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Standard Guide for Conducting Acute Toxicity Tests on Aqueous Ambient Samples and Effluents with Fishes, Macroinvertebrates, and Amphibians[1]

This standard is issued under the fixed designation E1192; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide covers procedures for obtaining laboratory data concerning the adverse effects of an aqueous effluent on certain species of freshwater and saltwater fishes, macroinvertebrates, and amphibians, usually during 2 day to 4 day exposures, depending on the species, using the static, renewal, and flow-through techniques. These procedures will probably be useful for conducting acute toxicity tests on aqueous effluents with many other aquatic species, although modifications might be necessary.

1.2 Other modifications of these procedures might be justified by special needs or circumstances. Although using appropriate procedures is more important than following prescribed procedures, results of tests conducted using unusual procedures are not likely to be comparable to results of many other tests. Comparison of results obtained using modified and unmodified versions of these procedures might provide useful information concerning new concepts and procedures for conducting acute toxicity tests on aqueous effluents.

1.3 This guide is based in large part on Guide E729 where addition details are provided for test elements that may be applicable to the ambient and effluent toxicity testing described in this method. The major differences between the two guides are (*1*) the maximum test concentration is 100 % effluent or ambient sample, (*2*) testing is not chemical-specific, and (*3*) the holding time of effluent and ambient samples is often considerably less than that for chemicals and other test materials. Because the sample is often a complex mixture of chemicals, analytical tests cannot generally be used to confirm exposure concentrations.

1.4 Selection of the technique to be used in a specific situation will depend upon the needs of the investigator and upon available resources. Static tests provide the most easily obtained measure of acute toxicity but should not last longer than 48 h. Renewal and flow-through tests may last longer than 48 h because the pH and concentrations of dissolved oxygen and effluent are maintained at desired levels and degradation and metabolic products are removed. Static tests might not be applicable to effluents that have a high oxygen demand or contain materials that (*1*) are highly volatile, (*2*) are rapidly biologically or chemically transformed in aqueous solutions, or (*3*) are removed from test solutions in substantial quantities by the test chambers or organisms during the test. Flow-through tests are generally preferable to renewal tests, although in some situations a renewal test might be more cost-effective than a flow-through test.

1.5 In the development of these procedures, an attempt was made to balance scientific and practical considerations and to ensure that the results will be sufficiently accurate and precise for the applications for which they are commonly used. A major consideration was that the common uses of the results of acute tests on effluents do not require or justify stricter requirements than those set forth in this guide. Although the tests may be improved by using more organisms, longer acclimation times, and so forth, the requirements presented in this guide should usually be sufficient.

1.6 Results of acute toxicity tests should usually be reported in terms of a median lethal concentration (LC50) or median effective concentration (EC50). In some situations, it might be necessary only to determine whether a specific concentration is acutely toxic to the test species or whether the LC50 or EC50 is above or below a specific concentration.

1.7 This guide is arranged as follows:

[1] This guide is under the jurisdiction of ASTM Committee E50 on Environmental Assessment, Risk Management and Corrective Action and is the direct responsibility of Subcommittee E50.47 on Biological Effects and Environmental Fate.

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1.8 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.* Specific hazard statements are given in Section 7.

1.9 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D4447 Guide for Disposal of Laboratory Chemicals and Samples

E724 Guide for Conducting Static Short-Term Chronic Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Molluscs

E729 Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians

E943 Terminology Relating to Biological Effects and Environmental Fate (Withdrawn 2023)[3]

E1203 Practice for Using Brine Shrimp Nauplii as Food for Test Animals in Aquatic Toxicology (Withdrawn 2013)[3]

E1604 Guide for Behavioral Testing in Aquatic Toxicology

E1706 Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates

E1733 Guide for Use of Lighting in Laboratory Testing

IEEE/ASTM SI 10 American National Standard for Use of the International System of Units (SI): The Modern Metric System

3. Terminology

3.1 *Definitions:*

3.1.1 *acute test, n*—a comparative study in which organisms, that are subjected to different treatments, are observed for a relatively short period usually not constituting a substantial portion of their life span.

3.1.2 *dilution water, n*—non-toxic aqueous exposure media (that is, water) used to reduce the concentration of a test substance in aquatic toxicity tests and is used as the control water.

3.1.3 *reconstituted water, n*—a dilution water that is prepared by adding sea salt or appropriate amounts of reagent-grade salts to water, which is usually prepared using deionization, distillation, or reverse osmosis, so that the concentrations and ratios of the major ions in the dilution water are similar to those in comparable natural surface waters.

3.2 The words “must,” “should,” “may,” “can,” and “might” have very specific meanings in this guide. “Must” is used to express an absolute requirement, that is, to state that the test ought to be designed to satisfy the specified condition, unless the purpose of the test requires a different design. “Must” is only used in connection with factors that directly relate to the acceptability of the test (see 13.1). “Should” is used to state that the specified condition is recommended and ought to be met if possible. Although violation of one “should” is rarely a serious matter, violation of several will often render the results questionable. Terms such as “is desirable,” “is often desirable,” and “might be desirable” are used in connection with less important factors. “May” is used to mean “is (are) allowed to,” “can” is used to mean “is (are) able to,” and “might” is used to mean “could possibly.” Thus the classic distinction between “may” and “can” is preserved, and “might” is never used as a synonym for either “may” or “can.”

3.3 The term “effluents” refers to aqueous discharges regulated under the National Pollutant Discharge Elimination System (NPDES) collected at the sampling point specified in the NPDES permit.

