United States Environmental Protection Agency | Office of Emergency and Remedial Response Washington, DC 20460 | Office of Research and Development Cincinnati, OH 45268

Superfund | EPA/540/S-95/501 | July 1995

EPA

Engineering Bulletin
Biological Toxicity Testing

Purpose

Section 121(b) of the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) mandates the U.S. Environmental Protection Agency (EPA) to select remedies that "utilize permanent solutions and alternative technologies or resource recovery technologies to the maximum extent practicable" and to prefer remedial actions in which treatment "permanently and significantly reduces the volume, toxicity, or mobility of hazardous substances, pollutants, and contaminants as a principal element." The Engineering Bulletins comprise a series of documents that summarize the latest information available on selected treatment and site remediation technologies and related issues. They provide summaries of and references for the latest information to help remedial project managers (RPMs), on-scene coordinators (OSCs), contractors, and other site cleanup managers understand the type of data and site characteristics needed to evaluate a technology or other remedial tool for potential applicability to their Superfund or other hazardous waste site. Those documents that describe individual treatment technologies focus on remedial investigation scoping needs. Addenda will be issued periodically to update the original Bulletins.

Abstract

Biological toxicity testing is an important tool in performing ecological assessments at Superfund sites. Site managers* legislatively mandated to protect the environment can use biological toxicity testing to support decisions made at any stage of the remedial process. Providing information that chemical-specific testing alone cannot supply, these tests evaluate the aggregate toxic effects of all contaminants in a medium. They also can be an important indicator of increased toxicity caused by incomplete treatment of contaminated media.

This Engineering Bulletin is intended to provide site managers with information on ecological assessment and biological toxicity testing, applicability of biological toxicity testing, planning effective biological toxicity assessments, descriptions of test methods, limitations, current trends, and sources of additional information. Additional emphasis has been placed on terminology and references for biological toxicity test methods in order to provide a basic understanding from which to seek additional information as needed. This Bulletin is not intended to be a comprehensive review of toxicity test methods. RPMs and OSCs are encouraged to contact the Biological Technical Assistance Group representative for their region for additional information.

Ecological Assessment and Biological Toxicity Testing

CERCLA and the National Oil and Hazardous Substances Pollution Contingency Plan (NCP) require that remedial actions at hazardous waste sites protect human health and the environment. Site managers are also responsible for compliance with all applicable or relevant and appropriate requirements (ARARs), including numerous statutes and regulations enacted to protect natural resources. In response to these mandates, the Office of Emergency and Remedial Response (OERR) and the Office of Waste Programs Enforcement (OWPE) issued a joint memorandum in December 1988 directing Regional Offices to perform "thorough and consistent" ecological assessments at all Superfund sites. EPA followed up the memorandum by publishing the Risk Assessment Guidance for Superfund, Volume II, Environmental Evaluation Manual [1, pp. 1-57]**. The manual defines an environmental evaluation, or more precisely an ecological assessment, as a "qualitative and/or quantitative appraisal of the actual or potential effects of a hazardous waste site on plants and animals other than people and domesticated species." Ecological assessments at Superfund sites should supply site managers with the information necessary to determine potential and actual threats to the natural environment caused by current con-

* For brevity, the term "site managers" will be used to denote RPMs, OSCs, potentially responsible party (PRP) contractors, and other site remediation professionals. In cases where the information presented only applies to one or more of these groups, the included groups will be identified.

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ditions, remedial actions, and contaminants remaining at a remediated Superfund site. An ecological assessment generally is composed of four interconnected activities, which are described in the following subsections: problem formulation; exposure assessment; ecological effects assessment; and risk characterization [2, p. 3]. Figure 1 depicts these activities and their components. As shown in Figure 1 and discussed below, toxicity testing is an integral component of the ecological effects assessment activity.

Problem Formulation

Problem formulation includes development of assessment objectives and assessment endpoints. Assessment objectives are usually qualitative statements identifying the environmental values to be investigated. A typical assessment objective would be to determine if areas of a river flowing through the site have reduced populations of game fish, possibly due to contamination of the river water. To be

