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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:

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

outside the scope of this guide to provide the tolerance limits for all water quality conditions for all species that can be used for in-situ testing. Tolerance limits are provided for selected species as examples and points of reference (6.4).

4.2 The approach can be used to characterize exposure and effects over space and time. The primary measurement end points are bioaccumulation of chemicals in bivalve tissues to assess biological availability or bioaccumulation potential, and sublethal effects, like growth, to assess adverse biological effects. The bioavailability of chemical(s) in water, sediment, and food and associated biological effects are determined by the relative differences in these exposure and effects end points among stations over time.

4.3 In practice, the two most commonly measured effects end points are survival and growth. Survival is the easiest effects end point to measure and provides an estimate of toxicity in exposures of any duration that is determined to be appropriate to meet the study needs (see 8.10). The survival end point may be insensitive for some chemicals but can provide important corroborative effects information. Sublethal endpoints like growth are generally more sensitive. Growth can be estimated from changes in whole-animal wet-weight, shell length, tissue weight, or shell weight, with baseline tissue and shell weights for the entire test population estimated from a subsample of that population. Reproduction is another sensitive end point, but is more difficult to measure in bivalves.

4.4 Bioaccumulation and growth are compared among test stations for ranking purposes, among reference and treatment stations, or among stations for temporal and spatial variability as well as short- and long-term trends. It is also possible to use the data to construct dose-response relationships (**6**, **7**) and to identify sources of point and non-point discharges by comparing bioaccumulation and biological effects at various distances away from suspected sources of contamination in a gradient approach (**23**).

5. Significance and Use

5.1 The ecological importance of bivalves, their wide geographic distribution, ease of handling in the laboratory and the field, and their ability to filter and ingest large volumes of water and sediment particles make them appropriate species for conducting field bioassays to assess bioaccumulation potential and associated biological effects. The test procedures in this guide are intended to provide guidance for conducting controlled experiments with caged bivalves under "natural," site-specific conditions. It is important to acknowledge that a number of "natural" factors can affect bivalve growth and the accumulation of chemicals in their tissues (Section 6, Interferences). This field bioassay can also be conducted in conjunction with laboratory bioassays to help answer questions raised in the field exposures. The field exposures can also be used to validate the results of laboratory bioassays.

5.2 The ultimate resources of concern are communities. However, it is often difficult or impossible to adequately assess the ecological fitness or condition of the community or identify and test the most sensitive species. Bivalves are recommended as a surrogate test species for other species and communities for the following reasons: (*1*) They readily accumulate many chemicals and show sublethal effects associated with exposure to those chemicals (**2**); (*2*) they accumulate many chemicals through multiple pathways of exposure, including water, sediment, and food (**24**, **25**, **26**, **27**, **28**, **29**), and (*3*) caged bivalves have been shown to represent effects on the benthos more accurately than traditional laboratory tests (**30**, **31**). Although bivalve species might be considered insensitive because of their wide use as indicators of chemical bioavailability, it has been suggested that sensitivity is related to the type of test, end points being measured, and duration of exposure (**2**). In relatively short-term toxicity assessments in which survival is typically determined as the measurement end point, bivalves may appear to be more tolerant to and less affected by chemicals because of their ability to close their valves for short periods and avoid exposure (**32**, **33**, **34**, **35**). However, studies comparing the mortality end point in bivalves and other test species have found bivalves to be equally (**36**, **37**) or more sensitive (**38**, **39**) than the other species (Table 1). When the bivalve growth end point was compared to the mortality end point in other test species, the bivalve growth end point was more sensitive (**20**, **30**, **31**, **40**, **41**).

TABLE 1 Relative Sensitivity of Bivalves Compared to Other Test Species

Bivalve Species	Species Compared	Exposure	End Point	Sensitivity
Anodonta grandis (**37**) (giant floater; currently *Pyganodon grandis*)	daphnia, fathead minnow, rainbow trout	municipal effluent	LC-50	equal
Anodonata imbecilis (**38**) (paper pondshell; currently *Utterbackia imbecilis*)	daphnia	pulp and paper mill effluent	10-d vs 7-d mortality	more
Anodonata imbecilis (**36**) (paper pondshell; currently *Utterbackia imbecilis*)	daphnia, midge, fathead minnow	metals	7-d mortality	equal
Musculium transversum (**39**) (fingernail clam)	17 different species	ammonia	20-d mortality	more sensitive than 16 species
Mercenaria mercenaria (**30**, **31**) (hard clam)	2 amphipods, microtox	sediment	7-d growth, 10-d mortality	more
	Caged *Mercenaria* more sensitive than lab *Mercenaria* (**30**, **31**)			
Mullinia lateralis (**40**) (dwarf surf clam)	amphipod	sediment	7-d growth, 10-d mortality	more
Mytilus galloprovincialis (**20**) (Mediterranean mussel)	amphipod	in-situ water column	84-d growth, 10-d mortality	more, [tissue TBT]

5.2.1 Chronic tests designed to monitor sublethal end points, such as growth, are recommended because bivalves generally show increasing sensitivity with increasing exposure period. Sublethal end points measured in bivalves that have demonstrated high levels of sensitivity include growth (**3**, **20**), reproduction (**21**), DNA damage (**42**, **43**), metallothioneins and other biochemical markers (**44**, **45**, **46**).

5.2.2 There are many field monitoring programs in the US which use bivalves, including the NOAA Status and Trends Program (**47**), the California Mussel Watch (**48**), and the California Toxics Monitoring Program, a freshwater monitoring program (**49**). Similar field-monitoring programs exist in other countries. Numerous laboratory studies throughout the world have examined bioaccumulation and biological effects in bivalves. The existing databases which have compiled bioaccumulation and effects in bivalves and other species (**8**, **9**) make it possible to use tissue residues associated with effects in bivalves as surrogates to estimate effects in both water column and benthic organisms in many freshwater, estuarine, and marine environments.

