



Designation: E1415 – 22

Standard Guide for Conducting Static Toxicity Tests With *Lemna gibba* G3¹

This standard is issued under the fixed designation E1415; 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 adverse effects of a test material added to growth medium on a certain species of duckweed (*Lemna gibba* G3) during a 7-day exposure using the static technique. These procedures will probably be useful for conducting toxicity tests with other species of duckweed and other floating vascular plants, although modifications might be necessary.

1.2 Special needs or circumstances might also justify modification of this standard. 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 tests with duckweed.

1.3 The procedures in this guide 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 tests on temperature and pH and on such other materials as aqueous effluents (see also Guide E1192), leachates, oils, particulate matter, sediments and surface waters. These procedures do not specifically address effluents because to date there is little experience using duckweeds in effluent testing and such tests may pose problems with acclimation of the test organisms to the receiving water. Static tests might not be applicable to 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 toxicity tests performed using the procedures in this guide should usually be reported in terms of the 7-day IC50 based on inhibition of growth. In some situations it might

only be necessary to determine whether a specific concentration unacceptably affects the growth of the test species or whether the IC50 is above or below a specific concentration. Another end point that may be calculated is the no observed effect concentration (NOEC).

1.5 The sections of this guide appear as follows:

Title	Section
Referenced Documents	2
Terminology	3
Summary of Guide	4
Significance and Use	5
Hazards	6
Apparatus	7
Facilities	7.1
Test Chambers	7.2
Cleaning	7.3
Acceptability	7.4
Growth Medium	8
Test Material	9
General	9.1
Stock Solution	9.2
Test Concentration(s)	9.3
Test Organisms	10
Species	10.1
Source	10.2
Stock Culture	10.3
Procedure	11
Experimental Design	11.1
Temperature	11.2
Illumination	11.3
Beginning the Test	11.4
Duration of Test	11.5
Biological Data	11.6
Other Measurements	11.7
Analytical Methodology	12
Acceptability of Test	13
Calculation of Results	14
Report	15

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

1.7 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.

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 Nov. 1, 2022. Published November 2022. Originally approved in 1991. Last previous edition approved in 2012 as E1415 – 91 (2012), which was withdrawn January 2021 and reinstated in November 2022. DOI: 10.1520/E1415-22.

2. Referenced Documents

2.1 ASTM Standards:²

- 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
- E1218** Guide for Conducting Static Toxicity Tests with Microalgae
- 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 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 Section 13). *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*, *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 of Terms Specific to This Standard:

3.2.1 *frond*—individual leaf-like structure on a duckweed plant.

3.2.2 *IC50*—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 growth or reproduction), 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 Guides **E729** and **E1023**. For an explanation of units and symbols, refer to Practice **IEEE/ASTM SI 10**.

4. Summary of Guide

4.1 In each of two or more treatments, plants of *Lemna gibba* G3 are maintained for 7 days in two or more test chambers using the static technique. In each of the one or more

control treatments, the plants are maintained in growth medium to which no test material has been added in order to provide a measure of the acceptability of the test by giving an indication of the quality of the duckweed and the suitability of the growth medium, test conditions, handling procedures, and so forth, and the basis for interpreting data obtained from the other treatments. In each of the one or more other treatments, the duckweed plants are maintained in growth medium to which a selected concentration of test material has been added. Specified data concerning growth of duckweed in each test chamber are obtained during the test and are usually analyzed to determine the IC50 or NOEC based on inhibition of growth.

5. Significance and Use

5.1 The term duckweed commonly refers to members of the family Lemnaceae. This family has many species world-wide in 4 genera. This guide is designed for toxicity testing with one particular clone of one species of duckweed that has been extensively studied, *Lemna gibba* G3, although other species such as *Lemna minor* or *Spirodela* spp. can probably also be tested using the procedures described herein.

5.2 Duckweeds are widespread, free-floating aquatic plants, ranging in the world from tropical to temperate zones. Duckweeds are a source of food for waterfowl and small animals and provide food, shelter, and shade for fish. The plants also serve as physical support for a variety of small invertebrates. Duckweed is fast growing and reproduces rapidly compared with other vascular plants (1).³ Under conditions favorable for its growth, it can multiply quickly and form a dense mat in lakes, ponds, and canals, primarily in fresh water, but also in estuaries. It also grows well in effluents of wastewater treatment plants and has been suggested as a means of treating wastewaters (2). A dense mat of duckweed can block sunlight and aeration and cause fish kills (3).

5.3 Duckweed is small enough that large laboratory facilities are not necessary, but large enough that effects can be observed visually.

5.4 Because duckweed is a floating macrophyte, it might be particularly susceptible to surface active and hydrophobic chemicals that concentrate at the air-water interface. Results of duckweed tests on such chemicals, therefore, might be substantially different from those obtained with other aquatic species.

5.5 Results of toxicity tests with duckweed might be used to predict effects likely to occur on duckweed in field situations as a result of exposure under comparable conditions.

5.6 Results of tests with duckweed might be used to compare the toxicities of different materials and to study the effects of various environmental factors on results of such tests.

5.7 Results of tests with duckweed might be an important consideration when assessing the hazards of materials to aquatic organism (see Guide **E1023**) or when deriving water quality criteria for aquatic organisms (4).

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