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Catalogue of Standard Toxicity Tests for Ecological Risk Assessment

This Bulletin, which serves as a companion to "Using Toxicity Tests in Ecological Risk Assessments" (*ECO Update* Vol. 1, No. 1), consists of an annotated list of standardized aquatic, sediment, terrestrial, and microbial toxicity tests currently in use at Superfund sites. Future Bulletins will address new approaches to measuring toxicity, as they become available.

In addition to the standardized approaches described in this Bulletin, the literature describes many other toxicity tests. In some cases, an investigator may identify a non-standard test that appears more relevant to a site and its contaminant picture than the standardized tests. Before deciding to use the non-standard test, the investigator should ascertain that the test is both repeatable and logical. The BTAG can assist the investigator in making a sound decision.

Most of the terms used in this document are defined in the companion Bulletin. Reference numbers in the text indicate source documents (listed at the end of this Bulletin) that more fully describe test protocols. In the case of acute aquatic toxicity tests, the catalogue also directs the reader to sources describing additional standardized tests.

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Aquatic Toxicity Tests

The aquatic toxicity tests described here are the most commonly used tests and are highly standardized. Investigators[1] should have little difficulty identifying laboratories capable of performing them.

While these tests have the advantage of wide acceptance and well-established protocols, other tests can contribute to the ecological risk assessment of Superfund sites. The references at the end of this Bulletin include sources of information about many such tests. The Regional Biological Technical Assistance Group (BTAG)[2] also is a source of information about toxicity tests that are especially well suited for use at a particular site.

Acute Freshwater Toxicity Tests

The following two tests measure the lethality of water samples to freshwater organisms, indicating the toxicity of water samples. They are described in Reference 5.

[1] The term "investigator" refers to the individual charged with responsibility for designing and/or carrying out any part of an ecological risk assessment. Investigators can include government scientists, contractors, or university scientists. However, the site manager (remedial project manager or on-site coordinator) retains ultimate responsibility for the quality of the ecological risk assessment.

[2] These groups are sometimes known by different names, depending on the Region, and not all Regions have established BTAGs. Readers should check with the appropriate Superfund manager for the name of the BTAG coordinator or other sources of technical assistance in their Region. A more complete description of BTAG structure and function is available in "The Role of BTAGs in Ecological Assessment" (ECO Update Vol. 1, No. 1).

The *Daphnia pulex* or *Daphnia magna* acute toxicity test evaluates acute toxicity of a sample to a water flea belonging to the genus *Daphnia*. The test uses a static or static-renewal design and lasts 24, 48, or 96 hours. Observations at 24, 48, and 96 hours permit the calculation of 24-, 48-, and 96-hour LC values.

Two documents describe additional acute freshwater toxicity tests. *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms* (Reference 6) describes acute toxicity tests with three species of freshwater fish and three invertebrate species. The test organisms include both warm-water and cold-water species. The guide includes range finding and definite tests.

The second document, *Annual Book of ASTM Standards: Water and Environmental Technology* (Reference 1), contains guidelines for acute toxicity tests and sediment elutriate tests using freshwater fish, macroinvertebrates, and amphibians. The guidelines describe static, static-renewal, and flow-through techniques and recommend that investigators consider EC_{50}s along with lethality.

Chronic Freshwater Toxicity Tests

These tests measure both lethal and sublethal effects over the life cycle or partial life cycle of freshwater organisms, providing information useful in assessing the potential long-term effects of contamination. Originally developed for the NPDES permitting program, the tests have since been used in assessing the toxicity of water associated with waste sites. All of these tests are described in Reference 8.

The ***Ceriodaphnia dubia* survival and reproduction test** estimates chronic toxicity of a sample to *Ceriodaphnia dubia*, which is a water flea. The test uses the static-renewal design and lasts for seven days, monitoring both the survival of test organisms and the number of offspring they produce.

The **fathead minnow (*Pimephales promelas)* larval survival and growth test** uses the static-renewal design and lasts for seven days, tracking the survival of test organisms and their increase in weight.

The **fathead minnow *(Pimephales promelas)* embryo-larval survival and teratogenicity test** assesses the chronic toxicity of a sample to minnows, beginning as embryos and extending to the larval stage. The test uses the static-renewal design and lasts for seven days, noting both the survival of the fish and the induction of terata.

