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Standard Guide for Conducting In-situ Field Bioassays With Caged Bivalves[1]

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1. Scope

1.1 This guide describes procedures for conducting controlled experiments with caged bivalves under field conditions. The purpose of this approach is to facilitate the simultaneous collection of field data to help characterize chemical exposure and associated biological effects in the same organism under environmentally realistic conditions. This approach of characterizing exposure and effects is consistent with the US EPA ecological risk assessment paradigm. Bivalves are useful test organisms for in-situ field bioassays because they (*1*) concentrate and integrate chemicals in their tissues and have a more limited ability to metabolize most chemicals than other species, (*2*) exhibit measurable sublethal effects associated with exposure to those chemicals, (*3*) provide paired tissue chemistry and response data which can be extrapolated to other species and trophic levels, (*4*) provide tissue chemistry data which can be used to estimate chemical exposure from water or sediment, and (*5*) facilitate controlled experimentation in the field with large sample sizes because they are easy to collect, cage, and measure (**1**, **2**)[2]. The experimental control afforded by this approach can be used to place a large number of animals of a known size distribution in specific areas of concern to quantify exposure and effects over space and time within a clearly defined exposure period. Chemical exposure can be estimated by measuring the concentration of chemicals in water, sediment, or bivalve tissues, and effects can be estimated with survival, growth, and other sublethal end points (**3**). Although a number of assessments have been conducted using bivalves to characterize exposure by measuring tissue chemistry or associated biological effects, relatively few assessments have been conducted to characterize both exposure and biological effects simultaneously (**2**, **4**, **5**). This guide is specifically designed to help minimize the variability in tissue chemistry and response measurements by using a practical uniform size range and compartmentalized cages for multiple measurements on the same individuals.

1.2 The test is referred to as a field bioassay because it is conducted in the field and because it includes an element of relative chemical potency to satisfy the bioassay definition. Relative potency is established by comparing tissue concentrations with effects levels for various chemicals with toxicity and bioaccumulation end points (**6**, **7**, **8**, **9**, **10**) even though there may be more uncertainty associated with effects measurements in field studies. Various pathways of exposure can be evaluated because filter-feeding and deposit-feeding are the primary feeding strategies for bivalves. Filter-feeding bivalves may be best suited to evaluate the bioavailability and associated effects of chemicals in the water column (that is, dissolved and suspended particulates); deposit-feeding bivalves may be best suited to evaluate chemicals associated with sediments (**11**, **12**, **13**, **14**). It may be difficult to demonstrate pathways of exposure under field conditions, particularly since filter-feeding bivalves can ingest suspended sediment and facultative deposit-feeding bivalves can switch between filter- and deposit feeding over relatively small temporal scales. Filter-feeding bivalves caged within 1 m of bottom sediment have also been used effectively in sediment assessments from depths of 10 to 650 m (**5**, **15**, **16**). Caged bivalve studies have also been conducted in the intertidal zone (**17**). The field testing procedures described here are useful for testing most bivalves although modifications may be necessary for a particular species.

1.3 These field testing procedures with caged bivalves are applicable to the environmental evaluation of water and sediment in marine, estuarine, and freshwater environments with almost any combination of chemicals, and methods are being developed to help interpret the environmental significance of accumulated chemicals (**6**, **7**, **9**, **18**, **19**). These procedures could be regarded as a guide to an exposure system to assess chemical bioavailability and toxicity under natural, site- specific conditions, where any clinical measurements are possible.

1.4 Tissue chemistry results from exposures can be reported in terms of concentrations of chemicals in bivalve tissues (for example, µg/g), amount (that is, weight or mass) of chemical per animal (for example, µg/animal), rate of uptake, or bioaccumulation factor (BAF, the ratio between the concentration of

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

a chemical in bivalve tissues and the concentration in the external environment, including water, sediment, and food). Tissue chemistry results can only be used to calculate a BAF because caged bivalves in the field are exposed to multiple sources of chemicals and can accumulate chemicals from water, sediment, and food. Toxicity results can be reported in terms of survival (**3**, **20**), growth rate (**3**, **20**), or reproductive effects (**21**, **22**) after a defined exposure period.

1.5 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 standardized tests. Comparisons of results obtained using modified and unmodified versions of these procedures might provide useful information concerning new concepts and procedures for conducting field bioassays with bivalves.

1.6 This guide is arranged as follows:

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

1.8 *This standard may involve hazardous materials, operations, and equipment – particularly during field operations in turbulent waters or extreme weather conditions. 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.