5.3 Bivalves are an abundant component of many soft bottom marine, estuarine, and freshwater environments. Intertidal marine bivalves make up a significant portion of many habitats and provide habitats for many additional species. It is important to monitor freshwater bivalves for the following reasons: they are among the first taxa to disappear from benthic communities impacted by chemicals; they have been shown to be more sensitive than several other major taxa in laboratory tests.(**50**) The threatened and endangered status of many freshwater bivalve species also make them an important group to monitor.

5.4 If practical, the species to be used in a field bioassay should be one that is endemic to the area under investigation. In many cases, the specific area under investigation may not support bivalves due to a variety of factors including high concentrations of chemicals, competition or predation, or lack of suitable habitat or substrate. Under these conditions, it may be desirable to use a species that would normally be found in the environment if all conditions were favorable; however, it may be necessary to use a surrogate species, that is, a species that can tolerate the environmental conditions but is not normally found in the area, if native species are unavailable in the test area.

5.5 Bivalves generally utilize one of two primary modes of feeding: filter-feeding or deposit feeding. However, all known deposit-feeding bivalves are facultative in that they can either deposit- or filter-feed. Filter-feeders assimilate dissolved organics as well as suspended particulate matter, including plankton and suspended sediments, from the water column and have the potential for exposure to chemicals associated with this ingested material. Facultative deposit-feeding bivalves can be exposed to chemicals associated with sediments as they ingest sediments. They also ingest particulate material from the water column as they filter feed. As such, bivalves are capable of integrating exposure to chemicals dissolved in water and sorbed on sediment particles on the bottom or in suspension. It should be acknowledged that bivalves transplanted in the overlying water above sediment or transplanted directly on or in sediment may not exclusively accumulate or be affected by chemicals in a particular medium. That is, bivalves in or on sediment may still filter and accumulate chemicals from overlying water. Conversely, bivalves transplanted in the water column may filter suspended sediment and accumulate chemicals from that sediment. Bivalves can also assimilate chemicals as they ventilate overlying water.

5.6 Field bioassays are conducted to obtain information concerning the bioavailability of chemicals in the water column or bedded sediments and subsequent biological effects on bivalves after short- and long-term exposure to water and sediment under site-specific conditions. These bioassays do not necessarily provide information about whether delayed effects will occur, although a post-exposure observation period could provide such information. Sublethal post-exposure observations may include gonad development, spawning success, gamete survival, and development. The decision to conduct post-exposure studies in the field or in the laboratory depends on the observations being made, test conditions required, and experimental logistics.

5.7 The in-situ exposures described in this guide could be followed by laboratory measurements, such as scope for growth (**2**), filtration rate (**51**), byssal thread production (**52**, **53**, **54**), and biomarkers (**55**, **56**).

5.8 The bivalve field bioassay can be used to determine the spatial or temporal trends of chemical bioavailability in water and sediment and effects due to exposure to those chemicals. Spatial comparisons of parameters of concern can be made by distributing the caged bivalves along physical and chemical gradients at scales commensurate with the desired level of discrimination. For example, station locations might be distributed along a known physical or chemical gradient in relation to the boundary of a disposal site (**57**, **58**, **59**, **60**, **61**), sewage outfall (**62**), or effluent pipe or at stations identified as containing elevated concentrations of chemicals in water or sediment as identified in a reconnaissance survey (**3**, **63**, **64**). This can be accomplished by placing caged bivalves along horizontal transects or at different depths in the water column. Temporal comparisons can be made by conducting before-and-after studies. For example, the effectiveness of dredge activities, effluent diffuser construction, effluent reduction, or remedial action can be determined by conducting field bioassays before the action, during the action, and after the action.

5.9 The relative bioavailability of chemicals from the various pathways of exposure (that is, aqueous phase, suspended particulate matter, sediment) and subsequent effects can be determined by simultaneously deploying bivalves with different feeding strategies and making supplementary measurements. A combination of filtration and the use of sediment traps followed by chemical analysis of the various environmental compartments can be used to identify the relative contribution of the aqueous phase, suspended particulate matter, and sediment. Lipid bags or semi-permeable membrane devices (SPMDs), which predominantly collect the dissolved fraction of chemicals, could also be used (**65**, **66**, **67**, **68**, **69**, **70**). The bioaccumulation of chemicals and effects among different bivalve species deployed either side-by-side, at exposure and

reference locations, or before and after exposures can be compared and used to help explain the spatial variability of chemical contamination, particularly if the different species are placed in different locations (that is, in the water column, on top of the sediments, within the sediments) as determined appropriate for the study design. This field assessment approach could be supplemented with laboratory studies designed to answer specific questions regarding dissolved versus particulate pathways of exposure.

5.10 Results of bivalve field bioassays might be an important consideration when assessing the hazards of materials to aquatic organisms (see Guide E1023) or when deriving water or sediment quality guidelines for aquatic organisms (**17**, **71**). Bivalve field bioassays can be useful in making decisions regarding the extent of remedial action needed for contaminated sites. They also provide a convenient method for manipulative field experiments, hypothesis testing, and monitoring specific sites before, during, and after cleanup operations (**63**, **64**).

6. Interferences

6.1 As with all bioassay procedures, there are limitations to the methods described in this guide. However, these limitations should not be considered as a reason for not using the methods described in this guide.

