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Toxicity bioassays for ecological risk assessment in arid and semiarid ecosystems

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I. Introduction

Ecological risk assessment (ERA) is a tool used to estimate adverse effects on the environment from chemical or physical stressors. ERA can evaluate historical releases or the potential impact of proposed facilities or new chemicals. This review will consider ERA in the context of historical releases of chemical stressors in dry environments. Arid and semiarid ecosystems present special challenges for application of risk assessment tools. One key problem is that most toxicity bioassays have been developed for aquatic environments or mesic ecosystems. However, roughly one-third of the earth's land area is characterized by dry conditions. In the United States, many large Federal facilities are located in arid and semiarid environments. For example, a recent evaluation of ecological resources at major U.S. Department of Energy (USDOE) facilities showed that almost 90% of the land surveyed is characterized as arid and semiarid (McAllister et al. 1996). It is anticipated that ERA will be the main tool used by USDOE to make waste-management and restoration decisions in these dry systems (McAllister et al. 1996).

The problem formulation phase is a key aspect of an ERA. The U.S. Environmental Protection Agency (USEPA) characterizes this phase as the identification of ecosystem components at risk and specification of the endpoints used to assess and measure that risk (USEPA 1997). Assessment endpoints are expressions of the valued resources to be considered in an ERA, while measurement endpoints are the actual measures of data used to evaluate the assessment endpoint. For example, consider the risk posed by contaminated soil to biota in a desert grassland. In addition to decomposing much of the photosynthetically fixed carbon, the subterranean termite population in this desert community may largely support lizards and other higher-trophic level consumers (see, e.g., Whitford et al. 1995). To evaluate the community's ecological stability, a reasonable assessment endpoint would be the termite population's sustainability. Quantification of toxicant impacts on termite survival and reproduction (measurement endpoints) would be accomplished using a critical component of ERA, the toxicity bioassay.

Toxicity bioassays are an important line of evidence in an ERA because these tests provide relevant and direct measures of toxicity of contaminated media to biota. By employing toxicity bioassays, the investigator can substantially diminish uncertainty regarding biological availability, uptake, and biological toxicant effects. Considering the potentially enormous scope of work involved with assessing ecological risks in the western and southwestern United States alone, it is imperative that ERA components such as toxicity bioassays be applicable to the environmental conditions of dry environments. As indicated above, these tests are conducted under conditions that fall within the range of average temperate environments; that is, 20^{o}C, well watered, and moderate to high humidity, high soil moisture, and high soil organic matter content. Few toxicity bioassay tests use organisms representative of, or relevant to, arid ecosystems or employ conditions that mimic the environment of arid systems. Everts (1997) recently noted this substantial data gap in a call for arid-system ecotoxicology research — a pertinent request given the considerable areal extent of arid zones in the United States and worldwide.

This review evaluates current bioassays for their applicability to arid systems and also recommends nonstandard toxicity tests that should be further evaluated for dry environments. The applicability of these tests is based on criteria such as test standardization and relationship of the test measurement to the assessment endpoint. In addition, this review summarizes available bioassays based on taxonomy of the test organisms to facilitate presentation of our findings. However, ecological relevance of the bioassay is based on the species functional role (e.g., detritivory) (Van Gestel 1998), and emphasis is placed on this aspect of the organisms, rather than the taxonomy, to better relate the test measure to assessment endpoints of interest.

II. General Considerations

This section provides certain general information on toxicity bioassay tests. This information is useful in understanding basic considerations in bioassay testing with regard to test species selection and selection of single or multispecies test systems.

A. Test Species Selection

The choice of test species is a major consideration in conducting terrestrial ecotoxicity tests (Laskowski et al. 1998). When selecting from among available test organisms, the investigator should choose species that are relevant to the overall assessment endpoints, representative of functional roles played by resident organisms, and sensitive to site contaminants (USEPA 1994a). In addition, test species of greatest utility will have the following characteristics: rapid life cycles, uniform reproduction and growth, ease of culturing and maintenance in the laboratory, uniformity of population-wide phenotypic characteristics (e.g., parthenogenic species[1] producing genetically identical clones), and similar routes of exposure to that encountered in the field (ASTM 1998; Laskowski et al. 1998). Specific applicability of these criteria depends on the type of organisms and assessment endpoints under consideration at a given site of concern.

In arid and semiarid environments, relevance of test organisms to resident organisms may be the limiting criterion for extracting useful information from existing toxicity bioassays. Established guidelines and historical information make the use of standard test organisms an attractive option for site assessment. Circumstances exist, however, in which well-described species are unavailable or are not applicable to site conditions. If standard test organisms are used despite being unrepresentative of a site's biota, the investigator must account for these discrepancies when interpreting test results.

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[1] Consideration of population genetic uniformity with regard to parthenogenic species is limited to the test duration that brackets a single cohort of parthenogenic clones. Use of genetically identical clones is a convenience in toxicity testing because this practice can potentially reduce test-system variability. Relative to sexually reproducing species, however, greater confidence in extrapolating test results with parthenogenic species to parthenogenic populations in the wild is unwarranted because the test population is not necessarily representative of populations in the field.

Alternatively, nonstandard species may be used that more accurately represent the functional roles played by local flora or fauna. Functional categorization of test species, based on what the organism represents to the protected resource, is a key consideration for selecting test species. The cost of performing tests with nonstandard species can be higher because of the need to fill data gaps, including defining optimal conditions for the organism's growth, establishing the organism's sensitivity to site-related contaminants, and establishing control condition responses. However, the extra investment may be justified by obtaining information on toxicant effects on resident species (USEPA 1994a).

Whenever possible, the general approach recommended in this review is to identify species that are appropriate candidates for toxicity testing. Additional information on the functional role of these species in arid environments is also provided to help gauge the organism's ecological relevance to the study system. In evaluating test species for ecotoxicity assessment, it is illustrative to consider organisms that represent extremes in ecological relevance, standardization, and sensitivity. The criterion "ecological relevance" is subjective and based on the investigator's judgment regarding a species' applicability or meaningfulness to the site being assessed. Ecological relevance can be based on the likelihood of occurrence of the test organism at the site, the functional role that the organism represents, and the relationship of the test species to the biological resource being assessed. Standardization has been defined as "the extent to which the study follows specific protocols recommended by a recognized scientific authority for conducting the method correctly" (Menzie et al. 1996). Examples of accepted standard toxicological testing protocols are those published by the USEPA, American Society for Testing and Materials (ASTM), or those found consistently in peer-reviewed scientific literature. In their weight-of-evidence approach, Menzie et al. (1996) rank methods that have been used in three or more peer-reviewed publications as having repute equal to that of accepted standard methods (e.g., ASTM published methods). Sensitivity refers to the test species' proclivity of response to the physical or chemical stressor of concern.

B. Test Systems

1. *Single species*. Much of the ecotoxicity literature is based on the response of single species to single or multiple chemicals. If numerous species are evaluated, it is usually as a composite or battery of individual single-species tests. Single-species tests are employed for pragmatic reasons; namely, in an attempt to reduce uncertainty associated with test results. The use of these tests in laboratory settings allows for precise control of experimental variables and relatively unambiguous establishment of cause and effect relationships between chemical stressors and organism-level responses. Knowledge of this type is essential, both for understanding how stressors cause adverse biological effects and for elucidating the strategies adopted by organisms to tolerate stress (Maltby 1999). The impetus for performing simplified laboratory tests stems from contrasting goals of providing interpretable test results with the need to estimate toxicant impacts on the structure or function of environmental assessment endpoints. Single-species tests are easily standardized, and the test results may be conveniently interpreted in a regulatory framework (Karr 1993). Single-species tests are the most widely used and perhaps most important tool available to ecotoxicologists for assessing chemical stressor impacts (Weyers and Schuphan 1998).

The strengths of single-species tests, such as simplified and interpretable systems, are also weaknesses when considering their lack of environmental realism. Contaminants may cause direct mortality, but may also negatively affect an organism's behavior, reproductive potential, food sources, and habitat. The most relevant ecological effects when considering environmental toxicity are impacts to mortality, morbidity, and reproduction. These factors and other important variables, such as differences in toxicant bioavailability in the field, are often inadequately simulated in the laboratory. For example, predacious organisms experimentally exposed to contaminated soil are often fed "clean" prey. This practice ignores the potentially important exposure route known as bioaccumulation (or in some instances, biomagnification).

2. *Multispecies*. The use of model terrestrial systems (microcosms) offers a potentially useful compromise between the simplicity of laboratory tests and the complexity of the field (Hopkin 1997). Inexpensive test systems with field communities of soil microinvertebrates (nematodes, mites, springtails, and other microarthropods) have been used to investigate the effects of toxicants in terrestrial systems (Parmelee et al. 1993, 1997). Community-level chemical effects on soil microarthropods, such as disruption of food web structure or alteration of other biotic or physical processes, can be measured in these systems. For example, in microcosm tests using trinitrotoluene (TNT), Parmelee et al. (1993) found that at the highest exposure concentration (200 ppm TNT), neither total nematode nor total microarthropod abundance was significantly affected, but there was a significant decrease in the population of oribatid mites. While the ecological ramification of this shift in community composition may be open to interpretation (e.g., the innate functional redundancy of the ecosystem may compensate for the loss of oribatid mites in a model system), the susceptibility of the system to further perturbation may consequently be increased.

Terrestrial model ecosystems (TMEs) offer an important means of assessing direct and indirect toxicant effects and multispecies interactions at varying levels of community complexity. This complexity, however, can make the interpretation of TME results more difficult. Additional research is required before this technique can be used routinely in ecotoxicity assessments. Weyers and Schuphan (1998) recently made important contributions in this area by studying the inherent variability associated with the biotic component of their study systems. Information on natural variability can be used to facilitate interpretation of dose-response relationships obtained from treated TMEs.

3. *In Situ Testing and Biomonitoring*. The most realistic type of toxicity testing involves exposing organisms to contaminated media (e.g., soils) in the field, wherein plants and animals are subject to both realistic conditions and natural variations in exposure (Suter 1993). This testing protocol is most applicable to sessile organisms (e.g., plants) and is considerably more difficult to use with animals because of their mobility. Given the potentially wide range of studies and the site specificity associated

with using a field-testing approach, the focus here is on laboratory toxicity bioassays. Further information on the use of in situ testing is provided in the USEPA's suggested protocol for conducting field studies for ERA (USEPA 1994c).

Biomonitoring is another tool frequently used to assess damages to natural resources. As Karr (1993) points out, biological monitoring integrates and evaluates cumulative impacts from a variety of natural and anthropogenic stressors. With this approach, resource condition and the relative degree and direction of degradation are evaluated. Given the field aspect of biological monitoring, the results of these programs may include the effects of contaminant-unrelated stressors, such as climate, and natural ecological patterns, such as population cycles, disease outbreaks, and predation effects. While attention to these factors in the study design will serve to minimize the confounding effects, considerable natural variation exists in these assessments. Not surprisingly, monitoring results are not replicable, and critical background data for characterizing the monitoring conditions are often incomplete or expensive to collect. Many sources can be consulted for biomonitoring techniques (e.g., Karr and Chu 1997) and this subject will not be covered further in this review.

III. Soil Processes and Microorganisms

Bacteria and fungi are directly responsible for decomposition of organic matter in terrestrial ecosystems (Aber and Melillo 1991; Seastedt 1984). As primary decomposers, microorganisms occupy a critical position in the soil food web, and virtually all nutrient cycling passes through this group of organisms to higher trophic levels (Kennedy 1999). It is through microbial activity that nutrients locked in organic matter are made available for new growth. Microorganisms form the base of heterotrophic food webs and are involved in virtually all ecosystem processes (Paul and Clark 1989; Schlesinger 1991). Because of their importance to ecosystem functioning, microorganisms and microbial processes have become increasingly well studied with regard to the ecotoxicological factors that affect their biology (Van Beelen and Doelman 1997).

The functional role of microorganisms in arid soils has received considerably less attention than their counterparts in more mesic or aquatic ecosystems, but some generalities can be made regarding arid-system microbial ecology. Bacteria require a film of moisture around soil particles for their movement and activity. Without water, bacteria either perish or enter a dormant state to survive arid conditions in the form of cysts. Unlike bacteria, fungi maintain biomass and, therefore, function in arid environments (Whitford 1989). For example, Schnürer et al. (1986) found that during a one-week period of soil drying, there was no change in fungal biomass but a marked decrease in bacterial biomass. In a more long-term experiment, Parker et al. (1984) demonstrated that as soils dry, bacterial growth and bacterial biomass decrease rapidly, but that fungi continue to grow. These results suggest that in seasonally dry or arid environments, soil food webs are based primarily on fungi rather than on soil bacteria (Whitford 1989). Bursts of bacterial activity do occur in association with soil wetting in arid environments; however, it is unclear to what extent such activity influences decomposition and mineralization of organic matter in characteristically dry soils. Regardless of the dominant microbial taxa involved, tests directed toward measuring rates of organic matter decomposition are the most common means of assessing the status of microbial function in soils. Quantifying decomposition can help to unveil the complexities of organismal interactions with other biota and their physical/chemical surroundings. Organic matter decomposition is perhaps the most unifying and inclusive process in soil ecosystems. The major chemical processes occurring during decomposition are carbon mineralization and nitrogen mineralization (Paul and Clark 1989).

