the biogeochemistry of the site, as these compounds may be biodegradable or toxic to indigenous organisms.
Physical treatment (heat, particle size reduction via mixing)	Increases the rate or release of contamination from the solid phase, thereby increasing the potential bioavailability.	Physical treatment may have unintended negative effects on indigenous biota and other natural processes.

of the bioavailability information. If inclusion of bioavailability information makes little difference to the decision, a risk manager would not find this extra level of analysis helpful and, in fact, such information could detract from rather than support decision-making.

There are a number of factors that determine whether or not consideration of bioavailability processes in risk assessment will make a difference for a particular situation. These factors may be grouped into three general categories: chemical, site (setting), and regulatory. Consideration of these factors will help risk managers and assessors judge the value of assessing bioavailability processes in detail at a particular site. The five basic factors that determine whether or not consideration of bioavailability processes in risk assessment will make a difference for a particular situation are:

1. when the contaminant is a risk driver for the site;
2. when default assumptions about bioavailability processes or parameters are not appropriate for the site;
3. when a significant difference in the remediation goal is possible if bioavailability processes are considered;
4. when future conditions at the site are not likely to change and can be estimated with confidence; and
5. when there is potential for regulatory and public acceptance of consideration of bioavailability processes.

Chemical is a Risk Driver

Consideration of bioavailability processes will be most important for chemicals that pose or will pose the greatest risk to human health or the environment, or both, at a particular site—the "risk drivers." Such chemicals are frequently persistent, bioaccumulative, or toxic (and usually some combination of these characteristics is required). However, the most important factor in determining whether a chemical is a risk driver depends on the degree of overlap between the exposure at the site and the chemical's threshold for effect.

Chemicals that persist in the environment (i.e., those with long half-lives) are particularly important from a bioavailability perspective. Persistent chemicals have the potential to become widely distributed, which can result in prolonged exposure, greater likelihood for transfer across environmental media, and greater accumulation in organisms, resulting in greater risk. Table 5-2 gives the persistent chemicals of current greatest concern as identified by the United Nations Environment Programme and EPA. Clearly not all persistent chemicals will be important for the purposes of assessing bioavailability. For example, potassium is an element that is ubiquitous in the environment and highly persistent. However, under most situations it does not pose a risk because concentrations are less than those required to cause adverse effects. Not surprisingly, there are no docu-

TABLE 5-2 Persistent Chemicals for which Production Controls are Established or are being Sought in the United States and by the United Nations

Priority Persistent Bioaccumulative and Toxic Pollutants—EPA	Persistent Organic Pollutants United Nations Environment Programme
Aldrin/Dieldrin	Aldrin
Benzo(a)pyrene	Furans
Chlordane	Chlordane
DDT, DDP, DDE	DDT
Hexachlorobenzene	Hexachlorobenzene
Alkyl lead	Heptachlor
Mercury and its compounds	Endrin
Mirex	Mirex
Octachlorostyrene	Dieldrin
PCBs	PCBs
Dioxins and furans	Dioxins
Toxaphene	Toxaphene

mented cases of conducting bioavailability assessments for potassium. However, since it is a required element that is often limiting, plants and animals have mechanisms to bioconcentrate potassium, such that long-term continuous exposure could potentially result in adverse effects (as have been found in some clams). As always, it is the degree of exposure relative to the onset of adverse effects in the organism that will determine whether a persistent chemical is a risk driver in a given situation.

Chemicals that bioaccumulate in organisms warrant special bioavailability consideration because of the potential to cause great harm via food web amplification. This is especially important when the target organism is a threatened or endangered species. Compounds like PCBs, certain pesticides, selenium, and mercury are known to biomagnify as they pass up the food chain. Organic chemicals with very large values of octanol–water partition coefficients (K_{ow}) will tend to bioconcentrate in the tissue of aquatic organisms. Often the root source of persistent chemicals that are amplified up the food chain is contaminated sediment or soil. Thus, decisions about the bioavailability of such chemicals in sediment or soil have important implications for bioaccumulation and food web transfer.

As the concern at sites with contaminated soil or sediment is usually risk from long-term exposure, the chronic toxicity of chemicals is usually the focus of assessment. Chemicals that exhibit the greatest potency with respect to chronic human health effects (e.g., cancer) or ecosystem effects (e.g., species reproduction) are usually risk drivers if present in sufficient abundance (i.e., sufficiently near the threshold for effect).

Finally, bioavailability process evaluations will be most useful when soil or sediment *concentrations* of the risk-driving chemical are driving remedial decision-making. In many cases, however, regulatory agencies rely upon a wide range of other criteria for managing contaminated soils and sediments, including the presence and thickness of free product and hot spots (which may be highly mobile and thus available) and aesthetic criteria. In situations where these factors will drive cleanup decisions (often manifested in immediate removal actions like excavation), the value of refining risk estimates to include bioavailability process information may be limited.

Default Assumptions are Inappropriate

As discussed in Chapter 2, risk assessment incorporates numerous assumptions that may be inappropriate or incorrect for a given site. For example, state soil standards have often been developed by assuming a direct pathway from the soil to the human or other receptor. Modification of contaminant concentration via fate and transport processes is neglected or considered only minimally. Sediments and surficial soils provide obvious opportunities for transport, exposure, and entry of a contaminant into an organism, while buried or encapsulated material clearly will have impeded transport to humans and biota for many exposure scenarios. If the physical setting appears to cut off the pathway, or present a pathway that provides for substantially impeded release and transport, then consideration of the relevant bioavailability processes will be warranted. Bioavailability process considerations may be of greatest value in guiding decision-making where the threat of transport and exposure is low.

Another common conservative assumption is that total chemical concentration in the solid phase correlates with negative effects in receptors. However, for some chemicals there is clear evidence that the total chemical concentration correlates poorly with receptor response (for example, elemental mercury—EPA, 1996a). In cases where such chemicals are perceived as potential risk drivers at early stages of assessment, consideration of bioavailability processes will usually be warranted. If limited bioavailability can be established early in the process, there may be no need to evaluate exposure further (although it also should be established that the conditions limiting bioavailability will not change with time). Deviating from a conservative default assumption in this way must be done cautiously, and must include tests that determine the form of the chemical present, because there may be strong or weak correlation of total chemical concentration with receptor response depending on biogeochemical conditions. A good example of such a chemical is chromium, a redox active element whose toxicity depends on the biogeochemical conditions in soils and sediments. The reduced form, chromium(III), has low toxicity due to poor membrane permeability and noncorrosivity, while chromium(VI) is highly toxic due to strong oxidation characteristics and ready membrane permeability. Chromium(III) and chromium(VI)

also have different chemical reactivities and thus different fate and transport characteristics. Biogeochemical processes can mediate chromium(III)–chromium(VI) transformation, thus greatly affecting bioavailability and toxicity. Thus, tests to determine which form of the metal is present are critical for determining cleanup goals and an appropriate remedy.

There are many other default assumptions made during human health and ecological risk assessment (see Chapter 2) that might be replaced by site-specific information about bioavailability processes. Fortunately, most states allow for site-specific risk assessment in cases where the appropriateness of default cleanup goals is challenged. With the flexibility to perform a site-specific risk assessment, the key regulatory issue then becomes the type of bioavailability process assessment allowed and the level of scientific rigor that must be associated with results for the assessments to be potentially acceptable.

A Significant Difference in Remediation Goals is Possible

Consideration of bioavailability processes in a risk assessment for a particular chemical is usually worthwhile only if there is potential for the revised exposure assessment to change the estimated risk (and thus the cleanup goal) to an extent greater than its uncertainty bounds. Conditions that increase the likelihood of a sufficiently large change in exposure and remediation goals are as follows.

First, chemical concentrations must be of the same order of magnitude as proposed action levels. As discussed in Chapter 2, bioavailability considerations for soils and sediments, in the few cases where data are available, have tended to adjust cleanup goals (acceptable contaminant concentrations) by factors of two to three. Thus, experience suggests that if contaminant concentrations are of the same order of magnitude as proposed action levels, the adjustment of cleanup goals by factors of two or three may be sufficient to keep exposure in the acceptable range. (Also, this experience indicates that adjustments by factors of two to three have potential to be accepted.) If contaminant concentrations are many times higher than the initial cleanup goals, then it is possible that no reasonable amount of bioavailability information will have a meaningful impact on environmental decision making.

Second, a revised exposure assessment may significantly change the estimated risk for those chemical–receptor combinations where small differences in concentration correspond to large differences in toxicity—that is, where the response versus dose plots are steep. Generally such plots indicate intense receptor sensitivity to the chemical. Where small differences in bioavailability that lead to small differences in exposed dose will translate into large differences in risk, refining the exposure assessment with bioavailability considerations may be worthwhile. (It should be noted that for this type of chemical there is also the need for extremely high precision in bioavailability estimates,

because small errors in determining exposure will result in large errors in the estimated risk.)

The overall risk at a site and costs for soil or sediment remedial actions often depend on the total amount of contaminant mass present. Bioavailability considerations can have a major impact on a site comprised of large amounts of material at low concentrations. This is because small adjustments in acceptable concentrations translate to large differences in the overall amount of material treated and remediation costs. This may include broadly contaminated areas such as estuaries.