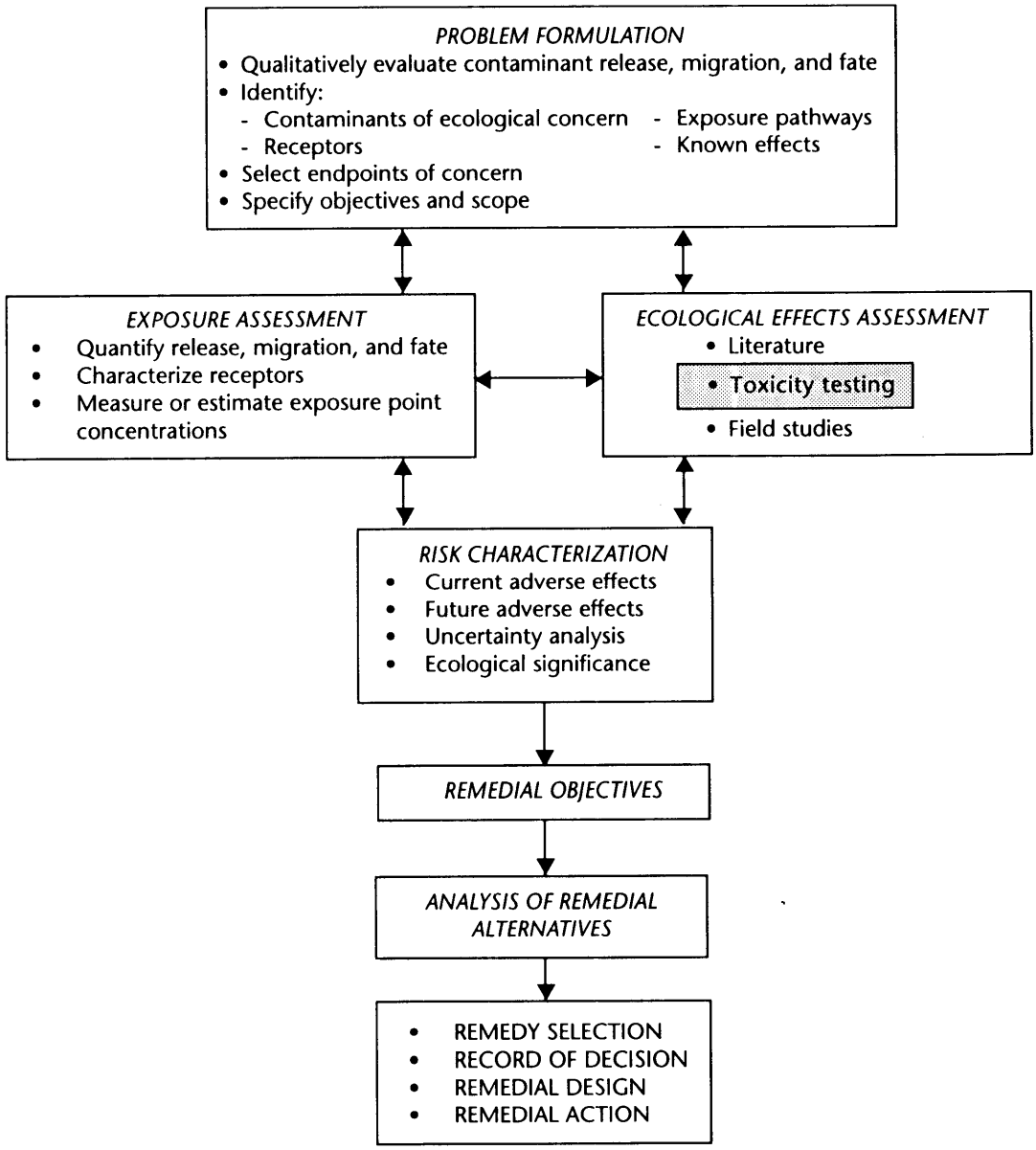

Figure 1. Ecological assessment of Superfund sites: Overview. Source: (2, p.3)

useful in guiding ecological assessments, assessment objectives must be translated into quantifiable assessment endpoints. Identification of potentially affected areas of the river where the concentration of contaminants in the surface water could result in a greater than 10 percent reduction in bass populations could be an assessment endpoint for the example assessment objective.

Exposure Assessment

Exposure assessment quantifies the magnitude and routes of contaminant exposure to which ecological receptors are subjected. Using the above example, an exposure assessment would determine the concentration of contaminants in the surface water, sediment, and food sources for bass populations upstream, within the boundaries, and downstream of the site.

Ecological Effects Assessment

Toxicity testing is conducted within the ecological effects assessment. Toxicity tests expose test organisms to soil, water, or sediment and evaluate the effects of the medium on the survival, growth, reproduction, behavior, or other attributes of the test organisms [3, p. 1]. These attributes are usually referred to as indicators. The quantitative expression of an indicator (i.e., the results of a biological toxicity test) is called a measurement endpoint. Common measurement endpoints used in toxicity testing are listed in Table 1 [4, pp. 9-10]. Measurement endpoints for an assessment should be relevant to the assessment objectives. Use of toxicity tests with measurement endpoints such as LC_{50} and NOEC for fathead minnows (standardized tests for fish toxicity testing) and LC_{50} for prey species that spend a portion of their lives in river sediment would be appropriate for the cited example.

Measurement endpoints are combined with other components of the risk assessment to evaluate the assessment endpoints. Given the potential complexity of ecological interactions at Superfund sites, multiple measurement endpoints often will be required to evaluate a single assessment endpoint. The most useful assessment endpoints are those for which there are well-developed measurement endpoints, test methods, field measurement techniques, and predictive models [5, p.24]. Using the above example, the relationship of indicators, measurement endpoints, assessment endpoints, and assessment objectives is shown in Figure 2.

Risk Characterization

Risk characterization involves a direct comparison of the results of the ecological effects assessment with the results of the exposure assessment, drawing conclusions in support of the assessment objectives. The data collected in the exposure assessment for areas upstream, within the boundaries, and downstream of the site are compared to determine the distribution of contaminants. The relative effects of these contaminants, determined by the ecological effects assessment, are then overlaid upon the contaminants distribution. This process generates a risk description that includes conclusions on the ecological risks, uncertainties associated with the conclusions, and interpretations of the ecological significance of the observed effects. For the cited example, the risk characterization could conclude that game fish populations are probably being affected by contaminants in three areas of the site, as evidenced by greater than 10 percent reductions in bass populations; and although sport fishing may be affected, the abundance of these fish in other areas of the site should preclude overall degradation of that part of the river ecosystem flowing through the site. More detailed information on performing ecological assessments can be obtained by consulting the "Sources of Additional Information" section of this Bulletin. RPMs and OSCs are especially encouraged to utilize their Regional contacts for the Biological Technical Assistance Group (BTAG) listed in that section.

Table 1. Common Measurement Endpoints Used in Toxicity Testing

NOEC	No-Observed-Effect Concentration. (The highest concentration of a contaminated medium at which no statistically significant effect relative to negative controls was observed in test organisms.)
LOEC	Lowest-Observed-Effect Concentration. (The lowest concentration of a contaminated medium at which a statistically significant effect relative to negative controls was observed in test organisms.)
MATC	Maximum Acceptable Toxicant Concentration. (The maximum concentration at which a contaminated medium can be present and not be toxic to the test organism. The MATC is normally calculated using the geometric mean of the lowest concentration for which an adverse effect was observed [LOEC] and the highest concentration that did not yield any adverse effects [NOEC].)
EC_{50}	Median Effective Concentration. (The concentration of a contaminated medium that produces a designated effect on 50 percent of the test organisms.)
LC_{50}	Median Lethal Concentration. (The concentration of a contaminated medium that produces mortality in 50 percent of the test organisms.)

