DRAFT Standard Guide for

Conducting In-situ Field Bioassays with Marine, Estuarine and Freshwater Bivalves

1.0 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. Caged bivalves can also be used as part of an integrated exposure-dose-response assessment strategy (Salazar and Salazar 1998). In this approach, exposure is defined as the external concentration of chemicals in water and sediment, dose is defined as the internal concentration of chemicals in bivalve tissues, and response is defined as a biological effect like survival or growth that has been clearly linked with adverse effects on individuals or populations (Bayne et al. 1985, Widdows and Donkin 1992). The simultaneous, synoptic measurement of bioaccumulation and effects endpoints in the same organism permits the calculation of dose-response relationships (McCarty 1991, McCarty and Mackay 1993). If external chemical exposure is also measured, concentration-response relationships can also be calculated. Collectively, dose-response and concentration-response relationships provide a more integrated assessment strategy. The dose-response data can be associated with chemical exposure data from water or sediment.

Biochemical measurements, such as percent lipids, percent water, or percent carbohydrates that are commonly measured in bivalve tissues as part of chemical analyses, can serve as corroborative evidence of effects. However, other more specialized biochemical measurements commonly referred to as biomarkers are generally considered indicators of exposure and can also be used as corroborative evidence of effects (Black et al. 1996; Black and Belin 1998; Couillard et al. 1995a, b; Steinert and Pickwell 1988).

Bivalves are used 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 dose-response data which can be extrapolated to other species and trophic levels, (4) provide dose 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 (Bayne et al. 1985, Widdows and Donkin 1992). 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 or sediment, dose can be estimated with bioaccumulation of chemicals in bivalve tissues, and effects can be estimated with survival, growth, and other sublethal endpoints (Salazar and Salazar 1996). Although a number of assessments have been conducted to 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 (Salazar and Salazar 1991, 1995; Widdows and Donkin 1992). This guide is specifically designed to help minimize the variability in dose and response measurements by using a practical minimum size range and compartmentalized cages for multiple measurements on the same individuals.

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 endpoints. Since filter-feeding and deposit-feeding are the primary feeding strategies for bivalves, various pathways of exposure can be evaluated. Filter-feeding bivalves may be best suited to evaluate the bioavailability and associated effects of chemicals in the water column (i.e., dissolved and suspended particulates); deposit-feeding bivalves may be best suited to evaluate chemicals associated with sediments (Luoma 1995, Luoma and Fisher 1997). 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-meter of bottom sediment have also been used effectively in sediment assessments from depths of 10 to 650 m (Salazar and Salazar 1995, Forlin et al. 1996a,b). Caged bivalve studies have also been conducted in the intertidal zone (Salazar and Salazar 1997). These procedures 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 currently being developed to help interpret the environmental significance of accumulated chemicals (Di Toro et al. in review, McCarty 1991, McCarty and Mackay 1993, Jarvinen and Ankley 1999, Bridges et al. 1996). 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 these short- and long-term exposures can be reported in terms of absolute concentrations of chemicals in bivalve tissues (e.g., µg/g), amount (i.e., weight or mass) of chemical per animal (e.g., µg/animal), rate of uptake, or bioconcentration (from water) or bioaccumulation (from water and sediment) factors. Bioconcentration factors (BCF) or bioaccumulation factors (BAF) can only be calculated if the concentration of chemicals in water or sediment (i.e., the external chemical

exposure) are also measured as well (Meador et al. 1995, Salazar and Salazar 1996). A BAF is the ratio between the concentration of a chemical in bivalve tissues versus the concentration in the external environment. Since caged bivalves in field bioassays are exposed to multiple sources of chemicals and can accumulate chemicals from water, sediment, and food, this ratio represents a BAF and not a BCF. BCFs can be estimated by measuring the concentration of chemicals in filtered water samples to remove chemical exposure from sediment particles and food and then calculating the ratio of chemicals in water, sediment particles and food versus the chemicals in bivalve tissues. Toxicity results can be reported in terms of survival (Salazar and Salazar 1996, 1998), growth rate (Salazar and Salazar 1996, 1998), or reproductive effects (Bright 1991, Blaise et al. 1999) after exposure for some defined period. Field surveys can be designed to provide either a *qualitative* reconnaissance of the distribution of bioconcentration or toxicity in water or sediment or a *quantitative* statistical comparison of toxicity and bioconcentration among stations or relative to a reference or control station.

