



Designation: E1295 – 22

Standard Guide for Conducting Three-Brood, Renewal Toxicity Tests with *Ceriodaphnia dubia*¹

This standard is issued under the fixed designation E1295; 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 data concerning the adverse effects of an effluent or a test material (added to dilution water, but not to food) on *Ceriodaphnia dubia* Richard 1894, during continuous exposure throughout a portion of the organism's life. These procedures should also be useful for conducting life cycle toxicity tests with other Cladocera (Guide E1193), although modifications will be necessary.

1.2 These procedures are applicable to most 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, pH, dissolved ions, and on such materials as aqueous effluents (see also Guide E1192), leachates, oils, particulate matter, sediments (see also Guide E1706), and surface waters. Renewal tests might not be applicable to materials that have high oxygen demand, are highly volatile, are rapidly biologically or chemically transformed, or sorb to test chambers. If the concentration of dissolved oxygen falls below 4 mg/L or the concentration of test material decreases by more than 20 % in test solution(s) at any concentration between renewals, more frequent renewals might be necessary.

1.3 Other modifications of these procedures might be justified by special needs or circumstances. Results of tests conducted using unusual procedures are not likely to be comparable to results of many other tests. Comparisons of results obtained using modified and unmodified versions of these procedures might provide useful information on new concepts and procedures for conducting three-brood toxicity tests with *C. dubia*.

1.4 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.

Current edition approved Jan. 1, 2022. Published April 2022. Originally approved in 1989. Last previous edition approved in 2013 as E1295 – 01(2013). DOI: 10.1520/E1295-22.

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*A Summary of Changes section appears at the end of this standard

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 8.

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

D1193 Specification for Reagent Water

D3978 Practice for Algal Growth Potential Testing with *Pseudokirchneriella subcapitata*

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

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

E1193 Guide for Conducting *Daphnia magna* Life-Cycle Toxicity Tests

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

E1733 Guide for Use of Lighting in Laboratory Testing

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

SI10–02 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 has 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 Section 14). “Should” is used to state that the specified condition is recommended and has to be met in most tests. Although a 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 For definitions of other terms used in this standard, refer to Guide E729, Terminology E943, and Guide E1023. For an explanation of units and symbols, refer to SI10-02 IEEE/ASTM SI 10.

3.3 Definitions of Terms Specific to This Standard:

3.3.1 brood, *n*—the young neonates released at the time of adult molt by the young/adult animal originally exposed to the control and test solutions.

3.3.1.1 Discussion—The number of young in each brood are typically counted over each 24 h period of the test and should increase over the duration of the test. Animals may be transferred to fresh control or test solution before completing the release of a brood, resulting in split broods. Care is needed when interpreting the results to determine the number of broods released during a test.

4. Summary of Guide

4.1 At the beginning of the test, at least ten *C. dubia* less than 24 h old, and within 8 h of age, are introduced individually in separate test chambers, and exposed to control water and a least one (preferably 2 or more) toxicant concentrations. One control treatment must be used but more may be used. Control treatments may include standard laboratory water only, or some combination of standard water(s) and uncontaminated site water, to provide a measure of organism survival and reproduction based on specific test water conditions, such as hardness, alkalinity, and so forth. Specified data on the concentration of test material and the survival and reproduction of *C. dubia* are collected and analyzed to determine the effect of the tested concentration (% effluent or ambient water) on *C. dubia*.

4.2 Table 1(1)⁴ contains a summary of the conditions used when conducting a three-brood test with *C. dubia*. Table 2 and Section 14 list the requirements that need to be met for a test to be deemed acceptable.

5. Significance and Use

5.1 *Ceriodaphnia* was first used as a toxicity test organism by Mount and Norberg (2). Introduced for use in effluent and ambient water evaluations, *Ceriodaphnia* have also been a valuable addition to single chemical test procedures.

5.2 Protection of a population requires prevention of unacceptable effects on the number, weight, health, and uses of the individuals of that species, or species for which the test species serves as a surrogate. A three-brood toxicity test is conducted

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.

⁴ Boldface numbers in parentheses refer to the list of references at the end of this guide.