The **algal *(Selenastrum capricornutum)* growth test** identifies both **biostimulatory**[3] and chronic toxic effects of a sample to a one-celled freshwater alga. The test uses the static design and lasts 96 hours, most commonly monitoring cell density (cells per mL). Alternative measures include biomass (weight of living matter), chlorophyll content, or light absorbance.

Acute Marine Toxicity Tests

These tests measure short-term lethality to marine and estuarine organisms. Protocols differ little from acute toxicity tests for freshwater organisms, with the marine/estuarine tests incorporating the appropriate species substitutions and test condition adjustments.

The **static acute toxicity test using larvae of bivalve mollusks** evaluates the acute toxicity of test media to one of four species of bivalve mollusks (invertebrates such as clams, with two piece shells). The test lasts 48 hours and notes abnormal shell development. (See Reference 1.)

The **static acute toxicity test using silversides (*Menidia* species) or sheepshead minnow (*Cyprinodon variegatus)*** evaluates the acute toxicity of test media to these fish species. The screening test lasts 24 hours and the definitive test 48 hours. Both record mortality as a lack of movement. (See Reference 6.)

Two documents describe additional acute marine toxicity tests. The *Annual Book of ASTM Standards: Water and Environmental Technology* (Reference 1) contains guidelines for tests using marine and estuarine fish and macroinvertebrates. This volume presents a variety of tests including static, static-renewal, and flow-through designs. Test durations vary from two to eight days, depending on the species selected.

The second document, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms* (Reference 6) provides protocols for tests using four species of marine and estuarine fish and one invertebrate species. The test organisms include both warm-water and cold-water organisms. Range finding and definitive tests are represented in the guide. Duration and test conditions vary with the species and specific test selected.

Chronic Marine Toxicity Tests

The following tests measure both lethal and sublethal effects over the life cycle or partial life cycle of marine and estuarine organisms, providing information about the potential long-term effects of contamination. The tests using fish resemble the survival, growth, and teratogenicity tests for freshwater fish, with the appropriate adjustments of test conditions and species substitutions. All of these tests are described in Reference 7.

The **inland silverside (*Menidia beryllina)* larval survival and growth test** is a static-renewal test that lasts for seven days and measures the survival and increase in weight of inland silverside larvae.

The **sheepshead minnow *(Cyprinodon variegatus)* larval survival and growth test** uses the static-renewal design and lasts for seven days, monitoring the survival of test organisms and their increase in weight.

[3] Biostimulation refers to excessive growth of algae, a condition not likely to occur at Superfund sites.

The **sheepshead minnow *(Cyprinodon variegatus)* embryo-larval survival and teratogenicity test** assesses the chronic toxicity of a sample to minnows, beginning as embryos and extending to the larval stage. The test uses the static-renewal design and lasts for nine days, recording both the survival of the fish and any development of terata.

The **mysid *(Mysidopsis bahia)* survival, growth, and fecundity test** evaluates the chronic toxicity of a sample to shrimp, beginning as juveniles and extending through their sexual maturation. The test uses the static-renewal design and lasts for seven days, monitoring survival, weight gain, and egg production.

The **sea urchin *(Arbacia punctulata)* fertilization test** evaluates toxicity to the eggs and sperm of *Arbacia punctulata*. The test exposes dilute sperm suspensions to a water sample for one hour. Then eggs are added, and 20 minutes later the test terminates. The technician then calculates the percent fertilization.

The algal *(Champia parvula)* sexual reproduction test uses a static design and lasts five to seven days, exposing a mixture of male and female algae to the sample for two days and then transferring them to a nontoxic medium. At the end of the test, the test organisms are scored for their production of cystocarps, the structures that result from fertilization.

Sediment Toxicity Tests

Because of the paucity of data concerning sediment toxicity, tests designed specifically to evaluate the toxicity of sediments are in their early stages of development. Currently, protocols exist for chronic toxicity tests only. As research continues, new tests should become available to investigators.

For additional information on sediment toxicity tests, consult ASTM's *Standard Guide for Conducting Sediment Toxicity Tests with Freshwater Invertebrates* (Reference 4). In addition, *Protocols for Short Term Toxicity Screening of Hazardous Waste Sites* by Greene et al. (Reference 5) describes a lettuce seed germination assay and a lettuce root elongation assay that can be used to measure the toxicity of elutriates.