Standardization in microbial toxicity tests is often a problem because each site contains its own indigenous microflora (Van Beelen and Doelman 1997). In recognition of the vast microbial diversity in soils, some countries have focused on universal soil processes as being well suited for toxicity testing. For example, the Canadian Council of Ministers of the Environment selected nitrification as a process to be considered for the derivation of environmental soil quality guidelines (Siciliano and Roy 1999a). But even in this guideline, there is no strict standardization of the microflora because there is no prescribed soil (Van Beelen and Doelman 1997). A site-specific approach is required for using microbial toxicity tests because of the unique features and complexity of soils and their native microbial communities.

Even if a site-specific approach is followed, understanding the effects of site toxicants on microflora remains challenging. The genomic plasticity and rapid reproductive rates characteristic of microbial populations are problematic when evaluating contaminated sites because microorganisms can quickly evolve resistance to introduced toxicants (Efroymson and Suter 1999). In an evaluation of metal-impacted sediments at the Milltown Reservoir Superfund site, the local bacteria were found to be highly resistant to heavy metals relative to bacteria from an uncontaminated site (Markwiese et al. 1998). This resistance was induced and greatly enhanced in the laboratory in just a few generations (Markwiese 1999). Although only one bacterial process was measured, it was shown that the process was dependent upon interactions among several species (Markwiese and Colberg 2000). Each of these species presumably had unique tolerance levels to heavy metal pollution, which makes interpretation of microbial bioassay results difficult. Numerous investigations have documented similar instances of toxicant resistance in terrestrial soil microbial communities (Doelman 1985; Trevors et al. 1985). Generally speaking, bioassays performed on resistant microflora can seriously compromise the test results used to assess toxicant sensitivity for the microfloral community as a whole (Van Beelen and Doelman 1997).

It is important to note that microbial communities are typically characterized at the process level (e.g., soil respiration or decomposition), with little attention given to responses at the organismal level (e.g. individual or population responses) (Kennedy 1999). Process-level measurements, although critical to understanding the ecosystem, may be insensitive to community-level stress because of the redundancy of these functions within an ecosystem (Kennedy 1999; Rutgers and Breure 1999; Siciliano and Roy 1999b). Functional redundancy across broad taxonomic groups enables substantial shifts in community composition without remarkable change in rates of decomposition or community respiration (Kaputska 1999). Consequently, microbial toxicity test results can be difficult to interpret (Kaputska 1999; Sheppard 1999). However, the rapidity with which tests can be run, their ease of use, and the importance of microbial processes to ecological functioning may, in some instances, make these attractive systems for gauging toxicant impacts in soil environments (Efroymson and Suter 1999; Hull et al. 1999). Various microbial test systems are discussed below and summarized in Table 1.

A. Soil Litterbags

Toxicant effects on carbon mineralization can be quantified in several ways. One of the simplest techniques is to enclose preweighed plant litter in a mesh bag, bury it, and after a period of time, collect and weigh the bag's contents, comparing the mass loss relative to similarly bagged litter in reference soil. As described by Heath et al. (1964), the litterbag method offers a simple and meaningful tool for measuring decomposition in terrestrial systems. This method is of high ecological relevance because the actual rates of decomposition with (presumably) native material can be measured in situ, providing real-time data for toxicant impacts on decomposition at a study site. In addition, various functional and taxonomic groups can be chemically restricted (e.g., with fungicides, bacteriocides, and pesticides) or physically restricted (e.g., the mesh size of the bag can exclude certain size groups of microarthropods) in order to evaluate their contribution to the process. An aspect of the litterbag method that detracts from its ecological relevance is simply that the litter is not in direct contact with contaminated soil and, thus, presents a microenvironment different from buried litter in native surroundings.

Santos et al. (1981) performed an elegant experiment using the litterbag method to evaluate decomposition in the arid soils of New Mexico's Chihuahuan Desert. The application of the insecticide chlordane, which eliminates mite populations but does not affect bacteria, fungi, or nematodes, resulted in a 40% decrease in decomposition relative to controls. By eliminating mites that preyed on bacteriophagous nematodes, the nematode population exploded, thus severely restricting the population of microbial decomposers in this system. This simple experiment provided important insights into the structure and functioning of decomposition in arid soils. Over the last four decades, the litterbag method has proven to be a valuable tool in soil ecology. Information on recent modifications to the litterbag method is provided by De Jong (1998).

B. Carbon Mineralization

Microbial respiration is one of the primary physiological measurements of microbial activity (Regno et al. 1998). Respiration is defined as either carbon dioxide (CO_2) production or oxygen consumption. To provide an indication of microbial carbon mineralization (i.e., the conversion of organic carbon to CO_2), it is important that measurements be collected at stable pH and that appropriate controls be employed, because the dissolution of carbonate minerals in soils can also lead to CO_2 generation.

Carbon mineralization is often measured with the substrate-induced respiration technique (SIR), as modified by Cheng and Coleman (1989). This technique is based on the addition of a labile substrate, such as glucose, to induce a maximal respiratory response (CO_2 production) from the soil microbiota (Kuperman 1996). At the U.S. Army's Aberdeen Proving Ground in Maryland, Kuperman and Carreiro (1997) used the SIR technique to examine ecotoxicological impacts of heavy-metal contamination on the soil ecosystem. Strong correlations were established between total heavy-metal concentrations, SIR, total fungal (hypha) length, and total microbial biomass. From this, the authors concluded that heavy-metal contamination of soil decreased the abundance and activity of microorganisms involved in organic matter decomposition and nutrient cycling at this site.

C. Pollution-Induced Community Tolerance

The pollution-induced community tolerance (PICT) approach is based on shifts toward tolerant microbial communities in the presence of contaminants (Rutgers and Breure 1999). In this case, microbial community shifts are characterized by carbon substrate utilization. The carbon substrates are contained in a multiwell Biolog® plate consisting of a freeze-dried medium, a standard set of carbon substrates (1 substrate per well, up to 96 wells, and 1 blank) and a redox dye for monitoring microbial activity. An aliquot of the microbial community is uniformly inoculated in all wells of the plate and incubated under controlled laboratory conditions. When the community exhibits catalytic properties, the carbon substrate is oxidized and the redox dye (tetrazolium violet) turns purple. Each substrate in the Biolog® plate represents a different test system and provides information on the microbial oxidation reaction (binary 0 or 1, rate constants, etc.) that is specific to the inoculated microbial community (Rutgers and Breure 1999).

Originally developed for algal communities, PICT has recently been applied to the evaluation of terrestrial soils (Bååth et al. 1998; Rutgers et al. 1998; Rutgers and Breure 1999). After extraction from soil, a PICT analysis is carried out by inoculating microorganisms in a series of plates with increasing levels of a contaminant. The relative sensitivity of the microbial community for toxicant exposure is typically calculated as the toxicant concentration causing a 50% decrease in activity (substrate utilization) in comparison to its untreated control (i.e., the toxicant's EC_{50}). Tolerance is manifested as an enhanced ability to use substrate (higher EC_{50}) relative to an unexposed population. The concept of PICT encompasses the phenomenon that, given variation in species' sensitivities, toxic effects reduce the survival and growth rate of the most sensitive organisms within a population, thus increasing the average tolerance of the community (Rutgers et al. 1998).

D. Soil Enzymes

Soil ecotoxicological evaluations have employed enzyme assays to measure impacts on microbial populations. The commonly measured enzymes are beta-glucosidase, carboxymethylcellulase (carbon-acquiring enzymes), n-acetylglucosaminidase (nitrogen acquiring enzyme), and acid/alkaline phosphatases (phosphorous acquiring enzymes) (Bardgett et al. 1994; Kuperman 1996). Enzyme activity provides an indication of microbial heterotrophy and mineralization in soil ecosystems. To be useful in the current context, these measures need to be related back to measures that demonstrate the function of an intact microbial soil community. This is not a straightforward task, and Kaputska (1999) notes that, of the various microbial toxicity measures, enzyme assays are least amenable to interpretations of ecological significance.

E. Microtox

The Microtox test procedure does not measure soil processes. Rather, the test directly measures chemical toxicity to the luminescent marine bacterium *Vibrio fischeri* (formerly *Photobacterium phosphoreum*). Toxicity is quantified by measuring the reduction in luminescence in response to chemical stressors in liquid or solid samples. Originally developed for evaluating effluents and elutriate samples, the procedure has been modified for directly testing solid matrices such as sediments and soils (Kwan and Dutka 1992). This test is easy to run, and results can be obtained in a matter of hours. The test also has widespread acceptance and well-established protocols (USEPA 1994b). The Microtox procedure has been used to evaluate soils contaminated with inorganic and organic chemicals such as metals and high explosives (Simini et al. 1995), crude oils (Dorn et al. 1998), and diesel fuels (Marwood et al. 1998).

Despite widespread use of the Microtox system and endorsement by the USEPA (1994b), the test has serious shortcomings with regard to assessing arid soils. Foremost among these is the near-complete lack of correspondence between the measurement endpoint (luminescent output of a marine bacterium) and the microbial processes occurring in dry soil. Moreover, a relatively small aliquot of soil is added to the liquid test medium (Kwan and Dutka 1992), thus creating a low proportion of solid to solution phases. Contaminant bioavailability in the resultant dilute soil slurry is, therefore, not representative of the true bioavailability in dry soils.

IV. Terrestrial Plants

Plants capture the sun’s energy, thereby providing the basis for most of life on earth. Plants modify the landscape, and, thus, create the biotic lattice upon which ecological communities are structured. The ability to predict the impacts of toxicants on plants enables investigators to identify potential thresholds of contamination above which floral establishment, growth, and reproduction may be compromised, and above which direct mortality is a concern. In all biotic communities, an alteration of the floral structure of the environment (e.g., by altering the population structure for key plant species) can lead to significant impacts on the overall composition of the community.

Many plant species and numerous phytotoxic assessment endpoints have been used to characterize toxicant impacts on vegetation. Terrestrial vascular plants can be among the most sensitive organisms to a variety of chemicals (Marwood et al. 1998; Miller et al. 1985). Plant toxicity bioassays offer unique advantages compared to tests with animals or microbes. Wang (1991) suggests that general advantages of plant toxicity tests include (1) ready availability for tests, in that seeds can be purchased in bulk and that most have shelf lives of a year or more; (2) ready activation for testing (seed germination); and (3) minimum maintenance costs between tests. These characteristics make plants well suited for assessing contaminated soils. Because plant toxicity tests evaluate seed germination and seedling growth, they are directly relevant to plant recruitment on the basis of seeds set in disturbed, contaminated soil.

Plant bioassays have been effectively employed in toxicity screening studies and in more advanced stages of ERAs. The sites at which terrestrial plant bioassays have been applied include industrial facilities such as ordnance, pesticide, and chemical manufacturing sites (Linz and Nakles 1997); oil refineries (Ramanathan and Burks 1996); military facilities (Kuperman 1996; Simini et al. 1995); Superfund sites (Rader et al. 1997); and heavy metal mining and smeltering sites (Kaputska et al. 1995). Soil contaminants associated with these facilities include heavy metals, chlorinated solvents, petroleum fuels, munitions, pesticides, polycyclic aromatic hydrocarbons (PAHs), and other potential toxicants (Linz and Nakles 1997). Various assays have proven cost-effective for initially characterizing contaminated sites. They are increasingly used to evaluate post-remediated soil, making them valuable monitoring tools for assessing the efficacy of remediation technologies (Baud-Grasset et al. 1993; Dorn et al. 1998; Marwood et al. 1998).

A. Current Practice

Many stages in a plant's life cycle can and have been used in phytotoxicity assessments. The first days of seedling growth are emphasized because this period is often the most sensitive in plant development (ASTM 1994). Under favorable conditions, germinating seeds and seedlings undergo rapid changes in metabolic activity, nutrient transport, and cell division and environmental stressors have the greatest potential for exerting adverse effects during this early growth phase (Wang 1987). For this reason, the seedling growth test is the most common type of plant toxicity test (Suter 1993).

