# 3. METALS RISK ASSESSMENT RECOMMENDATIONS

The metals risk assessment recommendations are a formulation of the primary concepts and factors that should be considered when conducting assessments of the ecological or human health risks associated with metals exposures. The fundamental metals principles, outlined in Section 2.1, that should be considered throughout metals risk assessment are integrated into these recommendations as appropriate. The recommendations are not intended to provide a prescriptive step-by-step guide on conducting metals risk assessment. Rather, they are intended to promote the consistent application of the various tools and methods currently available to risk assessors so that assessments take into consideration the unique properties of metals. Moreover, these recommendations are presented with the intent that they be used in parallel with currently available Agency guidance for human health and ecological risk assessment.

Section 4 provides the risk assessor with an expanded discussion of the underlying issues and methods that form the basis for the recommendations in Section 3. In addition, the metals recommendations rely on the final metals issue papers, which have been developed by scientists commissioned under contract by EPA to identify current information, tools, and methods in metals science. The final metals issue papers are available on the EPA Web site at http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=86119. Section 5 reviews research needs based on the limitations in the currently available tools and methods; it also reviews gaps in our understanding of metals behavior and effects on humans and the environment.

## 3.1. HUMAN HEALTH RISK ASSESSMENT RECOMMENDATIONS

Assessing the risks of metals to human health is similar to conducting assessments of organic substances. However, risk assessors should pay particular attention to the metal-specific principles discussed in Section 2. These include the influence of environmental chemistry on speciation, bioavailability, natural and ambient background levels of metals in the environment, and the ubiquitous presence of metal mixtures. Additionally, certain properties associated with toxicokinetics and toxicodynamics, carcinogenesis and non-cancer, and the sensitivity of particular subgroups, are unique to metals and should be incorporated into the risk assessment process. Data from standard toxicity tests should be interpreted appropriately in light of these metal-specific attributes. Tools are available for addressing most of these metal-specific attributes to one degree or another during a human health risk assessment.

### 3.1.1. Fate and Transport

Transport or movement of metals through the physical environment (soil and sediment, surface and ground water, and air) is a function of the source characteristics (e.g., discharge point), the nature of the metal species or compound (e.g., chemically reactive or recalcitrant; dissolved, complexed, or solid/precipitate), and the physical/chemical characteristics of the receiving environment. Generally, the fate and transport of metals are not greatly affected by macrobiota (e.g., via tracking), but the influence of local microbial processes can be significant (including mobilization in the root zone, and methylation) (see Section 4.1.9) Therefore, such analyses are equally relevant to human health and ecological risk assessments. Consequently, recommendations pertaining to the fate and transport of metals are provided in Section 3.2. for the aquatic environment (e.g., water, sediment, associated biota), and in Section 3.3. for the terrestrial environment (e.g., air, soil, associated biota).

### 3.1.2. Exposure Assessment

Assessing human exposures involves evaluating how people can be exposed to metals and in what amounts. Specific steps include (1) identifying the forms (speciation) and concentrations of metals in the media to which people are exposed (e.g., soil, water, air, or biota, accounting for setting-specific biogeochemical conditions); (2) describing how people contact these media, by what specific routes they are exposed (e.g., incidental ingestion or inhalation); (3) determining the appropriate exposure metric (e.g., oral intake, air concentration, or blood concentration), and quantifying that metric (e.g., estimating the amount ingested, or determining the blood concentration); and (4) characterizing the uncertainty and natural variability associated with these estimates, where possible.

#### 3.1.2.1. *Background*

Metals, being part of the periodic table of the elements, constitute a portion of the natural background to which all humans are exposed. Metals are an important constituent of the diet and the Earth’s crust. For example, 95% of the general population’s exposure to copper is through the diet, and concentrations of natural levels of organic arsenicals in seafood can be sufficiently high to cause the urinary arsenic concentration to resemble that of an occupational exposure. Risk assessments addressing anthropogenically introduced metals should separate such introductions from natural levels, or levels associated with other contributions beyond the scope of the assessment, and account for the possibility of cumulative exposure. The former is critical for informing risk management decisions. The latter, considering cumulative exposures, is technically challenging because of potential differences between the bioavailability of naturally occurring metals and anthropogenically introduced metals. In addition to the human health-specific recommendations that follow, risk assessors are encouraged also to refer to the background discussion in the terrestrial risk assessment recommendations (Section 3.3) as it is applicable to human exposures.

***Recommendations:***

- Consideration of background exposures is important in risk assessments and may have a variety of aspects. For example, when considering soil ingestion pathways, if feasible, it is important to account for the potential differences in the bioavailability of naturally occurring metals and metals more recently introduced via anthropogenic activities. See section 4.5.4 for further discussion.

- If blood, urine, or other tissues are being used to assess exposure to a metal from a particular source (e.g., arsenic in drinking water), consideration of other contributing exposures is important.

### 3.1.2.2. *Air Pathway and Inhalation Exposure*

For most metals, inhalation exposure is to metals associated with particles. For particle size exposures, the size of the particles is an important factor in potential for respirability, subsequent dosimetry and health risk. The particle size of interest for inhalation exposure is generally those particles less than or equal to 10 microns in diameter ($PM_{10}$); larger particles do not penetrate far into the respiratory tract and can be cleared to the ingestion route. Additionally, toxicity of a metal can vary depending on its valence state or with the compound. In developing inhalation exposure estimates, attention should be given to the form of metal as compared to the form used in the dose-response assessment (e.g., RfC, IUR). Measuring or estimating the total amount of a metal without regard to speciation may introduce uncertainties into inhalation exposure and risk. See Section 4.2.2.2 for further discussion.

***Recommendations:***

- For inhalation assessments for metals that rely on monitoring data (e.g., using area or personal samples), consideration of particle size is important, and the primary focus generally should be on metals associated with particles that are less than or equal to 10 microns in diameter ($PM_{10}$).

- As feasible, inhalation exposure estimates should be specific to the metal speciation in order that there can be pertinent correspondence with the form of the metal used for the dose-response assessment (e.g., in deriving the RfC or IUR estimate).

### 3.1.2.3. *Soil, Dust, and Dietary Exposure Pathway*

Humans can be exposed to metals in soil and dust primarily via incidental ingestion, or via inhalation. Diet plays a dominant role in common human exposures to metals (via plant and animal uptake and food chain transfer). The dermal pathway is not considered a major pathway for metal intake because the relatively low lipid solubility of most metals limits absorption through the skin. However, some metals may produce skin toxicity (e.g., allergic contact dermatitis), which would require consideration of dermal exposure. See Section 4.2.2.3 for further discussion.

***Recommendations:***

- Owing to relatively low lipid solubility of most metals, dermal absorption from soil and dust is generally low and could be ignored in most metal assessments.

- Depending on the exposure situation, portal of entry dermal sensitization can be an issue. The assessor should consider dermatitis at least in a qualitative sense as a health endpoint of concern, particularly for nickel, chromium and arsenic.

- Exposure from incidental soil or dust ingestion should be adjusted for bioavailability before adding to intake from oral exposure pathways (U.S. EPA, 2004a). See Section 4.2.4 for further discussion.

- Concentrations of metal in food (i.e., dietary intake) should be adjusted to account for relative bioavailability differences; a default value of 100% can be used for metals that are biologically incorporated in food. For diets that contain metals deposited onto food (e.g., dust or other particles), bioavailability should be assumed to be the same as metals in soil. See Section 4.2.4 for further discussion.

### 3.1.2.4. *Water Pathway and Oral Exposure*

Humans consume water specifically treated for human consumption (i.e., drinking water); thus, the exposure context of metals in human drinking water will be very different from that of exposures to ambient water. Inorganic forms of metals in drinking water will consist of the more bioavailable, water-soluble species.

Estimation of intake of metals in drinking water requires information about concentrations of metals in the finished water and the amount of water consumed. Metal concentrations in drinking waters are measured at the distribution point for municipal water delivery systems. The contribution of metals from pipes (either from the distribution system to the home or within the home) is rarely assessed. Water delivered from private wells or ambient surface waters may contain higher levels of organic carbon or other ligands to which metals can bind, thereby requiring an adjustment to account for differential bioavailability of the dissolved metals. These factors can be incorporated into site-specific assessments, but local data will need to be collected on a case-by-case basis. See Section 4.2.2.5 for further discussion.

***Recommendations:***

- Regional differences in bioavailability of metals due to variation in water characteristics (e.g., hardness), the contribution of household distribution systems to total metal load at the tap, and lack of information from households on private wells should be included as uncertainties in national-level risk assessments.

- It is recommended that site-specific assessments use measured metal concentrations at the tap.

- Although people can be exposed to metals dissolved in ambient surface water during swimming, other recreational activities, or various occupational activities, dermal absorption can be considered a negligible exposure pathway.

### **3.1.2.5.** ***Integrated Exposure Approaches***

The many pathways that might be associated with metals exposure represent an important consideration for the risk assessor. For lead, EPA has developed the Integrated Exposure Uptake Biokinetic (IEUBK) model to address these pathways. The model simulates the transfer of lead into the blood, with consideration of absorption and elimination as a result of inhalation and oral exposures. Similar models for other metals have not yet been developed. While similar models for other metals have not yet been developed, there are a variety of exposure models that could potentially be applied in metals risk assessment (e.g., SHEDS, DEPM, TRIM, RESHRAD). These models, and their applications and limitations, are discussed in Section 4.2.4.1.