1.2 Other modifications of these procedures might by 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.

1.5 This guide is arranged as follows:

1.6 The values stated in the International System of Units (SI) (the Modernized Metric System) units are to be regarded as standard.

1.7 *This standard may involve hazardous materials, operations, and equipment - particularly during field operations in turbulent waters. This standard does not purport to address all of the safety problems 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.* Specific hazard statements are given in Section 7.

2.0 Referenced Documents

2.1 *ASTM Standards:*

D 1129 Terminology Relating to Water
D 3976 Practice for Preparation of Sediment Samples for Chemical Analysis
D 4447 Guide for Disposal of Laboratory Chemicals and Samples
E 380 Practice for Use of the International System of Units (SI) (the Modernized Metric System)
E 724 Guide for Conducting Static Acute Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Molluscs
E 729 Guide for conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians
E 943 Terminology Relating to Biological Effects and Environmental Fate
E 1022 Guide for Conducting Bioconcentration Tests with Fishes and Saltwater

Bivalve Mollusks
E 1023 Guide for Assessing the Hazard of a Material to Aquatic Organisms and Their Uses
E 1192 Guide for Conducting Acute Toxicity Tests on Aqueous Effluents with Fishes, Macroinvertebrates, and Amphibians
E 1342 Guide for Preservation by Freezing, Freeze-drying, and Low Temperature Maintenance of Bacteria, Fungi, Protista, Viruses, Genetic Elements, and Animal and Plant Tissues
E 1391 Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing
E 1525 Guide for Designing Biological Tests with Sediments
E 1688 Guide for Determination of the Bioaccumulation of Sediment-Associated Contaminants by Benthic Invertebrates

3.0 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 satisfied 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."

3.1.1 For definitions of other terms used in this guide, refer to Terminology D 1129, Guide E 729, Terminology E 943, and Guide E 1023. For an explanation of units and symbols, refer to Practice E 380.

3.2 *Descriptions of Terms Specific to This Standard:*

3.2.1 *automated biomonitoring* - automated, continuous monitors which employ a biological organism or material as a primary sensing element. The secondary sensing element, that from which the final measurement is obtained or derived, may be electrical, optical, or a combined electrical-optical-chemical system. It typically measures some physiological or behavioral function of the organism, such as valve closure in bivalves.

3.2.2 *bioaccumulation* - the net accumulation of a substance by an organism as a result of uptake from all environmental sources (includes water, sediment, and food).

3.2.3 *bioaccumulation factor* (BAF) - 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 (i.e., water, sediment, food), unless noted otherwise.

3.2.4 *bioassay* - an experiment that includes both an estimate of toxicity and an estimate of relative potency.

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

3.2.6 *bioconcentration* - the net accumulation of a substance by an aquatic organism as a result of uptake directly from aqueous solution.

3.2.7 *bioconcentration factor* (BCF) - a term describing the degree to which a chemical can be concentrated in the tissues of an organism in the aquatic environment as a result of exposure to water-borne chemical. At steady state during the uptake phase of a bioconcentration test, the BCF is the value which is equal to the concentration of a chemical in one or more tissues of the exposed aquatic organisms divided by the average exposure water concentration of the chemical in the test. (BCFs are usually calculated so that the volume of solution, for example 1 L, is about comparable to mass of tissue, for example, 1 kg, and the BCF is reported without units.) Historically, BCF has been measured on a wet-weight basis, although correcting for water content of tissue and reporting on a dry-weight basis would be more accurate.

3.2.8 *biomarker* - a biological measure (within organisms) of exposure to, effects of or susceptibility to, environmental stress using molecular, genetic, biochemical histological, or physiological techniques.

