Designation: E724 – 98 (Reapproved 2012)

Standard Guide for

Conducting Static Acute Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Molluscs[1]

This standard is issued under the fixed designation E724; 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 describes procedures for obtaining laboratory data concerning the acute effects of a test material on embryos and the resulting larvae of four species of saltwater bivalve molluscs (Pacific oyster, *Crassostrea gigas* Thunberg; eastern oyster, *Crassostrea virginica* Gmelin; quahog or hard clam, *Mercenaria mercenaria* Linnaeus; and blue mussel, *Mytilus edulis* Linnaeus) during static 48-h exposures. These procedures will probably be useful for conducting static acute toxicity tests starting with embryos of other bivalve species (**1**)[2] although modifications might be necessary.

1.2 Other modifications of these procedures might be justified by special needs or circumstances. Although using procedures appropriate to a particular species or special needs and circumstances is more important than following prescribed procedures, results of tests conducted by using unusual procedures are not likely to be comparable to results of many other tests. Comparison of results obtained by using modified and unmodified versions of these procedures might provide useful information concerning new concepts and procedures for conducting 48-h acute tests starting with embryos of bivalve molluscs.

1.3 These procedures are applicable to most chemicals, either individually or in formulations, commercial products, or known mixtures. With appropriate modifications these procedures can be used to conduct acute tests on temperature, dissolved oxygen, and pH and on such materials as aqueous effluents (see also Guide E1192), leachates, oils, particulate matter, sediments, and surface waters. Renewal tests might be preferable to static tests for materials that have a high oxygen demand, are highly volatile, are rapidly biologically or chemically transformed in aqueous solution, or are removed from test solutions in substantial quantities by the test chambers or organisms during the test.

1.4 Results of acute toxicity tests with embryos of bivalve molluscs should usually be reported as the EC50 based on the total incompletely developed and dead organisms. It might also be desirable to report the LC50 based only on death. In some situations, it might only be necessary to determine whether a specific concentration is acutely toxic to embryos or whether the EC50 is above or below a specific concentration.

1.5 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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[2] The boldface numbers in parentheses refer to the list of references at the end of this guide.

1.6 *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 and health practices and determine the applicability of regulatory limitations prior to use.* Specific hazard statements are given in Section 6.

2. Referenced Documents

2.1 *ASTM Standards:*[3]

E380 Practice for Use of the International System of Units (SI) (the Modernized Metric System)[4]

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

E943 Terminology Relating to Biological Effects and Environmental Fate

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

E1367 Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Estuarine and Marine Invertebrates

E1391 Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing and for Selection of Samplers Used to Collect Benthic Invertebrates

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

3. Terminology

3.1 *Definitions:*

3.1.1 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" statement 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."

3.1.2 For definitions of other terms used in this guide, refer to Guide E729, Terminology E943, and Guide E1023. For an explanation of units and symbols, refer to Practice E380.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *embryo*—used herein to denote the stages between the fertilization of the egg and the trochophore **(2)** (Figs. 1 and 2).

3.2.2 *larva*—used herein to include the trochophore and the straight hinge stage **(2)** (Figs. 1 and 2).

4. Summary of Guide

4.1 Adult saltwater bivalve molluscs are brought into the laboratory, cleaned of detritus and fouling organisms, and identified to species. If the gonads are not ripe, the bivalves are conditioned to bring them into a suitable reproductive state. Bivalves with ripe gonads are maintained under conditions that keep the gonads ripe without inducing undesired spontaneous spawning or resorption of gametes. In order to start a test, spawning is induced by using one or more stimuli which may be physical (for example, temperature), biological (for example, heat-killed bivalve sperm), or chemical (for example, serotonin).

4.2 In each of two or more treatments, embryos and the resulting larvae of one species are maintained for 48 h. In each of one or more control treatments, the embryos and resulting 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 organisms and the suitability of the dilution water, test conditions, handling procedures, and so forth, and (*b*) the basis for interpreting data obtained from the other treatments. In each of one or more other treatments, the embryos and resulting larvae are maintained in dilution water to which a selected concentration of test material has been added. The 48-h EC50 is calculated based on the proportion of live larvae with completely developed shells in chambers containing the test material to live larvae with completely developed shells in the controls at the termination of the 48-h test.

5. Significance and Use

5.1 An acute toxicity test is conducted to obtain information concerning the acute effects of a short term exposure of organisms to a test material under specific experimental conditions. An acute toxicity test does not provide information concerning whether delayed effects will occur.