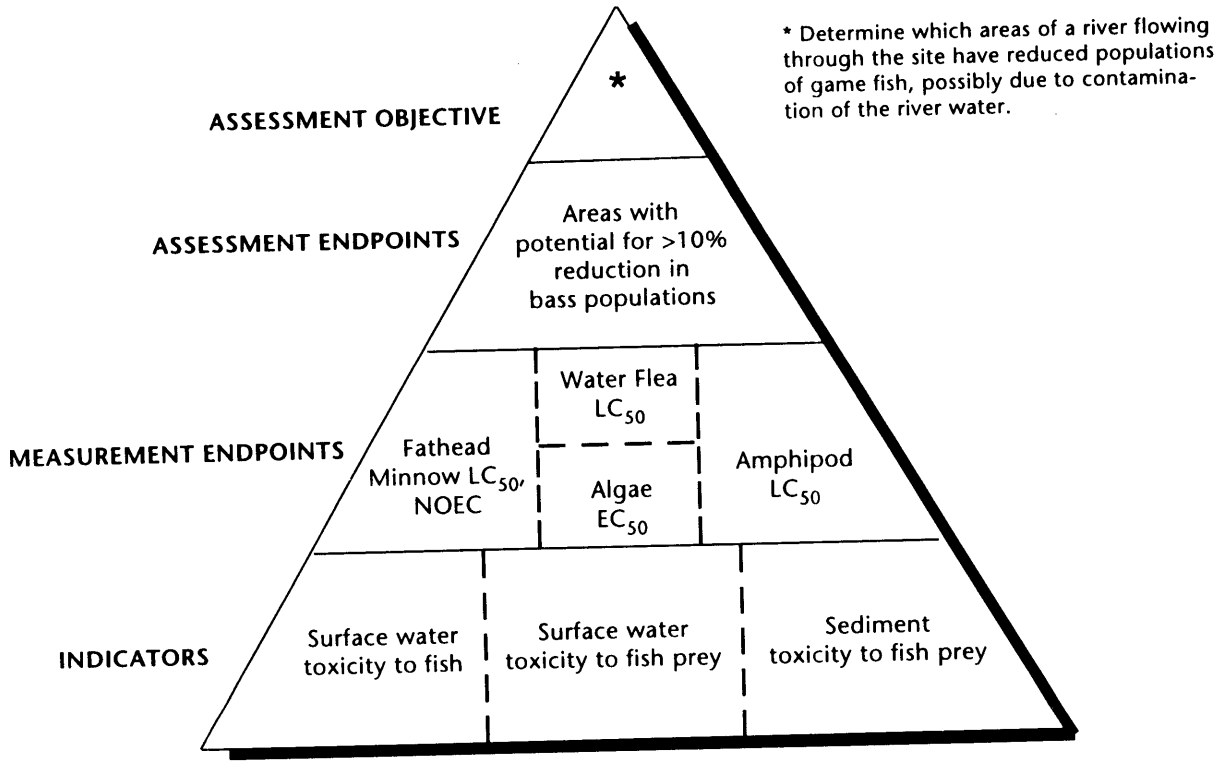

Figure 2. Relationship of indicators, endpoints, and objectives in the ecological assessment process.

Applicability of Biological Toxicity Testing

Biological toxicity testing is an important tool that is potentially applicable to any stage of the site remediation process. It can be applied to the initial stages of site prioritization, used in waste and site characterization, employed in the establishment of cleanup standards, used in the selection of treatment technologies, and finally, utilized in site monitoring during and after remediation. Figure 3 lists these applications and their corresponding stages in the remedial process [6, p. 8]. This section discusses these and other applications of biological toxicity testing.

Site Prioritization

When determining which contaminated sites should be addressed first, collection of information that allows the ranking of sites according to relative risk is an important process. Although ARARs and chemical analysis are typically used to prioritize sites, toxicity tests can identify sites that are impacted by contaminants that preliminary chemical screening may not identify. For example, pentachlorophenol (PCP) is a common contaminant at wood-preserving sites. Analytical quantification of PCP is difficult, as evidenced by an estimated quantitation limit in water of 50 parts per billion (ppb) [7, p. 8280A-32]. The presence of interfering contaminants can potentially raise the limit by an order of magnitude. Also, the acceptable spike recovery limit under the EPA Contract Laboratory Program is 9 to 103 percent [8]. When chemical testing is used alone, the reported low concentrations of PCP may indicate that the site is of lower priority. In contrast, the Microtox™ bioassay is highly sensitive to PCP, showing an EC_{50} at concentrations as low as 80 ppb. The addition of biological toxicity testing therefore could indicate that the site actually is of high priority from an ecological risk perspective.

Toxicity tests can be employed to classify the type of toxic effects produced by the mixture of contaminants at a site. They can be designed to provide information on acute or chronic toxicity of contaminants associated with media at a site. Acute toxicity tests generally indicate the test organisms' rapid response to contaminants, using a test indicator of survival. These tests can provide rapid screening information useful in site prioritization. Chronic toxicity tests generally measure the test organisms' longer-term response to contaminants including survival, changes in growth rates, reproductive capacity, biochemical and physiological functions, behavior, incidence of genetic mutations, tumors, and cancer [9, p. 6]. These tests can identify sites that did not display acute toxicity but should be further investigated due to their chronic toxicity. Use of toxicity tests in conjunction with chemical analyses in support of ARARs facilitates a more accurate site discovery and notification process.

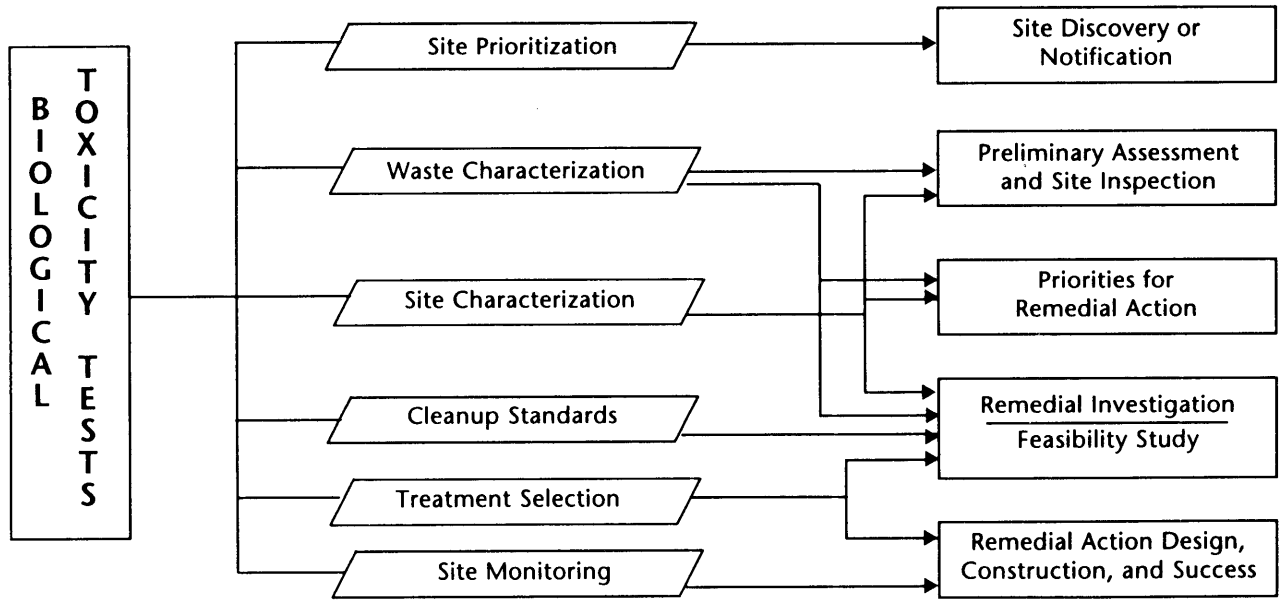

Figure 3. Potential role of biological toxicity testing in the NCP site remediation process. Source: (6, p.8)