6.2 Results of bivalve field bioassays will depend, in part, on natural factors, including temperature, food supply, other physical and chemical properties of the test environment, selection of adequate reference areas, species selected, condition of the test organisms, exposure technique, and handling of the bivalves prior to deployment. Taking bivalves out of their habitat and weighing and measuring them may be stressful to the bivalves. The degree of handling, holding time, and differences between water and sediment conditions at the collection site versus the transplant site may also be stressful. Careful handling and appropriate acclimation can minimize these stresses.

6.3 Condition of the test organisms is critical to the success of the field bioassay. The most important consideration is spawning cycle because of possible interferences on bioaccumulation and growth and with subsequent data interpretation. Generally, chemicals are lost during spawning, resulting in potential underestimation of chemical bioavailability (**72**). Conversely, the energy used for gonad development and spawning can make bivalves more sensitive to chemicals, reduce their growth rates, and overestimate potential toxicity. Tests should be conducted with populations that will most likely not spawn during the exposure period. The spawning cycle of candidate test species should be evaluated prior to developing the study design, and species that do not spawn during the proposed exposure period should be selected.

6.4 Temperature, conductivity, hardness, pH, and dissolved oxygen concentrations of the test environment could affect both bioaccumulation and biological effects. These water quality parameters should be monitored over the course of the study to quantify the exposure conditions and the potential effects of temperature. As a general guide, examples of temperature tolerance for the most commonly used species are provided in Table 2. Temperature conditions during the exposure period can be quantified using in-situ monitoring devices. These devices can be attached to the deployment cages and set to collect temperature data at specified time intervals for the duration of the test.

6.5 Lack of acclimation to deployment water quality conditions could be an interference. If water quality conditions differ at collection and deployment sites, it may be necessary to acclimate the test organisms gradually to the deployment conditions. This transition is particularly important near the bivalve's tolerance limits and may be accomplished using serial water dilutions until the proper water quality conditions (for example, temperature, salinity, and pH) are reached. Acclimation for temperature should proceed no faster than 3°C in 72 h (Guides E1022 and E1688). Once acclimated, bivalves should be maintained under these conditions for a minimum period of time. Holding bivalves for extended periods under laboratory conditions can induce stress because bivalves are particularly sensitive to temperature, nutrition, and water flow. If test specimens are held for an extended period of time in the laboratory, the effect of this holding can be assessed by comparing soft tissue weights, or other indicators of bivalve health, to that of bivalves of the same size group freshly collected from the field. Alternatively, bivalves could be acclimated in the field under conditions similar to the proposed transplant sites.

6.6 Food supply is important because it affects both biological availability and associated biological effects. Food availability may be more difficult to quantify during the test than temperature or other physical factors. Until in-situ monitoring devices for chlorophyll and other nutrient sources are developed, it is suggested that food availability be estimated at least three times during the study (that is, beginning, middle, and end of test). The measurements made (that is, chlorophyll-a, particulate or total organic carbon, and suspended solids) will depend on the feeding strategy of the test species.

6.7 Current speed is important for filter-feeding bivalves because currents regulate the food supply to the test organisms. Currents are also important to facultative deposit-feeding and filter-feeding bivalves in the benthos because flushing may reduce the potential effects of chemicals by dilution with clean water from outside the assessment area. Currents can be quantified during the exposure period with a continuously recording, in-situ current meter or quantified intermittently during the suggested sampling intervals used to measure food availability.

6.8 Salinity is particularly important in estuarine areas, where salinity can range from 0 ppt at the head of a river to 33 ppt at the mouth. Salinity should be evaluated prior to species selection. If there is a wide salinity range, it may be necessary to identify two or more bivalve species for the assessment: one species for the lower end of the salinity range and another for the upper end of the salinity range. It is recommended that both species be deployed in the area where salinity is in the middle, as this provides a means to compare results between species.

TABLE 2 Temperature (°C) and Salinity (Parts per Thousand (ppt)) Tolerance Limits for Selected Bivalve Species (Months when spawning may occur and species distribution are also shown)