***Recommendation:***

- The IEUBK model for lead in children is recommended for use in all site-specific assessments. It is available on line at http://www.epa.gov/superfund/programs/lead/ ieubk.htm. The IEUBK model may be used to account for lead accumulation resulting from differential rates of uptake and elimination. This model is not applicable to other metals.

### 3.1.2.6. *Bioavailability*

Bioavailability of metals is an important consideration in human health assessments, particularly when assessing risk of metals in soil. Metals may form organic complexes in water, bind to soil substrates or be incorporated into soil matrices, or form complexes with food materials, and the bioavailability of the metal in the study on which the dose-response assessment is based (e.g., animal feed, water, or corn oil) may differ from that of the metal in the matrix being assessed (e.g., soil). Different approaches have been used to address bioavailability, depending on the data available. These approaches have been advanced particularly with regard to soil ingestion. EPA's OSWER has developed a relative bioavailability approach to adjust the RfD. Further discussions on bioavailability issues are presented in Sections 2 and 4.2.4.2., and in McGeer et al. (2004), NRC (2002), and U.S. EPA (2004).

***Recommendations:***

- To address relative bioavailability, the preferred approach is to use information directly relevant to the conditions being assessed, e.g., data from animal toxicology studies that use the metal form encountered in that environment.

- Where this information is not available (e.g., in screening-level assessments), the recommended approach is to assume that the bioavailability of the metal in the medium being assessed is the same as that of the metal in the study on which the dose-response (e.g., RfD, RfC, or cancer slope factor) was based.

- For higher level, definitive risk assessments, a medium-specific default absorption factor for the metal may need to be used, and in such cases the factor should be as representative as possible of the conditions being assessed.

- For lead, the juvenile swine model presently is the preferred animal model to use for development of relative bioavailability factors (U.S. EPA, 2004a).

- When validated, in vitro methods for determining lead relative bioavailability in soils may be used in place of animal studies (U.S. EPA, 2004a).

### 3.1.3. Effects Analysis

#### 3.1.3.1. *Physiologically Based Pharmacokinetic (PBPK) and Physiologically Based Pharmacodynamic (PBPD) Modeling*

Combined use of PBPK and PBPD models provides understanding of the complex relationships between exposure and target organ effects. These models are valuable risk assessment tools for purposes of interspecies, high dose/low dose, rout to route, and exposure scenario extrapolation. PBPK models that include a fetal compartment are particularly valuable for human health risk assessment of metals, as transplacental exposures cannot be directly measured from environmental measurements. An additional specific recommendation regarding the IEUBK model is provided in Section 3.1.2.5.

***Recommendations:***

- When using PBPK models or other dosimetric adjustments, absorption/distribution and kinetic factors should be considered explicitly. The models require special considerations for cellular uptake, interaction with nutritionally essential and nonessential metals, protein-binding behavior and function, incorporation into bone, metabolism, and excretion (see Table 4-11 in Section 4.2.6).

- Application of a PBPK model to risk assessment should satisfy the following key criteria: (1) identifying toxic or active form(s) of the metal, (2) selecting the appropriate dose metric, and (3) identifying the appropriate target organ or cells on the basis of the health effect of greatest concern.

- These available models for lead (see section 4.2.6.1) can be used to estimate fetal exposure but are limited in that they rely on assumptions of a steady state between maternal and fetal blood lead concentrations, which is violated if the mother is no longer exposed to lead during pregnancy. This assumption should be stated in the risk characterization.

- Similar models for other metals are not available at this time, so transplacental transfer cannot be estimated for other metals.

#### 3.1.3.2. *Essentiality*

Some trace elements, such as cobalt, copper, iron, and zinc (Table 4-12 in Section 4.3.2), are necessary for biological functions and the normal development of humans, other animals, and plants. These metals are required for organism health at one range of concentrations and can be toxic at higher quantities. For essential elements that exhibit biphasic dose-response curves, adverse effects resulting from deficiency should be considered as well as those that result from excessive exposure.

***Recommendations:***

- In setting reference values (Reference Concentrations [RfCs]/RfDs) the Recommended Daily Allowance (RDA) should be taken into consideration.

### 3.1.3.3. *Toxicity Testing*

At least five transition metals or metalloids—arsenic (through drinking water exposure), cadmium, chromium VI, beryllium, and nickel (from pulmonary or, in some cases, dermal exposures) — are accepted as human carcinogens in one form or another or in particular routes of exposure (NTP, 2002). Inorganic lead compounds are considered probable human carcinogens by EPA's IRIS program, and IARC has concluded there is limited evidence of carcinogenicity to humans. Target organ sites for metals as carcinogens are summarized by Waalkes (1995). Many noncarcinogenic effects can also be caused by exposures to metals. See Section 4.3.5 for further discussion.

***Recommendations:***

- It is recommended that metals risk assessments follow the same approach for carcinogenicity assessment as used for all other substances (U.S. EPA, 2003b), with modifications as indicated by unique metal-specific data or methodology that may exist.

- In parallel, the same general approach applied to assess non-cancer endpoints for other chemicals should also be followed for metals, except where modifications are warranted.

For example, an extensive model has been developed to evaluate lead, but a completely parallel model does not exist for assessing a wide range of other metals and other chemicals. For arsenic, unlike many other chemicals, the standard toxicity test data for rodents are not the primary basis for assessing human toxicity.

### 3.1.3.4. *Metals Mixtures*

Metals are normally found in the environment as mixtures, and risks may be mitigated or enhanced by any associated interactions. There are generally three classes of interactions between metals: (1) between nutritionally essential metals; (2) between non-essential metals;

and (3) between essential and non-essential metals. Additionally, the relative dose and mixture composition, as well as mode of action of each metal in the mixture, is especially important in determining overall health consequences. One form of interaction, termed “molecular” or “ionic” mimicry, is an important consideration in evaluating the health effects of metals. Selenium, for example, may play a protective role with regard to ingested arsenic. See Section 4.3.6 for further discussion.

***Recommendations:***

- For metal mixtures, as for other mixtures, the assessor is referred to EPA’s guidelines and supplemental guidance for the assessment of mixtures (US EPA, 1986a; US EPA, 2000b).

- Unless other information is available, the default approach is to assume dose additivity for individual metals that produce the same effects by similar mode of action.

- In the case of metals with known differences in critical effects, separate effect assessments are encouraged for each metal. Interactive effects (e.g., synergism, antagonism) should be included if information as available or acknowledged as a source of uncertainty.

- While additional research is needed to explore the issues of metal mimicry and its effect on assessment, site assessments should consider the available information on metal mimicry related to the metal(s) of interest, including that referenced in this framework.

#### 3.1.3.5. *Sensitive Subpopulations and Life Stages*

Metals have a relatively robust literature on susceptible populations. For example, the efficiency of intestinal uptake of zinc declines in the elderly, absorption of cadmium may be greater in premenopausal women with depleted iron stores, and various genetic polymorphisms have been associated with the effects of metals, including copper, iron, lead, and nickel. See Section 4.3.7 for further discussion.

***Recommendations:***

- For metals, as with other agents, Agency risk assessments should consider subpopulations with differing sensitivities that may arise as a result of differential exposure or susceptibility.

## 3.2. METALS RISK ASSESSMENT RECOMMENDATIONS FOR AQUATIC ENVIRONMENTS

This section presents recommendations for assessing exposure and effects of metals in aquatic systems. Many of the suggested methods for fate and transport apply to human as well as ecological risk assessments. Recommendations given are specific to aquatic biota residing in the water column and sediments, unless otherwise noted. Particular methods and models described in the recommendations are discussed in more detail in Section 4.4, the metal issue papers, and referenced citations.

### 3.2.1. Fate and Transport

Approaches for evaluating the fate and transport of metals in aquatic environments include measurements and models. Selection of methods will depend on the nature and scale of the problem. For example, for applications where metals have already been released to the environment (e.g., at a hazardous waste site), measurements of the distribution of the metals among environmental compartments is often an important starting point for the assessment. For applications where decisions need to be made on wasteload allocations, such as for determination of Total Maximum Daily Loads (TMDLs), models play a central role; these are typically supported by measurements. For national assessments, generic models that capture a range of conditions may be most appropriate. A suite of measurement and modeling tools exists. Selection of the most appropriate combinations should be commensurate with the problem at hand. In many cases, relatively simple approaches will suffice, and in others more sophisticated methods are warranted. A complicated model is not necessarily a "better" model to use than a simple one; the appropriateness for use of a specific model needs to be decided on a case-by-case basis.

Measurement programs that support evaluations of fate and transport are addressed in other Agency guidance and are not addressed here; however, these should be considered an appropriate part of assessments. Measurement approaches for evaluating the bioavailability of metals are discussed later in this subsection. Recommendations related to the use of fate and transport models for the assessment of metals in aquatic environments are provided below.

An important parameter in modeling the transport and fate of metals in aquatic systems is the partition coefficient. The partition coefficient sets the distribution of the metal between the dissolved and sorbed phases; this, in turn, has important implications to the magnitude of particulate and diffusive transfers between the water column and sediment, and in assessing metal bioavailability. However, assuming equilibrium conditions is a potential oversimplification in some situations (e.g., in the vicinity of a point source discharge to a receiving water or during a pulse exposure), and this could be a significant source of uncertainty in the exposure assessment. See Section 4.4.1 for further discussion.

***Recommendations:***

- The reviews by U.S. EPA (1997) and Paquin et al. (2003), include up-to-date information with regard to the availability of models appropriate for use in evaluating fate and transport of metals in aquatic environments. These reviews also include descriptions of example applications of many of the models that are discussed. These reviews and Section 4.2.2.1 provide a good starting point for selecting among available models.

