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Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians[1]

This standard is issued under the fixed designation E729; 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.

This standard has been approved for use by agencies of the U.S. Department of Defense.

ε[1] NOTE—Sections 3 and 4 were editorially reorganized, and 7.8 and 8.2.1.1 were editorially corrected in February 2023.

1. Scope

1.1 This guide (1)[2] describes procedures for obtaining laboratory data concerning the adverse effects (for example, lethality and immobility) of a test material added to dilution water, but not to food, on certain species of freshwater and saltwater fishes, macroinvertebrates, and amphibians, usually during 2 to 4-day exposures, depending on the species. These procedures will probably be useful for conducting acute toxicity tests 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 such as meeting specific study goals, regulatory needs, or to accommodate specific test organism life stages. Although using appropriate procedures is more important than following prescribed procedures, results of tests conducted using unusual or novel 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 tests.

1.3 This guide describes tests using three basic exposure techniques: static, renewal, and flow-through. Selection of the technique to use in a specific situation will depend on the needs of the investigator and on available resources. Tests using the static technique provide the most easily obtained measure of acute toxicity, but conditions often change substantially during static tests; therefore, static tests should not last longer than 96 h, and test organisms should not be fed during such tests unless the test organisms are severely stressed without feeding over 48 h. Static tests should probably not be conducted on materials that have a high oxygen demand, are highly volatile, are rapidly transformed biologically or chemically in aqueous solution, or are removed from test solutions in substantial quantities by the test chambers or organisms during the test. Because the pH and concentrations of dissolved oxygen and test material are maintained at desired levels and degradation and metabolic products are removed, tests using renewal and flow-through methods are preferable; test organisms may be fed during renewal and flow-through tests. Although renewal tests might be more cost-effective, flow-through tests are generally preferable.

1.4 Acute tests may be performed to meet regulatory data requirements or to obtain time-independent estimates of toxicity.

1.4.1 If the objective is to obtain data to meet regulatory requirements, it may be necessary to limit the number of observation times based on stipulations of the regulatory agency and cost considerations.

1.4.2 If the objective of an acute toxicity test is to determine a time-independent (that is, incipient, threshold, or asymptotic) toxicity level, an appropriate number of observations must be taken over an exposure duration of sufficient length to establish the shape of the toxicity curve or allow the direct or mathematically estimated determination of a time-independent toxicity value (1), or both.

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 toxicity tests do not require or justify stricter requirements than those set forth herein. Although the tests may be improved by using more organisms, longer acclimation times, and so forth, the requirements presented herein should usually be sufficient.

1.6 Results of acute toxicity tests should usually be reported in terms of an LC50 (median lethal concentration) or EC50 (median effective concentration) at the end of the test, but it is

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

desirable to provide information concerning the dependence of adverse effects on both time and concentration. Thus, when feasible, flow-through and renewal tests should be conducted so that LC50s or EC50s can be reported from 6 h to an asymptotic (time-independent, threshold, incipient) value, if one exists. In some situations, it might only be necessary 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:

1.8 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

1.9 *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.

1.10 *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:*[3]

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

E943 Terminology Relating to Biological Effects and Environmental Fate (Withdrawn 2023)[4]

E1023 Guide for Assessing the Hazard of a Material to Aquatic Organisms and Their Uses

E1191 Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids

E1192 Guide for Conducting Acute Toxicity Tests on Aqueous Ambient Samples and Effluents with Fishes, Macroinvertebrates, and Amphibians

E1203 Practice for Using Brine Shrimp Nauplii as Food for Test Animals in Aquatic Toxicology (Withdrawn 2013)[4]

E1563 Guide for Conducting Short-Term Chronic Toxicity Tests with Echinoid Embryos

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

E2455 Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels

IEEE/ASTM SI 10 Standard for Use of the International System of Units (SI) (the Modernized Metric System)

3. Terminology

3.1 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.”

3.2 *Definitions:*

[3] 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.