Species and Reference	Temperature Range	Salinity Range	Spawning	Distribution
Corbicula fluminea (Asian clam) **(73)**	2–25	0–5	may be continuous, usually twice/year spring/early summer; later summer	All west, gulf, and east coastal United States to DE River; NM; OH & MS River systems
Dreissena polymorpha (Zebra mussel) **(74)**	<0–35	0–6	May to September	Canada and Northeastern United States; Great Lakes, St. Lawrence River; MS, OH, IL & TN River drainages; NY Canals, Hudson River, Finger Lakes
Elliptio complanata (Eastern Elliptio) **(75, 76, 77)**	0–30	0–3	most June to July; some May to September	Gulf St. Lawrence to GA; Great Lakes, except Lake Michigan & Lake Erie
Pyganodon (Anodonta) grandis (floater mussel) **(75)**	0–30	0–3	most April to May; some to late August	Canada Interior & St. Lawrence River drainage; Hudson Bay, MI and MO Rivers drainages; NM, CO, TX, Mex
Rangia cuneata (Atlantic Rangia) **(78, 79)**	8–32	0<19	VA: early April to summer; FL: July-November; LA: Mar-May and late summer to November; Mexico: February-June and September to November	Gulf of Mexico coast from northwest FL to Campeche, Mexico; along Atlantic coast to NJ
Argopecten irradians (Bay scallop) **(79)**	>7 >30	>14–28	mid-Atlantic: mid-April through early September; NY: June and July; NC and FL: August and December	Atlantic coast; Cape Cod to Gulf of Mexico
Crassostrea gigas (Pacific oyster) **(79)**	4–24	25–35	July to August	Pacific coast; Pacific Northwest
Crassostrea virginica (Eastern oyster) **(80)**	-2–36	5–32	Gulf of Mexico: April-October; Malpeque Bay, PEI: July-August; Bideford River Estuary, PEI: July	Gulf of Mexico to Cape Cod
Macoma balthica (Baltic clam) **(81, 82, 83)**	-2–23	5–30	June-August (Europe); July-September (United States)	Greenland to France; Baltic and Wadden Seas; UK; N. Canada to Chesapeake; AK to San Francisco Bay
Mercenaria mercenaria (Hard clam) **(84)**	<0–35	12–35	March-November depending on latitude and temperature. Peaks in July	Atlantic and Gulf coasts; abundant MA to VA
Mya arenaria (Soft-shell clam) **(85)**	-1.7–32	10–32	June-September; once/year north of Cape Cod, twice/year south of Cape Cod	Atlantic coast from Labrador to SC; less in FL; AK and CA
Mytilus californianus (California mussel) **(79)**	7–28	25–33	Continuous throughout year; peaks in July and December	AK to southern CA
Mytilus edulis (Blue mussel) **(86)**	0–27	5–33	differs between populations; some low-level throughout year; first in early summer, second in the fall	Atlantic coast, from Labrador to Cape Hatteras, NC
Mytilus galloprovincialis (Mediterranean mussel) **(87)**	8–25	10–33	Similar to *M. edulis*, but several weeks later when temperature is maximum	Mediterranean, Europe, Atlantic France and British Isles, Japan, East China to Korea, Australia, South Africa; southern CA to OR
Mytilus trossulus (Pacific blue mussel) **(88)**	0–29	4–33	July to September	Baltic Sea; west Coast, Central CA to AK; east Coast, Canadian Maritimes
Ostrea lurida (Olympia oyster) **(89)**	6–20	NA–25	Spring to fall: peaks in spring in south, mid summer in mid-range and north	Southeast AK to Baja California
Protothaca staminea (Littleneck clam) **(90)**	0–25	20-32	BC, Canada, January to March; AK, mid-July; southern CA, June	Aleutian Islands, AK to Cape San Lucas, Baja California
Venerupis japonica (Manila clam) **(79)**	13–21	24–31	Washington: once/year May-September; peaks in June/July	British Columbia to CA

6.9 Possible interferences influencing retrieval of test organisms from the field include caged bivalves being washed away during storm events, buried by underwater sediment shifts, theft, vandalism, fouling, disease, and consumption by predators.

6.10 Depending on the environment under assessment, it is possible for the bivalve cages, including the external predator mesh (see 11.3) and the mesh bags, to become fouled with both epiphytic plant and animal growth. Fouling occurs most frequently in highly productive embayments or areas with restricted flow, such as marinas. Excessive fouling can reduce or eliminate flow of water through the cage material, resulting in highly stressful conditions to the test bivalves. If such conditions are anticipated, the deployed cages should be examined for fouling at regular intervals during the exposure period. Fouling organisms can be removed from the exterior surfaces of the cages by hand or with a stiff brush. If the cages are heavily fouled and it is difficult to remove the attached biomass with brushing or scraping, the bivalves should be transferred to clean, unfouled cages for the remainder of the exposure period.

6.11 Possible interferences associated with interpretation of tissue chemistry data include the use of inappropriate analytical procedures. It is critical to use the most appropriate method for each chemical analysis. For example, when measuring the suite of PAH-alkylated homologs, it is essential to use sufficient silica gel to clean up excess lipids in the sample. A more specific approach for these analyses developed as part of the Exxon Valdez oil spill assessment program included advanced methods specific to that group of researchers. These methods

are recommended for bivalve tissues when source identification through chemical fingerprinting is necessary (**91**, **92**, **93**, **94**).

6.12 Natural variability in the concentrations of chemicals of concern in water or sediment coupled with intermittent chemical discharges may increase the difficulty in interpreting exposure concentrations in these pathways. However, weekly measurements of chemicals in the water column coupled with measurements of bioaccumulation and growth have proven effective in explaining the environmental significance of these variables (**3**, **20**). In practice, it is usually difficult to sample with that frequency, and water samples are generally taken only at the beginning and end of the test. Since the variability in sediment chemistry is generally less extreme than in water, collecting sediment samples for chemical analysis at the beginning and end of test may be sufficient to characterize exposure conditions (Practice D3976). However, sediments may also be highly variable on a small spatial scale (**95**).

6.13 In assessing effects of effluents with high organic loads, it is possible that the organic enrichment from the effluent will increase bivalve growth rates and make it more difficult to assess the adverse effects of associated chemicals. Differentiating between the positive effects of nutrient enrichment and the adverse effects of toxic chemicals is best accomplished by maximizing the number of stations in the assessment area, deploying caged bivalves at various depths, and maximizing the number of effects end points. The processes involved could be better characterized and understood by using various biomarkers in addition to the bioaccumulation and effects end points (**96**).

7. Hazards

7.1 Water and sediment might be contaminated with unknown concentrations of many potentially toxic materials. Any potentially contaminated water or sediment should be handled in a manner to minimize exposure of personnel to toxic compounds. Therefore, skin contact with all potentially toxic sediments and overlying water should be minimized by such means as wearing appropriate protective gloves, laboratory coats, aprons, and glasses particularly when washing equipment or placing hands into test water, effluents, sediment, or cleaning solutions. Respirators may also be necessary in some hazardous waste sites or during oil spills.

7.2 Water and sediment, particularly in effluent areas, might contain organisms that can be pathogenic to humans. Special precautions when working in these areas might include immunization prior to deployments and the use of bactericidal soaps after working in the water and touching sediments.