- Relatively simple steady-state and analytical solution models may be appropriate for use in the screening level. Use of conservative assumptions at this level of analysis may provide insight about whether or not it will be necessary to complete a more detailed, definitive risk assessment.

- Relatively complex and sophisticated time-variable models are appropriate for use in higher level, definitive assessments. These models, in particular, should always be used by an analyst who is experienced in the use of models and familiar with the structure of the model being employed. Predictions of fate and transport of metals in aquatic systems may be accomplished by using integrated models that include hydrodynamic, sediment transport, and chemical transport algorithms, or by using stand-alone hydrodynamic and/or sediment transport models that should then interface with a chemical fate model. The advantage of the former approach is that integration of the hydrodynamic, sediment, and chemical transport results takes place in a seamless manner with limited need for intervention by the analyst. This is in contrast to use of the stand-alone models, where the output of one model needs to be formatted in such a way that it is amenable to use by the subsequent models that are to be applied. An advantage of the latter approach is that it can in some instances reduce the need to rely on lengthy model run times.

- Most of the available transport models do not currently include chemical speciation subroutines. In such cases, chemical equilibrium models such as MINTEQ serve as useful alternatives for characterizing the forms of the metal that are present.

- Water quality analyses often require probabilistic results, as the Water Quality Criteria (WQC) specify not-to-exceed concentrations for a once-in-3-year return period. Steady-state models are not directly amenable to evaluation of a return period for WQC exceedences. When using these models, a Monte Carlo analysis can be conducted to generate a large number of model inputs and subsequent solutions, which can then be analyzed statistically to characterize the probability of an exceedence of the effect level of interest. Time-variable models that generate long-term time series results (e.g., 20 years) can also be statistically analyzed to evaluate the frequency of exceedences.

- The risk assessor should consider the potential for situations where nonequilibrium conditions exist, as this may have important implications to the partitioning of a metal between dissolved and particulate phases and to the characterization of metal speciation and bioavailability that is provided by a chemical equilibrium model (see Section 4.1.4).

- An important parameter in modeling the transport and fate of metals in aquatic systems is the partition coefficient. The partition coefficient sets the distribution of the metal between the dissolved and sorbed phases; this, in turn, has important implications to the magnitude of particulate and diffusive transfers between the water column and sediment, and in assessing metal bioavailability. However, assuming equilibrium conditions is a potential oversimplification in some situations (e.g., in the vicinity of a point source discharge to a receiving water or during a pulse exposure), and this could be a source of uncertainty in the exposure assessment. See Section 4.1.4 for further discussion.

- Special care should be taken when modeling metals (e.g., chromium) and metalloids (e.g., mercury and arsenic) that can readily change oxidation state or undergo transformation. Such changes affect physical and biological properties. Although many of the same transport models can be used, input parameters will require modification.

### 3.2.2. Water Column Exposure, Bioavailability, and Effects

Potential exposure routes for aquatic species include inhalation/respiration, dermal absorption, and dietary (from either food or incidental sediment ingestion). However, owing to the diversity of aquatic organisms, the extent to which a metal is taken up by any one of these exposure routes is difficult to define for all relevant routes. With those limitations in mind, most of the focus on exposure and effects has been on the binding of metals to the gill surface for short-duration exposures and the resultant toxicity.

Approaches for evaluating exposure and effects include measurements and chemistry-based exposure models. Laboratory and in situ toxicity tests and field assessments of biota have all been used to evaluate metals. These will continue to have an important place in developing chemical-based methods and in assessing conditions at particular water bodies. These approaches have been described in other Agency guidance and are not repeated here.

Accounting for bioavailability should be an essential part of metal assessments; however, a host of factors can influence the amount of metal that interacts and is taken up across biological surfaces, and water quality can have a dramatic influence on toxicity. Depending on the level of assessment, different methods and approaches can be used to incorporate bioavailability.

Ambient Water Quality Criteria (AWQC) are developed to support the Clean Water Act, and since the 1980s aquatic life criteria for several cationic metals have been expressed as a function of water hardness to address the relationship of certain cations on toxicity. However, water hardness adjustments do not account for other important ions and ligands that can alter metals bioavailability and toxicity. The Water Effect Ratio (WER) was developed in the 1990s to address this issue directly. This is an empirical approach to make site-specific bioavailability adjustments to criteria (U.S. EPA, 1994). This approach relies on comparing toxicity measurements made in site water with those made in laboratory water to derive a WER. The WER is then used to adjust the national criterion to reflect site-specific bioavailability.

Recent developments in understanding the physiology and toxicology of metals have enabled further advances in incorporating bioavailability into assessments. Different forms of the dissolved metal have differing bioavailabilities; free metal ions generally are the most bioreactive, and complex forms generally are much less so. The relationship between speciation and bioavailability has been developed as the Free Ion Activity Model (FIAM) (Campbell, 1995), and geochemical modeling software such as CHESS, MINEQL, WHAM, and MINTEQ are powerful tools in understanding speciation. The development of speciation profiles for a dissolved metal concentration via modeling and/or direct measurement will help to provide information at a particular site of interest and can be used on a comparative basis between sites to reduce the variability associated with different exposure conditions.

Although the link between bioavailability of metals and factors influencing speciation (such as pH, temperature, and organic and inorganic anionic complexation) are of prime importance, other abiotic factors, particularly cations, influence metal bioaccumulation and toxicity. Dissolved cations such as $Na^+$, $K^+$, $Ca^{2+}$, and $Mg^{2+}$ can competitively inhibit metal uptake and the recent development of integrated toxicity prediction models. Biotic Ligand Models (BLMs) have successfully combined abiotic speciation, cationic competition, and bioaccumulation at the presumed site of toxic action (as reviewed by Paquin et al., 2002).

Applicability of the BLM is being extended to chronic toxicity through, for example, the use of the Ion Balance Model (IBM) of Paquin et al. (2002a, b, c). Although the approach, which was initially applied to silver, may ultimately provide a way to predict effects due to metals over varying exposure durations, further development and testing are required.

Overall, the BLM approach has wide application in terms of understanding bioavailability in relationship to toxicity because it incorporates speciation in the exposure medium, bioaccumulation, and toxic impacts in a robust approach that has been possible to apply in a variety of contexts to account for differences in bioavailability. For example, the BLM has recently been incorporated as part of the revision of the WQC for copper, it has been used in the context of risk assessment, and it is being applied as an alternative to the application of WER approaches for setting site-specific discharge and cleanup objectives. When considering the application of this approach, as with all models, care should be taken to understand and explicitly account for the assumptions and potential sources of uncertainty. It should be recognized that the BLMs are being developed only for a subset of metals (e.g., copper, nickel, cadmium, silver, lead, and zinc) and are based on experimental data from a limited number of test species. Additionally, most BLMs predict acute toxicity, although a few are being developed that predict chronic toxicity (e.g., the IBM). Considerable research efforts are ongoing, and it is likely that our understanding of metal bioavailability and the method for integrating this knowledge into prediction models will improve quickly in the coming years. See Section 4.5.7 for further discussion.

***Recommendations:***

- Methodologies for evaluating the bioavailability and toxicity of metals in water include hardness adjustments, site-specific WERs, and use of the FIAM and BLM. Selection of an appropriate approach will depend on the metal of interest and the availability of data. The FIAM and BLM have been shown to offer dramatic improvements over traditional approaches, and these models are preferred for metals for which adequate supporting data have been developed.

- Hardness should be used as a normalizing function for metal toxicity only when information on speciation is lacking.

- The BLM offers a mechanistic approach for relating the bioavailability of metals to toxicity. It can be used to develop or revise water quality criteria (e.g., as in the case of copper), in risk assessments, and as an alternative to the WER approaches for setting site-specific discharge objectives.

- Quantitative Ion Character Activity Relationships (QICARs) are available for select species and may be used to extrapolate availability and/or toxicity for a metal for which data are limited. However, the QICAR approach may require validation prior to each application.

### 3.2.3. Background

Background concentrations of metals may contribute to and exacerbate toxicological effects and accumulations of metal from various exposure sources or, conversely, it may result in adaptation of organisms to higher metal concentrations and result in increased tolerance to exposure. Thus, consideration of background metal concentration is an important part of risk assessment.

Concentrations of metals in waters of the U.S. vary tremendously. Thus, use of a single number to represent all areas within the U.S. is discouraged. See Sections 4.5.4.1 and 4.5.4.2 for further discussion.

Regarding the discussion on the 'added risk approach", this approach has been used in Europe, but has recently been reported to be unreliable by the European Commission Health and Consumer Protection Directorate General, Scientific Committee on Toxicity, Ecotoxicity, and the Environment (CSTEE) (equivalent to EPA SAB) (EC Health and Consumer Protection/CSTEE, 2004). They report: "The CSTEE is of the opinion that current knowledge on the geographic distribution of metal background concentration in aquatic systems is insufficient to correctly implement the added risk approach… and,.. not accounting for bioavailability in both the MPA (maximum permissible addition, i.e., water quality criteria as an amount added to background) and Cb fraction (background fraction) results in the incorrect assessment of the risks of metals and thus prevents the establishment of science-based EQS (environmental quality standard). The CSTEE suggests that an accurate assessment of the risks (or EQS) posed by metals should be done by establishing - on a site-specific-, watershed/basin- or regional basis - both the bioavailable total fraction in the environmental compartment/medium ($Ec_{bioavailable}$ - background) and the bioavailable total no effect concentrations ($PNEC_{bioavailable}$)."