ASTM (1994, 1998) lists numerous measurement endpoints for the early seedling growth test, including the number of seedlings to emerge, percent survival, plant height, radicle (root) length, and dry weights of aboveground and belowground vegetation (Table 2). These endpoints are used to generate estimates of the effective concentration (ECx) affecting a percentage of the test organisms (x). Effective concentrations are often listed as the no-observed-effect concentration (NOEC) and the lowest-observed-effect concentration (LOEC). Qualitative endpoints include the severity of phytotoxicity, abnormal changes in growth and development, and abnormal changes in plant morphology, as compared with study controls. Additional endpoints reported in the literature are increases in plant biomass and percent survival after various exposure durations, dry weight at flowering, percent flowering, and leaf growth (Linz and Nakles 1997), among others (see, e.g., Tyler et al. 1989).

For Superfund sites, general consensus has not been reached on either test duration or endpoints, but the USEPA (1994b) recommends the 5-day lettuce (*Latuca sativa*) seed germination and root length tests. While results from early seedling growth tests can be used to estimate the potential adverse impact of test chemicals released onto the land, Suter (1993) notes that the relationships of these short-term responses to plant demographics (e.g., plant survival and reproduction) have not been established. Assessments spanning the spectrum from seed germination and plant establishment to the production of new seeds would provide more definitive phytotoxicity information by considering effects on photosynthesis, flowering, or advanced growth stages. A standardized complete life-cycle test using the turnip (*Brassica* *rapa*) has been developed and can be run from start to finish in a little over 1 month (Shimabuku et al. 1991; ASTM 1998). In general, however, examples of whole-life-cycle tests in phytotoxicity assessments are rare (Suter 1993).

Recent advances in plant molecular biology have demonstrated that chromosome aberration tests (Grant 1994; Kovalchuk et al. 1998) and biomarkers, such as antioxidant enzyme activity, can be sensitive indicators of toxicant-related stress. Results from a study conducted by Ferrari et al. (1999) indicated that plant antioxidant enzyme assays were more reliable than measures of plant growth or seed germination for permitting the early detection of plant injury in phytotoxicity tests. Although the development of plant biomarkers offers high specificity and sensitivity for assessing phytotoxicity, the general approach is probably still too costly and nonstandard to employ in routine assessments (Ferrari et al. 1999).

In addition to the criteria outlined in Section I (test species selection), ASTM (1994) specifies the following: seeds should have a germination response within several days; should be compatible with the testing environment; should be of economic or ecological importance; and should represent dicotyledonous and monocotyledonous species, the two major groups of plants. None of the plants recommended in ASTM 1994 are considered representative of arid ecosystems species. And, as noted in ASTM (1998), the majority of plants routinely used in phytotoxicity tests have been limited to species of agricultural importance. A recent update of ASTM methodology for terrestrial plant toxicity testing lists nearly 100 plant taxa that have been used in phytotoxicity testing (ASTM 1998). As in the previous guidance document (ASTM 1994), few of these taxa are adapted to dry conditions or are ecologically similar to indigenous species in the arid Southwest.

Table 3 lists several species that could be employed in arid-system risk assessments and uses the Los Alamos National Laboratory (LANL) as an example facility in a semiarid environment. As noted in Section I, the specific tests used in experimental phytotoxicity assessment are largely standardized. However, the responses of test species can vary from the well characterized to the poorly understood, depending upon the scrutiny the species has received from the technical communities. It is with regard to this latter aspect of standardization that plant species are ranked in Table 3.

Rader et al. (1997) provided a recent example of balancing species-selection options in a study on the phytotoxicity of metal-contaminated soils in Montana. The authors evaluated four types of plants, ranging from species that were well-studied and of limited ecological relevance to the site, to highly relevant species for which little toxicological information existed. While of limited applicability to resident organisms, lettuce (*Latuca sativa*) and radish (*Raphanus sativus*) were chosen because they have been shown to be quite sensitive to a broad range of soil toxicants (Miller et al. 1985). Although no toxicity data existed for redtop bentgrass (*Agrostis gigantea*), this species was chosen to represent site flora because it is the dominant organism at the Montana study site. Barnyard grass (*Echinochloa crusgalli*) was selected as an intermediate between standardized organisms and those with high ecological relevance. Barnyard grass has a wide distribution and has been the focus of prior phytotoxicity investigations. Except for a few time-consuming modifications to the testing protocol made for *A. gigantea*, this species proved to be a valuable addition to the battery of plant species; it was the most sensitive of all the organisms for seedling emergence time. This information was useful for predicting potential impacts of toxicants on native flora, especially with regard to establishment and recruitment. *E. crusgalli*, on the other hand, was most sensitive with regard to root length. This study demonstrated the utility of employing nonstandard species to derive data relevant to site-specific organisms in phytotoxicity assessments.

Blue grama (*Bouteloua gracilis*) is an example of an ecologically relevant plant species that should be considered for assessments in the southwestern and western United States. It grows well under very dry conditions, requiring 8 in. or less annual precipitation. Blue grama is one of the predominant native grasses in arid lands of the southwestern and western United States, and it meets several of the criteria for preferred test species — namely, it is abundant, ecologically important in arid grasslands (Vossbrinck et al. 1979), an economically valuable range grass, extremely drought-tolerant, and appears to possess growth characteristics that would facilitate laboratory testing (Huffman and Jacoby 1984; Knight et al.

1993; Morgan and Knight 1991). Stephenson et al. (1997) previously studied blue grama in a comprehensive battery of phytotoxicity bioassays aimed at selecting species representative of select Canadian environments.

Huffman and Jacoby (1984) examined the effects of various herbicides on blue grama seed germination in a 28-day laboratory study. Of the seeds that germinated, 90% did so within 7 days, and 97% were completed by 14 days. The anticipated duration of the study is useful to consider in planning a phytotoxicity study. However, only 57% of all *B. gracilis* seeds germinated. Some of the species recommended by ASTM (1998) have lower typical germination percentages (e.g., carrot, 55%), but the percentage for *B. gracilis* is still lower than most species (ca. 75-80%). The investigator responsible for designing a phytotoxicity study with blue grama must recognize these aspects of plant physiology and plan tests accordingly. For example, the investigator may adopt a sample design with a larger number of replicates to help distinguish experimental effects from natural variation. Although Huffman and Jacoby (1984) found that blue grama was the least sensitive of three native grass species (including buffalograss and sideoats grama) exposed to the herbicide picloram, they cite literature indicating that blue grama is almost five times more sensitive than plant species more commonly employed in toxicity testing (e.g., red fescue, Kentucky bluegrass, smooth bromegrass, orchardgrass [ASTM 1998]).

Further ecological realism can be incorporated into later phytotoxicity testing stages by creating moisture conditions representative of the arid-ecosystem study site. For example, the Morgan and Knight (1991) study on herbicidal, dose-dependent depression of photosynthesis in mature blue grama plants can be easily modified to examine toxicant effects related to water limiting conditions. It is recommended that the investigator collect literature relevant to the testing of a particular species, and perform pilot tests with the plant, prior to embarking on more involved experimentation.

B. Future Considerations

ASTM (1998) recognizes that some circumstances require relatively uncharacterized plant taxa to achieve more ecologically relevant phytotoxicity testing, and offers the following guidance on species selection:

1. *Purchasing Seeds.* Landscaping companies and firms dealing with land restoration offer seeds for nonstandard test species. When seeds are obtained through this route, it is important to gather as much information as possible about the seed lot, especially with regard to details on germination. In addition, it is imperative that enough seeds from the same lot be available for running the experiment (i.e., enough for all experimental treatments and controls).

2. *Collecting Seeds or Other Plant Material.* Seeds can be collected from the field, but it is critical that only seeds from the species of interest be collected. Details regarding location, field conditions (e.g., possibility of drought conditions), time in growing season, and potential confounding factors (e.g., previous pesticide and or herbicide use in the area) must be recorded for the collection event. These same record-keeping concerns apply to the collection of seedlings and cuttings.

3. *Species Selection.* A rigorous protocol for evaluating potential species in phytotoxicity testing was developed by Stephenson et al. (1997). Tests included the early seedling growth and seed emergence bioassays. Boric acid, an effective and nonselective biocide, served as the reference toxicant; two control soils (artificial and field-collected) were used; and endpoints included mortality, shoot length, root length, shoot wet mass, root wet mass, and shoot and root dry mass. The most sensitive endpoint for detecting a statistical effect of the toxicant concentration was root length. Root length, shoot length, and shoot wet mass were equally sensitive to test soils. Anomalies revealed by visual inspection provided reliable information on growth conditions, making this a quick and efficient means of qualitative screening. In addition to toxicant sensitivity, species selection criteria included time to germination/emergence; source,

availability, and quality of seed; root formation; life history/phenology traits; and critical variable requirements such as pH, nutrients, and mycorrhizal associations.

Considering the personnel and maintenance costs involved in performing extended laboratory studies, rapid growth is an ideal characteristic of test species. Desert and drought-adapted plants may be particularly well suited for phytotoxicity studies in this regard. Relatively short growing seasons and sporadic rainfall provide strong selective pressure on plants to quickly maximize growth and reproduction during favorable conditions (Inouye 1991). Desert annuals, in particular, are characterized by rapid germination and rapid growth. Plants of this type have some of the highest photosynthetic rates ever recorded (Mooney et al. 1976). Such characteristics favor the development and standardization of desert species as potential test organisms.

4. *Data Interpretation.* Interpretation of results of phytotoxicity testing based on relatively unstudied species must be tempered by ecological characteristics of the species utilized (ASTM 1998). It is important that study and data collection objectives be addressed early on to distinguish ecotoxicological effects specific to the species under consideration. If the investigator has limited knowledge of a species, experiments to characterize variability innate to germination, growth, etc., should be conducted as a companion set of tests (ASTM 1998). This testing is accomplished by running rigorous control tests with enough replicates to achieve the desired statistical test power.

V. Terrestrial Invertebrates

Aber and Melillo (1991) note that the quantity of essential nutrients entering an ecosystem each year is generally low compared to the amount cycling within the system, and that plant production depends on the internal recycling of nutrients. Dead vegetation can be one of the largest biomass fractions in arid ecosystems, and it represents one of the most reliable nutrient and energy resources therein (Van der Valk 1997). A distinctive feature of energy flow in deserts is that a majority of the plant biomass is broken down through abiotic factors and by arthropod detritivores (Haverty and Nutting 1975; Pearce 1997; Van der Valk 1997; Whitford et al. 1982a). Bacteria and fungi are typically responsible for decomposition of dead vegetation (Seastedt 1984; Aber and Melillo 1991); their activity in arid environments, however, can be limited to sporadic and brief periods of adequate soil humidity (Whitford 1989; Van der Valk 1997). As a result, decomposition of organic matter is more arthropod-linked in deserts than in mesic environments. In addition, dead soil animals are rapidly mineralized, and their nutrients are made available to the system (Seastedt and Tate 1981). With relatively short life cycles, soil-dwelling invertebrates are part of the rapid turnover of nutrients in terrestrial systems.

Even though soil animals often account for less than 10% of soil respiration in most terrestrial environments (Seastedt 1984), soil fauna largely regulate the decomposition process through their influence on the decomposer flora by means of feeding activities (Seastedt 1984). Investigators should keep in mind that although decomposers serve important functions in the ecosystem, there may be difficulties associated with communicating the rationale for selecting decomposers to evaluate ecological risk. Most decomposer taxa are small-bodied and maintain a relatively cryptic lifestyle which obscures the role that they play in nutrient cycling and soil development. Thus, the assessment endpoint (e.g., carbon cycling) must be clearly distinguished from the measurement endpoint (e.g., abundance of microarthropods and nematodes in soil). Soil-dwelling microfauna play important symbiotic roles in the transfer of microflora that are integral to the nutrient cycling process, and serve as a food source for a large fraction of secondary consumers (Van der Valk 1997). Arthropod detritivores are also important in developing soil structure, which in turn is critical to the establishment and persistence of other species (Pearce 1997; Whitford 1996). Terrestrial invertebrates are of high ecological relevance in dry environments; Table 4 summarizes considerations for using invertebrate bioassay test systems.

A. Earthworms and Nematodes

1. *Earthworms*. For the soil environment, ecotoxicity data requirements in most countries are limited to earthworms (Van der Valk 1997). In the United States, the only faunal test for soil toxicity screening of hazardous waste sites that is endorsed by the USEPA is the earthworm (*Eisenia foetida*) 14-day survival assay (USEPA 1994b). As outlined by ASTM (1997), *E. foetida* was chosen as a soil test organism because (1) it is easily bred in the laboratory (Gibbs et al. 1996; Kula and Larink 1997); (2) it is the earthworm species most commonly used in laboratory experiments; (3) it has been studied extensively, producing a large data set on the toxicity and bioaccumulation of a number of compounds (Gibbs et al. 1996; Miller et al. 1985; Wilborn et al. 1997); and (4) it has been approved for use by the European Economic Community and the Organization for Economic Cooperation and Development (OECD) (Van Gestel and Van Straalen 1994).