Waste Characterization

Toxicity tests evaluate the aggregate toxic effects of all contaminants in the medium of concern. Identification and quantification of individual compounds in a complex mixture of contaminants can be prohibitively expensive and provide no information on additive, synergistic, or competitive interactions of the compounds. Biological toxicity tests, such as fish bioassays, have been used since the 1960s to determine the toxicity of complex aqueous wastes, including municipal and industrial effluents [10, p. 331]. At Superfund sites that formerly produced chemicals, little scientific literature may be available on the toxicity of the chemicals produced that are now contaminating the site. Toxicity tests can evaluate the toxicity of substances whose biological effects have not been well characterized. Over 10 million chemicals had been documented in the American Chemical Society's Chemical Abstracts Service as of April 1990. The "environmental" toxicity of only a small portion of these compounds (mostly pesticides and herbicides) is known [11, p. 266]. Various modeling approaches, such as structural-activity models, may be used to predict the availability and toxicity of compounds, but toxicity test results are needed to verify the toxic effects of compounds under specific environmental conditions. Information on the biological effects of wastes at a site can be important to the preliminary assessment and site inspection (PA/SI), remedial action prioritization (RAP), and remedial investigation and feasibility study (RI/FS) stages of the remedial process.

Site Characterization

Toxicity tests can be a cost-effective approach to characterizing the distribution of contaminants potentially affecting resident organisms throughout a site. Determining the distribution of site contaminants at a Superfund site by chemical analysis is very expensive and may not show the spatial relationship of contaminants and biosensitive areas. Many of the standard acute toxicity tests are comparable in cost to comprehensive chemical analyses [12]. More sophisticated chronic toxicity tests provide additional information useful in site characterization, but can be substantially more expensive. Biological toxicity testing used in support of site characterization provides information useful to the PA/SI, RAP, and RI/FS stages of the remediation process.

Cleanup Standards

Toxicity test results can be used to help ensure that cleanup standards established for a site will be protective of the environment. Cleanup standards that are based upon human risk assessments or technology performance may not be protective of all organisms at a site. The use of toxicity tests as a component in the cleanup standard-setting process provides a more complete picture of the overall protection derived from remediating site contamination to a specific level.

Toxicity tests can be used, in some cases, to determine which compounds are contributing to the observed effects on organisms at a site. Using a toxicity identification evaluations (TIE) approach, investigators can manipulate test conditions to selectively affect certain compounds and compare these results to results from unmanipulated tests. For example, a chelating agent can be added to one set of water tests and the results compared to results from tests where the agent was not added to the medium. If performed with the proper controls, a reduction in toxicity in

the chelated tests would indicate a likelihood that the toxic effect is at least partially due to compounds that can be removed by chelation (e.g., certain metals). This type of information would allow establishment of cleanup standards for specific groups of compounds based upon toxicity tests. EPA has developed a three-phase approach to TIE that progressively narrows the focus of the evaluation from toxicity characterization, through toxicity identification, to toxicity confirmation. This approach currently can be used for water and sediment samples. For further information, the reader is referred to the corresponding EPA documents [13][14][15][16] [17].

Toxicity tests can help determine the potential for a remediated site to support a viable ecological community. CERCLA and the NCP define natural resources to include biota and their supporting resources and designate natural resource trustees charged with their protection. CERCLA Section 104(b)(2) requires EPA to promptly notify the appropriate natural resource trustees of the potential for natural resource injuries resulting from releases under investigation. If natural resources are damaged, the trustees are allowed to file for monetary compensation. Toxicity testing that indicates the potential for restoration of natural resources provides site managers with valuable information for negotiating with natural resource trustees concerning the potential for natural resource damage assessments. Selection of remedial alternatives that protect and restore natural resources often reduces the chances of costly and time-consuming natural resource damage proceedings that may delay negotiated settlements [18, p. 1-9]. Toxicity tests used in support of the development of cleanup standards provide valuable information for the RI/FS stage of remediation.

Treatment Selection

Toxicity tests can be an important tool in the evaluation of different treatment technologies investigated through treatability studies. Remedy screening treatability studies are generally performed to determine the potential feasibility of several remedial technologies. Remedy selection treatability studies are usually employed to develop performance and cost data on a smaller group of treatment technologies [19, pp. 8-10]. Toxicity tests performed on the medium of interest before and after remedy screening treatability studies can identify which technologies are potentially effective at reducing the toxic properties of contaminated soil, sediment, and water. When used in the evaluation of remedy selection studies, toxicity testing can help determine the degree of treatment required to reduce toxic effects to an acceptable level. Cost of treatment estimates can then be generated.

Toxicity tests have been used to evaluate bench-scale treatment technologies for several mine drainage remediation projects. Acute toxicity tests have been used to augment bench-scale treatment investigations of mine drainage from a drainage tunnel near Leadville, Colorado. Forty-eight-hour water flea and minnow tests were used to evaluate five different chemical/physical treatment techniques. Results from these tests were used to determine which of the techniques cost-effectively maximized the removal of metal toxicity. Based upon the toxicity tests and other chemistry and engineering information, a treatment system was recommended and constructed.

Similar tests were used to evaluate the effectiveness of seven different artificially constructed wetlands in reducing toxicity of effluents from mine drainage in Idaho Springs, Colorado. Toxicity testing also was used to evaluate the effectiveness of modifications to the pretreatment of drainage prior to entering these pilot-scale wetlands. Biological toxicity tests used in the treatment selection process support decisions made during the RI/FS and remedial action design and construction processes.

Site Monitoring

Toxicity tests can be an integral component of remedial and post-remedial monitoring. Remedial actions, such as excavation, can increase soil loading to adjacent water bodies. Dredging of contaminated sediment can resuspend particles, potentially impacting downstream organisms. In order to monitor the effects of these activities, biological toxicity tests can be employed. Some remediation technologies have the potential to produce compounds that are more toxic than the original contaminants (e.g., intermediate metabolites during bioremediation). Biological toxicity testing can be used to monitor for these concerns [10, p. 336][20, pp. 105-112]. The long-term effectiveness of remedial actions can be monitored through periodic toxicity testing. Depending on the cleanup goals established, either acute or chronic tests can be employed. If the remediation was not completely successful, it is important to identify problems and implement corrective actions as soon as possible [6, p. 11]. Toxicity testing can also be incorporated into long-term monitoring of remediation residuals that remain on site (e.g., solidified or thermally treated soil).