Finally, in some cases consideration of bioavailability processes can expand options for remediation. This may be of particular interest to regulatory authorities when use of conventional conservative assumptions and default values necessitate solutions that have environmental or public use costs that negate some of the environmental or public health benefit. For example, if conventional remedial decision-making procedures point to a solution involving soil or sediment removal and treatment that will result in damaging a valuable resource such as a wetland or other habitat, or a boating area, regulators may be interested in finding a less destructive option. This is exemplified by the remedy chosen at the Gary, Indiana, Lagoons Superfund site, where soil is contaminated with PCBs, BTEX[1], and PAHs. In this case, soil excavation was significantly scaled back in order to not disturb an adjacent wetland ecosystem after it was determined (during site-specific investigations) that the PCBs adjacent to the wetlands posed less of a risk than would be imposed by excavation.

Future Conditions are Not Likely to Change

Consideration of bioavailability processes will make the greatest difference in decision making if the estimated risk can be projected into the future with certainty. This will be possible to do with confidence when the pathway of concern, site conditions, and key bioavailability processes are not likely to change with time. Obviously, there are many factors that may change the bioavailability or toxicity of a compound in the future. This may in fact be beneficial, as in the case of some organic compounds in soil for which aging is shown to decrease the compound release rate and extent. In this case, bioavailability decreases with time. Alternatively, changing the future conditions may lead to an increase in bioavailability via the modification of the geochemical setting, changes in the exposure pathway of concern, and the introduction of different receptors. Likewise, organisms can change the form of a chemical (e.g., when a chemical is eaten it may become more bioavailable to the predator). Examples of these changes are given below in Table 5-3. If an assessment of the potential future changes introduces a large degree of uncertainty, it is unlikely that evaluation of

[1]BTEX refers collectively to benzene, toluene, ethylbenzene, and xylene(s).

TABLE 5-3 Examples of Factors That May Affect the Availability of Soil and Sediment Contaminants over Time

Factor	Causes and Possible Effect on Contaminant Availability
Physical disturbance	This can result from human activities (e.g., land use change and associated soil excavation) or natural phenomena (e.g., earthquakes, volcanoes, floods, wave action). Depending on the degree of physical disturbance, contaminants that were previously unavailable may become more available.
Changes in pH or ionic strength	This may occur as a result of natural changes (plant growth) or human activities (disposal of waste materials) in the vicinity of contamination. Changes in pH can affect the speciation and consequently the availability of many metals as well as the binding of organic compounds to solids.
Aging or weathering	Aging refers to physical and chemical changes in the bonds between contaminants and solids as their contact time increases (see Chapter 3). These processes generally reduce the bioavailability of contaminants from soils and sediment over time.
Moisture	Natural and anthropogenic changes in the hydrologic regime (droughts and floods) near a contaminated site can change the moisture content of soils. Increasing moisture content may favor transfer of chemical contaminants from soil to bioreceptors.
Temperature	Temperature change can be induced by certain remediation strategies such as thermal treatment. In general higher temperatures increase the desorption of volatile chemical contaminants from solids. In some cases, higher temperatures may change reaction conditions resulting in a transformation that influences bioavailability.
Biota	Chapter 3 discusses various processes by which organisms help release contaminants from solid phases (bioturbation, excavation, siderophore action) or transform contaminants in solution (e.g., methylation of mercury). These processes often affect bioavailability of chemicals by changing the redox environment in which the chemical resides.

SOURCE: Adapted from Menzie et al. (2000).

bioavailability processes will yield results with sufficient certainty to impact decision-making.

Regulatory and Public Acceptance is Possible

The potential for results from bioavailability process analyses performed in risk assessment to support remediation decision-making depends on the regulatory domain and public acceptance. Before undertaking a bioavailability process assessment, the likelihood of acceptance of the results by regulators and the

public needs to be evaluated. Conditions for which regulatory and public acceptance of bioavailability information is most likely are described below.

If site conditions, the contaminant of interest, and the default cleanup objectives are similar to those at other sites where remedial action is needed or underway, investment in an assessment of bioavailability processes may be warranted because regulators and the public will have familiarity with the problem. Acquisition of process data and knowledge and application of new measurement tools for bioavailability assessment may help with formation of more cost-effective solutions. An example is the swine test to assess bioavailability of lead in soil, which was applied by EPA to test soils at the Palmerton Zinc Pile Superfund Site (see Box 2-5). The results of the swine testing did not affect the remediation decision at the Palmerton site, as they pointed to acceptable lead soil concentrations in the range estimated by the default assumptions. However, the experience gained at the Palmerton Site and elsewhere led to subsequent applications of the swine testing at approximately 20 other lead-contaminated sites, including several high-volume waste sites. Remediation decisions were influenced by the swine test results at some of these sites (Weis, 2000).

A bioavailability assessment is difficult to justify if a relevant regulatory body has a policy stance against explicit consideration of particular bioavailability processes. Some state environmental agencies and EPA regions have included in guidance to their remediation project managers and risk assessors recommendations or policy directives to refrain from consideration of certain bioavailability processes in estimating exposure (see Table 2-8).

In contrast, some state environmental agencies and EPA regions have developed guidance for consideration of bioavailability processes in risk assessment. EPA Region 10, for example, developed guidance for bioavailability considerations in human health risk assessments for arsenic contaminated soil (see Chapter 2). Washington state has very recently amended its Model Toxics Control Act to allow for incorporation of new scientific information which could be used to modify the "gastrointestinal absorption fraction" and other bioavailability default assumptions (G. McCormack, Washington Department of Ecology, personal communication, 2003). While this has only been done in a few states as of this writing, and for a limited range of bioavailability processes and contaminants, the existence of guidance signifies openness to bioavailability process evaluation.

NEXT STEPS

The preceding chapters have shown that there is a variety of physical, chemical, and biological processes that determine the availability of contaminants in soils and sediments to ecological receptors; that consideration of these bioavailability processes is inherently part of the risk assessment process; that validated measurement techniques and models exist for some bioavailability processes, but not for many others; and that uncertainty about how to measure and

describe some key bioavailability processes has led to limited use and regulatory acceptance of comprehensive bioavailability process evaluation in risk assessment. This chapter has identified soil and sediment contamination scenarios in which consideration of bioavailability processes can have a significant impact on remediation planning and decision-making. Clearly, limitations in measurement tools, models, and understanding serve as impediments to comprehensive assessment of bioavailability processes for many contaminated soil and sediment sites. Yet, just as clearly, there are substantial opportunities for consideration of bioavailability processes to advance risk-based remediation.

Various actions are needed to make progress in using bioavailability processes in risk assessment and decision-making at individual sites, in acknowledging bioavailability processes in regulations and creating appropriate guidance for management of contaminated soils and sediments, and in better understanding bioavailability processes on a mechanistic level.

In Risk Assessment and Decision-making at Individual Sites

In order for bioavailability processes to be considered more explicitly in risk-based management at individual sites, key issues that represent obstacles need to be addressed aggressively. These include (1) selecting appropriate bioavailability process measurement and modeling tools; (2) assessing and (when possible) reducing uncertainty in understanding, models, and parameters for particular bioavailability processes; (3) developing coordinated long-term monitoring of bioavailability processes critical to the risk-based remedial plan implemented; and (4) involving community groups in remediation planning at early stages.

Tools Selection

Chapter 4 described numerous existing and emerging measurement and modeling tools important for bioavailability processes, and it gave the criteria on which the merits of individual tools should be judged and validated. Bioavailability analyses are necessarily site-specific, and it is important that tools appropriate for the particular site and context be selected for assessment of bioavailability processes. Because development of tools relevant to bioavailability is a rapidly growing field with new techniques becoming available on a regular basis, there can be considerable confusion regarding which tools and how many of them to choose in order for the results to be useful in decision making.

In the last five years, scientists, risk assessors, and EPA have advocated relying on a weight-of-evidence approach as a way of making decisions in the face of limited information and imperfect tools. Although the term "weight-of-evidence" is used in different ways by different groups, two concepts associated with the term have important ramifications for choosing bioavailability tests.

First, "weight-of-evidence" can be used to refer to selecting individual bioavailability measurement tools based on the strength of the information that they produce, as well as on how that information will be used for risk-based decision making (Menzie et al., 2000). In this regard, four principles have been outlined that would give a specific tool greater weight: (1) soil–chemical relevance, (2) receptor relevance, (3) pathway relevance, and (4) acceptance or validation of the tool. These factors align closely with discussion earlier in this chapter (pages 377–383) and the criteria outlined for tool validation in Chapter 4. As discussed in Menzie et al. (2000), the degree to which these four factors are satisfied increases user confidence in the tool. Early attempts to explicitly consider bioavailability processes in risk assessment have frequently used inappropriate tools (that usually were not relevant and/or validated), which has contributed to concern about inclusion of bioavailability in risk-based decision-making for contaminated soils and sediments.