Chronic Freshwater Sediment Toxicity Tests

The following tests assess the chronic toxicity of sediment samples to three freshwater invertebrates. For these tests a layer of water overlies the sediment sample. The design may either be static or involve flow-through replacement of the overlying water. Test duration varies with the objectives of the study but generally does not extend beyond 30 days. These tests are described in Reference 2.

Hyalella azteca sediment toxicity tests evaluate sediment toxicity to Hyalella azteca, an amphipod that swims in the water column and burrows in the sediment surface. Short-term tests last 10 days or fewer and evaluate the survival, growth, and development of the test organisms. Longer tests can last up to 30 days allowing the evaluation of the effect of the sediment on reproduction behavior, sexual development, egg production, and the development of offspring.

***Chironomus tentans* and *Chironomus riparius* sediment toxicity tests** evaluate sediment toxicity to midge larvae. The larvae burrow into sediment to build a casing within which they mature. Tests lasting 10-14 days evaluate the effect of exposure on survival and growth. Longer tests assess the effects of toxicity on development and reproduction.

Chronic Marine Sediment Toxicity Test

The ten-day static sediment toxicity test using marine and estuarine amphipods measures the acute toxicity of marine sediments to amphipods that burrow in the sediment. This test also can evaluate sublethal effects, such as emergence from a highly toxic sediment and the inability to re-burrow into clean sediment at the termination of the assay. (See Reference 3.)

Terrestrial Toxicity Tests

Compared with aquatic toxicity tests, few protocols exist for evaluating the toxicity of soils. However, several techniques currently in development should soon be standardized, increasing the number of options available.

Some investigators have tried to overcome the lack of standardized terrestrial toxicity tests by preparing elutriates and analyzing these by means of aquatic toxicity tests. However, this approach does not account for the toxicity of contaminants that remain sorbed to soil particles. On the other hand, analyzing elutriates with aquatic toxicity tests can prove useful when exploring the mobility of contaminants.

These tests are described in Reference 5.

The **earthworm *(Eisenia foetida)* survival assay** estimates toxicity of soil or solid waste to earthworms. The test uses the static design and lasts 14 days, monitoring the survival of the test organisms. This assay usually involves the use of soil samples, but can be conducted in sediment diluted with artificial soil. An alternate test design—exposing earthworms to artificial soil mixed with water samples or elutriate dilutions—makes this assay useful in assessing water samples or elutriates.

The **lettuce *(Latuca sativa)* seed germination assay** employs a static design and lasts, as specified in the protocol, 120 hours (5 days). As in the earthworm test, the test can use sample soil or sediment diluted with artificial soil, or artificial soil wetted with a water sample or an elutriate of soil or sediment.

The **lettuce *(Latuca sativa)* root elongation assay** also uses a static design and lasts, as specified in the protocol, 120 hours (5 days). This test monitors both seed germination and seedling length. In this test, the technician places the seeds on pieces of filter paper wetted with either a water sample, an elutriate sample, or a sample dilution.

Microbial Toxicity Tests

Microbial toxicity tests assess toxic effects on the microbial community and can serve as cost-effective and rapid screening indicators. They are described in Reference 4.

The ATP-TOX system test measures a sample's effect on bacterial growth. For this test, bacteria are suspended in a water sample or soil or sediment elutriate. After several life cycles, a technician estimates the density of bacterial growth using a method that gives the test its name: since each bacterium has a fairly constant concentration of the chemical compound adenosine triphosphate (ATP), measuring the ATP content of a suspension of bacteria provides a reliable estimate of the bacterial population.

The Microtox^{R} test measures the toxicity of water samples or elutriates to *Photobacterium phosphoreum*, a species of bioluminescent marine bacteria. Some contaminants inhibit the bacteria's metabolism, decreasing the intensity of light emitted. Other contaminants stimulate the bacteria and cause an increase in luminescence.

Conclusion

As discussed in the companion Bulletin to this document, toxicity tests represent one set of tools that can be used to evaluate possible adverse ecological effects at Superfund sites. To be effective, these tests must be planned and evaluated carefully, in the context of an overall ecological risk assessment designed to meet specific objectives. Site managers are urged to consult with their Regional BTAGs to ensure that tests appropriate to specific circumstances are selected and that the tests are conducted in such a manner as to be useful in supporting remedial decisions.

References

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