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Standard Guide for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Mollusks[1]

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1. Scope

1.1 This guide describes procedures for obtaining laboratory data concerning bioconcentration of a test material added to dilution water—but not to food—by freshwater and saltwater fishes and saltwater bivalve mollusks using the flow-through technique. These procedures also should be useful for conducting bioconcentration tests with other aquatic species, although modifications might be necessary.

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, the results of tests conducted using unusual procedures are not likely to be comparable to those of many other tests. The comparison of results obtained using modified and unmodified versions of these procedures might provide useful information concerning new concepts and procedures for conducting bioconcentration tests.

1.3 These procedures are applicable to all chemicals that can be measured accurately at the necessary concentrations in water and in appropriate tissues. Bioconcentration tests are usually conducted on individual chemicals but can be conducted on mixtures if appropriate measurements can be made. Some techniques described in this guide were developed for tests on non-ionizable organic chemicals (see 11.1.2.1) and might not apply to ionizable or inorganic chemicals.

1.4 Results of bioconcentration tests should usually be reported in terms of apparent steady-state and projected steady-state bioconcentration factors (BCFs) and uptake and depuration rate constants. Results should be reported in terms of whole body for fishes and in terms of total soft tissue for bivalve mollusks. For fishes and scallops consumed by humans, some results should also be reported in terms of the edible portion, especially if ingestion of the test material by humans is a major concern. For tests on organic and organometallic chemicals, the percent lipids of the tissue should be reported.

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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1.6 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.7 *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 7.*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.*

1.8 *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]

D1129 Terminology Relating to Water
D1193 Specification for Reagent Water
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
E1191 Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids
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
E1241 Guide for Conducting Early Life-Stage Toxicity Tests with Fishes
E1295 Guide for Conducting Three-Brood, Renewal Toxicity Tests with *Ceriodaphnia dubia*
E1688 Guide for Determination of the Bioaccumulation of Sediment-Associated Contaminants by Benthic Invertebrates
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]
E2122 Guide for Conducting In-situ Field Bioassays With Caged Bivalves
E2455 Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels
SI 10 IEEE/ASTM SI 10 Standard for Use of the International System of Units (SI) (the Modernized Metric System)

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 used only in connection with factors that relate directly 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” is rarely a serious matter, violation of several will often render the results questionable. Terms such as “is 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 Terminologies D1129 and E943 and Guide E729. For an explanation of units and symbols, refer to Standard SI 10.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *apparent steady-state bioconcentration factor, n*—a BCF that does not change significantly over a period of two to four days at a uniform concentration (as defined in 11.10.3.2) of the test material in the solution containing the organism, that is, the BCF that exists when uptake and depuration are equal and bioconcentration (net accumulation) is zero for two to four days.

3.2.2 *bioaccumulation, n*—the net accumulation of a substance by an organism as a result of uptake from all environmental sources.

3.2.3 *bioconcentration, n*—the net accumulation of a substance by an aquatic organism as a result of uptake directly from aqueous solution.

3.2.4 *bioconcentration factor (BCF), n*—the quotient, at any time during the uptake phase of a bioconcentration test, of the concentration of a material in one or more tissues of an aquatic organism at that time, divided by the effective average exposure concentration at that time of the same material in the solution which contains the organism, in units of volume of solution per mass of organism. (BCFs are usually calculated so that the volume of solution, for example, 1 L, is about comparable to the mass of tissue, for example, 1 kg, and the BCF is reported without units.)

[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.

3.2.5 *depuration, n*—loss of a substance from an organism as a result of any active or passive process.

3.2.6 *depuration curve, n*—the line obtained by plotting the measured concentration of a test material in aquatic organisms versus time during the depuration phase of a bioconcentration test.

3.2.7 *depuration phase, n*—the portion of a bioconcentration test after the uptake phase and during which the organisms are in dilution water to which no test material has been added.

3.2.8 *depuration rate constant, n*—the mathematically derived value(s) that expresses how rapidly test material is eliminated from previously exposed aquatic organisms when placed in dilution water to which no test material has been added, usually expressed in units of reciprocal time.