Soil ecotoxicity studies regularly utilize *Eisenia* spp. for the reasons mentioned above, even though these organisms are not true soil dwelling species. Common habitats of *Eisenia* are compost piles and dung heaps, which means that *Eisenia* test results require an extrapolation from an organic rich media to make inferences for the soil community. Problems with ecological relevance of earthworm ecotoxicity results are more serious for arid and semiarid soil ecosystems. Earthworm-derived data are not relevant for arid ecosystems, where termites, ants, and tenebrionid beetles fulfill the functions that earthworms have in more humid soils (Van der Valk 1997). Consequently, the use of earthworms as the sole representative test species for arid and semiarid ecosystem toxicity testing cannot be recommended. However, situations may occur in which earthworm bioassays are performed in tandem with less standardized test species/systems for purposes of comparing and benchmarking new test results. The use of Enchytraeid worms in tests in arid and semiarid systems (see Van Gestel and Van Straalen 1994) deals with many of the concerns noted for *Eisenia*, and these organisms are similarly unsuitable for ecotoxicological assessment in such ecosystems.

2. *Nematodes*. Four of every five multicellular animals on the planet are nematodes (Bongers and Ferris 1999). These organisms occupy any niche in terrestrial environments that provides an available source

of organic carbon (Bongers and Ferris 1999). In addition to their sheer abundance, nematodes may be of high ecological relevance at contaminated sites because their permeable cuticle provides direct contact with soil contaminants in their microenvironment (Bongers and Ferris 1999), and nematodes do not rapidly migrate from stressful conditions. They occupy key positions in soil food webs, feeding on most soil organisms, and, in turn, serving as a food source for many others (Bongers and Ferris 1999; Yeates and Bongers 1999).

In arid and semiarid environments, abiotic factors are important influences on nematode activity. Soil particles are typically covered with a thin film of water, and it is within this film that nematodes are active (Houx and Aben 1993). If the water film dries up, nematodes become inactive and enter a state of anhydrobiosis, where they remain dormant until suitable moisture conditions are reestablished (Whitford 1989; Zak and Freckman 1991). When fully hydrated, nematodes have a considerable influence on decomposition by grazing on the bacteria and fungi that break down plant and animal matter. This grazing activity can greatly influence litter decomposition in arid systems (Santos et al. 1981; Whitford et al. 1982a) and increase the amount of nutrients in the environment at any given time (Anderson et al. 1981).

Characterization of nematodes generally focuses on trophic categories (e.g., Bongers and Bongers 1998) because of difficulties in identifying worms at the species level (Zak and Freckman 1991). On the basis of literature reports of feeding activity and esophageal morphology, nematodes can be placed into four trophic groups: bacterivores, fungivores, omnivore-predators, and plant feeders (Zak and Freckman 1991). Nematodes are gaining increasing recognition in laboratory ecotoxicological studies because of several factors that make them promising test species, including ease of handling and culturing, rapid generation times, and sensitivity to many chemicals (Kammenga et al. 1994).

Nematodes exist in an aqueous environment, and laboratory investigations of toxicant impacts on nematodes often employ a liquid medium for testing (e.g., Kammenga et al. 1994). While this type of information on toxicant sensitivity is crucial to characterizing a test organism, extrapolation of test results to arid soils, can be problematic. Van Gestel and Van Straalen (1994) note that information on soil sorption of toxicants can be coupled with porewater chemical concentrations for extrapolating results from laboratory to field settings; however, they also point out that this type of extrapolation has yet to be validated. To assess chemical toxicity to nematodes, more realistic soil testing can be achieved using artificial soils (Kammenga et al. 1996) or field soils (Donkin and Dusenbery 1993).

Given the current limitations in our understanding of nematodes, ecotoxicologists and soil scientists have adopted assessment endpoints other than traditional metrics (e.g., mortality) to evaluate nematode populations. Bongers and Bongers (1998) use ratios of adult to juvenile nematodes, termed the maturity index (MI), for characterizing soil stressors. For example, use of the MI can provide meaningful results if population fitness is strongly influenced by juvenile survival (Kammenga et al. 1996). The MI has been shown to be a sensitive metric for monitoring natural and anthropogenic stressors for some nematode populations (Bongers and Bongers 1998; Bongers and Ferris 1999; Yeates and Bongers 1999).

In an assessment of heavy-metal-contaminated soil at a military facility, Kuperman (1996) found that nematodes were strong indicators of pollution in the study area. This investigation showed that most trophic groups, as well as total numbers of nematodes, were affected in the areas of highest metal concentrations. In one sampling period, it was observed that total nematode numbers were reduced by 85% relative to the local background site. Fungivorous nematodes appeared to be most affected, while herbivores were the least impacted. From these results, one might expect that fungal biomass was negatively impacted by heavy metal contamination, whereas plants may have been relatively unaffected. Further research at this site, however, indicated that plant biomass was low in metal-impacted areas (Kuperman and Carreiro 1997), and that fungal-nematode relationships could not be established relative to soil contaminants (Kuperman et al. 1998). These carefully conducted studies indicate the level of complexity introduced by potentially confounding factors (e.g., lowered soil pH in relation to heavy metal contamination) when moving from laboratory to field ecotoxicological assessments.

Because of their high density in soils, short generation time, and anhydrobiotic tolerances, nematodes are one of the first groups of invertebrates to begin grazing on bacteria and fungi associated with litter decomposition in desert soils (Santos et al. 1981). While nematodes play a major role in arid-ecosystem nutrient cycling, their contribution to the decomposition and mineralization processes is restricted by water availability. In the arid environments characteristic of the southwestern and western United States, investigators have found that nematodes can be anhydrobiotic in soil and litter much of the time (Vossbrink et al. 1979), and that soil microarthropods (e.g., mites) tolerant of lower soil-water potentials (Whitford 1989) are the principal microbial grazers. Although nematodes may be promising candidates for future ecotoxicity studies in semiarid climates, it is questionable whether their ecological relevance will be equivalent to that of more drought tolerant arthropods in strictly arid ecosystems.

B. Ants and Termites

1. *Ants*. Ants are abundant in most ecosystems of the world, both in terms of biomass as well as species numbers (MacKay 1991). Their diversity in arid regions is amazingly high, although present knowledge of their distribution and occurrence in most deserts is far from complete (Sømme 1995). It is clear that ants play a major role in arid ecosystems, simply because of their numerical abundance; this is especially true for seed predators (Sømme 1995).

Certain characteristics of ants may make them valuable bioindicators of environmental quality. Relevant attributes of ants include (1) active throughout most of the year, (2) relative abundance, (3) high species richness, (4) many specialists, (5) occupation of higher trophic levels, (6) easily sampled, and (7) easily identified (Lobry de Bruyn 1999; Majer 1983; Whitford et al. 1999). Given their relative insensitivity to short-term fluctuations in climate (Whitford et al. 1999), ants should be particularly suitable for identifying ecosystem perturbations (e.g., chemical contamination) that manifest as effects beyond the background variability in natural systems. Bengtsson and Rundgren (1984) examined abundances and diversity of ground-living invertebrates, including ants, beetles, spiders, harvestmen, and slugs, in the vicinity of a brass mill in Sweden. They found that total species numbers were lowest closest to the mill. Relative to the other invertebrates, ants were abundant at all sampling sites. However, ant abundance was also inversely related to metals concentrations in soil litter. While this observation lends credence to the idea of using ants as ecotoxicity bioindicators, MacKay (1991) notes that primary production is the principal factor determining the number of ant species in a given habitat. Because Bengtsson and Rundgren (1984) do not provide vegetation data in their study, it is unclear whether reduced ant abundance near the brass mill is related to metal toxicity or to a depauperate site flora.

Whitford et al. (1999) recently evaluated the potential of ants to serve as indicators of ecosystem health, biodiversity, disturbance, and biotic recovery in North American desert grasslands. They selected a range of sites that were exposed to varying degrees of livestock grazing intensity, as well as sites that had been subject to a variety of treatments in attempts to restore desert grasslands in shrub-dominated areas. The study demonstrated that exposure to both chronic disturbance, such as continuous and steady grazing, and acute disturbance, such as herbicide application, had little effect on the ant communities studied. Whitford et al. (1999) conclude that ants cannot be used as indicators of exposure to stress, ecosystem health, or rangeland rehabilitation success. In addition, ants were not useful indicators of faunal biodiversity in rangeland ecosystems. Despite their abundance in dry systems, ants may not be promising candidates for ecotoxicity testing because of their apparent insensitivity to stress and potential difficulties associated with laboratory maintenance. However, ants may be useful in biomonitoring studies because of their lack of sensitivity to some physical stressors.

2. *Termites*. Termites are diverse and widespread in desert ecosystems (Whitford 1986; MacKay 1991; Whitford et al. 1995). The biomass of termites in desert grasslands exceeds the biomass of domestic livestock therein (Haverty and Nutting 1975), possibly by an order of magnitude (Whitford 1986). In addition to sheer numerical abundance, termites can be considered as key ecological components of arid environments because they greatly modify biotic and abiotic processes on a vast scale (Whitford 1986). For example, their modification of the soil through extensive burrowing and sorting of soil particulates increases porosity and improves penetration by rainwater, making the microenvironment more hospitable for the termites themselves, as well as plants and other soil organisms (Whitford 1995; Pearce 1997). Termites are intimately tied with soil characteristics and moisture gradients and experiments in the

Chihuahuan desert documented plant biomass reductions and alterations to floral community structure in plots where termites were removed (Whitford et al. 1982b).

Although adept burrowers, many of the termites found in semiarid and arid areas seek shelter by nesting in the lower stem, stump, or roots of woody plants, woody debris deposited by historical flooding, and in soil under rocks. Termites may develop complicated networks of aboveground shelter tubes and belowground subterranean tunnels which interconnect multiple feeding sites, provide access to soil moisture, and regulate temperature and humidity homeostasis. The nests which may be in wood or soil are sophisticated structures which provide an essential focal point for reproductive activities, including protection of the reproductive castes, as well as an economically efficient focal point of the colonies' trophic investment energies in feeding of the young instars in nursery areas.

Termites are generally the primary detritivore in warm desert systems, actively consuming standing dead vegetation, plant litter and feces, although the specific aspects of feeding and biotic symbioses or commensalisms are not understood. In desert grasslands, termites consume 50 percent or more of all photosynthetically fixed carbon (Whitford 1991). Termites also serve as an important food source for higher trophic levels. In the Kalahari, Australian and North American Deserts, termites make up a significant fraction of the diets of lizards (Whitford 1986). Termites are critical desert community members in terms of energy and nutrient cycling, food chain relationships and community structure (Haverty and Nutting 1975; Whitford 1986; Whitford 1995; Pearce 1997).

Water mining is an extremely important activity for desert termites. In most cases, subterranean termites gather the majority of their water from free sources. This means that they obtain water that is retained in soil, under rocks, captured in underground tunnels, retained around plant roots and other soil heterogeneities, or from free sources of surface or groundwater. *Heterotermes* and *Reticulitermes* avidly build shelter tubes above ground, suggesting that these termites must have sufficient access to some source of free water so that they can secrete the wet saliva needed to construct these tubes. Likewise the building of mud sheeting by *Gnathamitermes* and the packing of mud into dung by *Bolitotermes* (Myles) (new genus: *Termitidae*) suggest access not merely to soil humidity but to moist or wet soil.

Reticulitermes are known to carry water in the crop and regurgitate it in enclosed areas to maintain termitarium humidity, or to moisten food sources when feeding. Some species in arid parts of Africa (*Hodotermes*) are known to use their salivary reservoirs for water storage, even though the crop is more commonly used as a distendible water storage organ. Since soil moisture would appear to be a critical need, it is hard to rule out the possibility of deep level foraging for water, perhaps many meters if necessary. *Hodotermes* of Africa have been known to attain depths of 14 meters (Pearce 1997).