1.9 *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]

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

D3976 Practice for Preparation of Sediment Samples for Chemical Analysis

D4447 Guide for Disposal of Laboratory Chemicals and Samples

E724 Guide for Conducting Static Short-Term Chronic Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Molluscs

E729 Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians

E943 Terminology Relating to Biological Effects and Environmental Fate

E1022 Guide for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Mollusks (Withdrawn 2022)[4]

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

E1367 Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Estuarine and Marine Invertebrates

E1391 Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing and for Selection of Samplers Used to Collect Benthic Invertebrates

E1525 Guide for Designing Biological Tests with Sediments

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

E1847 Practice for Statistical Analysis of Toxicity Tests Conducted Under ASTM Guidelines (Withdrawn 2022)[4]

E2455 Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels (Withdrawn 2022)[4]

SI10-16 IEEE/SI 10 American National Standard for Metric Practice

[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.

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

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 a 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. "Should" is used to state that a 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 Terminology D1129, Guide E729, Terminology E943, and Guide E1023. For an explanation of units and symbols, refer to SI10-16.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *bioaccumulation, n*—the accumulation of a chemical in an organism.

3.2.2 *bioaccumulation factor (BAF), n*—the ratio of tissue chemical residue to chemical concentration in the external environment. BAF is measured at steady state in situations where organisms are exposed from multiple sources (that is, water, sediment, food), unless noted otherwise.

3.2.3 *bioassay, n*—an experiment that includes both an estimate of toxicity and an estimate of relative potency.

3.2.4 *bioavailability, n*—the fraction of the total chemical concentration in water, on sediment particles, and on food that is available for bioaccumulation.

3.2.5 *biomonitoring, v*—use of living organisms as "sensors" in water or sediment quality surveillance to detect current conditions or changes in an effluent or water body or to identify exposure to chemicals and risks to aquatic life.

3.2.6 *chemical concentration, n*—the ratio of the weight or volume of chemicals to the weight or volume of a test sample.

3.2.7 *chemical content, n*—mass of chemical per whole animal (for example, µg/animal) can be used to normalize the expression of chemical uptake per unit time by eliminating the effects of growth on changing tissues masses.

3.2.8 *chemical fingerprinting, v*—the use of specific patterns in the ratios of chemicals accumulated in bivalve tissues to identify chemical sources; for example, the ratio of PAH alkylated homologs to parent compounds.

3.2.9 *compartmentalized cage, n*—a rigid or flexible mesh cage with individual compartments for holding bivalves in a controlled position so that multiple measurements can be made on the same individual organism. The compartmentalized cage helps maximize water flow around individual test organisms and provides even exposure to the test environment.

3.2.10 *growth dilution, n*—a process whereby the rate of accumulation is exceeded by the rate of tissue growth so that when the concentration is expressed on mass of chemical per mass of tissue over time, it appears as though depuration or elimination is occurring because the concentration (µg/g) is decreasing.

3.2.11 *reference station, n*—a station similar to the test station(s) in physical and chemical characteristics and with relatively little to no contamination by the particular chemical(s) under study. A reference station should ideally contain only background concentrations of chemicals characteristic of the region.

3.2.12 *scope for growth, n*—an integrated physiological measure of the energy status of an organism at a particular time, based on the concept that energy in excess of that required for normal maintenance will be available for the growth and reproduction of the organism.

3.2.13 *shell length, n*—the distance from the tip of the umbo to the distal valve edge.

3.2.14 *site, n*—a geographical area within a somewhat defined boundary that is being studied. The size of a site is dependent on the extent of suspected perturbation, generally on the order of 0.1 to 50–km^2. Part of the vagueness in size is due to variability in spatial scale and inadequate results from preliminary reconnaissance survey that clearly define the boundary of suspected stressors.

3.2.15 *steady state, n*—the state in which fluxes of material moving bidirectionally across a membrane or boundary between compartments or phases have reached a balance. An equilibrium between the phases is not necessarily achieved.

3.2.16 *station, n*—a specific sampling location or area within a site. The size of a station can vary from a single point with one cage to an area of approximately 10 by 10 m including several cages. Vagueness in size is due to variability in spatial scale and experimental design. Several stations in a small geographic area could comprise a site.

3.2.17 *tissue loss magnification, n*—the process whereby the tissue mass is lost during the exposure period and the chemical mass remains constant over time, so that when the concentration is expressed on mass of chemical per mass of tissue over time, it appears as though bioaccumulation is occurring because the concentration (µg/g) is increasing.

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

3.2.19 *whole-animal wet-weight, n*—the wet weight (g) of the entire bivalve, including water trapped between the valves.

4. Summary of Guide

4.1 This guide describes procedures for exposing marine, estuarine, and freshwater bivalves to chemicals in water, sediment, and food in the field under natural in-situ field conditions. The purpose of this guide is to provide a standard approach for in-situ testing with bivalves. Because of its application to a wide variety of species, many of which have a range of tolerance limits for water quality conditions, it is