3.2.9 *biomonitoring* - use of living organisms as “sensors” in water or sediment quality surveillance to detect changes in an effluent or water body or to indicate whether aquatic life may be endangered.

3.2.10 *chemical concentration* - the ratio of the weight or volume of chemicals to the weight or volume of a test sample.

3.2.11 *chemical content* - mass of chemical per whole animal (e.g., 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.

3.2.12 *chemical fingerprinting* - the use of specific patterns in the ratios of chemicals accumulated in bivalve tissues to identify chemical sources; e.g. the ratio of PAH alkylated homologs to parent compounds.

3.2.13 *compartmentalized cage* - 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 provides maximum water flow around the individuals and provides even exposure to the test environment.

3.2.14 *depuration* - the loss of a substance from an organism as a result of any active or passive process.

3.2.15 *dose* - the chemical concentration measured in animal tissues that is used as a surrogate for the target dose at receptors of concern.

3.2.16 *growth dilution* - 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.

3.2.17 *reference station* - 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.

3.2.18 *replication* - for field bioassays with caged bivalves, there are two levels of replication. A chemical exposure replicate is formed by combining the tissues of all living bivalves from one cage (See Section 8.9.1). Each individual bivalve is considered a replicate for the effects assessment (See Section 8.9.2). The cages are not the sampling unit, they are a convenient way to arrange the individual bivalve replicates.

3.2.19 *scope for growth* - 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.

3.2.20 *shell length* - the distance from the tip of the umbo to the distal valve edge.

3.2.21 *site* - a geographical area with water and/or sediment 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 square kilometers. 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.

3.2.22 *steady state* - 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.

3.2.23 *station* - a specific sampling location or area within a site. The size of a station can vary from a single point with one cage (or sampling unit) to an area of approximately 10 x 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.

3.2.24 *tissue loss magnification* - 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 (ug/g) is increasing.

3.2.25 *uptake* - acquisition of a substance from the environment by an organism as a result of any active or passive process.

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

4.0 Summary of Guide

4.1 This guide describes procedures for exposing marine, estuarine, and freshwater bivalves to water, sediment, and/or water and sediment combined, 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 will be provided for selected species as examples and points of reference.

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

4.3 In practice, the two most commonly measured effects endpoints are survival and growth. Survival is the easiest effects endpoint to measure and provides an estimate of toxicity in short- or long-term exposures. The survival endpoint is relatively insensitive for many chemicals but can provide important corroborative effects information. Growth is a more sensitive sublethal endpoint. It can be determined 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 effects endpoint, but is more difficult to measure.

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 (McCarty 1991, McCarty and Mackay 1993) 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 (Applied Biomonitoring 1998, 1999a; Widdows et al. 1981).

4.5 It is highly recommended that the concentration of chemicals in water and sediment be determined, in addition to measuring the concentration of chemicals in tissues, to characterize total potential exposure; i.e., exposure from both water and sediment. These data can be used to establishing relationships between chemicals in various environmental compartments as a form of field validation.

5.0 Significance and Use

5.1 The test procedures in this guide are intended to conduct 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 6.0, Interferences). For this reason, it is highly recommended that supplementary measurements be made on those factors most likely to affect bioaccumulation and growth such as temperature, food (See Section 6.6), particulate matter, turbidity, salinity, dissolved oxygen, pH, and the velocity of water currents. These measurements are more easily made at shallower depths and become problematic at the greatest depths where caged bivalves have been deployed (i.e., 650 meters). Nevertheless, it is important to measure the chemicals in water or sediment, and this has been accomplished even at great depths with discrete samples (e.g. Forlin et al. 1996 a,b). In-situ temperature monitoring devices are available to measure temperatures for periods up to 1 year at depths up to 330 meters. Standardized procedures should be used to measure chemicals of concern in water and/or sediment. Other water or sediment parameters that can affect bioaccumulation and growth, such as temperature, salinity, dissolved oxygen, and pH, could also be measured as part of these field bioassays to provide site-specific information. These data can be used to help explain the measured exposure and effect endpoints. 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 Since the ultimate resources of concern are communities and it is often difficult or impossible to adequately assess community health or identify and test the most sensitive species, other assessment methods are needed. Bivalves are recommended

as a test species because they readily accumulate many chemicals and show sublethal effects associated with exposure to those chemicals (Widdows and Donkin 1992).