Toxicity testing was used at a site in Michigan to evaluate the removal of organic contaminants from soil after treatment by thermal desorption. Using an earthworm test, it was determined that the soil was as toxic after treatment as before. Further investigation showed that, while the toxicity attributed to organic contaminants was removed, the treatment had increased the bioavailability of manganese through the removal of organic matter to which it had previously been bound. Both aquatic and terrestrial toxicity tests have been used at several sites to evaluate the effectiveness of mine tailings remediation projects. At one site, aquatic tests were used to pin-point instream impacts from tailings and to evaluate the effectiveness of isolating tailings drainage and runoff from a creek. At another site, earthworm tests were used to determine the degree of residual toxicity remaining after mine tailings and roaster piles were removed.

These examples emphasize the need to consider all relevant data when determining whether cleanup goals have been achieved. A decision that cleanup goals have been met based upon only one type of test may lead to an

incorrect conclusion. Toxicity tests used in support of site monitoring can help ensure a thorough evaluation of remedial action design, construction, implementation, and success.

Planning Effective Biological Toxicity Assessments

Site managers charged with planning or reviewing biological toxicity testing should be aware of the elements that comprise effective biological toxicity assessments and the ideal characteristics of toxicity tests. Important elements discussed in the following subsections include: the objective, the reference site, the medium analyzed, the test organisms, the test methodology, the test site, the statistical analysis to be used to interpret the results, and the quality assurance/quality control (QA/QC) standards necessary to ensure the collection of valid data [3, p. 3]. These elements are interrelated; changes in one area affect the other areas. Site managers should review all elements if changes are made to any one.

The Objective

The development of clear, attainable objectives is the most critical element in a toxicity assessment. Objectives need to reflect the type and level of information required from the study. Each of the applications shown in Figure 3 may require a different set of objectives. For example, the objective of toxicity tests used in site characterization may be to identify the areas with the highest toxicity to organisms in order to prioritize remediation of the site. The other elements of the assessment would reflect this objective (e.g., the testing may evaluate both soil and water media, using acute toxicity test methodologies). If the assessment objective is to set toxicity-based cleanup standards for soil at the site, the other elements would focus on the evaluation of soil and the use of chronic toxicity test methodologies.

An important component of establishing assessment objectives is the development of data quality objectives (DQOs). DQOs are qualitative and quantitative statements specifying the quality of data needed to support test conclusions. They are developed in accordance with the intended end use of the data to be collected. The three stages of DQO development are: identify decision types, identify data uses/needs, and design the data collection program. For further information on development of DQOs, consult Data Quality Objectives for Remedial Response Activities, Development Process [21].

The Reference Site

Toxicity tests usually compare the response of one or more test species exposed to contaminated media with the species' response to media from an area unaffected by the site or other sources of contamination. This unaffected area, or reference site, should be situated as close to the Superfund site as possible without being impacted by onsite or offsite contamination. Upstream locations are often appropriate for surface water and sediment toxicity tests. Upwind, upgradient areas can be appropriate reference sites for terrestrial tests. Site managers should consider factors such as sediment and soil particle size, vegetation, slope, previous usage, the presence of fill material, and unrelated sources of contamination when choosing a reference site. Careful evaluation of these factors will allow investigators to match site characteristics as closely as possible, reducing the effect of noncontaminant differences on data comparisons.

In some cases, negative controls (i.e., a medium that is known to be nontoxic to the test organisms and is geochemically similar to the test medium) are used instead of a reference site. This approach provides a reasonable worst-case comparison by eliminating most of the non-contaminant factors that affect organisms at a reference site. Consequently, the difference in organism response between the test medium and the negative control may be greater than the difference in organism response between the test medium and the reference site.

The Medium

Toxicity tests most often evaluate the effects of contaminants in surface water, sediment, or soil. For the latter two, samples may be tested as bulk samples or processed first, using water as an extraction fluid to remove substances adsorbed to the solid particles, and the water extract used for the test. Bulk soil or sediment tests evaluate the toxicity of the medium itself, while water extract tests provide information about the potential toxicity of runoff, leachate, or water associated with sediment disturbances, such as dredging. Obtaining information on the medium being tested is important for characterizing the medium and in some instances, estimating contaminant availability. For water, important factors to consider include alkalinity, hardness, pH, temperature, biochemical oxygen demand, total dissolved oxygen, total dissolved solids, total organic carbon, nitrogen, and phosphorus. For sediment and soil, important factors include grain size distribution, bulk density, humic content, percent moisture, pH, and total organic carbon. These factors also must be considered when establishing control and reference site samples. The effect of any adjustments to the medium (e.g., increasing the total dissolved oxygen content or diluting samples to determine ranges of toxicity) must also be considered and should be kept to a minimum whenever possible.

Test Organisms

Information on specific test organisms is presented in the "Descriptions of Biological Toxicity Test Methods" section of this Bulletin. In most cases, use of standard organisms will be sufficient to meet assessment objectives. Organisms typically used for toxicity tests include: bacteria; algae; seeds; young plants; aquatic macroinvertebrates, such as amphipods, chironomids, and sediment worms; mollusks; sea urchins; aquatic vertebrates, such as amphibians and fish; and terrestrial invertebrates, such as earth-

worms [22, pp. 1-4]. When choosing standard organisms, it is important to consider species that are representative of resident organisms, sensitive to site contaminants, applicable to assessment endpoints, consistent with DQOs, and supportive of assessment goals.

The use of nonstandard organisms, such as contaminant sensitive, resident invertebrates or fish species, may provide better representation of the actual effects of site contaminants. Additional factors that are standardized when using designated test species, such as test conditions, organism age, positive identification, and physiological condition, must be examined more closely when using nonstandard organisms. When considering resident species for toxicity tests, species-specific factors should be considered. These factors include the species' potential for exposure, relative sensitivity to contaminants, role in the ecological functions of the site, potential for wildlife and human consumption, time spent on site, characteristics that contribute to the ease or difficulty of conducting the test, appropriateness as a surrogate for other species, and recognized value (e.g., importance as a game fish) [23, p. 3]. Furthermore, collection of some organisms may be regulated by Federal, State, and local regulations (e.g., threatened and endangered species regulations) that must be followed when gathering test species.

In order to accomplish most biological toxicity assessment objectives, it is usually necessary to use more than one test organism. For soil tests, one plant and one animal species can be used. When evaluating aqueous phases, it is recommended that at least one fish and one invertebrate species be used, unless the site is known to have only one contaminant and one of the groups of organisms is known to be insensitive to that contaminant [3, p. 8]. For sediment tests, a battery of tests has been recommended [24, p. 556]. In instances where this number of tests is not feasible, sediment evaluations should include at least two invertebrate species, including one that spends a portion of its life in sediment (e.g., the amphipod, *Hyalella azteca*).