While termites are essential components of dry ecosystems, they are also serious human pests. Termites can damage crops, buildings, pasture and forest resources as well as non-cellulose materials such as dam linings and electrical cables (Pearce 1997). Given their economic importance, considerable research has gone into understanding termite ecology and toxicology. Pearce (1997) discusses physical (e.g., drought) and chemical tolerances of termites with regard to choosing test species. Details on setting up colonies for soil-dwelling termites and additional references on termite culturing are also provided. Unlike many of the soil arthropods discussed in this section, basic information on toxicological test protocols exists for termites (Pearce 1997). Many of their pesticide sensitivities are well understood (Pearce 1997) and knowledge of their toxicological profile will facilitate further investigations of soil toxicity in dry ecosystems. In addition, useful information on collecting and identifying termites is available (Pearce 1997) and the evaluation of field sampling methods provided by Taylor et al. (1998) is particularly relevant to dry environments. It is clear that termites are promising subjects for arid system bioassays.

C. Springtails and Mites

1. *Springtails*. Collembola (springtails) are small, wingless microarthropods that live primarily in the soil litter layer. Soil collembola are primarily detritivorous, living on partially decomposed vegetation or microflora, especially fungi. Collembola contribute to soil organic matter breakdown and nutrient cycling by shredding of organic matter into fragments, feeding on humus and feces in the litter, and feeding on

decaying roots (Bardgett and Chan 1999; Efroymson et al. 1997b; Seastedt 1984). Collembola also serve as important prey items for many animals (Hopkin 1997). Along with the mites, collembola make up the majority of the soil litter arthropod fauna (Peterson and Luxton 1982; Seastedt 1984; Efroymson et al. 1997b). While more common in mesic environments, collembola are present and abundant in arid and semiarid ecosystems (Franco et al. 1979; Loring et al. 1988; Péfaur 1981; Santos and Whitford 1983; Steinberger and Whitford 1984; Wallwork 1972; Zak and Freckman 1991). However, Peterson and Luxton (1982) note that soils classified as "dry" contain the most depauperate collembolan fauna.

Collembola in arid zones have several adaptations that allow them to cope with desiccation. Collembolans are unable to regulate their water content at a specific level, but mechanisms, either physiological or behavioral, have evolved that reduce their rate of water loss (Hopkin 1997). For example, many collembolans stop feeding in response to low relative humidity and go into a dormant state, such as anhydrobiosis. In this state, the animals are able to survive dehydrating conditions that would kill those not in the anhydrobiotic state (Hopkin 1997).

Collembola have become popular test organisms in toxicity bioassays because they are easy to maintain in laboratory cultures, have uniform genomic structure (many species are parthenogenic), and have short generation times (about 6 months). For these reasons, species such as *Folsomia candida* have been proposed as useful indicators of disturbance in soil ecosystems (Moore and DeRuiter 1993). Hopkin (1997) notes that interclonal variation is not large, that the test is easy to carry out and requires little attention while running, and gives reproducible results. Indeed, tests with collembola, particularly those with *F. candida*, are close to becoming truly standard bioassays for soil organisms. Because tests can be adapted for the consideration of chronic toxicity using reproduction as an endpoint, bioassays with *F. candida* are generally more sensitive than acute earthworm mortality tests (Crouau et al. 1999). Some indication of the considerable number of chemicals that have been evaluated with collembolans is shown in Table 4.

According to Hopkin (1997), *F. candida* is typically maintained on moist plaster of Paris to which a small amount of powdered charcoal is added. The charcoal absorbs some of the metabolic wastes generated by the organisms and has the additional advantage of making it easier to spot nonpigmented springtails against the black background. A small amount of yeast is added, along with a few *F. candida*. These organisms can be readily obtained from cultures in other ecotoxicological laboratories. It is also a simple matter to create a monotypic strain by depositing just one animal on the culture medium (this only applies to parthenogenic collembola). The population builds up quite rapidly, so that after a few weeks there are hundreds of organisms in the container. Actual testing consists of adding groups of newly hatched juveniles to replicate chambers (e.g., plastic cups) with natural or artificial soil (see Chapter 2, Methods) in which they are left for a minimum of 3 weeks. After that time, surviving females have matured and have laid eggs. The soil is flooded with distilled water, and adult springtails and their offspring float to the surface. Each container is photographed from above onto transparency film and counted later. Recent developments in image analysis may greatly facilitate and streamline this aspect of the test (Krogh et al. 1998). Various measurements (e.g., weight, toxicant body burden) can be performed on harvested organisms. For further information on test conditions for Collembolans, the reader is referred to Wiles and Krogh (1998). *F. candida* is maintained in culture and under test conditions at 20^{o}C and high relative humidity, and the tolerance range of *F. candida* to desiccation is extremely narrow. For the most part, laboratory culture conditions are not applicable to arid and semiarid ecosystems, although relative humidity can reach saturation in soil and under forbs. Holmstrup (1997) investigated drought susceptibility in *F. candida* after exposure to sublethal concentrations of copper and two organic chemicals and found that decreasing the relative humidity increased toxicant susceptibility in *F. candida*. More relevant to arid system toxicity testing, control survival in the lowest humidity treatment (96.8% relative humidity) was extremely low, between 0 and 30%. Clearly, more drought resistant species of collembola must be utilized for ecologically meaningful bioassays in arid and semiarid ecosystems.

In a study on the abundance and diversity of collembola active in the soil surface of northern Chihuahuan Desert, Loring et al. (1988) found that the species *Folsomides americanus* was present and active. The authors noted that Isotomid collembolans were most abundant in the dry season. Hopkin (1997) described species in the genus *Folsomides* that effectively cope with water stress by lowering metabolic activity, reducing water and glycogen content, and filling transcuticular channels with hydrophilic substances, thus reducing passive evaporation. Because members of drought-tolerant *Folsomides* are closely related to the toxicologically well-characterized *Folsomia candida*, it may be worthwhile to evaluate the use of *Folsomides* to serve as representative microarthropods for arid and semiarid toxicity bioassays. This evaluation will require an initial investment in taxonomic identification techniques and basic research into the organism's biology.

2. *Mites*. The Acarina (mites) are a diverse group of arthropods (Efroymson et al. 1997b). Population data for the Acari considered at the taxonomic level of order have been compiled for about 180 different habitats (Peterson and Luxton 1982). Each suborder contains representatives living in a wide range of habitats (Zak and Freckman 1991; Efroymson et al. 1997b). With collembolans, mites usually account for about 95% of total microarthropods (numerically) in arid and semiarid systems. Oribatid mites are often the most numerically abundant group in forested, grassland, and desert ecosystems (Seastedt 1984).

Mites were the dominant arthropods, and oribatids accounted for more than 50% of the total arthropods present in the northern Mojave Desert (Franco et al. 1979). Mites constituted an even greater proportion of individual arthropods in the southern Mojave Desert (Wallwork 1972). Almost 90% of all individual arthropods collected were mites, and of these, oribatids were the most numerically abundant group, representing more than 50% of the total number of mite species. The oribatids were represented by only a few species, including *Joshuella striata* and *Eremaeus magniporus*. Franco et al. (1979) noted that these are the same genera (and probably the same species) as those found at the highly arid Nevada Test Site and are also common to other deserts in the southwestern United States. It is important to note, however, that Oribatids are common only in soils with relatively high organic matter (OM) content, that is, >5% OM. Oribatids are dominant in soils under thick leaf layers, such as under juniper, piñon, and oak. In desert grassland and in desert shrubland, Oribatids may be only a minor component of the soil fauna.

Oribatid mites are generally omnivorous and act to reduce the size of organic matter fragments. Their omnivory includes microflora involved in decomposition (Efroymson et al 1997b; Lebrun 1979). These feeding habits increase the rate of organic matter decomposition in soil systems. Fragmentation of litter into fine particles creates new surface area for microbial colonization and facilitates the infiltration of microflora into soil particulates. Oribatids also inoculate litter with new microflora through their feeding activity, by acting as "carriers" of the microflora to new substrates.

Santos and Whitford (1983) noted that in extremely dry and organic-carbon-depleted soils of North American hot deserts, Prostigmatid mites are the most abundant mite taxon. Prostimatids have mouthparts adapted to piercing tissue, and these animals do not ingest large particles of food (Seastedt 1984). Examples of this group include tydeid mites that feed on nematodes; this may act to regulate decomposition by regulating the population of bactivorous nematodes (Santos et al. 1981). The small tydeid mites are considered keystone species in arid ecosystems in that they exhibit direct and indirect effects on the survival of other species that are disproportionately large relative to their known abundance (Whitford 1996). Mites of the family Nanorchestidae also represent Prostigmatids; these organisms feed on soil algae and are the most abundant mite taxon in extremely arid and organic-carbon-depleted soils. Most mites, whether considered predatory or strictly herbivorous, are basically omnivorous in nature and, thus, can capitalize on available food sources in variable and unpredictable environments, such as arid and semiarid ecosystems.

The general conclusion regarding the dominant trophic function of soil microarthropods, and especially considering arid systems, is that fungal feeders like oribatids and fungiphagous prostigmatid mites predominate (Seastedt 1984; Behan-Pelletier 1999). Whitford (1989) notes that in dry soils with a water potential below –1.5 megapascals (MPa), most bacteria, protozoa, and many species of nematodes are not active and persist only in a state of anhydrobiosis. Because soil fungi grow at water potentials of –6.0 to –8.0 MPa, soil food webs in dry environments appear to be fungal based. Soil mites are among the few organisms that are active under extremely dry conditions and are thus the predominant grazers of live organic matter (fungi) in dry environments (Whitford 1989). Fungal grazers are hypothesized to increase decomposition rates by browsing on senescent hyphae and thus increasing fungal activity (Santos et al. 1981).

Lebrun (1979), noting species sensitivity and the intimate relationship that soil mites have with their environment, called for more use of mites in toxicity bioassays two decades ago. Since then, numerous studies on ecological and reproductive responses of oribatids to environmental changes have been performed (Behan-Pelletier 1999). Perhaps the most widely cited paper is that of Denneman and Van Straalen (1991). In that study, individuals of the oribatid mite *Platynothrus peltifer* were collected in the field and acclimated to laboratory conditions for 3 weeks, at which point they were added to experimental units (moistened sand covered with a layer of filter paper) and fed green algae. After one week, mites were fed the same algae spiked with heavy metals over a period of 3 months. Quantitative endpoints included reproduction (egg production), growth (weight), and survival. The authors found that metals decreased reproduction and growth, but not survival. Compared with springtails, *P. peltifer* is more sensitive to copper and lead (Denneman and Van Straalen 1991). The authors noted that mites meet several aspects of promising test species, such as ease of handling, measurable reproductive output, and monotypic genomic structure (these mites are only known to be parthenogenic). Furthermore, the authors cited the added benefits of low cost of materials and minimal space requirements for mite toxicity testing.

Denneman and Van Straalen (1991) generated useful ecotoxicological information, but the test conditions employed were not representative of terrestrial systems, much less arid environments. Investigations incorporating more ecological realism were carried out with *P. peltifer* as a member of an intact microarthropod community in natural soil spiked with copper (Streit 1984). It was shown that *P. peltifer* was the most sensitive of the six mite species present in the soil. In field studies, Strojan (1978a) noted that mites, especially the oribatids, were the most numerically dominant taxa in the three metal-impacted sites investigated. Relative to metal pollution, mites were among the most sensitive of the arthropods as indicated by decreased numerical abundance close to the polluting metal smelter. Ecological implications of reduced mite abundance included a significant reduction in leaf litter decomposition and a corresponding increase in standing mass of undecayed litter at the site (Strojan

1978b). Strojan (1978a) further noted that damage to decomposers is a potential source of instability to the entire ecosystem.

In the most realistic simulation of a soil environment, Parmelee et al. (1993, 1997) used natural communities of soil microfauna in a multispecies test system to examine the response of mites, isopods, nematodes, collembolans, and other microarthropods to metals, high explosives (TNT), phenolics, and polychlorinated biphenyls (PCBs). In both studies, the authors found that soil mites, especially the oribatids, exhibited the highest susceptibilities among the soil organisms tested. Although the experiments were carried out for almost 2 months, the authors concluded that 7 days after chemical application was the most appropriate time to sample microcosms. Additional data gleaned at later stages in the experiment were not sufficiently informative to warrant increasing the study length beyond 1 week. These studies also revealed a few benefits of using soil microarthropods as test organisms. Microarthropod extractors (devices used to extract the organisms from their experimental medium) were easy to use and did not require sophisticated equipment. Minimum training was required for personnel to recognize and correctly count total microarthropods, and the test could be carried out very quickly. However, community and trophic level analyses (e.g., recognition and correct characterization of esophageal morphology) required more advanced training.