On a broader scale, the term "weight-of-evidence" is used to refer to how one uses the combined results of multiple tests. Here the term is synonymous with providing "multiple lines of evidence" about bioavailability processes at a site. For example, this approach might combine empirical measures with measures of bioaccumulation, toxicity, and others parameters. EPA has recently provided guidance on how to use this approach to better identify stressors in aquatic ecosystems (EPA, 2000a) and how to collect sediments (EPA, 2001a), suggesting that the agency would be amenable to using this approach for bioavailability assessments. It is highly consistent with the Chapter 4 notion that each method has unique advantages and limitations and that an integrated suite of tools (see Box 5-4 for an example) is preferable to a single tool. Several recent publications discuss the tenets of this approach for use during human health and ecological risk assessment (Menzie et al., 1996; Burton et al., 2002a,b; Chapman et al., 2002).

The "multiple lines of evidence" approach provides an opportunity to make near-term progress at sites and to overcome some of the pessimism felt by the regulatory community regarding bioavailability because of the lack of mechanistic tools currently available. Its use is an implicit recognition that although our empirical techniques are not able to unambiguously predict bioavailability, they represent progress over the assumption that receptors are exposed to the total contaminant mass bound to soils or sediments. Nonetheless, because of the limitations of empirical tools in their ability to make predictions or be applicable to other sites, the multiple lines of evidence approach should be accompanied by substantial efforts to promote the development of more precise tools. This means employing measurements and models that relate directly to bioavailability process mechanisms to the maximum extent possible. Mechanistic knowledge and insight enables clearer explanation of existing site conditions and how they will respond to a particular remediation or management strategy as well as more confident long-term projections of reliability and durability of remediation and management solutions. For example, mechanistic models based on kinetics will allow

understanding of potential future contaminant release from the solid phase. Empirical knowledge based on measurements that aggregate processes has substantial limitations in this regard. When it is possible to choose tools that will provide better mechanistic understanding, this opportunity should be exploited and not bypassed in favor of conventional empirical assessment approaches.

Given the complexities of bioavailability processes identified in this report, it is likely that mechanistic tools and predictive model development will be a multi-decade effort. Adopting a multiple lines of evidence approach today would utilize and build on the currently existing battery of empirical tests, many of which may have future application for site-specific validation of mechanistic models of exposure and effects. As more robust methods evolve, the need for a multiple lines of evidence approach should diminish concomitant with our increasing ability to predict impacts, leading to greater acceptance of risk assessment that includes explicit consideration of bioavailability processes.

Assessment and Reduction of Uncertainty

At the present time, many bioavailability processes are hidden within default assumptions that are highly simplified and likely to be uncertain (although this uncertainty is generally not reported). More explicit, site-specific consideration of bioavailability processes in risk assessment can reduce this uncertainty. However, if there is (even perceived) substantial uncertainty associated with a bioavailability process that controls the ultimate estimated risk, there may be a tendency to not measure that process explicitly and instead to use conservative assumptions. For example, if the rate of contaminant desorption from a sediment is suggested for consideration in an ecological risk assessment, a slow rate of desorption may decrease significantly the concentration of contaminant predicted to occur in fish. Consideration of this bioavailability process will only be acceptable, however, if the desorption rate for current and projected site conditions can be measured with a fair degree of certainty. If the desorption rate and how it will change as site conditions evolve is not well understood, conservative assumptions such as release of all contaminant to the aqueous phase or equilibrium partitioning may be invoked.

For these reasons, it is important to recognize the uncertainty and variability in each bioavailability process descriptor and the potential for propagation of error in risk assessment. More substantive efforts to manage or reduce the uncertainties, especially for key bioavailability processes, have the potential to greatly reduce the degree of uncertainty in the overall risk assessment. The influence of bioavailability process uncertainty and variability on the overall risk can be assessed qualitatively, quantitatively through sensitivity analysis (deterministic risk evaluation), or through stochastic risk assessment. These approaches are discussed in greater detail in Box 5-6.

BOX 5-6
Methods for Assessing Uncertainty in Risk Estimates

Most risk assessments are performed in a deterministic manner, that is, with single values for the various parameters in exposure and toxicity models (Burmaster and Wilson, 1998). This results in a single value estimate of risk. All of the exposure and toxicity model parameters have some associated uncertainty and variability, however. Attempts to examine the effects of uncertainty and variability in critical parameter values for a particular risk assessment usually involve performance of sensitivity studies in which the value of a critical parameter is systematically varied while holding all other parameter values constant. This can be done for any number of parameters and is often done for several. The manner in which differences in model results are treated defines various kinds of sensitivity analyses (Cullen and Frey, 1999). Important changes in risk predictions that may result from simultaneous changes in two or more uncertain parameters can be missed with sensitivity analysis, however.

The uncertainty and variability in exposure and toxicity model parameter values can be taken into account more rigorously by describing some or all key model parameters with a probability distribution (Morgan and Henrion, 1990; Burmaster and Wilson, 1998; Cullen and Frey, 1999). The risk model is then run many times with different combinations of parameter values sampled from each of the distributions using any of a number of stochastic sampling strategies (e.g., Monte Carlo, Latin Hypercube). Resulting risk model outputs are compiled and used to construct a probabilistic distribution of risk. This process is known as stochastic risk assessment. It is used mostly in the research community at present, though EPA (1997a, 1997b; 2001b) and other organizations are encouraging its use in practice.

Simultaneous consideration of distributions for critical parameters in stochastic risk assessment provides a rigorous estimate of the uncertainty in the risk estimate, provided that the parameter distributions are reasonably well defined. This also provides other useful information and insights. For example, a study of the uncertainty in a site-specific risk assessment resulting from variable site physicochemical properties revealed greater uncertainty in risk for more mobile and less degradable compounds present as soil contaminants (Labieniec et al., 1997). The uncertainty in the site properties affecting transport and hence exposure had more importance for these compounds, and that was reflected in the predicted risk distribution.

A primary limitation to use of stochastic risk assessment is the lack of sufficient data to define the probabilistic distributions for exposure and toxicity parameters. Site physicochemical data are often quite limited, necessitating the assumption of distributions if a stochastic approach is to be employed. Similarly, toxicity data are often too limited or too dependent on specific test conditions for meaningful assignment of distributions to parameters such as reference doses, carcinogenic slope factors, or absorption fractions. In addition, there is the uncertainty associated with the selected exposure or toxicity model (including the extrapolation of animal results to humans). Nevertheless, there is much work under way to develop accurate distributions for exposure and toxicity parameters, such that stochastic risk assessment should become increasingly useful and routine as part of bioavailability assessment.

Long-Term Monitoring

A more rigorous evaluation of bioavailability processes during risk assessment will likely alter both the prioritization of remediation efforts at contaminated sites and decisions pertaining to the remedial technology(s) chosen at individual sites. These impacts on decision-making are profound and have the potential to change the current landscape of contaminated soils and sediments management. Whether these decisions provide long-term protection to humans and the environment will depend, in part, on how much is known about bioavailability processes over time. Thus, the consideration of bioavailability processes in risk assessment must include evaluation of future system states via coordinated and process-based long-term monitoring, including the potential for events that might reintroduce unacceptable exposure conditions. Events that could alter contaminant bioavailability at sites where contamination has been left in place include changes in land use, fluctuations in site geochemistry, or the introduction of a new sensitive receptor in the area. Most current bioavailability information is derived from studies shorter in duration than the time frames of interest in site management. These studies are conducted under more consistent physical, chemical, and biological conditions than would be expected at any particular contaminated site. Thus, our understanding of temporal changes in underlying bioavailability mechanisms is limited.

The need for long-term monitoring to enable confident assessment of system behavior over time is a recognized component of most remediation strategies and is generally not complicated in a conceptual sense. Long-term monitoring is also a statutory requirement for those sites regulated under Superfund where contamination is left on-site at levels above those necessary to allow unrestricted use of the land. In the case of bioavailability processes, there is almost no guidance on approaches for long-term monitoring that specifically target the *stability* of the contaminant "form" instead of total contaminant concentration. Furthermore, monitoring may need to shift from classical site monitoring of total contaminant levels to include the activities of receptors, changes in site-specific processes (e.g., geochemistry), plans for future land use, and other factors.

Depending on the certainty of the bioavailability assessment conducted, a range of monitoring efforts may be appropriate. No further action may be required in some cases where certainty is relatively high, extensive long-term monitoring may be needed at some sites, and a range of possibilities can occur in between. In addition, the need for long-term monitoring may decrease over time if reliable data indicate a high potential for future system stability and other statutory requirements are met. Box 5-7 discusses the development of site-specific monitoring tools for assessing bioavailability over the long term at a hazardous waste site. While there are often legal and practical constraints involved with the design of long-term monitoring programs, from a scientific perspective the stronger the commitment to monitoring the greater the payoff in terms of confidence in describing system performance.