3.4 The term “ambient samples” refers to water samples collected from the environment. Examples include surface waters, storm waters, leachates, and ground water.

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For Annual Book of ASTM Standards volume information, refer to the standard’s Document Summary page on the ASTM website.

[3] The last approved version of this historical standard is referenced on www.astm.org.

3.5 For definitions of other terms used in this guide, refer to Guide E729 and Terminology E943. For an explanation of units and symbols, refer to IEEE/ASTM SI 10.

4. Summary of Guide

4.1 In each of two or more treatments, test organisms of one species are maintained for 2 days to 8 days in one or more test chambers. In each of the one or more control treatments, the organisms are maintained in dilution water to which no effluent has been added in order to provide (*1*) a measure of the acceptability of the test by giving an indication of the quality of the test organisms and the suitability of the dilution water, test conditions, handling procedures, and so forth, and (*2*) the basis for interpreting data obtained from the other treatments. In each of the one or more other treatments, the organisms are maintained in dilution water to which a selected concentration of effluent has been added. Data on effects on the organisms in each test chamber are usually obtained periodically during the test and analyzed to determine LC50s or EC50s for various lengths of exposure.

5. Significance and Use

5.1 An acute effluent toxicity test is conducted to obtain information concerning the immediate effects on test organisms of a short-term exposure to an effluent under specific experimental conditions. One can directly examine acute effects of complex mixtures of chemicals as occurs in effluents and some ambient waters. Acute effluent toxicity tests can be used to evaluate the potential for designated-use or aquatic life impairment in the receiving stream, lake, or estuary. An acute toxicity test does not provide information about whether delayed effects will occur, although a post-exposure observation period, with appropriate feeding if necessary, might provide such information.

5.2 Results of acute effluent tests might be used to predict acute effects likely to occur on aquatic organisms in field situations as a result of exposure under comparable conditions, except that (*1*) motile organisms might avoid exposure when possible, (*2*) toxicity to benthic species might be dependent on sorption or settling of components of the effluent onto the substrate, and (*3*) the effluent might physically or chemically interact with the receiving water.

5.3 Results of acute effluent tests might be used to compare the acute sensitivities of different species and the acute toxicities of different effluents, and to study the effects of various environmental factors on results of such tests.

5.4 Acute tests are usually the first step in evaluating the effects of an effluent on aquatic organisms.

5.5 Results of acute effluent tests will depend on the temperature, composition of the dilution water, condition of the test organisms, exposure technique, and other factors.

6. Apparatus

6.1 *Facilities*—Although some small organisms can be held and acclimated in static or renewal systems, most organisms are held, acclimated, and cultured in flow-through systems. Test chambers should be in a constant-temperature room, incubator, or recirculating water bath. A dilution-water tank, which may be used to store receiving water, or a headbox is often elevated so dilution water can be gravity-fed into holding and acclimation tanks and test chambers. Pumps are often used to deliver dilution water and effluent to headboxes and tanks. Strainers and air traps should be included in the water supply. Headboxes and holding, acclimation, culture, and dilution-water tanks should be equipped for temperature control and aeration (see 8.3). Air used for aeration should be free of fumes, oil, and water; filters to remove oil and water are desirable. Filtration of air through a 0.22 µm bacterial filter might be desirable (**1**)[4]. The facility should be well ventilated and free of fumes. To further reduce the possibility of contamination by components of the effluent and other substances, especially volatile ones, holding, acclimation, and culture tanks should not be in a room in which toxicity tests are conducted, effluent is stored, test solutions are prepared, or equipment is cleaned. During holding, acclimation, culture, and testing, organisms should be shielded from disturbances with curtains or partitions to prevent unnecessary stress. A timing device should be used to provide a 16 h light and 8 h dark photoperiod. A 15 to 30-min transition period (**2**) when the lights go on might be desirable to reduce the possibility of organisms being stressed by large, sudden increases in light intensity. A transition period when the lights go off might also be desirable (see Guide E1733).

6.2 *Special Requirements*—Some organisms require special conditions during holding, acclimation, and testing. For example, burrowing mayfly nymphs should be provided a substrate suitable for burrowing (**3**); immature stream insects should be in a current (**4**); and amphipods, midge larvae, crabs, shrimp, and bottom-dwelling fish should be provided a silica-sand substrate. Nylon or stainless steel mesh can also be used to provide a substrate to which amphipods can cling. Because cannibalism might occur among many species of decapod crustaceans, the claws of crabs and crayfish should be banded, or the individuals should be physically isolated by means of screened compartments or held individually in test chamber during testing.

6.3 *Construction Materials*—Equipment and facilities that contact effluent samples, test solutions, or any water into which test organisms will be placed should not contain substances that can be leached or dissolved by aqueous solutions in amounts that adversely affect aquatic organisms. In addition, equipment and facilities that contact effluent samples or test solutions should be chosen to minimize sorption of effluent components from water. Glass, Type 316 stainless steel, nylon, and non-fluorocarbon plastics should be used whenever possible to minimize dissolution, leaching, and sorption, except that stainless steel should not be used in tests on metals in salt water. Concrete and rigid plastics may be used for holding, acclimation, and culture tanks and in the water supply, but they should be soaked, preferably in flowing dilution water, for a week or more before use (**5**). Cast iron pipe should not be used with salt water and probably should not be used in a

[4] The boldface numbers in parentheses refer to a list of references at the end of this standard.