***Recommendations:***

- While work is underway to define average (and ranges) of background concentrations for ecoregions, the recommended default is to use state averages where possible and to always define the range that might be encountered within the spatial scale being considered. See section 4.5.4.1 for further discussion.

- For site specific assessments, a physical and/or temporal boundary needs to be defined then background should be described, estimated, or measured. (See section 4.5.4.1 for more information.)

- Although consensus on a methodology has not been reached at this point, it is recognized that the bioavailability of the background , as well as the anthropogenic contribution, should be assessed in terms of both exposure and impact.

### 3.2.4. Bioaccumulation

Characterization of metal bioaccumulation is an important component of many Agency assessments that range in complexity (i.e., from screening-level to definitive assessments) and overall scope (i.e., from to site-specific assessments to national ranking and characterization). Assessments may consider bioaccumulation either in terms of the potential for the metal to directly affect the organism and/or as the basis for estimating exposure to consumers via a trophic pathway. Understanding this component is relevant for ecological risk assessments, and also for human health assessments when the food chain pathway is involved.

The prevalence and importance of bioaccumulation in Agency assessments has brought increasing attention on the scientific validity and uncertainty of the methods used to characterize and quantify metal bioaccumulation. To reduce uncertainty in metals assessments, robust connections should be established between the bioaccessible/bioavailable form(s) of metals in various exposure media, their accumulation, metabolism and distribution in tissues, and the form(s) of metals that exert their toxicity directly to the organism or indirectly to its consumers. However, for many metal-organism combinations, data are lacking on the mechanism(s) of absorption, distribution, metabolism, and excretion, as well as mode of action. Improved understanding of these issues would improve linkages between exposure and effects or accumulation and effects.

It has, however, been established that, within certain limits, organisms either regulate their uptake of metal, store metals in forms not toxic to the organism (but potentially toxic to their consumers), or are efficient metal excretors. Which one of these strategies is employed may differ by metal and organism, particularly for those metals that are essential micronutrients. For example, for aquatic organisms that regulate uptake or excretion of a nonessential metal, tissue levels of metals are most frequently inversely related to water concentrations. Where the organism is effectively regulating internal levels of an essential metal, the amount in the organism can remain constant over a certain range of increasing environmental concentration. Under both scenarios, toxicity will occur only when some threshold is reached where the organism's regulatory mechanisms become overwhelmed. BAFs/BCFs should be derived using mathematical relationships that represent the concentration in the organism or tissue as a function of the concentration in the exposure medium/media (for BCF/BAF) for each set of exposure conditions. It is important to emphasize that these relationships should always be based on the environmentally available or bioaccessible fraction of the metal in those media (e.g., water column or sediment), to normalize the relationships for site-specific differences in environmental chemistry and associated metal speciation.

Furthermore, with many metal-organism combinations, the bioaccumulation process cannot be quantified with sufficient rigor to enable unambiguous predictions of metal residues in tissues that are toxicologically meaningful for evaluating impacts to aquatic organisms. Generally, the linkage between bioaccumulation as measured by whole body concentration and the potential for toxicological impact (i.e., the hazard), is lacking. In the case of direct toxic impacts, the *rates* of metal accumulation are generally more meaningful than tissue residues. Thus, the latest scientific data on bioaccumulation do not currently support the use of BCF and BAF data as generic threshold criteria for the acute or chronic hazard potential of metals. See Section 4.5.8 for further discussion.

***Recommendations:***

- BAFs/BCFs should not be used as indicators of acute or chronic metal toxicity. Aquatic organisms have regulatory mechanisms for actively excreting excess metal and/or for storing excess metal in detoxified forms which makes these relationships highly uncertain.

- Assessment of metal bioaccumulation should consider bioaccessibility, bioavailability, essentiality, acclimation/adaptation, regulation of metals (uptake and internal distribution), detoxification and storage as well as background accumulation. While the ability to quantitatively address all of these factors may be limited at the present time, their potential impacts should at least be qualitatively addressed..

- The latest scientific data on bioaccumulation do not currently support the use of BCF and BAF values when applied as generic threshold criteria for the hazard potential of inorganic metals (e.g., for classification as a “PBT” chemical).

- Because BCFs/BAFs of metals are influenced by organism background concentrations and metabolism or storage mechanisms, they should be expressed as a function of water concentration.

- The BCF/BAF derivation should also account for the concentrations and forms of the metal in the exposure media, to reflect speciation, bioaccessibility, and bioavailability as indicated. These BCF/BAF values should be limited to site-specific applications.

- First order kinetic (single-compartment models), pharmacokinetic (multi-compartment models) and biotic ligand models may also be useful for estimating bioaccumulation.

- Bioconcentration and bioaccumulation models should be developed using the bioaccessible/bioavailable form of the metal, to normalize for differences in environmental chemistry. Where data from the literature include only total metal in the environmental media, speciation models may be used to estimate this fraction. If such models are unavailable, the risk assessor should clearly state that use of such data increases the uncertainty of the risk characterization.

- For site-specific risk assessments, the preferred approach for assessing metal bioconcentration and bioaccumulation is through direct measurement from biota at the site.

- Metals become toxic when regulatory mechanisms are overwhelmed and the concentration at a site of toxic action exceeds a toxic threshold (known as critical body [or tissue] residues, CBRs). Because BCF/BAFs are dependent upon exposure concentrations, they should not be used as predictors of CBRs.

### 3.2.5. Trophic Transfer, Biomagnification, and Dietary Toxicity

Assessment of the potential impacts associated with trophic transfer of metals requires an understanding of the linkages between exposure and bioaccumulation in both the prey organism and the predator (or consumer) organisms. Organisms that bioaccumulate elevated levels of metals and make them available for consumer organisms are of concern. Examples include phytoplankton that can bioaccumulate some metals to several orders of magnitude above water concentrations; organisms that detoxify and store metals; and organisms that accumulate metals from sediments and, when consumed by aquatic animals, can translocate sediment-bound metals into aquatic food webs. However, biomagnification of inorganic forms of metals across multiple (i.e., three or more) trophic levels in aquatic food webs is considered rare. Biodilution can also occur for metals as Chen and Folt (2000) observed for As and Pb.

Notably, concentrations in both the predator and prey organisms can arise from multiple sources and uptake pathways (e.g., water as well as sediment), thus rendering field measures of metal body burdens an ambiguous measure of trophic transfer in these situations (which, by definition, reflects contaminant transfer from the diet only). Although the accumulated metal in a prey organism may serve as a source of exposure and toxicity to predator species, the presence of a significant metal burden in the prey does not necessarily mean that this metal is bioavailable to the predator. The bioavailability of accumulated metals in prey species to their predators can vary significantly based on multiple factors, such as the form(s) of metal in tissues, the digestive physiology of the predator, and the tissues in which the metal is stored (e.g., exoskeleton vs. soft tissue). Development of analytical tools for quantifying the bioavailable fraction of accumulated metals to consumers (e.g., analysis of tissue fractions such as cytosolic metals) is currently an emerging area of research (e.g., Wallace and Luoma, 2003; Wallace et al., 2003). See Section 4.5.8 for further discussion.

***Recommendations:***

- Bioaccumulation and trophic transfer of metals does occur and can be an important source of exposure. In particular, consumers with high rates of metal uptake relative to elimination rates and high metal assimilation efficiencies are generally of greatest concern in aquatic food webs. However, biomagnification of inorganic forms of metals across three or more trophic levels in aquatic food webs is rare. Therefore, assessment of food web biomagnification alone is generally not an important aspect of classifying hazards or risks of inorganic metal compounds.

- Although the prediction of toxicity due to dietary exposure to inorganic metals is complicated by wide variation in the bioavailability and toxicity of accumulated metals, it is a factor that should be considered in metals assessments. Direct approaches to accomplish this include quantifying the bioavailable fraction of accumulated metals to consumers (e.g., analysis of tissue fractions such as cytosolic metals). Bioassay methods, such as feeding field-collected contaminated prey to predators under controlled conditions, offer another way to assess trophic transfer of metals, although such methods have not been widely standardized. These methods may be particularly useful for site-specific assessments, but they require knowledge of predator-prey relationships to be most applicable to field settings. Kinetic models also may be useful for predicting metal bioaccumulation in aquatic food webs (including trophic transfer); they show the most promise when applied and calibrated on a site-specific basis using experimentally derived kinetic parameters (e.g., uptake, assimilation, elimination, and growth dilution) that reflect typical environmental exposures. Efforts should continue to refine, evaluate, and apply models when warranted.

### 3.2.6. Sediment Exposure and Effects

Toxicity testing and benthic community assessments will continue to be important for evaluating exposure and effects of metals in sediments. Chemical-based approaches specific to evaluating metals in sediments have received increased attention. These include the simultaneously extracted metals-acid volatile solids (SEM-AVS) approach and use of interstitial pore water measurements. These approaches are focused primarily on estimating the toxicological potency (bioavailability) of metals in sediments to sediment-dwelling invertebrates. However, some uncertainties associated with estimating exposures to benthic invertebrates should be recognized. These include the creation of microenvironments by sediment organisms (e.g., irrigated burrows) and the influence of exposure through the diet sediment or metal-contaminated prey. For these reasons, the exposure pathway involving direct exposure to pore water is recognized as a simplification. Nevertheless, laboratory and field support exist for its application. See Section 4.5.10 for further discussion.