As discussed in Chapter 4, a number of methods have been or are being developed to assay the physical state of contaminants in soils and sediments. It is likely that a subset of these approaches will find routine use as monitoring tools. Also, it is likely that new methods will continue to be developed and incorporated into long-term monitoring strategies, such as the deployment of absorbents in the water column to measure potential bioavailability of contaminants over time. As methods are adopted and changed in the future, it is possible that the results of testing will alter the analysis of risk from what might be derived today. To that end, the introduction of bioavailability processes into risk assessment extends not only the time frame of monitoring, but also the time frame for decision-making. Regardless of the origin of change, it may be necessary to rethink risk assessments into the future and be prepared to respond accordingly to avoid unwanted exposure, or to stop ongoing activities that are no longer needed to reduce risk.

Community Concerns and Risk Communication

Experience has demonstrated that communities often have concerns about consideration of bioavailability processes in risk assessments for decision-making at hazardous waste sites. Perhaps most importantly, bioavailability assessments may be viewed as a "do-nothing" or "do-less" approach. Given that incorporation of bioavailability adjustments into risk assessments may raise acceptable contaminant concentrations in soil or sediment, it may be viewed as simply a justification for leaving more contamination in place. Second, in some cases evidence is often insufficient to justify the use of bioavailability process information. Because bioavailability process studies may not be conducted for the ultimate receptor of concern, or may yield results with considerable uncertainty, a community may not be confident that the scientific evidence is adequate to apply the results within their community. This can be exacerbated by the fact that standardized methods for evaluating some key bioavailability processes are lacking. Third, the long-term effectiveness of leaving "unavailable" contaminants in soils and sediments is unknown. Because the bioavailability of contaminants from soil or sediment may increase or decrease over time, or if site conditions change, exposure to the contaminants may increase or decrease in the future. Finally, monitoring requirements may be perceived as insufficient. The previous section discussed the need for monitoring of bioavailability processes over time to ensure that contaminant availability to receptors remains within an acceptable range. Given the potential cost of long-term monitoring, a community may not be confident that it will be conducted adequately, or for a sufficient period.

To date, bioavailability process evaluations at hazardous waste sites have been applied primarily for human health risks from metals in soils, and at a limited number of mining and smelting sites. Because only a small number of communities have had to grapple with bioavailability issues, it is uncertain which community concerns will predominate. Of the limited cases to date where com-

BOX 5-7
Monitoring Tools for Assessing Long-term Bioavailability in Leadville, Colorado

A Superfund site in Leadville, Colorado is characterized by high metal, pyritic alluvial tailings deposits along the Upper Arkansas River. The remedy at the site will involve amending the tailings with chemicals *in situ* to reduce the bioavailability of the metals as opposed to a more conventional soil removal and replacement. The threat posed by the tailings is primarily to the surrounding ecosystem because of the inability of the tailings to support a vegetative cover. The bare tailings along the banks of the river tend to erode into the river, resulting in damage to river biota.

The *in situ* amendment selected to reduce the bioavailability of the tailings includes application of municipal biosolids (224 Mt ha^{-1}) and limestone (224 Mt ha^{-1}). Surface application of this type of amendment has been shown to reduce surface as well as subsoil acidity, thereby reducing the solubility of the metals in the system (Brown et al., 1997). Biosolids also provide both inorganic and organic specific adsorption sites for metals (Zhenbin et al., 2001). In addition, the improvements in soil nutrient and physical properties associated with biosolids application will permit establishment of a vegetative cover on the tailings, and thereby reduce the potential for re-entrainment (Sopper, 1993).

By selecting an *in situ* amendment to reduce the bioavailability of the contaminants, project costs were reduced, allowing additional acreage to be treated. According to the project manager, a local repository for excavated soil was unavailable, and the costs to excavate and transport to a front range disposal facility were prohibitive. To date, about $1.25 million has been spent to treat 35 acres or about 42,000 cubic yards using the *in situ* remedy.

Remedial Assessment

EPA's Environmental Response Team, a division of Superfund, has been in charge of developing an appropriate monitoring scheme for the site. Addition of amendments to metals-contaminated soils to reduce the bioavailability of metals *in situ* is considered an "emerging" technology by EPA, and a standard array of tests has not been developed

Alluvial tailings deposits along the Upper Arkansas River outside Leadville. Surface salt consists of metal sulfates with zinc concentrations as high as 9 percent.

for performance evaluation. Scientists are attempting to develop appropriate tests and criteria to evaluate the effect of the amendment on the functioning of the ecosystem, focusing on the worst-case exposure pathways in order to be conservative.

The monitoring approach centers on soil function and biological activity in the remediated area as compared to both control uncontaminated and control contaminated areas. Increased soil function and biological activity is taken to be indicative of decreased bioavailability of the contaminants. The emphasis is on determining if the amendment has restored functionality to the system. Importantly, there has not been a corresponding effort to assess the speciation or fate of the metals. Ecosystem function is addressed in increasing orders of complexity—first soil functionality, then plant health, and finally the diversity and health of larger communities. The evaluation is being developed to answer the following questions. The tests being used to answer each question are listed below the question itself.

1. Is soil functioning impaired in treated plots?
   Microbial population counts, CO_2 evolution.
2. Are treated plots phytotoxic? Is there evidence of phytotoxicity?
   Plant germination and foliar tissue analysis conducted in a controlled environment setting.
3. Are dietary exposure levels of site contaminants sufficient to cause toxic effects, including reproductive impairment, to the herbivorous avian community that utilize the treated plots?
   Field collected plant metal concentration used in a dietary exposure model with a focus on willows.
4. Are dietary exposure levels of site contaminants sufficient to cause toxic effects, including reproductive impairment, to the insectivorous avian community that utilize the treated plots?
   Soil invertebrate tissue concentration (from lab studies) to model potential for insectivorous avian community.
5. Are dietary exposure levels of site contaminants sufficient to cause toxic effects, including reproductive impairment, to the carnivorous avian community that utilize the treated plots?
   Small mammal collection from amended areas, tissue analysis and total body burden of both herbivores and insectivorous mammals.
6. Are dietary exposure levels of site contaminants sufficient to cause toxic effects, including reproductive impairment, to the herbivorous small mammal community that utilize the treated plot?
   Small mammal collection, body burden combined with foliar tissue concentrations from field samples to use in an exposure model.
7. Are dietary exposure levels of site contaminants sufficient to cause toxic effects, including reproductive impairment, to the carnivorous small mammal community that utilize the treated plots?
   Soil invertebrate metal concentration from lab study for values to use to model shrew diet concentrations.

A functioning ecosystem will be viewed as effective proof that the bioavailability of the contaminants has been reduced as a result of the *in situ* amendment.

munities have been presented with bioavailability information (see Box 5-8), the responses have ranged from strong support (Oak Ridge, Tennessee) to acceptance (Bartlesville, Oklahoma) to strong objection (Aspen, Colorado).

Nonetheless, consideration of bioavailability process information for contaminated soils and sediments is inherently part of the risk assessment process, whether for protection of ecological receptors or human health. As discussed in Chapter 2, all risk assessments for soil and sediments contain implicit assumptions about bioavailability (a common default assumption being that the contaminant is equally bioavailable from soil or sediment as it was in the original laboratory toxicity study for the chemical). Thus, bioavailability does not present a risk communication problem unique from the risk assessment processes. The public should be introduced to the concept of bioavailability, and the consideration of bioavailability processes, as being a fundamental component of risk assessment no different from other exposure parameters or toxicity values used in risk assessment, and around which there may be considerable uncertainty.

Whether default assumptions about bioavailability processes are replaced with site-specific measurements will depend on whether such measurements are technically justifiable, and whether a good job of public outreach and communication is performed at a specific site. The quality of the risk communication (as outlined in Box 5-9) will determine whether the public is likely to evaluate the use of bioavailability processes on their scientific merits.

The technical components that should be included in any public communication program regarding application of bioavailability adjustments should include the following:

- factors that affect bioavailability from soils or sediments;
- the concepts of absolute bioavailability and relative bioavailability;
- the technical basis for the established toxicity values, and how bioavailability was handled in the derivation of those values;
- selection of a model for bioavailability studies and why it was chosen;
- how uncertainty was handled (e.g., different bioavailabilities in different animals in the study, uncertainty in overall study); and
- how the bioavailability information is incorporated into the risk assessment.

Specific interests or concerns of the community may dictate detail, or additional areas that need to be addressed.

The potential community concerns discussed above should be dealt with in a direct and honest manner by providing the public with timely information about any bioavailability studies that are proposed for a specific site, and their outcome and implications for the site. With respect to the concern that consideration of bioavailability processes is simply used to justify a “do-less” approach, it should be conceded that there is an element of truth in this. The reality is that bioavailability process studies are rarely undertaken simply to improve the accuracy

of a risk assessment. Rather they are generally performed to justify site cleanup goals that are more financially or technically feasible, and that involve leaving appreciable amounts of contaminant mass in place, while still being protective of public health and the environment. On the other hand, there can be a strong scientific basis for the incorporation of site-specific bioavailability process information into the risk assessment process; such technical information should be provided to the public. Bioavailability assessment may receive greater scrutiny, given its relative newness, than other site-specific studies that are performed at contaminated sites (e.g., soil ingestion studies in humans and wildlife, soil-to-house dust transfer studies, environmental exposure studies, or toxicity studies). The important fact to emphasize in communicating results is that all bioavailability process studies are aimed at exposure assessment, and are performed to reduce uncertainty regarding the magnitude of site risks and thereby support a more efficient cleanup strategy, or to support choices among different remedial alternatives.