5.2 Because embryos and larvae are usually assumed to be the most sensitive life stages of these bivalve mollusc species and because these species are commercially and recreationally important, results of these acute tests are often considered to be a good indication of the acceptability of pollutant concentrations to saltwater molluscan species in general. Results of these acute toxicity tests are often assumed to be an important consideration when assessing the hazard of materials to other

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

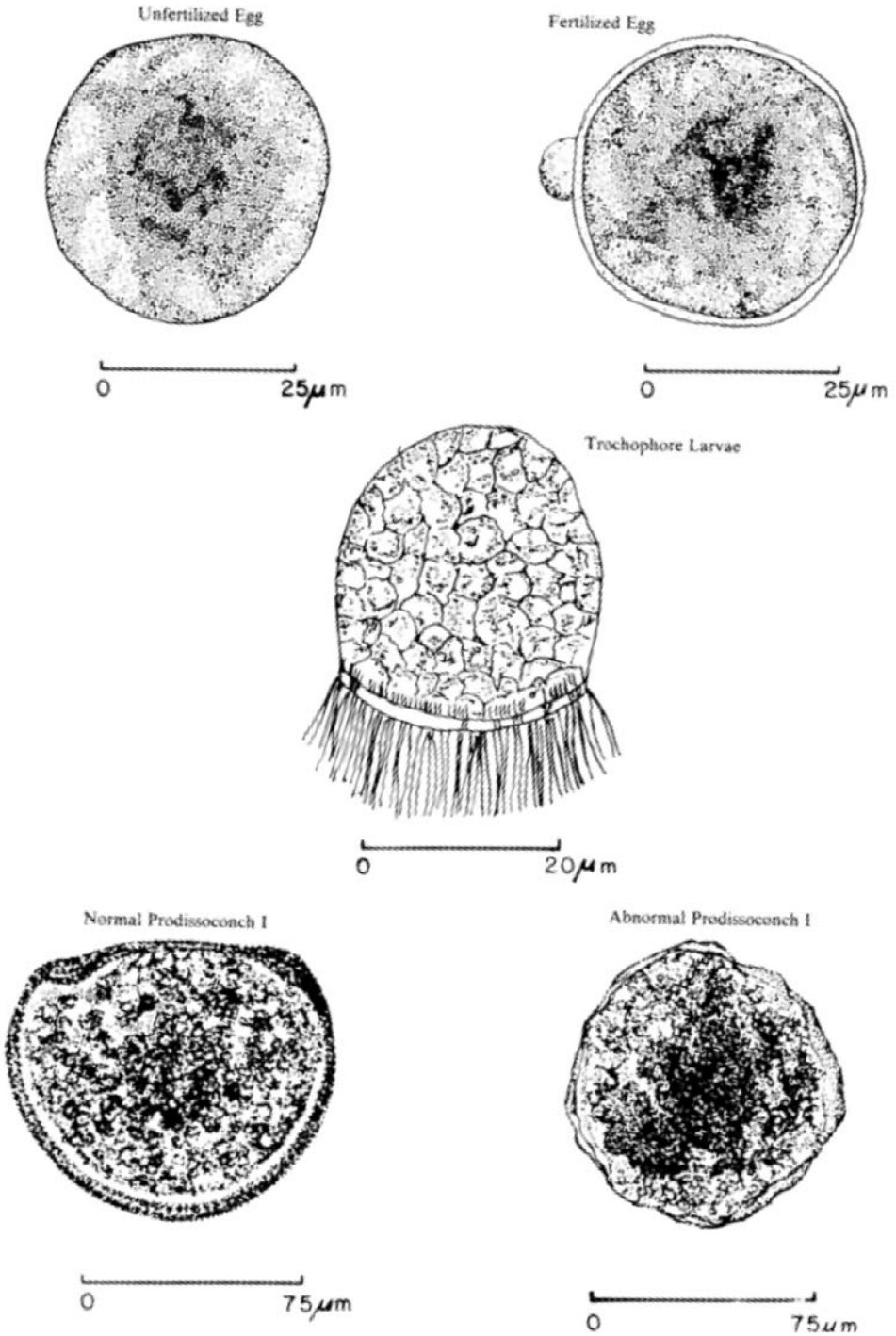

FIG. 1 Drawings Exemplifying Five Key Developmental Stages of Bivalve Larvae Occurring During the First 48 h of Development

saltwater organisms (see Guide E1023) or when deriving water quality criteria for saltwater organisms (**3**).

5.3 Results of acute toxicity tests might be used to predict acute effects likely to occur to aquatic organisms in field situations as a result of exposure under comparable conditions, except that toxicity to benthic species might depend on sorption or settling of the test material onto the substrate.

5.4 Results of acute tests might be used to compare the acute sensitivities of different species and the acute toxicities of different test materials, and to determine the effects of various environmental factors on results of such tests.

5.5 Results of acute toxicity tests might be useful for studying biological availability of, and structure activity relationships between, test materials.