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

3.2.1 *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.

3.2.2 *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.

3.2.3 *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.

3.2.4 *IC50, n*—a statistically or graphically estimated concentration of test material that is expected to cause a 50 % inhibition of one or more specified biological processes (such as shell growth of saltwater bivalve molluscs in acute shell deposition tests), for which the data are not dichotomous, under specified conditions.

3.3 For definitions of other terms used in this guide, refer to Terminology E943 and Guide E1203. 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 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 test material 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 test material has been added. Data concerning effects on the organisms in each test chamber are usually obtained periodically during the test and analyzed to determine LC50s, EC50s, or IC50s for various lengths of exposure.

4.2 Acute toxicity tests are generally used to determine the concentration of test material that produces a specific adverse effect on a specified percentage of test organisms during a short exposure, relative to the life-cycle of the test organism. Because death is an obviously important adverse effect and is easily detected for many species, the most common acute toxicity test is the acute lethality test. Experimentally, effect on 50 % of a group of test organisms is the most reproducible and easily determined measure of toxicity, and 96 h is often a convenient, useful exposure duration. Therefore, the measure of acute toxicity most often used with fishes, macroinvertebrates, and amphibians is the 96-h LC50. However, because immobilization is a severe effect and is not easy to distinguish from death for some species, the measure of acute toxicity most often used with daphnids and midge larvae is the 48-h EC50 based on death plus immobilization. The terms LC50 and EC50 are consistent with the widely used toxicological terms LD50 (median lethal dose) and ED50 (median effective dose), respectively. The terms LC50 and EC50 should be used whenever results are calculated based on the concentration of test material in dilution water, whereas the terms LD50 and ED50 should be used whenever results are calculated based on the quantity of test material that enters or is applied directly to test organisms. For toxic agents or tests for which neither concentration nor dose is appropriate, such as tests on temperature or with poorly water-soluble materials, the terms LL50 (median lethal level) and EL50 (median effective level) should be used, if the effect is dichotomous. For tests in which the effect is expressed as a percent inhibition compared to the control, for example, a percent inhibition in shell growth in acute 96-h shell deposition tests with saltwater bivalve molluscs (**2**), and not as the percentage of the individual organisms that were affected, the term IC50 should be used to denote the concentration that causes a 50 % inhibition compared to the control.

4.3 Acute toxicity tests in which test organisms are exposed to test solutions containing a test material can be conducted by at least four techniques:

4.3.1 In the static technique, test solutions and organisms are placed in chambers and kept there for the duration of the test.

4.3.2 The recirculation technique is like the static technique except that each test solution is continuously circulated through an apparatus designed to maintain water quality, and possibly remove degraded, but not undegraded, test material by such means as aeration, filtration, and sterilization and then returned to the test chamber.

4.3.3 The renewal technique is like the static technique except that test organisms are periodically exposed to fresh test solution of the same composition, usually once every 24 h or 48 h, either by transferring the organisms from one test chamber to another or by replacing nearly all the test solution.

4.3.4 In the flow-through technique, test solution flows through the test chamber on a once-through basis throughout the test.

4.3.4.1 Two procedures may be used. In the first a large volume of each test solution is prepared before the beginning of the test, and these solutions flow through the respective chambers. In the second and more common procedure, fresh test solutions are prepared every few minutes or hours just before they enter the respective test chambers. In both procedures a metering system controls the flow of test solution, and in the latter procedure the test solutions are prepared by the metering system. Both of the procedures may be used to conduct continuous-flow tests. Many tests conducted using the second procedure, however, are intermittent-flow tests because the metering system cycles and delivers test solution every few minutes or hours.

4.3.5 With any of these techniques a pump or stirrer can be used to create a current in the test chamber to accommodate particular species, but the current will often increase both aeration and volatilization.

4.4 In flow-through tests a “volume addition” is the introduction into the test chamber of a volume of test solution equal to the volume of solution in the chamber.