7.3 Sampled media potentially containing hazardous compounds should be properly disposed of properly (Guide D4447).

7.4 Developing and following a project-specific health and safety plan is recommended when conducting laboratory or field activities to protect human health and the environment.

8. Experimental Design

8.1 Field bioassays can be designed to provide either a qualitative reconnaissance or a quantitative assessment involving statistical comparisons of measured end points (that is, chemical concentration in tissues and effects end points) among stations. The object of a qualitative reconnaissance survey is to identify sites with the potential for bioaccumulation and associated biological effects. Qualitative surveys are often conducted in areas where little is known about contamination patterns. Quantitative assessments are conducted to test for statistically significant differences among stations.

8.2 Experimental design considerations, such as station location, number of stations per site, number of cages per station, and number of bivalves per cage, should be based on the purpose of the test and the procedure(s) used to analyze the results. Various experimental designs can be applied, with the most common used to:

(1) Compare bivalve tissue chemistry and growth at one or more stations to reference, background, or pre-test conditions.

(2) Compare bivalve tissue chemistry and growth among multiple stations to characterize patterns, trends, or gradients.

8.3 Experimental control of all test variables can be difficult to achieve in field tests that assess or monitor resident populations. The use of in-situ field bioassays allows the investigator to control the following: species; number; and size range of test animals, specific location(s) to be assessed, and exposure duration. Generally, the concentration of chemicals of concern and natural factors, such as temperature, salinity, dissolved oxygen, pH, current speed, and food supply, are not manipulated or controlled as they are in laboratory testing. However, temperature could be increased by heating dissolved oxygen by aeration, current speed by pumping, and food supply by adding nutrients. The intent of field bioassays is to determine chemical bioavailability and subsequent effects under natural, site-specific conditions, which includes intrinsic, site-specific variability. With an adequate number of stations, statistical testing can often identify the importance of these uncontrolled variables with respect to exposure and effects.

8.4 *Measurement End Points:*

8.4.1 At a minimum, biological effects should be characterized by measuring survival and growth. Survival may not always be a very sensitive indicator of effects in bivalves (**3**), but it is an important parameter to monitor. Several factors can affect survival, including handling prior to test initiation and physical-chemical factors at the deployment stations. Survival can be easily quantified, although it is possible to have some individuals missing at the end of the test due to shell decomposition. Under some circumstances, more individuals may be present at the end of the test than at the start. This would most likely be due to the settlement of juvenile bivalves during the course of the test. This can easily be accounted for as new recruits should be smaller than the test bivalves. All recruits should be removed prior to determining survival and assessing effects end points. Only effects measurements from surviving bivalves should be used to calculate summary statistics. It is possible for shells to stick together due to mucilaginous material or sediment within the shells, prohibiting a precise

determination of death. Thus, all dead bivalves may not be identified until the tissue removal process when the shells are opened to reveal the internal tissues.

8.4.2 Growth is a sensitive sublethal effects end point that is easy to measure and is recommended for all field bioassays. It is generally more sensitive than mortality, and reductions in growth have been related to adverse effects on bivalve populations (**1**). As many growth end points as are practical should be measured for assessing growth in a weight-of-evidence approach. For example, it has been shown that shell growth and tissue growth are decoupled. Measuring only one of these end points could provide misleading results and lead to a spurious interpretation of environmental effects on growth (**97**,**98**). Growth end points include, but are not limited to, whole-animal wet weight, shell length, tissue weight, shell weight (**20**). Whole-animal wet-weights and shell lengths are nondestructive measurements and can be made multiple times over the course of the exposure period. At a minimum, whole-animal wet-weights and shell lengths should be measured at the beginning and end of the test. Since tissue weights and shell weights provide a different perspective on animal health and may be related to different stressors, they should also be measured at the beginning and end of the test (**20**). Because these measurements are destructive, beginning-of-test tissue and shell weights for bivalves to be deployed can be estimated from a subsample of the bivalves distributed to the individual cages. The bivalves used to estimate beginning-of-test tissue and shell weights should be within the same size range as those identified for deployment. Because the initial tissue and shell weights are based on a subsample of the test population, the change in these metrics over the test period is an indirect determination and has some uncertainty. However, tissue and shell weights can provide the most discriminating measurements under certain conditions, particularly when growth rates are low (**20**).

8.4.3 Although tissue dry weights are less variable than wet weights, drying the tissues has some limitations. *(1)* It is more time consuming to dry all the tissues and make the weight measurements. *(2)* In a combined bioaccumulation and biological effects test, the same wet tissues can be used for chemical analysis and wet-weight measurements (drying tissues may destroy organic chemicals, and limit their ability to predict bioaccumulation potential). *(3)* A wet-weight approach has been used successfully (**20**), and may provide better correlations with other growth metrics. Nevertheless, if additional testing clearly demonstrates an advantage to measuring dry weights, or if particular studies require more emphasis on the accuracy of tissue weight measurements, it would be relatively simple to alter the procedures accordingly.

8.5 *Reference Stations*—The use of one or more reference stations may be used for field bioassays with caged bivalves. It is the responsibility of the investigator to determine the need for reference station(s) based on the experimental design. It may be difficult or problematic to identify a true reference in the field because of the variability in field conditions and the influence of natural factors on site-specific conditions. If reference stations are used, the physical and natural factors (that is, temperature, salinity, dissolved oxygen, vegetation, and currents) at the reference station(s) should be as similar as possible to those conditions at the area under investigation. Multiple reference stations may help account for natural differences and variability among uncontaminated areas. It may be more useful to employ a gradient design with decreasing chemical gradients in bivalve tissue chemistry associated with changes in growth rate rather than comparing treatments to reference conditions or upstream versus downstream sites.

