Designation: E 729 – 96 (Reapproved 2002)

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

This standard is issued under the fixed designation E 729; 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 Department of Defense.

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 during 2 to 8-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. 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 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. 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 and may last longer than 96 h; 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 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] This guide is under the jurisdiction of ASTM Committee E47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.01 on Aquatic Assessment and Toxicology.

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

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