The ecotoxicological test systems considered up to this point have been mesic in nature. Kay et al. (1999) recently evaluated soil microarthropods in North American desert rangelands for their potential as bioindicator species. Microarthropods were chosen as the indicator fauna because of their importance in ecosystem processes and because they are the only component of the soil fauna that remains active in dry soils (Whitford 1989). The investigators found that with the exception of herbicide-treated plots, all study plots were dominated by mites, specifically, bactivorous mites of the family Nanorchestidae. For the arid desert ecosystem they studied, Kay et al. (1999) concluded that the numerical responses of mites (abundance), especially nanorchestrids, appeared to provide a sensitive indicator of ecosystem health for communities characterized by drought conditions and drought-tolerant species.

Table 5 presents factors that may be useful for consideration in designing ecotoxicology tests with mites. The term "sensitivity" in the table refers to literature reports of sensitivity to chemical toxicants. For relatively organic-rich soils (e.g., in litter layers under piñon-juniper forests; >5% OM), the most promising candidates appear to be of the suborder Oribatida. They are present and numerically dominant in nearly all environments studied. Of mite taxa, oribatids have been the focus of virtually all ecotoxicological laboratory and field studies. They possess desirable characteristics for test organisms, such as measurable reproductive output, ease of culturing, and parthenogenic life history strategy, and they are ecologically relevant in arid and semiarid ecosystems. Moreover, oribatid populations decline rapidly when their habitat is damaged, a characteristic that allows detection of environmental degradation (Behan-Pelletier 1999). In more arid, organic-poor soils (<1% to 4% OM), Prostigmatid mites of the family Nanorchestidae are recommended as test organisms. These are among the few organisms that remain active in the virtual absence of measurable soil moisture (Santos and Whitford 1983). Particular mite species are not yet commercially available for ecotoxicological testing. Use of mite species will require collection from the field site, taxonomic identification by a trained professional, and successful culturing in the laboratory. Information on field sampling and laboratory maintenance of mites is presented by Van Gestel and Doornekamp (1998). The benefits derived and the relevancy of the information generated from use of soil mites may far outweigh the initial basic research costs.

D. Spiders, Beetles, Isopods, and Crickets

1. *Spiders*. A striking characteristic of hot deserts is the diversity of large predaceous arthropods that constitute a substantial proportion of the total arthropod fauna (Sømme 1995). Spiders represent a major fraction of predaceous arthropod diversity in arid and semiarid environments. Among arid-system spiders, non-web-building species predominate and include predators that hunt actively, as well as those that sit and wait for their prey. While relatively small body masses generally precludes spiders from representing a significant fraction of total animal biomass, spider biomass in deserts is still greater than

that of birds (Polis and Yamashita 1991). For an interesting but extreme example, Polis and Yamashita (1991) cite studies in which spiders were estimated to represent about 50% of the aboveground arthropod biomass in the Chihuahuan Desert near Portal, Arizona. The success of spiders can be attributed to unique physiological and ecological traits that make them particularly well suited to life in arid ecosystems.

While spiders are an abundant component of desert biota, they are, perhaps, less important to energy flow in arid ecosystems relative to other desert animals (e.g., detritivores). Polis and Yamashita (1991) cite two examples in particular. First, spiders have some of the highest efficiencies of all desert taxa at assimilating food energy into new biomass; second, they have very low metabolisms relative to other taxa. These features serve to decrease the amount of energy transferred within this trophic group and, potentially, decrease the relative magnitude of impacts on spiders to the ecosystem as a whole. With that said, however, spiders are an abundant component of the desert biota; sheer numbers may equate to their processing a substantial proportion of the energy in arid environments.

Given the prominent role of spiders in biotic interactions in terrestrial systems, they have been the focus of several toxicant impact assessments in recent years. In a review of invertebrate ecotoxicological test systems, Van Gestel and Van Straalen (1994) note that among arthropods, spiders seem to be a particularly sensitive group. For example, in a study on metal-polluted forest soils, Bengtsson and Rundgren (1984) found that spider abundances were impoverished close to the metal producing facility. Copper concentrations in spiders from this zone were higher than the corresponding body burdens in all other arthropods sampled. Spiders are upper trophic-level predators and may acquire toxic body burdens through ingestion of contaminated prey, as well as through direct contact with soil substrates. Hopkin and Marten (1985) found that spiders of the genus *Dysdera* accumulated very high levels of metals by feeding on terrestrial isopods from metal-polluted sites.

Everts et al. (1991) examined spider exposure to pesticide residues in soils. The authors found significant secondary effects associated with pesticide toxicity. Among these effects was increased mortality of exposed spiders from carabid beetle predation. More relevant to arid systems, however, was the finding of enhanced water loss after exposure to the pesticide. Everts (1997) notes that, in general, pesticide toxicity and temperature are positively correlated. The basis for this relationship stems from the fact that spiders always drink water when body fluids have been lost. At higher temperatures, spiders cannot compensate for the dehydrating effects of both the pesticide and temperature. Spider ecotoxicology has not reached the level where the test methodology has been standardized, especially with regard to arid-system species. However, numerous studies on other aspects of spider biology are common in the literature (e.g., Toft and Wise 1999) and may be useful in providing guidance on rearing and on other test conditions involving spiders.

2. *Beetles*. Beetles play many functional roles, and tenebrionids, one common family in arid and semiarid ecosystems, are detritivores. Tenebrionids are known to be extremely tolerant of dehydration and, as discussed by Sømme (1995), they are considered to be among the insects best adapted to life in the desert. While the numbers of species decline as desert conditions become more and more adverse to most forms of life, the relative numbers of tenebrionids increase (Sømme 1995).

Tenebrionid beetles are widespread throughout the arid regions of the Unites States and the rest of the world. Tenebrionids constitute the fifth largest family of beetles, with upward of 1,000 described North American species. Most of these North American species are western; only about 140 species occur in the East (Borror et al. 1989). Relative to other desert detritivores, tenebrionids are conspicuous and well understood taxonomically (Crawford 1991). Some of the more distinctive arid-system tenebrionids are those of the genus *Eleòdes*. These are the familiar black beetles that, when faced with perceived danger, tip their body to an angle of 45 degrees and almost seem to be standing on their head (Borror et al. 1989). Aptly labeled with the common name of “stinkbugs” (though not a true bug), these beetles emit a noxious reddish/black fluid (cantharidin) when disturbed or picked up.

Given their numerical abundance in deserts and their measurable contribution to desert faunal biomass, tenebrionid beetles are of potentially high relevance for toxicological testing in arid and semiarid environs. They may serve as excellent test organisms for ecotoxicological analyses, although information on toxicity testing tenebrionids in dry ecosystems is generally lacking. However, an abundance of information is available on tenebrionids (and other beetles) that are of agricultural importance in mesic systems, either because they are beneficial or because they are crop pests.

Carabid beetles, abundant in mesic and semiarid environments, have been the focus of several toxicological investigations (e.g., Heimbach et al. 1994; Heimbach and Baloch 1994). Heimbach and co-workers used carabid beetles to evaluate pesticide-contaminated soils by caging the beetles in field enclosures. The beetles were laboratory reared, and investigators took steps to acclimate them to field conditions before placing them in the enclosures. Similar strategies could undoubtedly be followed with tenebrionids to assess contaminated arid soils. Heimbach and Baloch (1994) also used laboratory-reared beetles in a lab study on pesticide efficacy in relation to temperature. The test substrate was sterile sand, and the pesticide was least effective at high temperatures, a general trend for pyrethroids. In contrast, most of the organophosphorous insecticides exhibit greater potencies at higher temperatures. The techniques employed by Heimbach are relatively straightforward, and there is every reason to believe that existing methodology for culturing and testing mesic-adapted beetles could be modified and applied to organisms that are more representative of arid ecosystems.

3. *Isopods*. Isopods are macroarthropod detritivores in arid systems and are widely distributed in warm deserts throughout the world (Crawford 1991). Fourteen species are found in the Saharan Desert alone (Sømme 1995). The isopod *Hemilepistus reaumuri* is common in the deserts of the North America, the Middle East, and North Africa (Sømme 1995). Shachak (1980) reports densities of *H. reaumuri* in the Negev Desert of up to 480,000 individuals per hectare. This density corresponds to a biomass of 19.2 kg/ha for just this isopod. In contrast, the total biomass of desert mammals worldwide is estimated to be 39.9 kg/ha (Polis and Yamashita 1991).

Assuming abundances equivalent to other desert arthropods (e.g., spiders), isopods may have a considerably greater impact on energy flow in arid ecosystems because of relatively low assimilation efficiencies (Van Straalen and Verwij 1991) and prodigious consumption rates. Hopkin (1990) reports that isopods in laboratory cultures can consume in excess of 35% of their body weight in a single 24-hour period. With that said, however, isopods are well adapted to variable conditions in food availability. Two attributes that translate into long-term viability for isopods are their long reproductive cycle and their ability to survive for up to 180 days without food (Drobne and Hopkin 1994).

Isopods are gaining recognition for their importance in ecotoxicological studies. Isopods are considered to be good candidates as standard test species because they are common, easy to handle, and generally respond quickly to environmental contamination (Paoletti and Hassall 1999). Isopods are also efficient at metal sequestration. For example, Hopkin (1990) notes that terrestrial isopods from sites contaminated with heavy metals can accumulate these metals to concentrations that are among the highest recorded in the soft tissue of any animal. While there is currently no agreement on standardization for the procedures in ecotoxicological tests using isopods, effects of chemicals on mortality, reproduction, food consumption, and assimilation are well documented (Drobne and Hopkin 1994). Most isopods are easy to maintain in the laboratory. Simple culture systems and several common species are readily available from commercial marketers of biological educational material and supplies (e.g., Carolina Biological Supply; http://www.carolina.com).

Some of the isopod-based test systems evaluated heavy metals (Hopkin 1990; Drobne and Hopkin 1994), high explosives (Gunderson et al. 1997), and PAHs (Van Straalen and Verweij 1991; Van Brummelen et al. 1996). Interestingly, toxicant-associated effects in these laboratory studies were generally minimal or nonexistent. For example, Van Straalen and Verweij (1991) concluded that the concentrations of benzo(a)pyrene eliciting toxicity in their experiments were so high, relative to contaminated soils in the Netherlands, that benzo(a)pyrene in contaminated soils is not expected to seriously affect isopod populations in that country. In a similar study using PAH concentrations representative of moderately contaminated sites, Van Brummelen et al. (1996) report that exposure to five different PAHs did not significantly affect brood size, weight of the mother after egg laying, or juvenile survival. In addition, Gunderson et al. (1997) found that isopods did better in the experimental (contaminated soil) treatments of high explosives than in (uncontaminated) controls. Drobne and Hopkin

(1994) found that at a critical threshold, cobalt significantly inhibited food consumption (as estimated by fecal production rates); however, they noted that more research should be conducted before information on this endpoint can be related to impacts on field populations.

Several terrestrial isopod species have been evaluated for ecotoxicological experimentation, but tests on arid-adapted isopods have apparently not been performed. It is unknown how species such as *H. reaumuri* (an arid-system isopod) will respond to particular toxicants; there is reason to believe that *H. reaumuri* will share desirable characteristics, such as ease of culturing, with better-understood species (e.g., *Porcello scaber* and *Oniscus asellus*). However, the apparent insensitivity of isopods to many hazardous wastes may diminish their utility as representative ecotoxicological test species in that test results could underestimate toxicant impacts to more sensitive detritivores. The reader is referred to Horning et al. (1998) for additional information on the culturing of isopods.

4. *Crickets*. Crickets are representatives of macroarthropod detritivores, a broadly inclusive functional group that includes millipedes, cockroaches, isopods, and many beetles. While detritivory is probably the most applicable category for crickets, omnivory may be applicable as well. In fact, crickets are known to exhibit high degrees of cannibalism (Crawford 1991). Crickets are widespread in most terrestrial systems. These organisms serve a valuable role in consuming and processing plant litter and are prey for other animals. In arid environments, Crawford (1991) recorded population densities of more than 80,000 individuals (*Rhaphidophorinae* and *Stenopelmatinae*) per hectare in the northern Chihuahuan Desert.

Laboratory ecotoxicology studies using crickets have been performed with the common house cricket (Gryllinae: *Acheta domesticus)* (Paine et al. 1993; Strayer et al. 1983). Strayer et al. (1983) investigated embryotoxicity and teratogenicity (generation of embryos with extra compound eyes) of soils contaminated with coal oils. Oviposition medium was prepared from various mixtures of control soil, sand, and water and spiked with known amounts of coal oil. For cricket eggs exposed to soils with 0.8% oil or greater (by volume), observed effects included 100% mortality and 2.3% teratogenicity. Paine et al. (1993) combined laboratory studies on *A. domesticus* with surveys of the field cricket *Gryllus* *pennsylvanicus*. The authors used uncontaminated soils and spiked them with the PCB Aroclor 1254. Significant mortality occurred in house crickets exposed to PCBs above 1,000 ppm (soil). Mortality was associated with PCB body burdens of 150 ppm (wet weight). However, a field investigation of the associated PCB-contaminated landfill revealed no evidence that *G. pennsylvanicus* was adversely affected in situ.