8.6 Natural population of bivalves could also be used for comparative purposes, but these comparisons should be made cautiously because there is evidence that caged bivalves can have different growth rates and different rates of accumulation than natural populations under certain conditions (**3**, **11**, **12**). It would be useful if growth rates of natural populations and caged bivalves were compared, if practical. Tissue concentrations at the onset of testing can also be used for comparison with tissue concentrations after exposures (**14**). This would be particularly relevant if reference stations cannot be used for bioaccumulation measurements.

8.7 *Statistical Design*—Field bioassays with caged bivalves can be used to support a variety of statistical designs. The experimental design is a function of the technical and environmental issues to be answered as well as the most appropriate statistical design for analyzing the data. The level of replication is a function of desired power and confidence. The following null hypotheses can be used to determine statistical differences in bivalve bioaccumulation and associated biological effects among stations as well as relationships between tissue chemistry, sediment or water chemistry, if measured, and measured effects:

(1) Null Hypothesis #1—There is no difference in bioaccumulation of chemicals of concern (as determined by tissue burdens) between test and reference station(s),

(2) Null Hypothesis #2—There is no difference in effects between test and reference station(s),

(3) Null Hypothesis #3—There is no relationship between effects end points in bivalves and tissues, water, or sediments containing chemicals of concern among stations.

(4) Null Hypothesis #4—There is no relationship between bioaccumulation and associated biological effects with distance from the suspected chemical source.

The preceding null hypotheses can also be used when it is appropriate to pool the stations to allow comparisons among sites. It may also be appropriate to apply these hypotheses to gradient designs in both horizontal and vertical planes in the water column or in bottom sediments.

8.8 *Replication*—The individual bivalves or the cage may be considered as the experimental unit. It is the investigator's responsibility to define the experimental unit and level of replication, which are appropriate for the study design. Additional guidance on statistical approaches can be found in other ASTM standards (E1847 and E1191). The distance between stations, or cages, is a function of the size of the area under investigation, the expected gradient or change in monitoring parameter(s), and the expected variability in conditions. Typically, stations can be placed 50 to 500 m apart. However, stations can be closer together, or further apart, as determined during development of the study design and hypotheses.

8.8.1 For the exposure assessment, a chemical replicate may be formed by combining the tissues of all living bivalves from one cage (see 9.3). Compositing may be necessary because, in most cases, individual bivalves do not contain sufficient tissue for chemical analysis. The cage can be used as a way to identify the bivalves to be combined for a chemical replicate. The number of chemical replicates prepared for each station depends on the level of replication desired for the bioaccumulation assessment. If statistical comparisons are desired, a minimum of three replicate tissue samples for each station is recommended. The number of bivalves required for each tissue sample is a function of the tissue mass requirements for the chemical analyses being performed and the tissue mass of the individual bivalves. The analytical laboratory performing the chemical analyses should be contacted to identify the amount of tissue required for each analysis. For example, if the analytical laboratory requires a minimum of 50 g wet tissue, and the average individual tissue weight is 0.5 g wet, then a minimum of 100 bivalves will be required for each chemical composite (that is, 100 bivalves per cage). With larger bivalves there may be sufficient tissue to conduct chemical analyses on individuals, particularly if only a few chemicals are being analyzed. This approach could improve the discriminating power of the assessment.

8.8.2 For the effects assessment, each individual bivalve may be considered a replicate, although concerns about individuals in the same experimental unit have been noted (Guide E1847). The bivalves within a predetermined size range are assigned to cages (see Section 11), and the cages are randomly assigned to stations. Independence among bivalves within each cage is assumed. In addition to the tissue chemistry biomass requirements, the minimum number of bivalves per cage should also consider the following with respect to effects end points: (*1*) the expected variance within cages, (*2*) the expected variance between cages, and (*3*) either the maximum acceptable width of the confidence interval on a point estimate or the minimum difference that is desired to be detectable using hypothesis testing.

8.9 *Statistical Analyses and Data Interpretation*—The calculating procedure(s) and interpretation of results should be appropriate to the experimental design. Procedures used to calculate results of these field bioassays can be divided into two general categories: those that test hypotheses regarding differences among stations, and those that establish relationships along suspected chemical gradients or between bioaccumulation and growth in the test organisms. No procedure should be used without careful consideration of (*1*) the advantages and disadvantages of various alternative procedures, and (*2*) appropriate preliminary tests such as those for outliers and heterogeneity. Preprocessing of data might be required to meet the assumptions of the analyses. All parameters measured at the end of the test (that is, whole-animal wet-weight, shell length, tissue weight, shell weight, and chemical concentrations in tissues) can be statistically analyzed. Summary statistics (for example, mean and standard deviation) can be calculated for each of these parameters on a station-by-station basis. The appropriate statistical test is a function of experimental design, hypotheses, and measurement end points. It is the investigator's responsibility to identify the appropriate statistical tests. In general, ANOVA and multiple comparison tests are used for hypothesis testing and comparison among stations. Linear regression analysis is generally used to establish relationships between bioaccumulation and growth end points along suspected chemical gradients and to establish relationships between bioaccumulation and growth. If statistical differences are found, a multiple range test can be used to determine which stations are different from the others. A textbook on statistical analyses of biological data can be referenced for appropriate tests and procedures (**99**, **100**, **101**).

