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Standard Guide for Behavioral Testing in Aquatic Toxicology[1]

This standard is issued under the fixed designation E1604; 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 covers some general information on the selection and application of behavioral methods useful for determining the sublethal effects of chemicals to fish, amphibians, and macroinvertebrates.

1.2 Behavioral toxicity occurs when chemical or other stressful conditions, such as changes in water quality or temperature, induce a behavioral change that exceeds the normal range of variability (**1**).[2] Behavior includes all observable, recordable, or measurable activities of a living organism and reflects genetic, neurobiological, physiological, and environmental determinants (**2**).

1.3 Behavioral methods can be used in biomonitoring, the determination of no-observed-effect and lowest-observed-effect concentrations, and the prediction of hazardous chemical impacts on natural populations (**3**).

1.4 Behavioral methods can be applied to fish, amphibians, and macroinvertebrates in standard laboratory toxicity tests, tests of effluents, and sediment toxicity tests.

1.5 The various behavioral methods included in this guide are categorized with respect to seven interdependent, functional responses that fish, amphibians, and macroinvertebrates must perform in order to survive. These functional responses include respiration, locomotion, habitat selection, feeding, predator avoidance, competition, and reproduction (**4**). These responses can be documented visually or through video or acoustic imagery. Electronically recorded information can be derived through manual techniques or through the use of digital image analysis software (**5**, **6**, **7**).

1.5.1 The functional responses are not necessarily mutually exclusive categories. For instance, locomotion, of some form of movement, is important to all behavioral functions.

1.6 Additional behavioral methods for any category may be added when new tests are developed as well as when methods are adapted to different species or different life stages of an organism.

1.7 This guide is arranged as follows:

1.8 The values stated in SI units are to be regarded as the standard. For an explanation of units and symbols, refer to IEEE/ASTM SI 10.

1.9 *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 precautionary statements are given in Section 9.

1.10 *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:*[3]

E729 Guide for Conducting Acute Toxicity Tests on Test

[1] This guide is under the jurisdiction of ASTM Committee E50 on Environmental Assessment, Risk Management and Corrective Actionand 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 standard.

[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.

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
E1241 Guide for Conducting Early Life-Stage Toxicity Tests with Fishes
E1383 Guide for Conducting Sediment Toxicity Tests with Freshwater Invertebrates (Withdrawn 1995)[4]
E1711 Guide for Measurement of Behavior During Fish Toxicity Tests
E1733 Guide for Use of Lighting in Laboratory Testing
E1847 Practice for Statistical Analysis of Toxicity Tests Conducted Under ASTM Guidelines
IEEE/ASTM SI 10 American National Standard for Metric Practice

3. Terminology

3.1 *Definitions*—The words "must," "should, "may," "can," and "might" have very specific meanings. "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 used only in connection with the factors that directly relate to the acceptability of the test. "Should" is used to state that the specified condition is recommended and ought to be met if possible. Although the 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 of Terms Specific to This Standard:*

3.2.1 *behavior*—the complex of observable, recordable, or measurable activities of a living organism.

3.2.2 *behavioral toxicity*—the phenomenon observed when a behavioral response varies beyond the range of normal as a result of exposure to chemical or other stressors.

3.3 For definitions of other terms used in this guide, refer to Guides E729, E1192, E1241, E1383, E1711, E1847, and Terminology definitions in Guide E943.

4. Summary of Guide

4.1 The potential toxicity of chemical substances in water, food, or sediments is assessed by measuring the behavior of fish, amphibians, and macroinvertebrates during exposure, using static, flow-through, or food exposure systems. The behavioral response of organisms exposed to chemical substances in water, effluents, food, or sediments is compared with the behavioral responses of control organisms. The behavioral responses measured during toxicity tests are highly sensitive to sublethal exposure. The behavioral measures are relevant to essential life functions that fish, amphibians, and macroinvertebrates often must perform in order to survive and include respiration, locomotion, habitat selection, feeding, predator avoidance, competition, and reproduction. Data are obtained to determine the effects of toxic substances on behavior from short (for example, 1 h) or long-term (partial to full life cycle) exposures.

5. Significance and Use

5.1 Protection of a species requires the prevention of detrimental effects of chemicals on the survival, growth, reproduction, and health of that species. Behavioral toxicity provides information concerning sublethal effects of chemicals and signals the presence of toxic test substances.

5.1.1 The behavioral responses of all organisms are adaptive and essential to survival. Major changes in the behavioral responses of fish, amphibians, and macroinvertebrates may result in a diminished ability to survive, grow, or reproduce and cause significant changes in the natural population **(8)**.

5.2 The results from behavioral toxicity tests may be useful for measuring injury in the assessment of damages resulting from the release of hazardous materials **(9)**.

5.3 Behavioral toxicity test methods may be useful for long-term monitoring of effluents **(10)**.

5.4 The results from behavioral toxicity data can be used to predict the effects of exposure on fish, amphibians, and aquatic invertebrates likely to occur in field situations as a result of exposure under similar conditions, including the avoidance of exposure by motile organisms **(11)**.

5.5 The results from behavioral toxicity tests might be an important consideration for assessing the hazard of materials to aquatic organisms. Such results might also be used when deriving water quality criteria for fish and aquatic invertebrate organisms.

5.6 The results from behavioral toxicity tests can be used to compare the sensitivities of different species, relative toxicity of different chemical substances on the same organism, or effect of various environmental variables on the toxicity of a chemical substance.

5.7 The results from behavioral toxicity tests can be used to predict the effects of long-term exposure.

5.8 The results of behavioral toxicity tests can be useful for guiding decisions regarding the extent of remedial action needed for contaminated aquatic and terrestrial sites.

5.9 The behavioral characteristics of a particular organism must be understood and defined before a response can be used as a measure of toxicity. The range of variability of any behavioral response of unexposed organisms is influenced by genetic, experiential, physiological, and environmental factors. Thus it is important to avoid selecting test organisms from populations that may vary significantly in these factors.

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