***Recommendations:***

- The SEM-AVS approach is the best available tool for screening analysis based solely on sediment chemistry. It is currently applicable to mixtures of copper, cadmium, zinc, lead, nickel, and silver.

- It is recommended that the use of SEM-AVS should be focused on identifying sediments where direct toxicity to benthos is *not* expected (i.e., SEM-AVS < 0); it is less powerful for predicting when toxicity will occur because it does not account for all factors that influence toxicity. A modification of the method is available to account for the presence of organic carbon within the sediment matrix.

- Consideration of uncertainties in the SEM-AVS approach include whether it is fully applicable to organisms living in microenvironments (e.g., irrigated burrows) or whether it adequately accounts for exposure via sediment ingestion. Some bioaccumulation of metal in benthic organisms may still occur when SEM-AVS < 0.

- The equalibrium partitioning (EqP) interstitial water approach may be combined with the SEM-AVS approach and direct measures of toxicity as part of multiple line-of-evidence approaches.

- The EqP interstitial water approach is best applied to identify nontoxic sediments; sediments that exceed this guideline may or may not show toxicity.

- The EqP interstitial water approach has been evaluated primarily for copper, cadmium, zinc, lead, nickel, and silver. Although the rationale may apply to other metals and metalloids, supporting data should be sought before making this extrapolation.

- For oxic sediments, additional tools that may be used to relate metal lability/availability in sediments to toxicity include: pH-dependent partition coefficients of metals (generally linear over a range of interstitial water pH values); chemical, acid, and biomimetic extraction methods.

### 3.2.7. Metals Mixtures

Some of the recommendations given above apply to exposures and acute effects of select mixtures of metals in surface water or interstital pore water; methods for assessing chronic effects of mixtures are not widely developed. There are no realistic means to rank mixtures of metals or individual metals within mixtures. Recommendations follow on assessing risks of mixtures in aquatic environments. See Section 4.5.3 for further discussion.

***Recommendations:***

- The Concentration Addition Model is recommended for assessing acute toxic effects of mixtures with fewer than six components. In this approach, all metals in a mixture are added together to predict toxicity; differing potencies are taken into account by converting chemical concentrations to an equitoxic dose using either Toxic Units (TUs) or Toxicity Equivalence Factors (TEFs) (for aquatic organisms, this can be accomplished through application of the BLM as a means of assessing binding of various metals to respiratory membranes). These calculations convert all metals to a single exposure/toxicity metric.

- The QICAR effects addition approach may be used to predict binary interactions of metals with joint independent action. The deviation from joint independent action can be predicted from the degree of similarity in binding tendencies for the paired metals.

## 3.3. METALS RISK ASSESSMENT RECOMMENDATIONS FOR TERRESTRIAL ECOSYSTEMS

As with any chemical, toxicity associated with metal exposures generally is estimated by using standard laboratory bioassays that are conducted in a manner similar to tests performed with organic substances. However, the response of organisms to metals is complicated by several factors that need not be considered for most organic substances. These include the natural background concentrations of the metals (including, but not limited to, the metal of interest) in either the test media or the site of concern, the acclimation of test animals to the culture conditions, whether or not the metal is an essential micronutrient, the potential interaction of the various metals, and the bioavailability of the metal. Recommended methods used to account for the influences of each of these factors that modify toxicity are presented here.

### 3.3.1. Fate and Transport

#### 3.3.1.1. *Atmospheric Chemistry and Behavior*

With mercury being a notable exception, most metals and metal compounds occur primarily in the particle phase of the atmosphere. For purposes of risk assessment, the size of the particles is important. The aerodynamic size and associated composition of particles determines their behavior in the mammalian respiratory system, and particle size is one of the most important parameters in determining atmospheric lifetime of particles, as well as playing a role in associated deposition behavior. For those metals that may undergo transformation, much of the atmospheric chemistry takes place in the aqueous phase (e.g., cloud droplets or water films on particles).

***Recommendation:***

- Models to estimate metal speciation in atmospheric aquatic aerosols are of limited availability, with the use of the Community Multi-Scale Air Quality Model (CMAQ) for mercury being a notable exception. See Section 4.1.7. for further discussion.

#### 3.3.1.2. *Soil Mobility*

Primary processes governing the environmental fate and transport of metals in the subsurface are advection, dispersion, matrix diffusion, and retardation (U.S. EPA, 1994a). Advection and dispersion are functions of the system rather than of the contaminant, so they are not specific to metals. Matrix diffusion, which is a function of the contaminant, is relatively unimportant and is omitted in most soil transport models. Retardation is contaminant and site specific. Because of this complexity, and the interactions between metals and environmental factors, proper characterization of soils is an important aspect of assessing and/or predicting the fate and transport of metals in terrestrial environments. Soil characteristics that can influence the fate and transport of metals include pH, the type and amount of organic matter (OM) in the soil matrix, soil particle size, soil compaction, and soil cohesiveness. Soil pH is among the most important properties to determine because it is considered the “master soil variable” that influences virtually all fate processes for metals in soil. These include solubility, precipitation, speciation, and sorption processes as well as microbial activity. See Section 4.4.1 for further discussion on soil transport models, and Section 4.4.1 for additional information on partition distribution coefficients ($K_d$).

***Recommendations:***

- A wide variety of analytical and chemical techniques may be used to characterize metal speciation. For particle-bound metals, direct measurement tools include x-ray absorption spectroscopy (XAS), x-ray diffraction (XRD), particle-induced x-ray emission (PIXE and μPIXE), electron probe microanalysis-scanning electron microscopy (EPMA-SEM), secondary ion mass spectrometry (SIMS), x-ray photoelectron spectroscopy (XPS), sequential extractions, and single-chemical extractions. See Section 4.1.8, Table 4-9, for further information about the applicability and limitations of each approach.

- Computer models may be used to predict speciation of metals in soil solutions. These include the Windermere Humus Aqueous Model (WHAM) (Tipping, 1998, 1994) and Non-Ideal Competitive Absorption (NICA) model (Gooddy et al., 1995). See Lumsdon and Evans (1995) for a good review of metal speciation models.

- Models using partition distribution coefficients ($K_d$) have significant inaccuracies for metals, and the application of single partition coefficient values for individual metals should be limited to site-specific assessments or to regional- and national-scale studies where bounds of potential $K_d$ values, or reasonably representative single values are adequate.

- The MINTEQ model can be used to generate generic partition coefficients that may be applied to regional or national mobility evaluations (epa.gov/ceampubl/mmedia/minteq/index.htm or lwr.kth.se/english/OurSoftware/Vminteq/).

- Linearity of log $K_d$ with pH may be assumed as a default approach for national-scale assessments. The value of log $K_d$ for metal cation adsorption can be assumed to increase linearly with pH, whereas the value of log $K_d$ generally decreases with pH for anion adsorption (Langmuir, 1997; Tessier, 1992). Partition coefficients tabulated as a function of pH are available and a useful way to proceed; non-pH-dependent values are available for lead (900), mercuric chloride (58,000), and elemental mercury (1,000) (U.S. EPA, 1999a).

- For higher level, site-specific assessments, metal adsorption may be estimated fairly accurately using literature information to estimate the sorption properties of metal oxides, clays, and OM, which are then used in diffuse layer sorption models. If greater accuracy or site specificity is required, it may be necessary to measure metal adsorption in laboratory experiments.

- For site-specific assessments, $K_d$ values developed for the site or for a similar location are generally preferred over generic or default values.

- If fluid flow in porous soil is isotropic and adsorption is fast, reversible, and linear, the Diffuse Layer (DL) model (also called the Generalized Two-Layer Model, or GTLM) in MINTEQA2 can be used to predict adsorption and precipitation behavior as a function of pH.

- PHREEQC, which has the DL metal adsorption model, can be used with the 3-Dimensional Flow, Heat and Solute Transport model (HST3D, a ground water flow and transport model), and the Chemical Transport (CHMTRNS) model also can be used to model metal transport through porous media.

#### 3.3.1.3. *Transformation in Soils*

Methylation and demethylation of organic mercury compounds in soils are mediated by the same types of abiotic and microbial processes that occur in aquatic systems. Because soils are primarily oxygenated systems, particularly in the root zone, conditions favorable to sulfide formation and bacterial methylation occur infrequently. With the exception of peat bogs and similar anoxic, highly saturated soils, methylation generally occurs only at very low rates in soils. Plants also can transform metals and metalloids taken up from the soil. The most notable example is selenium. Soluble inorganic oxanions of selenium are readily taken up by plants and converted to organoselenium compounds, such as selenomethionine, selenocysteine, dimethyl selenide, and dimethyl diselenide.

The dissolution and transformation of a metal compound in soil is influenced by chemical and physical properties of the compound and of the soil. Environmental parameters, such as temperature and humidity, have a strong influence on the rate of transformation. When metal salts are added to soil, the form of the salt dictates the rate and amount of soluble metal that will form in the pore water. For example, insoluble forms of metals (e.g., vanadium pentoxide [$V_2O_5$]) will transform to soluble free ion (V) at a slower rate than will soluble metal salts (e.g., $Na_3VO_4$). However, it should be kept in mind that the rate of formation of the free ion is not proportional to the dissolution rate of the salt, as aging reactions that take place at the same time as transformation and dissolution will remove the free ion from the pore water. See Section 4.1.9 for further information.

***Recommendations:***

- In areas with well-oxygenated soils where methylation rates are very low, the mercury in soils and plants can generally be assumed to be an inorganic form.

- In lieu of site specific information, selenium in oxygenated soils should be assumed to be in the inorganic form.