Into the Regulatory Arena

In relatively few cases has the replacement of default assumptions about bioavailability processes with site-specific measurements been incorporated as a matter of practice into protocols that govern risk-based decision-making in the regulatory arena for contaminated soils and sediments. Consideration of physical transport processes is sometimes permitted, if these processes are adequately characterized. Use of a dilution-attenuation factor for contaminant concentration mitigation along the pathway from source to receptor is also sometimes allowed. More often than not, however, the total contaminant mass in the source area is assumed to be available to the receptors of interest, and potential attenuation of exposure via fate and transport processes is neglected. This approach is conservative with respect to protection of public health and the environment, and it deals simplistically with the issue of uncertainty. In this regulatory environment, when even basic transport processes are not routinely considered, it is difficult to introduce new kinds of data and information pertaining to processes that affect exposure.

There is no question that risk assessment methods and models will evolve to encompass our improved scientific understanding of bioavailability processes. It will always be the case, however, that process-based methods for exposure analysis will only be applicable and useful in situations for which site characterization data are adequate. Thus, the existence of thoroughly validated measurement techniques and models for particular bioavailability processes will still not guarantee the ability to perform comprehensive, process-based exposure assessments in all cases. Regulatory requirements and constraints on risk-based management of soil and sediment sites necessarily must account for this reality.

BOX 5-8
Case Studies of Community Concerns Regarding Bioavailability

Oak Ridge, Tennessee

At the Oak Ridge site, the community was in favor of applying a mercury bioavailability adjustment to soils and sediments of the East Fork of Poplar Creek. In general, the community viewed extensive remediation of soils and sediments as disruptive and of questionable benefit. The situation at this site was somewhat unique in that the community was both highly informed and highly educated (due to the presence of Oak Ridge National Labs) and actively participated in evaluating the data and science used to assess risk and develop cleanup goals. The residents of Oak Ridge readily accepted the mercury bioavailability adjustment for soils and sediments, which was applied in the risk assessment and ultimately increased the cleanup level. (Sources: M. O. Barnett, Oak Ridge National Laboratory, Environmental Sciences Division, personal communication, 2000; NEPI, 2000.)

Bartlesville, Oklahoma

At the National Zinc site in Bartlesville, Oklahoma, oral bioavailability studies were conducted for lead, cadmium, and arsenic in soil, and the resultant data were used in the human health risk assessments for these elements (see Box 2-4). No concerns were voiced by the community, either at public meetings or as written comments, regarding the development and application of bioavailability adjustments. A number of factors were likely involved in the community acceptance of this issue, including (1) proactive engagement of the community by the Oklahoma Department of Environmental Quality, coupled with a concerted community awareness and risk communication program, (2) existence of an active Citizens Advisory Group that represented the community throughout the entire remedial investigation/feasibility study process, and (3)

It remains to be seen whether regulatory agencies will embrace the bioavailability concept in the short term. The resistance in some regulatory domains to allowing site-specific measurements of some bioavailability processes in risk assessment stems from many factors, including uncertain methodologies and lack of validation, public anxiety and suspicion about motives, and lack of precedent. These factors are not unique to the issue of bioavailability of contaminants in soil or sediment. Similar concerns arise in other contexts with proposals for new approaches for the protection of human health and the environment. Some examples are the application of innovative remediation technologies for site cleanup, or adoption of new methods for treating drinking water, or engineering manipulation of river or groundwater resources for ecosystem restoration. In each of these cases there is reluctance to make a substantial commitment to a new approach until more is known.

A viable way to move around these obstacles and achieve more widespread consideration of bioavailability processes in risk-based management of contami-

representation of the community by an expert (Dr. Frederick Oehme from Kansas State University) who reviewed and commented on the bioavailability study protocols and data interpretation.

For both of the sites, project managers commented that consideration of bioavailability processes in risk assessment posed no special risk communication problem relative to the overall challenge of communicating the role and results of risk assessment in project decision-making.

Aspen, Colorado

The Smuggler Mountain Superfund site in Aspen was placed on the National Priority List in 1986 due to elevated levels of metals, particularly lead, in soil in the vicinity of residences. The EPA's proposed remedial options, which included the removal of substantial amounts of soil and the deposit of funds in escrow accounts for future environmental cleanup, were opposed by the affected community, partly because the remedy for the site involved hauling tons of dirt. In addition, a blood-lead survey found that lead concentrations in the children living near the site were below that for the general population, leading to the claim that the lead at the site is not bioavailable and thus not harmful. Lead bioavailability studies in young swine that were conducted by EPA Region 8 on Aspen soils met with considerable opposition (Bernstein, 1991). However, this response was symptomatic of the already strained relations between EPA and the Aspen community at the time that the bioavailability studies were conducted, and may not have reflected public discord with the bioavailability studies themselves.

nated soils and sediments is to invoke an adaptive management approach. This paradigm embraces two ideas. The first is that there should be various pilot studies to experiment with different techniques to see if they work or not. The second is that agencies should use the results from such efforts to develop a common systematic approach to determine how and when to incorporate bioavailability concepts into regulations in a consistent manner.

Adaptive management applies findings from carefully monitored experiments to the adjustment of future management and policy decisions in light of changing conditions and new knowledge. This approach moves away from rigid requirements that require the selection of fixed goals and the means to achieve them. Adaptive management is receiving increasing attention and application to problems of regional ecosystem management (Gunderson et al., 1995; Lee, 1993; Walters, 1997). It is being promoted for wider use in water management programs such as in the Florida Everglades and in Glen Canyon on the Colorado River (NRC, 1999, 2001b) and has been tried in some forest and fisheries sectors

BOX 5-9
Tenets of Good Risk Communication

As consideration of a wider range of bioavailability processes becomes more common in risk assessments, there will be an associated responsibility for risk assessors and project managers to educate potential stakeholders regarding the key bioavailability processes and related measurements, their incorporation into site-specific risk assessments, and their ultimate effect on cleanup goals. This may require that regulatory agencies institute more comprehensive risk communication programs that emphasize both the learning and explaining activities of communication, while training risk managers and others engaged in communicating risk. Communicating scientific issues regarding public health risks has been an active field of study and practice since the early-1980s (Sandman, 1986). Risk communication has come to mean communication that supplies lay people with the information they need to make informed independent judgments about risks to health, safety, and the environment (Morgan et al., 1992). The basics tenets of risk communication include (Elder, 1997; NACCHO, 1995; Sandman, 1996):

- involving the community early in the process;
- communicating in a direct, honest, and timely manner;
- understanding and acknowledging the public's concerns and values;
- providing sufficient information for the community members to be able to make informed, independent decisions;
- building an effective working relationship with the community;
- providing a consistent and ongoing process for communication; and
- providing the community with influence in the decision-making process.

These principles of public communication hold equally well for the communication of bioavailability information as for any other type of scientific information.

One of the fundamental features of public communication is that to be successful it should be treated as a process, not as a single event or mechanism. Successful examples of public communication documented by Ashford and Rest (1999) had in common that each was a process designed to improve communication with the community, educate community members and build their technical skills, and facilitate specific participation by the community in the decision-making process. In a 1996 evaluation by the Agency for Toxic Substances and Disease Registry (ATSDR) of its community involvement efforts, it was concluded that in order to build an effective working relationship, community involvement should be viewed as a dynamic and developing relationship between community members and the ATSDR. This approach to public communication is particularly important when addressing issues of bioavailability, because these issues require a considerable amount of technical information to be transmitted, and may require some time for the studies to be designed, conducted, and interpreted. This provides an opportunity to work with interested individuals or organizations within a community (or technical experts who may represent the community) to reach consensus on the design and application of such studies.

(NRC, 1996; Taylor et al., 1997). It has also been proposed for management and remediation of PCB-contaminated sediments in rivers (NRC, 2001a) and for cleanup of hazardous waste sites (NRC, 2003). Adaptive management arose from concerns that conventional resource management approaches inadequately considered system dynamics and uncertainties, and that some problems in large-scale ecosystem and resource management can only be understood through experiments. In principle, the concept is not new. It is akin to the scientific method and engineering problem solving, as in "learning by doing." But it is not simply trial and error. The outcomes must be based on integrated scientific experimentation with attention to uncertainties and hypothesis testing to reduce these uncertainties. The adaptive management paradigm allows a way around the stakeholder, regulatory, and policy gridlock that characterizes cleanup at many contaminated soil and sediment sites.