3.2.9 *effective average exposure concentration, n*—the average concentration, at any time during the uptake phase of a bioconcentration test, of test material in the test solution during the preceding period of time equal to the shorter of (*a*) the length of the uptake phase to that point and (*b*) one half the time to apparent steady-state. Effective exposure concentrations cannot be calculated until after the time to apparent steady-state has been determined, unless the concentration of test material is constant.

3.2.10 *projected steady-state bioconcentration factor, n*—a BCF calculated for infinite time (*a*) from uptake and depuration rate constants derived using an appropriate compartmental model or (*b*) by fitting an appropriate equation to data concerning BCF versus time.

3.2.11 *uptake, n*—acquisition of a substance from the environment by an organism as a result of any active or passive process.

3.2.12 *uptake curve, n*—the line obtained by plotting the measured concentration of test material in aquatic organisms versus time during the uptake phase of a bioconcentration test.

3.2.13 *uptake phase, n*—the portion of a bioconcentration test during which organisms are exposed to test material intentionally added to dilution water. (Although uptake and depuration both occur during the uptake phase, uptake always predominates at the beginning, but depuration often becomes nearly equal to uptake at the end of the uptake phase. Occasionally depuration exceeds uptake during a portion of the uptake phase.)

3.2.14 *uptake rate constant, n*—the mathematically derived value(s) that express how rapidly test material is accumulated by aquatic organisms during the uptake phase of a bioconcentration test, in units of volume of solution per mass of organism per time.

4. Summary of Guide

4.1 Each of two groups of test organisms of one species is administered a treatment, consisting of an uptake phase and a depuration phase, using the flow-through technique. The control treatment, in which organisms are exposed during both phases to dilution water to which no test material has been added, provides a measure of the acceptability of the test by giving an indication of the quality of the test organisms and the suitability of the dilution water, food, test conditions, handling procedures, etc. In the other treatment the organisms are (*a*) exposed during the uptake phase to dilution water, to which a selected concentration of test material has been intentionally added, at least until either apparent steady-state or 28 days is reached and (*b*) exposed during the depuration phase to dilution water to which no test material has been added. During both phases of the test, representative organisms and water samples are removed periodically from each test chamber and analyzed for test material. Apparent steady-state and projected steady-state BCFs and uptake and depuration rate constants are usually calculated from the measured concentrations of test material in tissue and water samples. If it is desired to determine whether BCFs and rate constants are dependent on the concentration of test material in water, additional treatments, utilizing different concentrations of test material during the uptake phase, must be used.

5. Significance and Use

5.1 A bioconcentration test is conducted to obtain information concerning the ability of an aquatic species to accumulate a test material directly from water. This guide provides guidance for designing bioconcentration tests on the properties of the test material so that each material is tested in a cost-effective manner.

5.2 Because steady-state is usually approached from the low side and the definition of apparent steady-state is based on a statistical hypothesis test, the apparent steady-state BCF will usually be lower than the steady-state BCF. With the variation and sample sizes commonly used in bioconcentration tests, the actual steady-state BCF will usually be no more than twice the apparent BCF.

5.3 When both are determined in the same test, the projected steady-state BCF will usually be higher than the apparent steady-state BCF because the models used to calculate the projected BCF assume that the BCF steadily increases until infinite time.

5.4 The BCFs and rates and extents of uptake and depuration will depend on temperature, water quality, the species and its size, physiological condition, age, and other factors (**1**).[4] Although organisms are fed during tests, uptake by means of sorption onto food is probably negligible during tests.

5.5 Results of bioconcentration tests are used to predict concentrations likely to occur in aquatic organisms in field situations as a result of exposure under comparable conditions, except that mobile organisms might avoid exposure when possible. Under the experimental conditions, particulate matter is deliberately minimized compared to natural water systems. Exposure conditions for the tests may therefore not be comparable for an organic chemical that has a high octanol-water partition coefficient or for an inorganic chemical that sorbs substantially onto particulate matter. The amount of the test substance in solution is thereby reduced in both cases, and therefore the material is less available to many organisms.

[4] The boldface numbers in parentheses refer to the list of references at the end of this standard.