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Standard Guide for Conducting Static Toxicity Tests with Microalgae^{1,2}

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1. Scope

1.1 This guide covers procedures for obtaining laboratory data concerning the adverse effects of a test material added to growth medium on growth of certain species of freshwater and saltwater microalgae during a static exposure. These procedures will probably be useful for conducting short-term toxicity tests with other species of algae, although modifications might be necessary. Although the test duration is comparable to an acute toxicity test with aquatic animals, an algal toxicity test of short duration (72, 96 or 120 h) allows for examination of effects upon multiple generations of an algal population and thus should not be viewed as an acute toxicity test.

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 toxicity tests with microalgae.

1.3 These procedures are applicable to many 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 materials as aqueous effluents (see Guide E1192), leachates, oils, particulate matter, sediments, and surface waters. Static tests might not be applicable to materials that are highly volatile, are rapidly biologically or chemically transformed in aqueous solutions, or are removed from test solutions in substantial quantities by the test vessels or organisms

during the test. (1)³ However, practical flow-through test procedures with microalgae have not been developed.

1.4 Results of tests using microalgae should usually be reported in terms of the 96-h (or other time period) IC₅₀ (see 3.2.5) based on reduction in 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.

1.5 This guide is arranged as follows:

	Section
Referenced Documents	2
Terminology	3
Summary of Guide	4
Significance and Use	5
Hazards	7
Apparatus	6
Facilities	6.1
Equipment	6.2
Test Vessels	6.3
Cleaning	6.4
Acceptability	6.5
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
Culture	10.3
Quality	10.4
Procedure	11
Experimental Design	11.1
Temperature	11.2
Illumination	11.3
Beginning the Test	11.4
Gas Exchange	11.5
Duration of Test	11.6
Biological Data	11.7
Other Measurements	11.8
Determination of Algistatic and Algicidal Effects	11.8.5
Analytical Methodology	12
Acceptability of Test	13
Calculation	14
Report	15
Keywords	16

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² This standard guide is a document, developed using the consensus mechanisms of ASTM, that provides guidance for the selection of procedures to accomplish a specific test, but which does not stipulate specific procedures.

³ The boldface numbers given in parentheses refer to a list of references at the end of the text.

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 7.

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.

2. Referenced Documents

2.1 ASTM Standards:⁴

D1129 Terminology Relating to Water

D3731 Practices for Measurement of Chlorophyll Content of Algae in Surface Waters

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

D4447 Guide for Disposal of Laboratory Chemicals and Samples

E380 Practice for Use of the International System of Units (SI) (the Modernized Metric System) (Withdrawn 1997)⁵

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

E1733 Guide for Use of Lighting in Laboratory Testing

E1847 Practice for Statistical Analysis of Toxicity Tests Conducted Under ASTM Guidelines

3. Terminology

3.1 Definitions:

3.1.1 The words “must,” “should,” “may,” “can,” and “might” have very specific meanings in this guide.

3.1.1.1 *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”. Therefore the classic distinction between “may” and “can” is preserved, and “might” is never used as a synonym for either “may” or “can”.

3.1.1.2 *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).

⁴ 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.

⁵ The last approved version of this historical standard is referenced on www.astm.org.

3.1.1.3 *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.

3.2 Definitions of Terms Specific to This Standard:

3.2.1 *algicidal*—having the property of killing algae.

3.2.2 *algalstatic*—having the property of inhibiting algal growth.

3.2.3 *biomass*—the dry weight of living matter present in a population and expressed in terms of a given area or volume, for example, mg algae per liter. Because biomass is difficult to measure accurately, surrogate measures of biomass, such as cell counts, are typically used in this test.

3.2.4 *growth rate*—the increase in biomass per unit of time.

3.2.5 *IC50*—a statistically or graphically estimated concentration 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. Alternative values for inhibition, such as 10 % or 20 %, are referred to as IC10 or IC20.

3.2.6 *standing crop*—the algal biomass at the end of the test.

3.2.7 *yield*—the algal biomass at the end of the test minus the algal biomass at the beginning of the test.

3.3 For definitions of other terms used in this standard, refer to Guides E729 and E1023 and Terminology E943. For explanation of units and symbols, refer to Practice E380.

4. Summary of Guide

4.1 In each of two or more treatments, organisms of one species of microalgae are maintained in replicate test vessels using the static technique. The test duration is typically 96 h, but shorter periods (for example, 72 h) have been used for fast-growing algae and longer periods (for example, 120 h) may be necessary for slower-growing algae. In each of the one or more control treatments, the algae are maintained in growth medium to which no test material has been added in order to provide the following: a measure of the acceptability of the test by giving an indication of the quality of the algae 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 algae are maintained in growth medium to which test material has been added to achieve a selected concentration. Specified data on population growth are obtained during the test and are usually analyzed to determine the IC50 based on reduction in growth.

5. Significance and Use

5.1 Tests with algae provide information on the toxicity of test materials to an important component of the aquatic biota and might indicate whether additional testing (2) is desirable. Specific testing procedures under various regulatory jurisdictions follow procedures similar to those described in this Guide (3, 4). Users should consult with any specific regulatory