8.9.1 Power analyses performed on data from caged bivalve studies in Alaska indicate that between 100 and 300 mussels per station are sufficient to detect differences in weight on the order of 0.2 and 0.1 g wet, respectively. An environmental significance, or likely adverse effect to the community, is expected when both a statistically significant difference is observed ($\alpha = 0.05$) and there is a 10 to 25 % absolute difference between the test and reference/control station(s) (**5**).

8.10 *Test Duration*—For most studies, bivalves should be exposed to site-specific conditions for a minimum of 30 days. An exposure period of less than 30 days is not generally recommended unless the chemicals of concern are low molecular weight organic compounds, such as some PAHs. Equilibrium for most other chemicals, such as metals and high molecular weight organic compounds, is generally achieved in marine and freshwater bivalves within a period of approximately 60 to 90 days (**3**, **11**,**12**, **17**, **62**, **102**, **103**, **104**, **105**, **106**, **107**). If both exposure and effects end points are being measured, it may be advantageous to continue the test for 60 to 90 days to facilitate chemical equilibrium and provide sufficient time to allow adverse effects to manifest themselves. Extending the exposure period may also increase the ability to detect statistically significant differences among effects end points. Although unlikely, it is possible that deployment of caged bivalves in or on bottom sediments may reduce concentrations of some chemicals. This may be particularly important in very small areas with restricted circulation where bivalves are removing chemicals from sediment. Consistent sediment conditions during the deployment period can be verified by sampling the sediment before and after deployment. It is the responsibility of the investigator to verify concentrations of chemicals in sediment before, during, and after deployment if this is an issue of concern.

9. Apparatus

9.1 *Facilities*—Sources of water and power and the ability to be protected from rain, snow, and wind can be of considerable help in sorting the animals at the beginning of the test, making the appropriate measurements, and removing tissues for chemical analysis at the end of the test. Preparations can be made outdoors, but inclement weather can interfere with making accurate measurements. The portable analytical balance is particularly sensitive to wind although some protection can be provided by a wind barrier around the entire area, such as a lean-to, or a smaller barrier such as a box to protect the balance. Making weight measurements aboard boats or floating piers is not recommended, unless the measuring devices are

specifically designed for use on unstable platforms. Length measurements made with calipers are not affected by the instability associated with boats or floating piers.

9.2 *Construction Materials*—Equipment such as cages, predator mesh, holding tubs, and ice chests, that contact the test water, sediment, and organisms should not contain substances that can be leached or dissolved by aqueous solutions in amounts that can adversely affect test organisms or be accumulated in their tissues. In addition, equipment that contacts test water, sediment, and organisms should be chosen to minimize sorption of test materials from water. Glass, Type 316 stainless steel, nylon, high-density polyethylene, polycarbonate, and fluorocarbon plastics should be used whenever possible to minimize dissolution, leaching, and sorption, except that stainless steel should not be used in saltwater. Concrete may be used for cage anchors and rigid plastics (that is, PVC) may be used for cage frames. Plastic Frames and mesh bags should be soaked before use, preferably in flowing fresh or seawater, for at least 24 h to remove water soluble and volatile chemicals. Mesh bags, tubes, or trays used to create the compartmentalized cages for holding the mussels during deployment should be made from high-density polyethylene, polycarbonate, or fluorocarbon plastic. Plastic cable ties have many applications during cage construction, such as separating the individual bivalves when mesh bags are used and attaching cages to deployment moorings and lines. Plastic cable ties should not contain metal stops as these can corrode and break upon exposure to water. This corrosion can result in detachment or addition of chemicals. Brass, copper, lead, cast iron pipe, galvanized metal, and natural rubber should not contact water, sediment, or test organisms before or during the test.

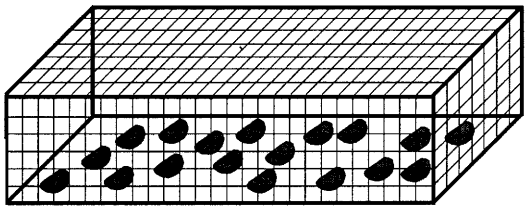
9.3 *Cages:*

9.3.1 The basic concept behind the cage design is to maximize water flow to the test animals. This is accomplished by using a mesh size large enough to maximize flow but small enough to contain the test animals. Cages with individual compartments are recommended for field studies with caged bivalves. The separation of individuals into individual compartments allows equal exposure to each bivalve (Fig. 1C). Compartmentalization facilitates tracking individuals throughout the test and eliminates the need to mark or notch individuals. Compartmentalization permits multiple growth measurements on individuals, ensures that an accurate record of measured end points can be maintained on individuals, and facilitates conducting tissue chemistry analysis on individuals if the individual bivalves contain sufficient biomass. Recording measurement data on an individual-by-individual basis increases the statistical power of the test. Each of the measurement end points, including tissue chemistry, can be paired during statistical analyses.

