



Designation: E1241 – 22

Standard Guide for

Conducting Early Life-Stage Toxicity Tests with Fishes¹

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1. Scope

1.1 This guide describes procedures for obtaining laboratory data concerning the adverse effects of a test material added to dilution water—but not to food—on certain species of freshwater and saltwater fishes during 28 day to 120 day (depending on species) continuous exposure, beginning before hatch and ending after hatch, using flow-through exposures. This guide will probably be useful for conducting early life-stage toxicity tests with some other species of fish, 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 early life-stage toxicity tests with fishes.

1.3 These procedures are applicable to all chemicals, either individually or in formulations, commercial products, or known mixtures, that can be measured accurately at the necessary concentrations in water. With appropriate modifications these procedures can be used to conduct tests on temperature, dissolved oxygen, and pH and on such materials as aqueous effluents (see Guide E1192), leachates, oils, particulate matter, sediments, and surface waters.

1.4 This guide is arranged as follows:

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1.5 *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 6 and 9.*

1.6 *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

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

D4447 Guide for Disposal of Laboratory Chemicals and Samples

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

E943 Terminology Relating to Biological Effects and Environmental Fate

E1022 Guide for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Mollusks

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

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)³

E1733 Guide for Use of Lighting in Laboratory Testing

E1847 Practice for Statistical Analysis of Toxicity Tests Conducted Under ASTM Guidelines (Withdrawn 2022)³

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

3. Terminology

3.1 The words “must,” “should,” “may,” “can,” and “might” have very specific meanings in this standard. “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:

3.2.1 For definitions of other terms used in this guide, refer to Terminology D1129, Guide E729, Terminology E943, Guide E1022 and Guide E1023. For an explanation of units and symbols, refer to SI 10.

3.2.2 *antagonism, n*—a situation which an effect of an exposure to multiple substances is less than would be expected if the known effects of the individual substances were added together.

3.2.3 *confounding, n*—a situation in which one or more other variables covary with the independent variable, making it impossible to determine the influence of the independent variable on the dependent variable.

3.2.4 *exposure, n*—when a test organism is in contact with a test material for a duration of time.

3.2.5 *synergism, n*—a situation in which an effect of an exposure to multiple substances is more than would be expected if the known effects of the individual substances were added together.

4. Summary of Guide

4.1 In each of two or more treatments, embryos and the subsequent larvae of one species of fish are maintained in two or more test chambers in a flow-through system for 28 to 120 days, depending upon species. In each of the one or more control treatments, the embryos and larvae are maintained in dilution water to which no test material has been added in order to provide (a) a measure of the acceptability of the test by giving an indication of the quality of the embryos and larvae and the suitability of the dilution water, food, test conditions, handling procedures, and so forth, and (b) the basis for interpreting data obtained from the other treatments. In each of the one or more other treatments, the embryos and larvae are maintained in dilution water to which a selected concentration of test material has been added. Specified data on the concentration of test material and the survival and growth of the embryos and larvae in each test chamber are obtained and analyzed to determine the effect(s) of the test material on the survival and growth of the test organisms.

5. Significance and Use

5.1 Protection of a species requires prevention of unacceptable effects on the number, weight, health, and uses of the individuals of that species. An early life-stage toxicity test provides information about the chronic toxicity of a test

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.