The strengths and limitations of the adaptive management approach (Lee, 1999; Walters, 1997) could apply to progressively incorporating bioavailability concepts into regulations as well as they do to managing forests or fisheries. To explain how such an approach might be used, it is instructive to think through a hypothetical example. AVS/SEM (see Chapter 2) is an approach that regulatory agencies around the world have considered incorporating into sediment quality guidelines for metals, although opinions differ widely as to the suitability of the method for use as a regulatory tool (EPA, 2000b). An adaptive way of incorporating the tool in regulations might involve the following:

1. Make the management decision that AVS/SEM methodology will be used to evaluate site cleanup at, for example, a large Superfund site or in a single region (like San Francisco Bay) for a finite period of time (e.g., five years).
2. Establish a conceptual model of how the method would be applied and specific methodologies for each step of the application. For example, a contaminated site might be dredged when sediments marginally exceed total metal guidelines, with the spoils being deposited in an area where AVS/SEM > 1.
3. Design hypotheses about the outcomes of the application. For example, one could hypothesize that exposure of resident organisms to metals in the disposal area (where AVS/SEM > 1) should not increase as a result of disposal of the marginally contaminated dredge spoils. Also, benthic communities in such an area should recover after spoil disposal similarly to if the dredged spoils were not contaminated.
4. Set up a formal experimental design, including marginally contaminated pilot sites, uncontaminated pilot sites, and non-dredge sites.
5. Monitor outcomes in the different experimental treatments, for example by assessing metal concentration and form, exposures in resident organisms, benthic community changes, or predator useage. Part of the goal is to better define the relationships between chemical concentrations and biological responses.

6. Study changes in the regulatory and stakeholder responses that occur as a result of the experiment over the five-year period.

7. Feed back the results of the experiment into the AVS normalization model to improve its predictive capabilities, and then make decisions about how and whether to implement such a strategy on a larger scale.

This would constitute an intermediate step between (1) removing and treating sediment with contaminant concentrations above a fixed acceptable concentration, determined via an ecological risk assessment with minimal consideration of bioavailability processes, and (2) not doing anything.

Another example is the adaptive management approach recommended for determining the efficacy of dredging and how much PCB-contaminated sediment to dredge from the Hudson River. The EPA has formulated a cleanup plan that involves a series of performance standards by which the cleanup will be evaluated regularly (EPA, 2001c). The plan attempts to accommodate concerns about increased bioavailability of PCBs during dredging. Performance indicators will include PCB concentrations in sediment, in the water column, and in fish, and the amount of dredged material that becomes suspended in the water column. Risks will be reevaluated, and cleanup plans and objectives will be adjusted as the performance monitoring information is acquired and interpreted.

The adaptive management paradigm, embracing various well-designed pilot studies, is a viable approach to moving new bioavailability process considerations into the field and the regulatory arena. The experiments could progress from small-scale to larger-scale, from short time frame to long time frame, and from narrow perspective to broad perspective. Assessment of risk can be performed simultaneously, and the influence on risk of the bioavailability process information developed can be elucidated.

Into the Scientific Arena

Expansion of bioavailability process considerations into risk assessment and remediation decision-making for contaminated soil and sediment sites requires improved scientific understanding and models for a number of key bioavailability processes. Also required are additional federal sources of funding for bioavailability research. Some specific research needs in these broad areas are outlined below.

Mechanistic Studies and Tool and Predictive Model Development

Much research on soil and sediment contamination has been driven by regulatory agendas, with associated emphasis on the need for simple measurements and models. The result is a knowledge base limited by substantial dependence on empirical measurements and models. Models for many bioavailability processes

have weak predictive capability, a serious limitation for their use in risk assessment. In the case of human health risk assessments, for example, much information on bioavailability of contaminants transported to human receptors comes from industry-funded studies at specific sites. Those are usually, and understandably, not conducted in a way that advances understanding of fundamental underlying processes.

Greater mechanistic understanding and predictive models of bioavailability processes are needed to improve the accuracy of risk assessments for contaminated soil and sediment sites. Investment in mechanistic understanding and models will prove more profitable in the long-term than reliance on empirical knowledge because models have greater predictive power for a broader range of situations. As part of this research effort it will be important to draw ties between mechanistic understanding and more operational tests for bioavailability. For example, there have been feeding studies with different lead minerals that revealed different relative bioavailabilities, and there have been measured differences in blood lead levels in humans from mining (primarily PbS) versus urban ($PbCO_3$ or PbO) sites (Steele et al., 1990; Cotter-Howells and Thornton, 1991; Davis et al., 1992, Freeman et al., 1992, Ruby et al., 1992). But there are almost no studies that quantitatively examine both the mineralogical form of the contaminant (using X-ray absorption spectroscopy) and biological uptake (using plants or small mammal bioassays). Chapter 3 discusses other areas in need of attention, including contaminant–solid interactions, the nature and effects of aging on contaminant release rates, the role of colloids, and the feeding ecology of animals. Research areas suggested by the present chapter include better understanding of whether and when associations between contaminants and soils and sediments can be made permanent. As a corollary, describing and measuring the "activity" of solid-phase-associated contaminants should be a future research goal, including understanding how naturally occurring chemical and biochemical reactions already mediate changes in the activity of solid-phase-associated pollutants. The results from such research are needed before bioavailability explanations can be used with confidence to determine the amounts of soil and sediment remediated.

Many of the tools discussed in Chapter 4 are still in development and require future research, including some with tremendous potential for better understanding bioavailability processes. In addition to developing new tools, existing tools require research to expand their applicability to more sophisticated processes and greater numbers and types of contaminated sites. Most tools have not undergone the type of validation outlined in Chapter 4 as necessary for ensuring their accuracy and usefulness, nor can their results be generalized to multiple sites. Finally, research is greatly needed to develop a systematic approach to identifying an appropriate suite of complementary tools for use at a particular site. Such an approach should assess the state of validation for particular tools and their performance in different experimental matrices and at different sites. This would help

ensure that all important bioavailability processes relevant to a particular site are studied. At the present time, individual bioavailability tools are frequently applied, producing information that is difficult to interpret in isolation, that is extrapolated to the field without adequate scientific justification, or that is not relevant to the key bioavailability processes at a site.

Chapter 3 stressed the need for better understanding bioavailability processes at the field-scale. As a corollary, field tests are critical to determining whether proposed measurement techniques and models can accurately describe and predict bioavailability process performance at relevant scales. There has been limited investment in well-designed field experiments in which the complexity of environmental conditions can be accurately represented. Because these studies are expensive, priority should be given to selected important, recurring soil and sediment contamination problems. To provide more regulatory confidence, these studies could be conducted strictly in a pilot context before adopting the techniques widely. In addition to providing the most rigorous scientific test platform for a bioavailability measurement or modeling tool, field testing also enables realistic assessment of implementation costs and regulatory and public acceptance of the results obtained.

Funding for Bioavailability Research

Significant advances in understanding of bioavailability processes will have to come from new research. There are several potential avenues for funding of this research by federal agencies with research missions and responsibilities for managing environmental contamination. These agencies include the National Science Foundation (NSF), the National Institutes of Health, EPA, DOE, and the Department of Defense (DoD). NSF has funded a variety of studies of bioavailability processes, principally those related to interactions between environmental contaminants and media and the movement of chemicals in the environment. The National Institutes of Health, through the Superfund Basic Research Program administered by the National Institute for Environmental Health Sciences, funds a few bioavailability process studies, as does DoD, principally through the Strategic Environmental Research and Development Program. DOE is conducting research on methods for assessment of bioavailability processes as they affect remediation.

Among federal agencies, the greatest commitment to bioavailability research has been made by EPA. Over the last decade, EPA has supported nearly 100 studies on bioavailability processes through its National Center for Environmental Research. The vast majority of these research projects have involved mobility of chemicals in the environment, uptake relevant to assessing ecological risks, and bioavailability processes that might affect bioremediation. Despite this research investment, progress in understanding these bioavailability processes is quite limited. For example, the number of bioavailability field trials or mechanis-

tic studies from EPA's Superfund program is surprising low. Bioavailability studies at complex hazardous waste sites could be instrumental in designing improved risk management at those sites.

Recently, EPA has evaluated research needs and prioritized research topics (EPA, 1999); bioavailability in human health risk assessment emerged as a high priority. For example, for soils the topic with the highest research priority was "Estimating Human Exposure and Delivered Dose." This topic included focus points such as "evaluating the bioavailability of contaminants in various soil matrices," "deriving dermal absorption factors for common soil contaminants," and "developing biotransfer and bioaccumulation factors for contaminants to facilitate estimates of exposure via the food chain." Despite this high priority, however, very little in the way of sponsored research on this topic is being funded by the agency. In fact, most of what is known about the potential oral bioavailability of contaminants from soil matrices, for example, comes not from agency-sponsored research projects, but rather from studies conducted by EPA Regions, states, and responsible parties on bioavailability of lead and arsenic from contaminated sites (e.g., EPA, 1996b; Casteel et al., 1997, 2001; Freeman et al., 1992, 1993, 1995; Roberts et al., 2002). These studies offer valuable observations regarding the absorption of contaminants from soils in specific situations, and some inferences on general behavior of absorption from soils might be gained from looking at these studies collectively. However, they are not an effective substitute for directed research because they have a different objective. The purpose of these studies was to obtain empirical measurements of relative bioavailability to support a human health risk assessment. For understandable reasons, this objective does not include an exploration of factors that might influence bioavailability processes, and therefore it is difficult to determine the extent to which these observations can be generalized or used to predict the results that might be obtained at different sites or under different conditions. Unless a greater commitment is made to fund bioavailability process studies from more of a research perspective, progress in developing information that can be utilized to advance human health risk assessments will be slow.