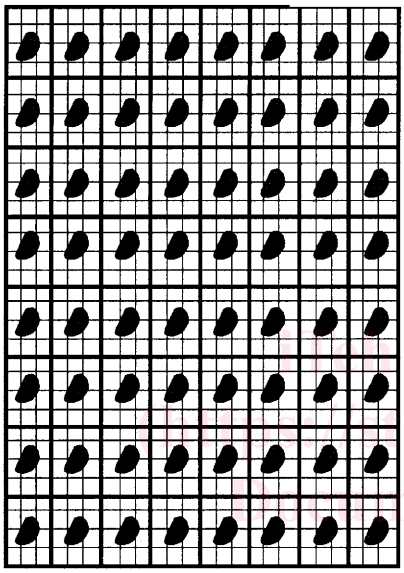
9.3.2 In its simplest form, in-situ field tests can be conducted with bivalves held in cages without compartments as shown in Fig. 1A (**108**). This approach is not recommended because it limits the ability to make multiple measurements on the same individuals throughout the course of the test. There are techniques for numbering individuals (**109, 110**), but this may be prohibitively time consuming if large numbers of animals are being caged. Numbering with different glues and epoxies could also introduce other potentially toxic chemicals. Cages can also be rigid with fixed compartments (Fig. 1B), as in plastic trays and wire baskets. Rigid cages with fixed compartments have been used in freshwater (**111, 112**) and marine (**3**) environments. Cages can also be a combination of flexible mesh material with compartments attached to a rigid frame (Fig. 1C), as with mesh bags attached to a PVC frame. This approach has been used in freshwater (**3**), estuarine (**64**), and marine habitats (**17**). The flexible mesh bags can also be attached to heavy plastic mesh that serves as a protective cage and an attachment point for the bags. The mesh bags used to hold the bivalves are created from tubular oyster culch netting similar to that used in bivalve aquaculture. The bivalves are separated within the mesh bags by placing a plastic cable tie or other restricting device between individuals. Different cage designs have also been tested to compare with the performance of natural bivalve populations. These include rigid cages with and without compartments, corrals that limit the movement of sediment-dwelling bivalves, and leashes where monofilament lines were glued to each bivalve shell (**113**).

9.3.3 The final dimensions of the deployment cages depend on the size of the individual test organisms and the number of organisms per cage. One advantage of using the flexible mesh bags and a PVC frame is that the size of the individual compartments and the overall cage size can be easily adjusted. Sufficient space should be provided in each compartment to allow test animals to open their valves and grow during the exposure period; the amount of space depends on the species used, the size of individuals at the start of the test, and expected increases in growth over the deployment period. For rigid cages, investigators should make the individual compartments large enough to accommodate expected growth during the test. A 6-in. (approximately 15-cm) diameter mesh material is recommended for smaller smoothed-shelled species like mussels and clams because there is less excess mesh at the point of constriction. For larger bivalves with rough shells and irregular shapes, such as oysters, it may be necessary to use a tubing of larger diameter. Because the flexible mesh is tubular in form, it is not necessary to adjust the width/height dimensions. The length of each compartment in the mesh bag (that is, the distance between constricting cable ties) should be large enough to accommodate valve opening and expected growth during the test. The mesh bag should be long enough to accommodate the desired number of bivalves per bag plus enough material to allow secure attachment to the PVC frame. Approximately 30 cm of mesh netting on either end of the bag is generally sufficient for attachment to a PVC frame constructed from ¾-in. (approximately 1.90-cm) material. The PVC frame should be approximately 5 cm longer than the space occupied by the bivalves positioned in the mesh bag. The width of the frame should be about 5 cm greater than the distance occupied by all mesh bags to be attached to the frame when laid side-by-side.

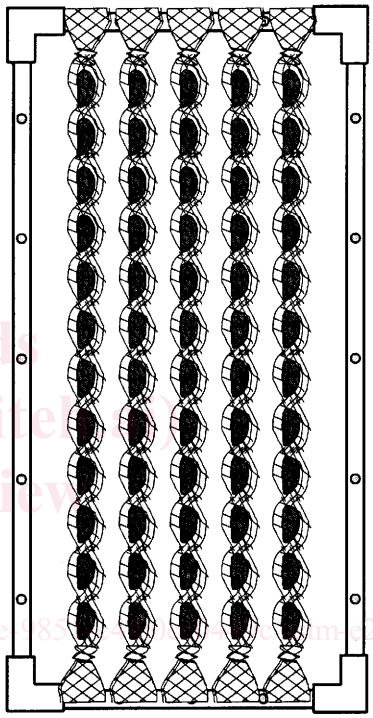
9.3.4 If PVC cages are to be deployed on top of sediments, pushed a short distance into the sediments, or positioned where neutral buoyancy is desired, the PVC pipe should be drilled approximately every 24 cm with a ¼-in. (approximately 0.64-mm) hole to allow water to enter the pipe and remove



A. Mesh basket (front view)



B. Compartmentalized mesh tray (top view)



C. PVC frame supporting mesh bags (side view)

FIG. 1 Possible Cage Types for In-situ Field Tests With Caged Bivalves

trapped air. The corners of the frame should not be drilled. Drilling the corners could weaken the overall structure of the frame. For water column deployments, flotation can either be increased or decreased depending on whether the PVC frames are drilled to allow a water ballast or left undrilled to add extra flotation.

9.4 *Cage Deployment Configuration*— The methods used to deploy cages and the type of mooring system depends on the experimental design identified for the specific media being assessed and substrates of opportunity. It may be useful to conduct a reconnaissance of the deployment area prior to setting out the cages to allow identification of potential deployment impediments and potential interference from the public. If floating or fixed piers are available in the assessment area, they could provide a potentially effective substrate for attaching bivalve cages. Figs. 2-5 provide various deployment configurations, and for simplicity, only rigid cages are shown. The PVC frames supporting bivalves in mesh bags can also be used in the same deployment schemes. Fig. 2 shows caged bivalves attached to floating (**3**) and fixed piers (**5**). Under most circumstances structures such as piers may not be available and open-water, nonstructural deployments should be used as shown in Fig. 3A (**3**) and Fig. 3B. A more direct assessment of bottom sediment is possible with fixed bottom deployments as shown in Fig. 4A (**3, 63**) and Fig. 4B (**5, 114**)). Caged bivalves can be placed directly on bottom sediment or on legs used to raise the cages above the sediments. Cages with legs can also be used to stabilize the unit and maintain position in high