Test Method

Information on specific test methods is presented in the "Descriptions of Biological Toxicity Test Methods" section of this Bulletin. Standard toxicity test methods have been developed for the evaluation of contaminated soil, sediment, and water by several organizations, including The American Society for Testing and Materials (ASTM), EPA, and private companies [4][25][26][27][28]. Biological toxicity test methods should supply the following information: scope and application; summary of method; sample collection, preservation, and handling; interferences; equipment (including test organisms); reagents; procedures; calculations; quality assurance/quality control measures; data validation and reporting (including statistical presentation); and health and safety considerations. The method should specify the use of one or more negative controls. A medium from an identified reference site is sometimes used for this purpose [25, p. 6].

Table 2 presents the characteristics of an ideal biological toxicity test [24, p. 543]. Depending on the objectives and level of effort required for the assessment, the test may only meet a portion of these characteristics. For example, toxicity tests designed to determine the chronic effects of sediment contaminants on sediment dwelling invertebrates will meet many of the characteristics, especially sensitivity, ecological relevance, and usefulness for regulatory standards. This type of test is not rapid or inexpensive compared to tests focusing on acute effects. Conversely, if the objective is to identify areas of the site with the highest sediment toxicity to aquatic macroinvertebrates, an acute toxicity test that is rapid (e.g., 48 hours), simple, and relatively inexpensive is more appropriate. The results of the acute test, however, may be less useful in developing regulatory or site-specific standards than the results of the chronic tests.

Table 2. Characteristics of an Ideal Biological Toxicity Test

- Rapid	- Discriminatory
- Simple	- Ecologically relevant
- Replicable	- Relatable to field effects
- Inexpensive	- Useful in developing, and relatable
- Standardized	to, regulatory standards
- Sensitive	

Source: (24, p.543)

Test Site

The majority of toxicity tests are performed at laboratories on samples of media collected and shipped from the site of interest. Some companies have mobile laboratory facilities that can be set up onsite, reducing the time between sample collection and testing. The advantages of laboratory testing include constant conditions, standardized protocols, and readily available equipment [3, p. 8].

In situ toxicity tests allow the test organisms to be in constant exposure to the medium of concern under actual site conditions. This type of test may provide a more realistic evaluation of contaminant toxicity, and if using species present on the site, can generate data that are directly applicable to the ecological risk assessment. Additionally, in situ tests do not invoke the regulatory issues or disposal requirements raised by offsite shipment of contaminated media. However, use of in situ toxicity tests provides little control over changing test conditions. For example, heavy rainfall during a toxicity test may increase stream flow, changing the chemical conditions to which test organisms are exposed. Also, testing designed to simulate a reasonable worst-case scenario may be disrupted by changing site conditions, calling into question the stringency of the test. For these reasons, site conditions during the test should be closely monitored.

Statistical Analysis

The type of statistical analysis used to evaluate toxicity test results depends on the test objectives and the measurement endpoint used. Measurements that estimate the effects from specific dilutions (e.g., LC_{50} and EC_{50}) can be

subjected to regression models that assume the greater the dilution of contaminants, the lesser the effect. Coefficients of variation can be calculated for these types of point estimates.

Measurements that compare test dilutions with controls and evaluate whether differences are significant (e.g., NOEC and LOEC) make use of hypothesis testing [3, p. 8]. Using the null hypothesis that there is no difference between a test dilution and the control, the test data should result in acceptance or rejection of the hypothesis at a confidence level determined by project DQOs or other requirements. A thorough discussion on the statistical analysis of toxicity test results is presented in other EPA documents [29][30].

QA/QC Standards

In order for test results to be defensible, it is necessary to have the appropriate level of supporting QA/QC. As stated earlier, toxicity test methods should specify QA/QC measures to be followed, starting from sample collection and concluding with report preparation. These measures should be consistent with the DQOs for the project. Biological toxicity assessment plans should have a separate section on project QA/QC. Large-scale efforts may need to have separate quality assurance project plans (QAPPs). The level of QA/QC effort is dictated by the end use of the data. EPA has divided data collection projects into four categories based upon data usage. Category I projects require the most rigorous and extensive QA; Category IV projects require the least. Most biological toxicity testing efforts fit within Category II or III, producing results that complement other inputs to a decision process, or producing results used to evaluate and select basic options, respectively. Two documents useful in establishing appropriate QA are Preparation Aids for the Development of Category II Quality Assurance Project Plans and the complementary document for the development of Category III QAPPs [31][32].

Descriptions of Biological Toxicity Test Methods

Biological toxicity tests should be chosen to accomplish the stated objectives of the study. Depending on those objectives, tests that differ in measurement endpoints, range of media concentrations, contaminant delivery scenarios, and organism selection may be chosen. The following subsections discuss these differences.

Measurement Endpoints

Acute toxicity tests measure an organism's short-term response (typically 1 to 5 days) to contaminants. The measurement endpoint for acute toxicity tests usually relates to survival of the test organisms [3, p. 2]. Chronic toxicity tests generally are more sensitive than acute tests and commonly expose test organisms to lower contaminant concentrations. They also typically require more financial resources and time to perform. (Chronic tests presented later in this Bulletin use exposure durations ranging from 2 to 90 days.) Measurement endpoints for chronic tests usually include survival, growth, reproductive impairment, nerve impairment, reduced or abnormal motility, development of structural abnormalities (teratogenicity), development of chromosomal abnormalities (genotoxicity and mutagenicity), and behavioral changes [3, p. 3]. Given the amount and types of data generated by chronic tests, their expense and duration are often justified. When designed properly, both acute and chronic toxicity tests provide valuable, statistically defensible results. Common measurement endpoints for acute and chronic toxicity tests were presented in Table 1.

Range of Media Concentrations

Biological toxicity tests can be divided into three categories based upon the range of media concentrations used in the tests. These categories are screening, range-finding, and definitive tests.

Screening tests generally examine the acute and chronic effects of undiluted samples on the test organisms. These tests can be useful for site prioritization and site characterization by distinguishing between areas of high toxicity and low/no toxicity. Since these tests are performed at one concentration (undiluted samples), they are generally less expensive than range-finding and definitive tests. Significant results from screening tests point out the potential need for definitive tests.

Range-finding tests generally examine the test organisms' acute response to a broad range of media dilutions. They commonly utilize three or more media dilutions and do not usually require replicate tests [27, pp. 5-7, 13-15]. Consequently, range-finding tests are usually more expensive than screening tests, but less expensive than definitive tests. Range-finding tests help identify appropriate dilutions for definitive tests.