In their examination of environmentally acceptable endpoints in soil, Linz and Nakles (1997) noted that crickets are considered relevant ecological receptors representative of soil arthropods because they come into direct contact with soil and are abundant in almost all terrestrial ecosystems. The authors also point out, however, that it is unknown to what extent cricket bioassays have been used in a regulatory setting (Linz and Nakles 1997).

VI. Mammals and Birds

In most terrestrial ecosystems, birds and mammals play key roles as high-level (secondary or higher) consumers and predators. In extremely arid environments, however, birds represent a minor part of the fauna (Polis 1991). As described by Polis and Yamashita (1991), birds constitute low average biomass and density relative to other vertebrates. For example, the density of birds averages 0.6 individuals per desert hectare, while the corresponding number of mammals is 43 per hectare. The average biomass (wet wt.) of birds per desert hectare is 0.02 kg, while the equivalent biomass of mammals is 1.4 kg/ha. Polis (1991) points to low aboveground biotic structural diversity in deserts as limiting bird population establishment. Given the depauperate avian fauna that is characteristic of extremely dry environments, efforts to test toxicants on arid-system birds seem unjustifiable (Van der Valk 1997). Birds are considerably more diverse in semiarid environments. For example, approximately 200 bird species are found within Los Alamos National Laboratory (LANL 1998). However, given the limited overall importance of birds in very dry systems, as well as their complicated maintenance requirements in the laboratory, the class Aves will not be discussed further as potential test organisms.

Hot deserts have a surprisingly rich mammalian fauna, and the diversity of rodents, is greater in deserts than in most other habitats (Polis 1991; Van der Valk 1997). Rodents are among the most efficient exploiters of resources such as seeds (Brown et al. 1979; Gutterman 1993), and they have numerous physiological and behavioral adaptations that enable them to survive and prosper in deserts. Because of their small size, rodents are able to burrow or exploit other environs to avoid environmental extremes (Van der Valk 1997). Everts (1997) cites literature indicating that desert-dwelling mammals have lower water loss rates than nondesert species. In fact, many desert rodents are able to meet their water demands solely by the metabolic water generated from the food they ingest.

Considering the importance of rodents in desert ecosystems, Van der Valk (1997) notes that standard mammalian toxicity studies, which are largely based on rodents, are highly relevant to desert mammals. Particular aspects of rodent physiology may make them more vulnerable to toxicant-induced stress than their counterparts in more mesic systems. For example, desert rodents are dependent on highly efficient renal-concentrating capacities to maintain water balance. Everts (1997) notes that heavy metals can cause substantial reductions of this capacity and cites studies in which metal exposure (20 ppm potassium dichromate) resulted in a threefold increase in urine excretion in rats. Pesticides and a variety of other chemicals may also induce polyuric responses in rodents (Everts 1997). The use of rodents as ecologically relevant indicators of mammalian toxicant stress in arid ecosystems is justified and the toxicological literature is replete with laboratory studies using rodents for assessing the sublethal and lethal effects of chemicals. Relative to using other test organisms (e.g., soil invertebrates), however, ecotoxicity testing with rodents will take longer to perform and will incur much higher costs. Because costs are higher, it is more difficult to utilize a large sample size, thus decreasing the investigator's ability to detect toxicant effects. Rearing and maintaining test organisms in the laboratory is much more resource intensive for rodents than for plants or invertebrates. In addition, there are ethical concerns regarding testing with vertebrate animals than with other, lower taxonomic groups. For these reasons, the use of rodents in ecotoxicity testing is generally discouraged unless there are good reasons for specifically evaluating the mammal population at a site.

Summary

Substantial tracts of land in the southwestern and western U.S. are undergoing or will require ERA. Toxicity bioassays employed in baseline ERAs are, for the most part, representative of mesic systems, and highly standardized test species (e.g., lettuce, earthworm) are generally not relevant to arid-system toxicity testing. Conversely, relevant test species are often poorly characterized with regard to toxicant sensitivity and culture conditions. The applicability of toxicity bioassays to ecological risk assessment in arid and semiarid ecosystems was reviewed for bacteria and fungi, plants, terrestrial invertebrates, and terrestrial vertebrates.

Bacteria and fungi are critical to soil processes — understanding their ecology is important to understanding the ecological relevance of bioassays targeting either group. Terrestrial bacteria require a water film around soil particles in order to be active, while soil fungi can remain active in extremely dry soils. It is therefore expected that fungi will be of greater importance to arid and semiarid systems (Whitford 1989). If microbial processes are to be measured in soils of arid environments, it is recommended that bioassays target fungi. Regardless of the taxa studied, problems are associated with the standardization and interpretability of microbial tests, and regulatory acceptance may hinder widespread incorporation of microbial toxicity bioassays in arid system risk assessments.

Plant toxicity bioassays are gaining recognition as sensitive indicators of soil conditions because they can provide a cost-effective and relatively rapid assessment of soil quality for both pre- and post-remediation efforts. Phytotoxicity evaluations commonly target germination because environmental stressors have the greatest potential for exerting adverse effects in the early stages of growth. In arid systems, seeds respond rapidly to precipitation events, and it is typically after germination has occurred that plants must cope with water stress. Consequently, seedling emergence studies should be conducted under nonlimiting moisture conditions characteristic of mesic-plant testing. Further ecological realism can be incorporated into advanced growth stages by creating moisture conditions representative of the

arid-system study site. While the choices of suitable plant species for assessing mesic-system soils are numerous, the choices for arid-system soils are limited. Guidance is provided for evaluating plant species with regard to their suitability for serving as representative arid-system flora.

Terrestrial invertebrates can survive and flourish in extremely dry conditions. They play key roles in ecosystem functioning in arid environments. Perhaps the biggest drawback to using terrestrial invertebrates for toxicity bioassays involves uncertainties associated with choosing appropriate test species. While several examples of standard species exist for mesic soils (e.g., the earthworm *Eisenia foetida* and the collembolan *Folsomia candida*), no analogous organisms are available for testing arid and semiarid soils. The aid of an expert taxonomist and some basic research are prerequisite to using ecologically relevant invertebrates.

The use of birds for ecotoxicity testing in arid and semiarid environments is not recommended. On the other hand, mammals (especially rodents) are well represented in arid ecosystems. Much of the ecotoxicity testing performed on rodents is generally applicable to arid-adapted species; few demonstrations of rodent ecotoxicity testing for dry environments exist. Relative to other organisms discussed (e.g., soil invertebrates), the use of mammals in toxicity bioassays faces several obstacles.

Terrestrial plants and soil invertebrates appear to be the most appropriate and feasible organisms for ecotoxicity testing in arid and semiarid environments. Potentially relevant test species for arid-system testing are often poorly characterized with regard to toxicant sensitivity and culture conditions. Table 6 presents examples of standard and nonstandard species with these considerations in mind, and the best estimate of regulatory acceptance for each of the organisms is suggested. If currently accepted bioassays are not appropriate for evaluating risks in arid and semiarid ecosystems, their use in conducting ERAs in such environments may result in inadequate expenditure of time and money to develop data that accurately characterize risks. The inapplicability of this technical tool will thus hamper the risk management decision-making process and result in flawed decisions.

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Table 1. Considerations regarding the use of microbial processes and microorganisms for ecotoxicological testing in arid and semiarid ecosystems

Methodology	*Ecological Relevance*	*Standar-dization*[b]	*Documented Sensitivity*	*Comments*
Litter bag	High[a]	Moderate	Primarily a tool of soil ecologists, although used to assess contaminated soils (see De Jong 1998; Heath et al. 1964; Santos et al. 1981).	Time-tested protocol for studying decomposition in soil systems. Simple to use, although no standard ecotoxicological protocol exists. Considered as having high ecological relevance because it measures the decomposition that is actually occurring in situ. Of the microbial tests presented, this procedure is most highly recommended for assessing chemical toxicity in arid and semiarid soil ecosystems.
Carbon Mineralization / Substrate-Induced Respiration (SIR)	Moderate - high	Moderate	Primarily a tool of soil ecologists (Cheng and Coleman 1989); however, the technique is gaining recognition for its utility in assessing contaminated soils (e.g., Kuperman 1996; Kuperman and Carreiro 1997).	Carbon mineralization is a basic metric of microbial activity and represents microbial respiration of organic matter in the decomposition process. The instrumentation used to quantify substrate-induced respiration is considerably more complex and costly than the litter bag method.
Pollution-Induced Community Tolerance (PICT)	Moderate-high	Moderate	Used as a tool by ecotoxicologists from a system (Biolog®) developed by microbial ecologists. To date, primarily used to assess metal toxicity (Bååth et al. 1998; Rutgers and Breure 1999; Rutgers et al. 1998).	PICT can be cost-effective and rapid, but it is as yet relatively untested and thus not fully standardized. PICT results are subject to interpretational differences, and the system is limited to bacterial analyses.
Soil enzymes	Moderate	Moderate	Used in the assessment of toxic impacts of metals in soil (Bardgett et al. 1994; Kuperman 1996; Kuperman and Carreiro 1997).	Indirectly representative of organic matter mineralization. Requires some analytical sophistication to accurately quantify enzymatic concentrations; its applicability as a direct metric for soil ecosystem function is questionable.
Microtox	Low	High	Used in the toxicity assessment of metals, high explosives, coal oils, and diesel fuels (to name only a few) in soil (Dorn et al. 1998; Marwood et al. 1998; Simini et al. 1995).	Microtox has the highest degree of standardization of all microbial tests. However, results of this test may not be applicable to understanding and assessing toxicity of dry soils.

[a] High ecological relevance; however, a definite confounding factor associated with the litter bag technique is that the test introduces "clean" material.

[b] Although most of these tests meet the highest criteria for standardization (i.e., equivalent to an American Society for Testing and Materials [ASTM]-published method) put forth by Menzie et al. (1996), the test variability associated with different soil matrices and compositions precludes scoring anything but the Microtox test in the "high" standardization category.

Table 2. Phytotoxicity measurement endpoints

Endpoints [a]	*Brief Description*	*Toxicant Sensitivity*	*Considerations*	*Relevance*
Seedling emergence [b]	Seeds are germinated in a soil matrix over a defined time period, usually a few days to at most one week. The soil matrix is either spiked or is representative of contaminated soils and evaluated relative to a control.[c]	Can be a sensitive measure of toxicant-induced stress, but it is generally less sensitive than measures of subsequent growth.	Phytotoxicity resulting from impaired photosynthesis, flower development, or later growth stages may be underestimated by this approach. Test duration should be approximately twice the time required for normal germination of the test species. Data are quantitative and easy to collect.	Provides an indication of potential inhibition of vegetative establishment in contaminated soil.
Root/shoot elongation	Plants are grown for a set period, usually a few days post-germination, in test soil then harvested, measured, and compared with a control.[c, d]	Root elongation is typically the most sensitive measurement endpoint affected by toxicant stress.	Measures of length can be collected quickly and objectively. This metric is quantitative and is applicable for any species that produces a linear root.	Measures inhibitory effects of toxicant(s) on growth.
Root/shoot biomass (dry or wet)	Plants are grown for a set period (days to weeks) in test soil and harvested; then the measured oven-dried or fresh weight is compared with a control.[c, d]	Generally less sensitive than measures of plant root/shoot length but more sensitive than germination.	Objective, quantitative measurement. Collection of these data is generally more labor-intensive than measures of length.	Measures inhibitory effects of toxicant(s) on growth.
Visual inspection of phytotoxicity	After emergence of seedling from test soil, the plant's condition (e.g., chlorosis, mottled leaves, presence of necrotic tissue) is noted on a daily basis.	Can be a sensitive and reliable metric for assessing various qualitative phytotoxic responses.	Entails minimal time and energy for the processing of many test units. The endpoint and the judgment of the effect's magnitude are subjective. The potential exists for acquisition of quantitative measures.	Can provide an early and accurate assessment of detrimental impact from soil contaminants.

[a] ASTM (1998) provides complete details of acceptable test conditions (e.g., lighting, temperature, etc.).

[b] Seedling emergence and growth of plant species in arid systems is largely dependent upon precipitation events (Gutterman 1993; Inouye 1991). Seeds respond rapidly to plentiful moisture, and it is typically only after germination that arid-system plants must cope with water stress. Given nonlimiting water conditions at the time of germination, seedling emergence studies should be conducted under nonlimiting moisture conditions similar to mesic-plant testing.