- Selenium in plants and soil invertebrates should be considered as an organic compound for food chain analysis.

- Volatilization of all metalloids (mercury, selenium, arsenic) and organometallics should be considered when conducting detailed site-specific fate and transport models.

- Relative rates of dissolution and aging should be considered simultaneously to accurately predict pore water concentrations.

### 3.3.2. Exposure Assessment

Terrestrial wildlife, plants, and invertebrates accumulate metals from direct contact with soil or sediment, from ingestion of contaminated food (plants or other animals), and from incidental soil or sediment ingestion. Pathways of exposure include movement from soils through the food web and, to a lesser extent, air deposition either into soils or directly onto terrestrial receptors (e.g., plants). Because of significant differences in exposure patterns, it is convenient to discuss methods by receptor group (invertebrates, plants, wildlife) rather than by pathways or environmental compartments, with the exception of the contribution of natural background to total exposure, which is applicable to all organisms.

#### 3.3.2.1. *Background*

EPA has provided detailed guidance on estimating background concentrations for site-specific assessments (U.S. EPA, 2002b, c, 2000c). Statewide average background soil concentrations are available in the U.S. EPA ecological soil screening levels (EcoSSLs) document (U.S. EPA, 2003c). Additional information on concentration of metals in soils at smaller spatial resolutions is provided in Shacklette and Boerngen (1984). Some metals (e.g., iron, copper, zinc ) are included in the State Soil Geographic Database (STATSGO) available at www.nrcs.usda.gov/technial/techtools/ stat_browser.html. These data can be grouped at whatever spatial scale is required, but they are not screened for whether they represent true background concentrations. See Section 4.5.4 for further information.

***Recommendations:***

- Use of a single number to represent background concentration for metals in all areas within the United States is of very little or no value owing to high variability across such a large geographic area.

- Averages (and ranges) of background concentrations for various ecoregions should be defined (Hargrove and Hoffman, 1999; Bailey, 1998; Omernick, 1986).

- If ecoregion-specific information is not available, a recommended default when conducting national-scale assessments is to use state averages.

- The range of background concentrations that might be encountered within the spatial scale being considered always should be defined.

- For a site-specific release of a highly bioavailable form of a metal, the background concentration may be a negligible contribution to ecological exposure and may be ignored in assessing ecological risk for that metal.

- For areas of contamination where added metals have aged significantly, reduced bioavailability should be considered. Thus, it becomes important to estimate exposure in terms of the bioavailable fraction.

- For site-specific risk assessments, it may not be necessary to consider background during the initial screen; bulk soil concentrations may be compared directly with derived toxicity thresholds for soil organisms.

- For metals that do not pass the initial screen (based on bulk soil measurements), adjustments for site specific bioavailability may be performed that will address the significantly lower bioavailability of contributions from background.

- Natural background levels should be taken into consideration during any remediation decisions because reducing soil metals below naturally occurring values will alter the plant and soil invertebrate community composition (potentially as dramatically as did the anthropogenically elevated soil levels).

#### 3.3.2.2. *Soil Invertebrates and Plants*

The assessment of exposure and effects of metals on terrestrial plants and soil invertebrates should involve evaluating the bioavailability of the metals to the organism, the accumulation of metals in tissues, and the toxicity of the metals. The accumulation of metals in plants or soil invertebrates may be used as an indication of toxicity but also is a consideration when evaluating exposure to animals that feed on them. See Section 4.5.4 for further information.

***Recommendations:***

- Bulk soil concentrations collected in the top 0–12 cm of soil can be used as an initial estimate of exposure for soil organisms for all types of assessments.

- It is recommended that bulk soil concentrations be adjusted to account for bioavailability factors in higher level, definitive assessments; bioassays using site soils are encouraged for areas that do not meet initial screening level values.

- In higher level, definitive assessments, the organic matter on top of the soil (the "duff") should be analyzed separately to provide further detail on exposure to detritivores (such as *Collembola*) and deeper soil-dwelling organisms (e.g., various species of earthworms).

- Normalizing methods may also be useful for addressing soil variability in soil toxicity tests. Two normalizing metrics that have been used are percent organic matter and cation exchange capacity (CEC). Notably, it should be remembered that CEC is a function, at least in part, of soil pH. Therefore, normalization can be done only among soils of similar pH ranges.

- Most accurate estimates of exposures are generally achieved through measuring or modeling concentration of metals in soil pore water.

- Use of metalloregions for national-level assessments is encouraged to account for natural background levels and consequent adaptation of soil organisms.

- In characterizing risks to soil invertebrates and plants, it is important to recognize that soil concentrations below natural background may be harmful to native biota.

#### 3.3.2.2.1. *Soil Invertebrates*

Ecological and anatomical differences among soil invertebrates influence exposure to and bioaccumulation of metals present in the soils. The soil ecosystem includes a complex food web of soil invertebrates (both hard- and soft-bodied invertebrates) that feed on each other, decaying plant material, and bacteria or fungi. For risk assessment purposes, however, exposure is typically described as a function of soil concentration rather than as a detailed analysis of movement of metals through the food web. Although this is a reasonable approximation for soft-bodied invertebrates (e.g., earthworms) whose exposure is primarily through soil pore water, there is more uncertainty in correlating soil concentrations with effects in hard-bodied invertebrates. These animals are exposed primarily through ingestion of food and incidental amounts of soil. Direct measurements, when possible, provide a means of ascertaining bioaccumulation for these hard-bodied species and can be used to judge the relationships between those residues and the bioavailable concentrations in soils and the tissue levels in soft-bodied animals such as earthworms. See Section 4.4.2.4.1 for further information.

***Recommendations:***

- For site-specific assessments, direct toxicity testing of the soil of concern often is the best method for assessing bioavailability and toxicity-to-soil biota (Fairbrother et al., 2002).

- Critical body residues (CBRs) provide an approach for judging the toxicity of metals to soil invertebrates based on tissue measurements. However, only a few CBRs have been developed for metals in soil invertebrates: cadmium and zinc in the springtail and cadmium in earthworms. See Section 4.5.9.2.

- For site-specific assessments, the concepts of pollution-induced community tolerance (PICT) may be useful for assessing effects on soil invertebrate communities. See Section 4.5.2.

#### 3.3.2.2.2. *Plants*

Plants access metals through the pore water and have both active and passive mechanisms for taking up or excluding metals, depending on internal concentrations and whether the metal is an essential micronutrient or whether it is mistaken for an essential micronutrient. Plants can be exposed to metals via aerial deposition onto leaf surfaces, trapping metals in hairs or on rough cuticular surfaces that may provide an exposure route for herbivores. See Section 4.4.2.4.2 for further information.

***Recommendations:***

- The “soil-plant barrier” concept—developed for evaluating biosolids—influences the potential for bioaccumulation as well as toxicity. General assumptions that may be used include the following: strongly acidic soils (less than a pH of 4.5) increase plant uptake of zinc, cadmium, nickel, manganese, and cobalt and increase the potential for phytotoxicity from copper, zinc, and nickel; in alkaline soils, the high pH (greater than 8) increases uptake of molybdenum and selenium; and lead and chromium are not absorbed by plants to any significant extent at any pH.

- The influence of soil chemistry on metal speciation should be considered when judging the potential for accumulation and/or toxicity to plants. Reduced soils can form sulfide, and sulfide forms low-solubility compounds for most metals, including lead, zinc, cadmium, copper, and nickel. For essential elements (e.g., zinc, copper, nickel), low-solubility species can result in deficiency syndromes. Upon oxidation of the soil, sulfide is quickly oxidized, and the metals are returned to more normal equilibrium reactions of aerobic soils.

- Aerial deposition of metals onto leaf surfaces can be assumed inconsequential for plant exposure, with the exception of volatilized forms of metals, in particular mercury and lead.

- General categories of uptake based on bioavailability of metals to plants are shown in Section 4.

#### 3.3.2.3. *Wildlife*

With some exceptions, risks of metals to wildlife species will typically involve estimating dietary exposures and relating these to Toxicity Reference Values (TRVs). For some vertebrates (e.g., especially amphibians), dermal exposures will be important. There also may be circumstances where inhalation exposures are important. Better understanding of when to consider dermal and inhalation pathways is needed. The recommendations given below emphasize the dietary route of exposure because this appears to be the most important pathway for most wildlife species and is better understood. This pathway includes food, water, and incidental soil or sediment ingestion. The relative importance of the dietary versus incidental soil ingestion pathways is dictated by (1) the degree to which food items are able to bioaccumulate metals from the soils, (2) the degree of incidental soil ingestion as a fraction of overall food intake, and (3) the relative bioavailability of the metal in the soil as compared to the metal in food items. See Sections 4.5.9 and 4.5.12 for further information.

***Recommendations:***

- Incidental ingestion of soil can be assumed to be an important route for exposure to wildlife when (1) the BAF from soil to food (e.g., to plants or soil invertebrates) is less than 1 and (2) the fraction of soil in the diet is greater than 5%.

- The same variables that restrict uptake by plants or other soil organisms can be assumed to reduce bioavailability to wildlife that ingest soil directly; an exception may be the lower pH that occurs in the digestive systems of many animals.

- Measured concentrations of metals in soil, surface water, and food items can be used in food chain models to estimate total exposure, or concentrations in food can be modeled using literature or site-specific bioaccumulation models. The recommendations given earlier for estimating bioaccumulation of metals in plants and invertebrates are applicable to estimating food concentrations for wildlife exposure models.