Although bivalve species might be considered insensitive because of their wide use as indicators of chemical bioavailability, sensitivity is related to the type of test, endpoints being measured, and duration of exposure (Widdows and Donkin 1992). In toxicity assessments in which survival is the endpoint, bivalves may not be the most sensitive species because of their ability to close their valves for short periods and avoid exposure (Doherty et al. 1987, Redpath and Davenport 1988, Trueman and Akberali 1981, Waller et al. 1994). However, recent studies comparing mortality endpoints between bivalves and other test species found bivalves to be equally (Keller and Zam 1991, Moore et al. 1993) or more sensitive (McKinney and Wade 1996, US EPA 1998) than the other species. Other studies comparing bivalve growth to mortality in other test species found bivalve growth to be more sensitive than mortality (Burgess and Morrison 1994, Van Dolah et al. 1998, Salazar and Salazar 1998), and growth endpoints in caged bivalves were more closely related to benthic community structure than any laboratory test endpoint (Hyland 1998).

Longer tests designed to monitor sublethal endpoints, such as growth, are recommended because bivalves and other organisms generally show increasing sensitivity with increasing exposure period. Sublethal endpoints measured in bivalves that have demonstrated high levels of sensitivity include growth (Salazar and Salazar 1996, 1998), reproduction (Bright 1991), DNA damage (Black et al. 1996; Black and Belin 1998), metallothioneins and other biochemical markers (Couillard et al. 1995 a,b).

There is a very large database in existence in the U.S. for field monitoring of bivalves such as the NOAA Status and Trends Program (O'Connor et al. 1994), the California Mussel Watch (Martin and Severeid 1984), and the California Toxics Monitoring Program, a freshwater monitoring program (State of California 1990). Similar field monitoring programs exist in other countries. Numerous laboratory studies throughout the world have examined bioaccumulation and biological effects in bivalves. The existing data bases correlating bioaccumulation and effects in bivalves with other species (Jarvinen and Ankley 1999, US ACOE 1999) makes it possible to use bivalves as surrogates 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. Freshwater bivalves are among the first taxa to disappear from benthic communities impacted by chemical pollution and have been shown to be more sensitive than several other major taxa (Naimo 1995). 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 for bioaccumulation and biological effects.

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, or a surrogate species, i.e., a species that can tolerate the environmental conditions but is not normally found in the area.

5.5 However, with the current invasion of unwanted exotic species such as zebra mussels (*Dreissena polymorpha*) and Asiatic clams (*Corbicula fluminea*) in freshwaters and clams (*Potamocorbula*) in marine waters, it is important to verify that these species are already found in the area of concern before considering their use in in-situ field bioassays. Zebra mussels, Asiatic clams, and *Potamocorbula* are good candidates for in-situ field bioassays and have been used successfully (e.g. Mersch and Pihan 1993, Mersch et al. 1996, Morrison et al. 1995, Reincke 1992), but these species should be used with caution. Planning for regional and local permits sometimes takes considerable periods of time and the planning process should therefore begin early. Other unwanted introductions include parasites or diseases from infected bivalves such as oysters (*Vibrio marinus*). Similarly, unwanted pests could be introduced from microscopic attached forms on the shells of transplanted bivalves such as the freshwater weed *Hydrilla hydrilla*. The other potential problem that is unique to freshwater bivalves is the introduction of glochidia stages that are parasitic on fish for a portion of their life cycle. Although this is common in areas where the freshwater bivalves naturally reside, those interested in preserving fish stocks and their habitats may consider this an unnecessary threat.

5.6 Bivalves generally utilize one of two primary modes of feeding: filter-feeding and 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/or 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.