[c] The control can be either positive, such as the use of a nonspecific toxicant like boric acid; negative, such as the application of carrier solution used to introduce toxicant; or preferably, a combination of both.

[d] Measures of plant length or biomass can only be interpreted relative to values for negative controls.

Table 3. Considerations for species selection in arid-system phytotoxicity testing: Los Alamos National Laboratory abbreviated example

Species	*Ecological Relevance*	*Standar-dization*	*Documented Sensitivity*	*Comments*
Lettuce (*Latuca sativa*)	Low	High	Metals, fuel hydrocarbons, high explosives, pesticides, phenolic compounds, herbicides. (Miller et al. 1985; Wang 1987; Adema and Henzen 1989; Schneider et al. 1996; Efroymson et al. 1997a; Gunderson et al. 1997; Marwood et al 1998; Dorn et al. 1998).	Lettuce could have higher relevance to the overall assessment if the ERA is tied into human health risk assessments. For example, this species may be considered as a crop that is accessible to wildlife. Not drought-tolerant.
Cucumber (*Cucumis sativa*)	Low	High	Metals, high explosives (Simini et al. 1995; Efroymson et al. 1997a).	See lettuce considerations.
Turnip (*Brassica rapa*)	Low	High	Metals, high explosives (Efroymson et al. 1997a; Gong et al. 1999).	See above considerations. Also, the availability of standardized whole-life-cycle methodology exists.
Wheat (*Triticum aestivum*)	Moderate	High	Metals, high explosives, fuel hydrocarbons, pesticides, herbicides (Miller et al. 1985; Chen 1993; Efroymson et al. 1997a; Dorn et al. 1998).	Represents the more drought-tolerant end of the range of standard test species.
Perennial ryegrass (*Lolium perenne*)	Moderate	High	Metals (Efroymson et al. 1997a).	See wheat considerations.
Poplar (*Populus deltoides* hybrid)	Moderate	Low-moderate	High explosives (Thompson et al. 1999).	Representative of mountain habitat tree species (e.g., *Populus tremuloides*) in northern New Mexico.
Blue grama (*Bouteloua gracilis*)	High	Low-moderate	Herbicides (Huffman and Jacoby 1984; Morgan and Knight 1991; Knight et al. 1993).	Highly drought-tolerant species and one of the dominant grasses in New Mexico and arid Southwest (McAllister et al. 1996).
Galleta grass (*Hilaria jamesii)*	High	Low	None known	Drought-tolerant species present and representative of LANL understory vegetation (McAllister et al. 1996).

Table 4. Considerations for selecting invertebrate taxa for ecotoxicological testing in arid and semiarid ecosystems

Organism	*Ecological Relevance*	*Standardi-zation*	*Documented Sensitivity*	*Comments*
Earthworms	Low	High	Metals, high explosives, PCBs, PAHs, crude oils, pesticides, herbicides (Streit 1984; Miller et al. 1985; Neuhauser et al. 1985; Menzie et al. 1992; Gibbs et al. 1996; Gunderson et al. 1997; Kuperman 1996; Efroymson et al 1997b; Kula and Larink 1997; Wilborn et al. 1997; Scott-Fordsman et al. 1998; Dorn et al. 1998).	Highest degree of test standardization of all soil invertebrates. Relative to soil microarthropods, earthworms are of little ecological relevance in semiarid and arid ecosystems. For these reasons, earthworms are not recommended for use as ecotoxicological test species.
Nematodes	High	Moderate-high	Metals, high explosives, PCBs, PAHs, pesticides, phenolic compounds (Van Kessel et al. 1989; Parmelee et al. 1993; Donkin and Dusenbery 1993; Kammenga et al. 1994; Kuperman 1996; Kammenga et al. 1996; Parmelee et al. 1997; SnowAshbrook and Erstfeld 1998).	Many studies have been performed on nematode ecotoxicology, but no standard protocol has been established. Nematodes are ubiquitous and may be excellent candidates for ecotoxicity testing for semiarid ecosystems. However, they are not recommended as representative organisms in extremely dry environments, because handling may be difficult. Identification and selection of suitable arid-system test species for laboratory testing will require taxonomic and ecotoxicological expertise.
Ants	High	Low	Metals (Bengtsson and Rundgren 1984).	Although one of the most abundant, active, and ecologically important arthropod taxa in arid ecosystems, ants may not be promising test species because of potential difficulties with their maintenance in the laboratory and their apparent insensitivity to stress.
Termites	High	Low	Termites have a number of well-understood sensitivities to insecticides, including inorganic boron compounds (Pearce 1997). Most documented sensitivity is associated with pest control studies.	Termites are one of the most important detritivores in arid systems. Their ubiquity and abundance, coupled with their extensive subterranean soil activities, make them a natural consideration for field bioassays in dry environments. Because of their economic importance as pest organisms, research on termite toxicology and culturing is fairly advanced relative to other soil arthropods (e.g., ants).
Springtails	Moderate-high	High	Metals, high explosives, phenols, PAHs, PCBs, chlorpyrifos, organotins, fungicides, pesticides (Strojan 1978a; Bengtsson et al. 1985; Tranvik and Eijsackers 1989; Paoletti et al. 1991; Crommentuijn et al. 1993; Parmelee et al. 1993; Tranvik et al 1993; Sheppard and Evenden 1994; Crommentuijn et al. 1995; Krogh 1995;	Springtails are nearly all soil-dwelling insects that have intimate associations with soil detritus, fungi, and bacteria. Many symbioses exist for this otherwise taxonomically depauperate group. *Folsomia candida* (Isotomidae) has an extremely well-characterized toxicological profile but is intolerant of dry conditions.

			Holmstrup 1997; Efroymson et al. 1997b; Parmelee et al. 1997; Sandifer and Hopkin 1997; Scott-Fordsman et al. 1997; Smit and Van Gestel 1996; Smit et al. 1997; Van Gestel and Hensbergen 1997; Frampton 1998; Smit and Van Gestel 1998; Crouau et al. 1999; Martikainen and Rantalainen 1999; Martikainen and Krogh 1999; Scott-Fordsman et al. 1999).	Members of the desiccation-resistant genus *Folsomides* or other Isotomids may prove to be good test species for bioassays applicable to arid and semiarid ecosystems. Identification and selection of suitable arid-system test species for laboratory testing will require taxonomic expertise, because these organisms are difficult to identify.
Mites	High	Low-moderate	Metals, PCBs, high explosives, phenols (Strojan 1978a; Streit 1984; Denneman and Van Straalen 1991; Parmelee et al. 1993; Parmelee et al. 1997).	Mites are the only microarthropods that are consistently active in extremely arid environments. Mites play key roles in decomposition processes in semiarid grasslands, shrublands, and arid deserts. Identification and selection of suitable arid-system test species for laboratory testing will require taxonomic and ecotoxicological expertise.
Spiders	Moderate-high	Low	Metals and pesticides (Bengtsson and Rundgren 1984; Hopkin and Marten 1985; Everts et al. 1991).	Spiders are important in arid systems but may be of less ecological relevance than arthropods with lower energetic assimilation efficiencies. Predatory roles of spiders, however, should not be overlooked.
Beetles	Moderate-high	Low-moderate	Pesticides (Heimbach et al. 1994; Heimbach and Baloch 1994).	In particular, tenebrionid beetles are abundant and play many functional roles in desert ecosystems. Although mealworms (a common tenebrionid taxon) are easily reared, native taxa should be considered.
Isopods	Moderate-high	Moderate-high	Metals, high explosives, PAHs (Hopkin 1990; Van Straalen and Verweij 1991; Drobne and Hopkin 1994; Gunderson et al. 1997; Van Brummelen et al. 1996).	Isopods are easy to handle and culture in the laboratory and are strong bioaccumulators of metals. While its potential as an ecotoxicological test species has not been defined, the isopod *Hemilepistus reaumuri* may be a particularly attractive representative for arid ecosystems. Relative to other invertebrates, however, isopods may be insensitive to chemical contaminants.
Crickets	Moderate-high	Low-moderate	PCBs, coal oils (Strayer et al. 1983; Paine et al. 1993).	Crickets are easy to handle and culture in the laboratory and are of moderately high ecological relevance in arid and semiarid ecosystems (roles as herbivores and detritivores). Their use as test species for these environments deserves further consideration.

Table 5. Considerations for using soil mites in ecotoxicological assessments

Mite taxon or species	*Documented sensitivity*	*Habitat*	*Notes*	*References*
Prostigmatid mites of the family Tydeidae	None reported	Chihuahuan Desert, Mojave Desert, White Sands National Monument, North America (probably widespread across arid Southwest).	Feed on bactivorous nematodes and thus facilitate decomposition in arid ecosystems. The investigator would need to have an active culture of nematodes to maintain a tydeid mite population in the laboratory.	Wallwork 1972; Franco et al. 1979; Santos et al. 1981; Santos and Whitford 1983; Steinberger and Whitford 1984
Prostigmatid mites of the family Nanorchestidae	Herbicide	Nanorchestidae can be numerically predominant in very dry and organic-matter-depleted soils. These organisms are ecologically relevant in sandy, desert-grassland soils.	Of potentially high ecological relevance in arid and semiarid ecosystems. Field report of herbicide sensitivity.	Franco et al. 1979; Santos and Whitford 1983; Steinberger and Whitford 1984; Walter 1987; Kay et al. 1999
Prostigmatid mites, family Nanorchestidae, genus, *Speleorchestes*	None reported	Only microarthropod found in open dunes in White Sands National Monument. Members of this genus are found in all North American hot deserts.	Recommended test genera for ecotoxicological assessments in extreme arid or organic carbon depleted environments. No toxicological information exists for this genus.	Wallwork 1972; Franco et al. 1979; Santos and Whitford 1983
Oribatid mites	None reported	White Sands National Monument, Chihuahuan Desert, Mojave Desert, North America (widespread across the arid Southwest).	Oribatids are common in arid systems and play key roles in the decomposition process, making them highly ecologically relevant in arid and semiarid ecosystems. Can be supported on easily culturable fungi.	Wallwork 1972; Franco et al. 1979; Santos and Whitford 1983
Primarily Oribatid mites	Metals	Mixed oak forests, Pennsylvania.	Field report of herbicide sensitivity in this family.	Strojan 1978a
Prostigmatid and Oribatid mites	Metals, PCBs, high explosives, phenolics	Mature oak/beech forest in Maryland.	Oribatid mites found throughout arid and semiarid United States. Laboratory report of sensitivity to many hazardous wastes.	Parmelee et al. 1993, 1997
Oribatid mite *Platynothrus peltifer*.	Metals (Cu, Pb)	High abundance in European oak/beech forests and pine forests.	See above.	Streit 1984; Denneman and Van Straalen 1991

Table 6. Recommended test species for toxicity testing in dry environments

Taxa	Standardization[a]	Benefits	Drawbacks
Plants			
Wheat (*Triticum aestivum*)	High	Well-characterized test species employed in many phytotoxicity evaluations. Relatively drought-tolerant compared with more traditional floral bioassay organisms (e.g., lettuce).	Highly cultivated crop species. May be of low ecological relevance to study site. Will likely require prestudy to characterize optimal postemergence growth, while remaining applicable to arid conditions.
Perennial ryegrass (*Lolium perenne*)	High	See wheat benefits.	Highly cultivated crop species. May be of low ecological relevance to study site. Will likely require prestudy to characterize optimal postemergence growth, while remaining applicable to arid conditions.
Blue grama (*Bouteloua gracilis)*	Moderate	Of high ecological relevance to arid and semiarid environs. Several ecotoxicological studies performed on this organism. Important study parameters such as germination rate and photosynthetic capacity are known.	Relatively unstudied compared with more traditional phytotoxicity test species. Will likely require pre-study to characterize optimal postemergence growth under arid conditions.
Invertebrates			
Isopod (*Porcello scaber*)	Moderate	Most isopods are easy to culture in the laboratory. Several species are commercially available for immediate delivery (www.carolina.com). Isopods have been the subject of several toxicological studies and are known bioaccumulators of metals.	Fairly well described, but not to the same extent as more traditional test species. *P. scaber* is representative of more mesic environments. Possibility that it is relatively insensitive to site contaminants. Will require prestudy to assess growth under arid conditions.
Mite	Moderate	Of high ecological relevance. Once the test organism is identified, it will likely be easy to culture and maintain in the laboratory.	Will require a field survey to identify mite abundance. In organic rich, semiarid soils, choosing the site's most abundant Oribatid mite species is recommended. In organic poor, arid soils, choosing the site's most abundant Prostigmatid (family Nanorchestid) mite species is recommended.

[a] Standardization refers to a regulator's likelihood of recognizing the suggested test species as a valid candidate for toxicity testing.