- Although bioaccumulation and trophic transfer of metals does occur, biomagnification (i.e., increases in concentration through the food web) is rare and may be assumed to be unimportant. Exceptions are the organometallic compounds, such as methyl mercury, that do exhibit biomagnification. It follows that with the exception of organometallic compounds, biomagnification is not an important consideration when evaluating the hazards of metals or when ranking metals based on hazard.

- CBRs reduce uncertainties because they account for site-specific bioavailability and multiple exposure pathways and should be used when available. However, very little information is available for metal CBRs in terrestrial wildlife; exceptions are methyl mercury, lead, selenium, and cadmium (Beyer et al., 1996). Tissue residues of other metals can be used to indicate that exposure is occurring but will not allow a determination of risk to be made.

#### 3.3.2.4. *Food Chain Modeling*

Food chain modeling is used to estimate the exposure of wildlife to metals based on ingestion of soil, food, and water, and may also be useful in human health risk assessments. The basic format of the model is the same as that for organic substances. Detailed explanations are available in several related documents, such as U.S. EPA (2002h), (2003c), and (2004d). See also Sections 4.4.3 and 4.5.12 for additional information.

- EcoSSLs guidance – http://www.epa.gov/ecotox/ecossl/pdf/ecossl_guidance_chapters.pdf
- Ecological Committee on FIFRA Risk Assessment Methods (ECOFRAM) – http://www.epa.gov/oppefed1/ecorisk/rra_chap_three.htm
- OAQPS Fate, Transport and Ecological Exposure Model (TRIM.FaTE) – http://www.epa.gov/ttn/fera/data/trim/tsdv2-sect7b-sept.pdf

***Recommendations:***

- Measured or modeled concentrations of metals in soil, surface water, and food items may be used in food chain models. Available food chain models vary in their complexity (e.g., ranging from the use of soil-biota relationships and trophic transfer factors to bioenergetic models that simulate transfers of metals via inhalation and ingestion.)

- Information on diet, foraging area, similar topics is found in U.S. EPA (1993).

- In using wildlife exposure estimates to assess wildlife risk in screening assessments, relative (not absolute) bioavailability generally should be assumed to be either 1 (default value) or an appropriate site-specific estimate. However, very little information is available on dietary bioavailability for most wildlife species, particularly owing to differences in digestive physiology and anatomy across the board and the diverse range of mammalian and avian species. General guidelines are provided for some metals (e.g., lead) in NRC (1980), and values derived for human risk assessments (e.g., for lead) in U.S. EPA (2004) may be useful for animal species that have digestive systems similar to those of humans (generally omnivores); such information would be inappropriate for herbivorous animals. If data for one species (e.g., humans, cows, chickens) are used for another species, the uncertainties associated with animal to animal extrapolations for absorption of metals should be described.

- Food chain modeling should be used in national criteria-setting or hazard-ranking exercises. Screening level approaches and default values are detailed in the U.S. EPA EcoSSL documentation (U.S. EPA, 2003c).

- For site-specific risk assessments, it is recommended to begin with using default parameters and then successively adding more site-relevant data as particular species-exposure route combinations are not screened out; for methodology, see Fairbrother (2003).

#### 3.3.2.5. *Bioaccumulation*

Characterization of metal bioaccumulation is an important component of many Agency assessments that range in complexity (i.e., from screening-level to definitive assessments) and overall scope (i.e., from to site-specific assessments to national ranking and characterization). Assessments may consider bioaccumulation either in terms of the potential for the metal to directly affect the organism and/or as the basis for estimating exposure to consumers via a trophic pathway. Understanding this component is relevant for ecological risk assessments, and also for human health assessments when the food chain pathway is involved.

The prevalence and importance of bioaccumulation in Agency assessments has brought increasing attention on the scientific validity and uncertainty of the methods used to characterize and quantify metal bioaccumulation. To reduce uncertainty in metals assessments, robust connections should be established between the bioaccessible/bioavailable form(s) of metals in various exposure media, their accumulation, metabolism and distribution in tissues, and the form(s) of metals that exert their toxicity directly to the organism or indirectly to its consumers. However, for many metal-organism combinations, data are lacking on the mechanism(s) of absorption, distribution, metabolism, and excretion, as well as mode of action. Improved understanding of these issues would improve linkages between exposure and effects or accumulation and effects.

It has, however, been established that, within certain limits, organisms either regulate their uptake of metal, store metals in forms not toxic to the organism (but potentially toxic to their consumers), or are efficient metal excretors. Which one of these strategies is employed may differ by metal and organism, particularly for those metals that are essential micronutrients. Where the organism is effectively regulating internal levels of an essential metal, the amount in the organism can remain constant over a certain range of increasing environmental concentration. Under this scenario, toxicity will occur only when some threshold is reached where the organism's regulatory mechanisms become overwhelmed. BAFs should be derived using mathematical relationships that represent the concentration in the organism or tissue as a function of the concentration in the exposure media (for BAF) for each set of exposure conditions. It is important to emphasize that these relationships should always be based on the environmentally available or bioaccessible fraction of the metal in those media (e.g., soil), to normalize the relationships for site-specific differences in environmental chemistry and associated metal speciation. See Section 4.4.3 for further information.

Furthermore, with many metal-organism combinations, the bioaccumulation process cannot be quantified with sufficient rigor to enable unambiguous predictions of metal residues in tissues that are toxicologically meaningful for evaluating impacts to soil organisms. Generally, the linkage between bioaccumulation as measured by whole body concentration and the potential for toxicological impact (i.e., the hazard), is lacking. In the case of direct toxic impacts, the *rates* of metal accumulation often are more meaningful than tissue residues. Thus, the latest scientific data on bioaccumulation do not currently support the use of BAF data as generic threshold criteria for the acute or chronic hazard potential of metals. See Section 4.5.6.3 for further information.

***Recommendations:***

- Measurements of metals in plant tissues, when possible, provide the most direct method for assessing bioaccumulation. When the data are to be used to evaluate exposure to higher trophic levels, measurements should be made of the appropriate edible tissues.

- Because BAFs of metals are influenced by organism background concentrations and metabolism or storage mechanisms, they should be expressed as a function of soil concentration.

- The BAF derivation should also account for the concentrations and forms of the metal in the exposure media, to reflect speciation, bioaccessibility, and bioavailability as indicated. These BAF values should be limited to site-specific applications.

- The latest scientific data on bioaccumulation do not currently support the use of BAF values when applied as generic threshold criteria for the hazard potential of inorganic metals (e.g., for classification as a "PBT" chemical).

- For national-level assessments, equations from Sample et al. (1999, 1998a, b) for uptake of metals from soil for soil invertebrates and for vermivorous wildlife (e.g., songbirds, voles, and shrews) and from Efroymson et al. (2001) for plant uptake may be used. However, the soil invertebrate models are not specific to soil type and therefore do not account for bioavailability factors such as pH, clay content, or cation exchange. Furthermore, they do not adequately predict chromium or nickel uptake.

- Soil parameter values from the 5th to 95th percentile of the area of concern should be used to bound the possible uptake rates (alternatively, Monte Carlo approaches can be used, assuming appropriate distributions for each parameter value).

- If using models for screening-level assessments, site-specific assessments may use measured soil parameter values. For detailed assessments, site-specific assessments may use either measured values of metals in soil organisms or site-specific bioassays to determine uptake rates (see Fairbrother, 2003).

- The highest accumulation of metals in plants occurs in the roots, and, except for hyperaccumulator species, most plant trophic transfer rates can be assumed to be <1.

- Plants are quite sensitive to some metals and may die before achieving levels high enough to be toxic to animals.

### 3.3.3. Toxicity Assessment

Because metals are naturally occurring substances, toxicity assessment for ecological receptors exposed to metals requires an understanding of both the natural mechanisms for tolerance for (or, in the case of micronutrients, the use of) metals and the toxicological responses that occur when exposure exceeds the capacity of the organism to regulate its body burdens. Interactions between metals in either their uptake or toxicity (such as cadmium/calcium/zinc, mercury/selenium, copper/molybdenum) also should be considered in toxicity assessments. Risk assessments for metals are further complicated by the need to express the dose-response (or concentration-response) functions of bioavailable units that are functionally equivalent to measures of exposure. Issues of essentiality, appropriate toxicity tests, and acclimation or adaptation to continued exposures also should be considered.

#### 3.3.3.1. *Adaptation and Acclimation*

Organisms have developed various mechanisms to cope with variable background metal concentrations, particularly for the metals that are essential elements. The genetic makeup of an organism defines its ability to cope with variable environmental conditions. The shifting of tolerance to a metal within the genetically defined limit of an organism is known as *acclimation* and generally involves physiological changes that may not always be passed on to offspring. Genetic *adaptation* results from increased survival of tolerant genotypes and subsequent changes in gene frequencies. Laboratory experiments conducted with $F_1$ generations obtained from metal-contaminated habitats provide the strongest evidence to support a genetic basis of tolerance (Klerks and Levinton, 1993), and new methods in toxicogenomics (e.g., microarrays) are providing additional insights. The ability of organisms to adapt or acclimate to metals in their environment should be considered when assessing chronic risks or determining the relative ranking hazards of metals. See Section 4.5.2 for further information.

***Recommendations:***

- For national-scale assessments, the country should be subdivided into metalloregions, such that protection levels, mitigation goals, and ranking results will be appropriate for the suite of species naturally present within each type of controlling environment.

- For site-specific assessments, the concepts of PICT can be applied.

- All reports of toxicity effects used in setting TRVs or benchmarks, whether for generally applicable national assessments or for site-specific assessments, should be critically examined for information about how test organisms were raised and housed.