OVERARCHING CONCLUSIONS AND RECOMMENDATIONS

Bioavailability process considerations are not uniformly or widely embraced by scientists, regulators, or the public because of a lack of scientific and technical understanding. Explicit consideration of bioavailability processes and modeling in risk assessment would help to adjust cleanup goals by more accurately identifying that fraction of contaminant total mass that has the potential to enter receptors. Also, bioavailability process understanding would help guide the selection of appropriate remediation technologies. It is clear that more numerous validated tools and models are needed and that there should be reliance on an integrated suite of tools that lead to mechanistic understanding rather than on a single tool or

wholly empirical approaches. Ultimately, bioavailability process considerations are likely to make a difference where less than an order of magnitude adjustments in chemical concentrations are sought compared to proposed action levels, and where investments in assessment of bioavailability processes lead, over time, to familiarity with specific issues. Where site-specific consideration of bioavailability processes leads to more contaminated material remaining on site, long-term monitoring is needed to assess treatment performance, validate models, and demonstrate that contaminant bioavailability is not increasing over time. The following overarching conclusions and recommendations summarize our current understanding of processes that affect whether chemical contaminants in soils and sediments are bioavailable to humans, animals, microorganisms, and plants.

Bioavailability processes are defined as the individual physical, chemical, and biological interactions that determine the exposure of plants and animals to chemicals associated with soils and sediments. First, in the broadest sense, bioavailability processes describe a chemical's ability to interact with the biological world. Second, bioavailability processes are quantifiable through the use of multiple tools. Third, bioavailability processes incorporate a number of steps not all of which are applicable for all contaminants or all settings. Fourth, there are barriers that change exposure at each step. Thus, bioavailability processes modify the amount of chemical in soil or sediment that is actually absorbed and available to cause a biological response.

Bioavailability processes are embedded within human health and ecological risk frameworks. The goal of bioavailability analysis is to reduce uncertainty in exposure estimates and thus improve the accuracy of the risk assessment. However, today "bioavailability" is commonly thought of in relation to one process only—absorption efficiency—such that a single "bioavailability" factor is used as an adjustment to applied dose. Most of the other bioavailability processes are hidden within the risk assessment process, and assumptions made about these processes are not clear. The knowledge base underlying many default assumptions about bioavailability processes is weak.

Mechanistic understanding of bioavailability processes is ultimately needed to improve the scientific basis of risk assessment. Thus, tools for measuring bioavailability processes that further mechanistic understanding and promote predictive model development are preferred over conventional empirical approaches. In the short term, empirical approaches are useful in generating site-specific information—provided that their results are analyzed using a weight-of-evidence approach and with an understanding that they will be replaced with more mechanistic tools as they are developed. At any given site, a suite of tools will be necessary to describe bioavailability processes in soils or sediments.

The potential for the consideration of bioavailability processes to influence risk-based decision-making is greatest when certain chemical, environmental, and regulatory factors align. Consideration of bioavailability processes is most likely to impact decision-making when the contaminant is, and is likely to remain, the risk driver; when the default assumptions made for a particular site are inappropriate; when significant change to remedial goals is likely (e.g., because large amounts of contaminated soil or sediment are involved); when conditions present at the site are unlikely to change substantially over time; and where regulatory and public acceptance is high. These factors should be evaluated before committing the resources needed for a detailed consideration of bioavailability processes.

Moving bioavailability concepts further into the hazardous waste arena will require specific actions at individual sites, further scientific research on critical bioavailability processes, and large-scale, coordinated testing of bioavailability tools and techniques at pilot sites. At individual sites, assessment of bioavailability processes must be accompanied by uncertainty analysis, process-based long-term monitoring to ensure that present assessments of bioavailability remain accurate and acceptable, and community involvement beginning at the early stages of remediation planning. Although bioavailability is not a unique risk communication problem, experience has demonstrated that communities often have concerns about consideration of bioavailability processes during risk assessments. In order to demonstrate the utility of explicitly considering bioavailability processes and to test new models and tools, *adaptive management* should be applied to select pilot bioavailability test sites. Adaptive management applies findings from carefully monitored experiments to the adjustment of future management and policy decisions in light of changing conditions and new knowledge.

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Appendixes

A

Acronyms

ABS	Absorption factor
AhR	Aromatic hydrocarbon receptor
APA	Administrative Procedure Act
ARAR	Applicable or relevant and appropriate requirements
ASTM	American Society for Testing and Materials
ASV	Anodic stripping voltammetry
ATP	Adenosine triphosphate
AVS	Acid volatile sulfide
BMF	Biomagnification Factor
BSAF	Biota Sediment/Soil Accumulation Factor
BTEX	Benzene, toluene, ethylbenzene, and xylene
CBR	Critical body residue
CDC	Centers for Disease Control and Prevention
CEC	Cation exchange capacity
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act
CWA	Clean Water Act
DAF	Dilution attenuation factor

DEQ	Department of Environmental Quality
DGT	Diffusive gradient in thin films
DOC	Dissolved organic carbon
EAE	Environmentally acceptable endpoint
EPA	Environmental Protection Agency
EPR	Electron paramagnetic resonance spectroscopy
EqP	Equilibrium partitioning
ESG	Equilibrium partitioning sediment guidelines
EXAFS	X-ray absorption fine structure
FTIR	Fourier transform infrared absorbance
HOC	Hydrophobic organic compound
IEUBK	Integrated Exposure Uptake Biokinetic Model
IR	Infrared absorbance
MCL	Maximum contaminant level
MGP	Manufactured gas plant
NAPL	Nonaqueous phase liquid
NCP	National Contingency Plan
NERL	National Exposure Research Lab
NMR	Nuclear magnetic resonance
NOAA	National Oceanic and Atmospheric Administration
NOM	Natural organic matter
NPL	National Priorities List
NRC	National Research Council
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyl
PCP	Pentachlorophenol
PCR	Polymerase chain reaction
PRG	Preliminary remediation goal
PTD	Polyethylene tube dialysis
RAF	Relative Absorption Factor
RAGS	Risk Assessment Guidance for Superfund
RCRA	Resource Conservation and Recovery Act

SAGE	Serial analysis of gene expression
SARA	Soil or Sediment Availability Ratio
SEM	Simultaneously extracted metals or Scanning electron microscopy
SERDP	Strategic Environmental Research and Development Program
SIMS	Secondary ion mass spectrometry
SPLP	Synthetic Precipitation Leaching Procedure
SPMD	Semipermeable membrane device
SPME	Solid phase microextraction
SQG	Sediment quality guidelines
SSL	Soil screening level
TCLP	Toxicity Characteristic Leaching Procedure
TOC	Total organic carbon
TRW	Technical Review Workgroup
USACE	U.S. Army Corps of Engineers
XANES	X-ray absorption near edge structure
XPS	X-ray photoelectron spectroscopy
XAS	X-ray absorption spectroscopy
XRD	X-ray diffraction

B

Committee Member and Staff Biographies

Richard G. Luthy, *Chair*, is the Silas H. Palmer Professor of Civil and Environmental Engineering at Stanford University. He received his Ph.D. in environmental engineering from the University of California at Berkeley and was on the faculty at Carnegie Mellon University, where he was the Thomas Lord Professor of Environmental Engineering and former head of the Department of Civil and Environmental Engineering. His research interests include physicochemical and microbial processes and applied aquatic chemistry with application to waste treatment and remediation of contaminated soil and sediment. He is noted for work on phase partitioning and the treatment and fate of hydrophobic organic compounds. Dr. Luthy chairs the NRC's Water Science and Technology Board and was a member of the NRC Committee on Innovative Remediation Technologies and the Committee on Intrinsic Remediation. He is a registered professional engineer, a diplomat of the American Academy of Environmental Engineers, and a member of the National Academy of Engineering.

Richelle M. Allen-King is a professor in the Department of Geology at Washington State University. She received a B.A. in chemistry from the University of California, San Diego, and a Ph.D. in earth sciences (hydrogeology) from the University of Waterloo, Ontario, Canada. Her research focuses on organic pollutants in the hydrologic cycle. She has particular expertise in studying the biogeochemical processes affecting pollutant fate and transport in groundwater. Dr. Allen-King is currently a member of the NRC's Water Science and Technology Board and the Science Advisory Board for the Washington State Department of Ecology's Toxic Cleanup Program.

SALLY L. BROWN is a research assistant professor in the College of Forest Resources, University of Washington. Prior to her appointment, she was a postdoctoral associate in the USDA Agricultural Research Service's Environmental Chemistry Laboratory in Beltsville, Maryland. Her research interests include the co-utilization of residuals to alleviate metal toxicity in soils and the restoration of metal-affected ecosystems; in situ remediation of lead-contaminated soils using a range of soil amendments; and identification of the mechanisms by which residuals reduce the phytoavailability of soil metals. Dr. Brown has been the project leader for a number of research and demonstration programs at highly metal-contaminated sites in the United States. She received her B.A. in political science from Williams College and her M.S. and Ph.D. in agronomy from the University of Maryland, College Park.