Definitive tests establish concentration-response relationships or NOECs between media concentrations and the responses of test organisms. These tests typically use a range of concentrations established by the range-finding tests. Replicate test units are used when employing definitive tests. These tests can be useful in waste characterization, development of cleanup standards, and site monitoring [3, p. 8].

It should be noted that most sediment and soil tests are currently conducted using undiluted samples only. This limitation is necessitated by the lack of established techniques for performing sediment and soil dilutions.

Contaminant Delivery Scenarios

There are three contaminant delivery scenarios used for aqueous-phase toxicity tests. Static tests utilize the same contaminated medium, with no additions of the medium throughout the test duration. Static-renewal tests deliver new test solution to the test organisms by replacing all or a portion of the aqueous phase at specified times during the

test. The flow-through method continuously delivers new aqueous phase to the test organisms, minimizing the abiotic loss of contaminants. Of the three approaches, flow-through systems require the most complex equipment and incur the greatest expense [3, pp. 5, 8].

Toxicity tests on soil are most commonly of the static design. Depending on the objectives of the study, however, aqueous extracts of the soil or the waste can be introduced to the soil and static-removal or flow-through tests can be employed. For example, the effects on earthworms of leachate from a solidified waste that is to remain at a site can be examined by pouring the leachate into the test chamber once (static), intermittently (static-renewal), or continuously (flow-through) depending on the exposure scenario of interest. A modification used for sediment testing is to periodically renew the overlying water while not renewing the sediment.

Organism Selection

Table 3 lists commonly used biological toxicity tests that support remedial activities at contaminated sites. The tests are identified by the organisms employed in the evaluation. The same species are often used for both acute and chronic testing, with conditions and duration being modified to differentiate between the two types of tests. Table 3 is not intended to be a comprehensive list of available tests. The focus of the table is on tests that allow estimations of the effects of contaminated media on populations (groups of the same species) and, to a lesser extent, communities (populations of different species) at a site. Effects at the ecosystem level (as evaluated in microcosm experiments) and effects of contaminated media on higher organisms (e.g., birds and mammals) are not covered in the table or this Bulletin. For more information on these aspects of biological toxicity testing, refer to Compendium of Ecological Risk Assessment Tools [33], and consult the appropriate Regional BTAG member listed in the "Sources of Additional Information" section of this Bulletin.

General Guidance

When choosing biological toxicity test methods, the objectives of the monitoring program and available data on contaminant concentrations should be considered. Acute tests are usually conducted when concentrations of contaminants are in the part per million (ppm) range; chronic test are usually conducted when contaminants are in the ppb range. The appropriateness of methods, however, can be greatly influenced by the characteristics of the medium being investigated. As previously discussed, water quality conditions, such as hardness, alkalinity, pH, and/or organic content will affect the toxicity of both organic and metal contaminants. For example, high organic content, hardness, or alkalinity will reduce copper toxicity, while high organic content can make certain organic contaminants more soluble and therefore, more bioavailable.

Of the tests listed in Table 3, both the minnow and water flea acute and chronic tests are commonly used for evaluating the toxicity of streams, lakes, leachates, and effluents. Acute tests using these organisms are recommended for use in support of feasibility studies where previous toxicity tests and extensive chemical analyses have not been performed. If no statistically significant mortality is observed in the acute tests, chronic tests using these organisms then should be considered. This approach will indicate whether acute or chronic risks may need to be examined in the subsequent stages of the remediation process.

This approach also should be used to evaluate other types of contaminated media as well. For marine and estuarine water, shrimp and fish (silverside or sheepshead minnow) acute and chronic tests are commonly used. For whole sediment testing, current EPA methods list amphipods and midge larvae for fresh water sediment and amphipods for marine and estuarine sediment [29][30]. For soil testing, acute earthworm and 4-day seed germination tests can be conducted when soil contaminants are in the ppm range. Genotoxicity, root elongation, and earthworm reproductive tests are appropriate when soil contamination is present in ppb levels.

Limitations

Biological toxicity testing should be recognized as one tool in the overall process of environmental assessment. When properly utilized it provides information on the potential effects of contaminated media on the ecological conditions of the site of interest. Toxicity tests are not intended to be absolute proof of environmental damage [1, p. 1]. In order to properly use the information obtained from toxicity tests, it is important to understand their limitations.

- Biological toxicity tests generally do not differentiate between the individual contaminants contributing to the response of the test organism. A review of pertinent literature, however, may indicate a test organism's relative sensitivity to a specific type of contaminant. For example, a well executed literature search would reveal that green algae, such as *Selenastrum*, are more sensitive to low concentrations of some heavy metals than higher level organisms, such as fish [24, p. 548]. By using several test organisms, and changing test conditions (i.e., using the TIE approach), it may be possible to determine which contaminants are producing the effects of concern.

- Most tests require the identification of a reference site that is presumably unaffected by the contaminants of concern. Incorrect selection of the reference site may cause underestimation of the toxic effects of the medium. This limitation may be addressed through the use of appropriate laboratory negative controls and the use of multiple reference sites.

- Biological toxicity testing may not be necessary for all sites. Sites which have contaminant concentrations below levels associated with documented acute or chronic effects may not need to be tested. Review of pertinent literature and site chemical data may prevent the performance of unnecessary toxicity tests.

- Different organisms display widely different responses to the same contaminant; this often necessitates the gathering of prior information on site contaminants and species sensitivity. The performance of multiple tests using different species may address this limitation.

- Ancillary test conditions (e.g., alkalinity of the test water) can adversely affect test results. Nonstandard test conditions also make the comparison of results between sites and over time difficult to interpret. Test conditions specified in standardized test methods, therefore, should be followed where possible. Also, additional negative controls should be used to address this limitation.

- In many cases, single toxicity tests will not provide sufficient information to assist site managers in evaluating the environmental conditions of the site. Several tests using different species are usually required for surface water testing [3, p. 9]. Choosing tests that use site-appropriate organisms may reduce the number of tests required.

- Toxicity test results, when not evaluated in light of additional information (e.g., taxonomic surveys, chemical analyses, and computerized toxicity modeling), present only a partial, and possibly incorrect, picture of environmental conditions at a site [53, p. 827]. Interpretation of results can be difficult if supporting information is unavailable. Since biological toxicity testing in support of site remediation is a developing field, site managers may not have adequate experience in results interpretation. The use of all available resources, including BTAG contacts, will help ensure proper site evaluation.