- For national-scale assessments, organisms should be acclimated to standard soils or (preferentially) to the various metalloregion soils.

- For site-specific assessments, acclimation should take place in soils with similar characteristics (e.g., percent clay, pH, OM, CEC) as the site of concern.

#### 3.3.3.2. *Essentiality*

Essentiality is the requirement for metals in normal organism metabolic function. It is one of the primary factors that differentiates risk assessment for metals and metal compounds from that of synthetic organic chemicals and, therefore, requires specific consideration in risk assessments. Some trace elements, such as cobalt, copper, iron, manganese, selenium, molybdenum, and zinc, are necessary for the normal development of plants and animals. Other metals, such as arsenic, cadmium, lead, and mercury, have no known functions. Others may have beneficial metabolic effects but have not been shown to be essential. For essential elements, TRVs should not be below the optimal concentration range (or safe intake range). If set too low (i.e., in the range where deficiency can occur), the determination of risk will be erroneous and organisms actually may be harmed owing to lack of essential nutrients. See Section 4.5.1 for further information.

***Recommendations:***

- Metals that plants and wildlife require as micronutrients for normal organism metabolic function are identified in Table 4-14 in Section 4.5.1.

- Derived toxicity threshold values for essential elements should be used in screening-level risk assessments for both national and site-specific applications, if they are no more than 10-fold lower than the nutritional requirements. Otherwise, the required levels should be used as a threshold for allowable exposures. See Section 4.5.1.

- Higher level, definitive assessments may require additional bioassays to characterize the biphasic dose-response curve and determine both required and excessive threshold levels.

- For wildlife, the literature on dietary requirements of essential elements for livestock can be consulted. The National Research Council has published useful summaries (NRC, 1994, 1980), and a recent publication updates this information (McDowell, 2003).

- Minimum concentrations required for plant growth are summarized in Epstein (1972, 1965) and Kabata-Pendias and Pendias (2001).

### 3.3.3.3. *Metals Mixtures*

Mixtures of metals (including metalloids and other contaminants) are commonly encountered in the natural environment as a result of anthropogenic inputs and the natural co-occurrence and enrichment of many metals. Interpretation of available information on the toxic effects of metal mixtures is complicated by differing measures and definitions of the bioavailable fraction of metals, whether it is the fraction that is available for uptake from the environment or at the site of toxic action. Two key questions should be addressed in risk assessments of metal mixtures: To what extent does each metal contribute to any observed effect? Are the effects significantly greater than or less than the sum of the individual component effects? See Section 4.5.3 for further information.

***Recommendations:***

- Metals that have the same mode of action initially can be assumed to be additive in effect.

- For metals with assumed additivity, either Concentration Addition or Effect Addition models can be used (see Section 4.5.3 for descriptions of these models).

- Effect Addition models, especially if based on body or tissue concentrations, are more accurate than Concentration Addition models but require reliable dose-response and bioaccumulation curves for all single metals and then careful testing of the models.

- National criteria for mixtures may not be possible because the combined effects depend on relative amounts of each metal and their relative bioavailability.

### 3.3.3.4. *Toxicity Testing*

Variability among soil toxicity test results is due in part to the influence of soil properties on bioavailablity of metals. Additionally, incorporation of sparingly soluble substances, such as many environmental forms of metals, into the soil matrix is difficult, and acclimation/adaptation of test organisms can further complicate test results. Use of soluble metal salts with the addition of organism to the test matrix immediately after mixing is not representative of most environmental situations, where aging and other physical/chemical processes affect metal speciation and uptake. However, a large body of literature on the toxicity of metals to soil organisms and wildlife already has been developed. The risk assessor should use these data, taking into account the test-to-test variability in soil chemistry parameters and differences between laboratory test matrices and real-world conditions. See Section 4.5.11 for further information.

***Recommendations:***

- Existing toxicity data for soil organisms (plants and invertebrates) should be adjusted on the basis of OM, pH, and (if available) CEC to account for bioavailablity differences before use in any type of risk assessment or hazard ranking.

- Field data may be compared with laboratory toxicity response information by measuring metals in soil pore water from field assessments and comparing such data to spiked laboratory soils. This will increase the accuracy of the comparison by increasing the similarity of the bioavailability of the measured fraction.

- The effects of metals on plants can be evaluated by using either toxicity tests or published data. Toxicity tests performed by adding bioavailable metal salts to soils may provide different results than seen in field conditions with long-term metal exposures. The differences in bioavailability could be corrected by either allowing the added metals in the bioassays to age or by expressing metals in test soils in the environment as the bioavailable fractions (for protocols, see Fairbrother et al., 2002). Alternatively, measurement of metals in pore water from either field or laboratory studies will increase the comparability of results. The guidance provided for development of EcoSSL values (U.S. EPA, 2003c) is useful for judging the applicability of literature studies to plant toxicity threshold determinations.

- Sequential extractions of soil samples also may be used to estimate the bioavailable fraction. These treat soil samples with a succession of reagents intended to specifically dissolve different and less available phases of metals. Many of these techniques are a variation on the classical method of Tessier et al. (1979), in which metal associated with exchangeable, carbonate-bound, iron-manganese bound, organically bound, and residual species are determined. Although these methods are preferable to total metal extraction, relating the sequential fraction extracted to metal bioavailability is open to debate. For the application and limitations of extraction techniques, assessors should review Beckett (1989), Kheboian and Bauer (1987), and Foerstner (1987).

- Toxicity of metals to wildlife species can be expressed either as dose-response relationships or as TRVs; the latter have received the most application in risk assessments. Toxicity information is usually obtained from the literature because case-specific toxicity studies are rarely done. The best sources of information on metal toxicity thresholds are NAS/NRC (1994, 1980), McDowell (2003), and the documentation supporting development of EcoSSL values (U.S. EPA, 2003c). The EcoSSL document also includes a general approach for screening studies for acceptability for use in derivation of TRVs for risk assessments. Uncertainty factors can be carefully applied if there is concern for extrapolation of data to species in a different taxonomic category (e.g., genus, family, class). General summaries for some metals are available in Beyer et al. (1996) and Fairbrother et al. (1996).

- Site-specific toxicity data can be developed for wildlife when the uncertainties associated with use of published data are large and the implications of the decisions warrant better information. These typically involve feeding studies with contaminated foods or soils contaminated with metals such as lead.

- Cross-species extrapolations should be conducted with some knowledge of animal physiology and specific responses to metals. Digestive physiology is the most important distinction because most metal exposures in wildlife are by the dietary route. However, other specific organismal responses should be understood as well. See Section 4.5.12.

- Use of critical tissue residues as an alternative approach to toxicity endpoints is conceptually sound but may require~~s~~ significant research to establish critical tissue levels. Exceptions include some essential elements in plants, where deficiency/sufficiency concentrations in foliage have been developed, and tissue levels of lead, selenium, and cadmium in wildlife (Beyer et al., 1996). See Sections 4.5.6.3 and 4.5.12.

#### 3.3.3.5. *Extrapolation of Effects*

Extrapolating the results of toxicity tests to untested species is necessary because of the paucity of data on the toxicity of metals to these receptors. However, extrapolation of results should be approached with caution owing to the large amount of uncertainty that can be introduced into the risk assessment process. Toxicological responses among species vary because of many physiological factors that influence the toxicokinetics (absorption, distribution, and elimination) and toxicodynamics (relative potency) of metals after exposure has occurred. These include (but are not limited to) differences in gut physiology, renal excretion rates, and egg production. Methods for extrapolating metal effects data among species are not unique to metals risk assessment. However, for metals, some species are able to regulate or store metals in their tissues without experiencing toxic effects (i.e., biota-specific detoxification), which makes extrapolations between species especially problematic. The essentiality of other metals adds additional complexity to interspecific toxicity extrapolations. See Sections 4.5.11 and 4.5.12 for further information.

***Recommendations:***

- Methods for extrapolating toxicity thresholds among species (e.g., species sensitivity distributions (SSDs) are the same for metals as those used for organic substances and are subject to the same assumptions of physiological similarities and degree of required margin of safety (for a review of methods, see Kapustka et al., 2004).

- All extrapolations should account for different requirements for essential elements and the factors that modify metal toxicity (e.g., acclimation, essentiality, mixtures).

- SSDs should be used for extrapolating effects data among species (Van Straalen, 2001; Posthuma, 2001).

- A review of cross-species extrapolation methods can be found in U.S. EPA (2003).

- Across-metal extrapolations (even within the same species) should be avoided unless mechanisms of action are known to be similar.

- For soil invertebrates, extrapolating toxic response to one metal to potential effects of a nontested metal can be done using QICARS (Kapustka et al., 2004; Lewis et al., 2000).

- For plants and wildlife, there is sufficient information about modes of action of most common metals to make informed judgments about relative toxicity, so empirical extrapolation models such as QICARS would be applicable only for initial screens of minor elements.

- Caution should be exercised when using published or measured toxicity data to evaluate risks to plants in particular regions. This is because naturally occurring levels of metals play an important role in biogeographic distributions of plants and animals and may, in fact, be limiting factors in species distributions or use of landscapes. Thus, it becomes very important to define the geospatial location of the area to which the assessment results will apply.

- For assessments conducted for regional or national assessments, criteria development, or ranking purposes, it should be acknowledged that results will be based on organisms and soil types that result in greatest bioavailability and sensitivity. Care should be taken, however, that the organism-environment combinations assessed are, in fact, compatible with real-world conditions.