DAVID A. DZOMBAK is a professor of environmental engineering at Carnegie Mellon University. He specializes in aquatic chemistry, especially interactions of aqueous chemical species with mineral surfaces; fate and transport of chemicals in surface and subsurface waters; water and wastewater treatment; *in situ* and *ex situ* soil treatment; and hazardous waste site remediation. Prior to 1989 Dr. Dzombak was a consulting engineer with Paul C. Rizzo Associates, Inc., where he conducted engineering investigation, analysis, and design related to remediation of uncontrolled waste disposal sites and development of new waste disposal facilities. He holds a Ph.D. in civil and environmental engineering from the Massachusetts Institute of Technology and M.S. and B.S. degrees in civil engineering from Carnegie Mellon University. Dr. Dzombak is a diplomat of the American Academy of Environmental Engineers, and from 1996–1999 served as board member and treasurer of the Association of Environmental Engineering and Science Professors.

SCOTT E. FENDORF is an assistant professor of soil and environmental chemistry in the Department of Geological and Environmental Sciences at Stanford University. His research focuses on understanding the movement of inorganic contaminants through soils and their impact on plants and animals. Dr. Fendorf studies the chemistry of the interactions of inorganic contaminants with water and mineral surfaces using spectroscopic techniques in idealized systems and with experiments in real soils systems. He received his B.S. in soil science from the California Polytechnic State University, San Luis Obispo, his M.S. in soil chemistry from the University of California, Davis, and his Ph.D. in soil and environmental chemistry from the University of Delaware.

JOHN P. GIESY is a professor of zoology in the College of Natural Sciences at Michigan State University. A former president of the Society of Environmental Toxicology and Chemistry, his primary research focus is on the fate and effects of trace contaminants, including metals, polyaromatic hydrocarbons, pesticides,

and industrial chemicals, in aquatic systems and wildlife populations. He studies accumulation by and effects of these classes of compounds on fish, birds, and mammals, considering the biochemical mechanism of action and population, community, and ecosystem-level effects. Dr. Giesy received his Ph.D. in limnology from Michigan State University. He has recently served on the NRC Committee on Risk-Based Criteria for Non-RCRA Hazardous Waste and the Committee on Remediation of PCB-Contaminated Sediments.

JOSEPH B. HUGHES is an associate professor and chair of the Department of Environmental Engineering at Rice University. Professor Hughes' research focuses on the ability of bacteria and plants to metabolize hazardous organic chemicals. In particular, his work addresses metabolic pathways and their control, interactions of physiochemical processes and biodegradation processes, and how to modify and enrich metabolic processes *in situ*. He has chaired numerous conference sessions dedicated to contaminant bioavailability, bioremediation, and natural attenuation and is a principal investigator of contaminant bioavailability in the anaerobic subsurface. Dr. Hughes received his B.A. in chemistry from Cornell College and his M.S. and Ph.D. in civil and environmental engineering from the University of Iowa.

SAMUEL N. LUOMA is a senior research hydrologist in the Water Resources Division of the U.S. Geological Survey, where he has worked since 1976. Dr. Luoma's research centers on sediment processes, both natural and human-induced, particularly in the San Francisco Bay area. Since 1992, he has published extensively on the bioavailability of metals to aquatic organisms, with emphasis on sediments. He has also helped refine approaches to determine the toxicity of marine and estuarine sediments. In 1999, he was invited to discuss how chemical speciation influences metal bioavailability in sediments for the European Science Foundation. He has served multiple times on the EPA's Science Advisory Board Subcommittee on Sediment Quality Criteria. Dr. Luoma received his M.S. in zoology from Montana State University, Bozeman, and his Ph.D. in marine biology from the University of Hawaii, Honolulu.

LINDA A. MALONE is the Marshall-Wythe Foundation Professor of Law at the College of William and Mary, where she has worked since 1988. Prior to that she taught law at the University of Arkansas School of Law. During her career, she has clerked for Judge Wilbur F. Pell, U.S. Court of Appeals for the Seventh Circuit, and practiced law at Alston, Miller & Gaines in Atlanta and at Ross, Hardies, O'Keefe, Babcock & Parsons in Chicago. Ms. Malone is the author of numerous publications, including a treatise called *Environmental Regulation of Land Use*, and a casebook, *Environmental Law*. She was also the associate editor of the Yearbook of International Environmental Law and a member of the Advisory Board of the National Enforcement Training Institute of EPA. She received

her B.A. from Vassar College, her J.D. from Duke University, and her L.L.M. from the University of Illinois.

CHARLES A. MENZIE is principal at Menzie-Cura & Associates, Inc. He is responsible for providing environmental and risk assessment services related to soil, sediment, surface water and groundwater contamination, industrial and municipal discharges, hazardous waste sites, and RCRA and Right-to-Know Law compliance. Dr. Menzie has been involved in evaluating how bioavailability information can be used to refine exposures to both human and ecological receptors. He served as chair of the New England Workgroup concerning how to incorporate bioavailability information into risk-based approaches, and he is the co-chair of a group evaluating how bioavailability might influence and modify the ecological Soil Screening Levels. He has also directed an investigation of the comparative anatomy and physiology of vertebrate digestive systems as these may influence bioavailability. Dr. Menzie received his B.S. in biology from Manhattan College, and his M.S. and Ph.D. in biology from the City University of New York.

STEPHEN M. ROBERTS is the director of the Center for Environmental and Human Toxicology at the University of Florida and is a professor with joint appointments in the Department of Physiological Sciences in the College of Veterinary Medicine and the Department of Pharmacology and Therapeutics in the College of Medicine. He received his Ph.D. from the University of Utah College of Medicine. He has previously served on the faculties of the College of Pharmacy at the University of Cincinnati and the College of Medicine at the University of Arkansas for Medical Sciences. Dr. Roberts has an active research program to examine mechanisms of chemical toxicity, primarily involving the liver and immune system. He has also studied the bioavailability from soil of multiple chemical types (both metals and organics) following multiple exposure pathways (dermal, inhalation, and direct ingestion). He served as chair of the Florida Risk-Based Priority Council and currently provides advice to the Florida Department of Environmental Protection on issues pertaining to toxicology and risk assessment.

MICHAEL V. RUBY is an environmental chemist at Exponent who specializes in evaluating the transport and fate of toxic pollutants and the availability of these compounds to both human and ecological receptors. He has more than 12 years of experience in designing and managing studies of source determination, exposure pathway evaluation, and bioavailability of organic and inorganic contaminants at a variety of historical and operating industrial facilities. Mr. Ruby has directed multidisciplinary projects, with emphasis on soil, sediment, and water quality issues, driven by human health and ecological risk assessment concerns. He has developed risk assessments, feasibility studies, and remedial strategies for sites affected by inorganic and organic contamination of surface water, groundwater,

soil, air, and sediments. He received his B.A. in chemistry from the University of California, San Diego, and his M.S. in physical chemistry at Stanford University.

TERRY W. SCHULTZ is a professor of environmental toxicology in the Department of Ecology and Evolutionary Biology at the University of Tennessee. He also holds appointments in the Department of Animal Science and Department of Comparative Medicine and the University's Center for Environmental Biotechnology. Dr. Schultz's research is centered on the elucidation of cellular mechanisms of acute toxicity; the development of rapid and inexpensive assays for the evaluation of environmental toxicity; the development of structure-activity models for predicting toxic potency and advancing the basic understanding of toxicology; and use of bacterial bioluminescent assays, protozoan population growth inhibition assays, and yeast recombinant systems for endocrine disruption. His current research involves bacterial, protozoan, algal, daphnid, and fish endpoints. He received his B.S. from Austin Peay State University, his M.S. from the University of Arkansas and his Ph.D. from the University of Tennessee.

BARTH F. SMETS is an associate professor of environmental engineering at the University of Connecticut, Storrs. He also holds a joint appointment in the Department of Molecular and Cell Biology. His research is in the area of environmental biotechnology and biodegradation of organic and xenobiotic compounds including nitroglycerin, nitrotoluenes, and polyaromatic hydrocarbons. He is particularly interested in understanding how microbial populations and communities encountered in engineered or natural systems function and change under continuous or occasional exposure to environmental stress. Dr. Smets received his M.S. in applied biological sciences/biotechnology, State University of Ghent, Belgium, and his Ph.D. in environmental engineering and science from the University of Illinois. In 1999, he received the Outstanding Junior Faculty Award in the University of Connecticut's School of Engineering.

LAURA J. EHLERS is a senior staff officer for the Water Science and Technology Board of the National Research Council. Since joining the NRC in 1997, she has served as study director for nine committees, including the Committee to Review the New York City Watershed Management Strategy, the Committee on Riparian Zone Functioning and Strategies for Management, and the Committee on Assessing the TMDL Approach to Water Quality Management. She received her B.S. from the California Institute of Technology, majoring in biology and engineering and applied science. She earned both an M.S.E. and a Ph.D. in environmental engineering at the Johns Hopkins University.