- The collection, shipment, and biological toxicity testing of media contaminated with hazardous waste is regulated under the treatability study samples requirements of 40 CFR 261.4(e-f). (These regulations allow the collection, shipment, and testing of up to 10,000 kilograms (kg) of each medium contaminated with non-acute hazardous waste and up to 2,500 kg of each medium contaminated with acute hazardous waste to proceed under reduced regulation. These regulations do specify packing, shipping, storage, and notification requirements for sample collectors and testing facilities.) [54, pp. 13, 38-40] [55, pp. 8365-8366]. Onsite and in situ testing may reduce regulatory requirements.

Current Trends in Biological Toxicity Testing

The use of biological toxicity testing in conjunction with predictive modeling has gained considerable interest in recent years. Models, such as EPA's LC_{50}, are being used to support field and laboratory toxicity testing by providing site managers with tools for estimating the biological effects of contaminated media [33, p. 243]. Additional information on models is presented in the "Sources of Additional Information" section of this Bulletin.

Refinement, standardization, and validation of additional biological toxicity test methods is an ongoing task at EPA, academic, and private laboratories. The Compendium of Ecological Risk Assessment Tools, produced in September 1993, list 56 laboratory study methods [33]. ASTM also continues to update its guides for conducting toxicity tests, incorporating new species and test procedures [25][26].

Sources of Additional Information

Given the complexity of many Superfund sites, the difficulty in interpreting toxicity data, and the relatively recent emphasis on quantitative ecological risk assessment, site managers are strongly encouraged to make use of biological toxicity testing resources. For RPMs and OSCs, the Regional BTAG contacts listed in Table 4 are valuable sources of information on biological resource issues. An updated list of contacts is periodically published in ECO Update, an EPA bulletin described in Table 5. The BTAG member should be contacted early in the remedial process. Following initial review of site data, the BTAG member can make recommendations on the need for biological toxicity testing. The BTAG contact also should be consulted when test plans, QAPPs, interim reports, and data summaries are delivered. BTAG comments on these documents can save time and money by pointing to the need for additional or fewer tests [56, p. 2].

RPMs and OSCs are also encouraged to utilize the Center for Technical Assistance on Ecological Assessment of Superfund and RCRA Sites. The Center is part of the Ecological Monitoring Research Division of the National Exposure Research Laboratory (NERL) in Cincinnati, OH. The Center supports the Regions by providing technical reviews, conducting aquatic and terrestrial ecological assessment studies, and performing ecotoxicity testing. Available assistance with ecological assessments includes collection and assessment of aquatic and terrestrial biological systems, assessment of physical habitat, and performance of ecotoxicity assessments. Assistance with ecotoxicity assessments includes toxicity testing of water, sediments, and soils with vertebrates, invertebrates, and plants. The Center has both in-field and laboratory toxicity testing capabilities. Most of the tests listed in Table 3 can be performed in support of Regional efforts. Additionally, the Center has constructed 12 artificial streams that can be modified to simulate a variety of site conditions. For further information on the Center, contact Dr. James Lazorchak at (513) 569-7076.

Non-governmental site managers are encouraged to consult experts in the academic and private sector, and work closely with the overseeing regulatory agency.

Table 5 lists additional sources of information on biological toxicity testing. Many of these sources are available to both EPA and the general public.

Table 4. U.S. EPA Regional BTAG Coordinators/Contacts

EPA Headquarters

Steve Ells
OWPE
USEPA (OS-510)
401 M Street, S.W.
Washington, DC 20460
(703) 603-8934
(703) 603-6724 FAX

Joseph Tieger
USEPA (OS-510W)
401 M Street, S.W.
Washington, DC 20460
(202) 260-3104

David Charters
Mark Sprenger
ERT
USEPA (MS-101)
2890 Woodbridge Ave.
Building 18
Edison, NJ 08837-3679
(908) 906-6825 - David
(908) 906-6826 - Mark
(908) 321-6724 FAX

Region 1

Susan Svirsky
Waste Management Division
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JFK Federal Building
Boston, MA 02203
(617) 573-9649
(617) 573-9662 FAX

Region 2

Shari Stevens
Environmental Services Division
USEPA Region 2 (MS-220)
2890 Woodbridge Ave.
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(908) 906-6994
(908) 321-6616 FAX

Region 3

Robert Davis
Technical Support Section
USEPA Region 3 (3HW13)
841 Chestnut St.
Philadelphia, PA 19107
(215) 597-3155
(215) 597-9890 FAX

Region 4

Lynn Wellman
USEPA Region 4 (4WD-OHA)
345 Courtland St., N.E.
Atlanta, GA 30365
(404) 347-3555 x6366
(404) 347-0076 FAX

Region 5

Brenda Jones
USEPA Region 5 (5HSRLT-5J)
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(312) 886-7188
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Jon Rauscher
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Region 9

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(415) 744-1916 FAX

Region 10

Julius Nwosu
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EPA Contact

Technical questions regarding this Bulletin may be directed to:

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Table 5. Additional Sources of Information on Biological Toxicity Testing

Sample Collection	Characterization of Hazardous Waste Sites--A Methods Manual: Volume II. Available Sampling Methods, Second Edition. EPA-600/4-84/076, December 1984. Compendium of Ecological Risk Assessment Tools. September 28, 1993. Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters. EPA/600/4-90/030, November 1990. Fish Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters. EPA/600/R-92/111, March 1993. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisims, EPA/600/4-91/002. December 1994. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisims. EPA/600/4-91/003, December 1994.
Predictive Models	LC_{50}. Model estimates LC_{50} values for species based upon experimental data. Contact the Center for Exposure Assessment Modeling, Environmental Research Laboratory, USEPA, Athens, GA 30613-0801. (706) 546-3130. FGETS (Food and Gill Exchange of Toxic Substances.) Model predicts bioaccumulation and survival of several types of fish exposed to pollutants. Contact the Center for Exposure Assessment Modeling, Environmental Research Laboratory, USEPA, Athens, GA 30613-0801. (706) 546-3130.
Databases	AQUIRE (AQUatic toxicity Information REtrieval.) Database containing toxicity information from reports published in the open literature. Contact the Scientific Outreach Program, Environmental Research Laboratory, USEPA, Duluth, MN 55804. (218) 720-5602. ASTER (ASsessment Tool for the Evaluation of Risk.) Toxicological database and predictive model containing effects data for pollutants in aquatic ecosystems. Contact the Scientific Outreach Program, Environmental Research Laboratory, USEPA, Duluth, MN 55804. (218) 720-5602.
Publications	ECO Update (Publication 9345.0-05I) and BTAG Forum (Publication 9200.325I). Intermittent bulletins providing information on toxicity testing and other ecological assessment topics. Contact BTAG Forum, USEPA, 303 Methodist Building, 11th and Chapline Streets, Wheeling, WV 26003. (304) 234-0245.

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