5.7 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. Results of these exposure and effects field studies can be useful for studying biological availability of chemicals, and structure-activity relationships, among stations or groups of stations or sites. 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 (i.e., at the reference or control station(s)) or in the laboratory depends on the observations being made, test conditions required, and experimental logistics.

5.8 The minimum effects endpoints that should be measured are survival and growth. Survival may not be a very sensitive indicator of effects in bivalves (Salazar and Salazar 1996), but it is a very 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 an 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 endpoints. The live/dead status of the bivalves should be determined before proceeding with other effects measurements. However, 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.

5.9 Growth is a sensitive sublethal effects endpoint that is easy to measure and is recommended for all field bioassays. It is more sensitive than mortality, and reductions in growth have been related to adverse effects on bivalve populations (Bayne et al. 1985). As many growth endpoints as are practical should be measured to be used in a preponderance-of-evidence approach for assessing growth. For example, it has been shown that shell growth and tissue growth are decoupled, and measuring only one of these endpoints could give a spurious interpretation to environmental effects on growth (Hilbish 1986, Lewis and Cerrato 1997). Growth endpoints include, but are not limited to, whole-animal wet weight, shell length, tissue weight, shell weight (Salazar and Salazar 1998). Whole-animal wet-weights and shell lengths are non-destructive 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 (Salazar and Salazar 1998). These measurements are destructive; tissue and shell weights at the beginning of the test can be estimated from a sample the bivalves 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 slow (Salazar and Salazar 1998). The total number of bivalves required to initiate a field bioassay will be the sum of the number to be deployed plus the number to be used at the beginning of the test for the tissue and shell weight measurements. Between 100 and 300 animals should be sacrificed at the beginning of the test to provide the initial tissue and shell weight estimates and to provide sufficient tissue for chemical analysis.

5.10 The in-situ exposures described in this guide could be followed by laboratory measurements, such as scope for growth (Widdows and Donkin 1992), filtration rate (Bayne 1976), byssal thread production (Salazar and Kenis 1973, Martin et al. 1975, Van Winkle 1970), and biomarkers (Huggett et al. 1992, McCarthy and Shugart 1990).

5.11 Although tissue dry weights have less variability than wet weights, this approach has some limitations: 1) it is more time-consuming to dry all the tissues and make the weight measurements; 2) if it is a combined bioaccumulation and biological effects test, the same tissues can be used for chemical analysis (drying tissues has the potential to destroy organic chemicals) and wet-weight measurements; and 3) a wet-weight approach has been used successfully (Salazar and Salazar 1998). 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.

5.12 Results of the bivalve field bioassay can be used to predict bioaccumulation and biological effects likely to occur in aquatic organisms under comparable field conditions. While this may only represent first order approximations in some cases, equilibrium partitioning theory, quantitative structure activity relationships, and critical body residue theory suggest that tissue burdens of chemicals associated with adverse effects may be similar across species (McCarty 1991, McCarty and Mackay 1993).

5.13 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 trends can be assessed by placing caged bivalves in the area under investigation at scales commensurate with the desired level of discrimination . Horizontal and/or vertical gradients of chemical bioavailability in the water column can be delineated by placing caged bivalves along horizontal transects or at different depths in the water column. Similarly, horizontal gradients of chemical bioavailability in sediments can be delineated by placing caged bivalves along one or more horizontal transects at various distances from suspected sources of contamination. Temporal trends can be determined by repeating a given study design at specific time intervals, at the desired level of discrimination.

5.14 The relative bioavailability of chemicals from the various pathways of exposure

(i.e., aqueous, 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 (Herve et al. 1995; Peven et al. 1996; Prest et al. 1992, 1995; Shigenaka and Henry 1995). The bioaccumulation of chemicals and effects among different bivalve species can be compared and used to help explain the spatial variability of chemical contamination, particularly if the different species are placed in different locations (i.e., in the water column, on top of the sediments, within the sediments). This field assessment approach could be supplemented with laboratory studies designed to answer specific questions regarding dissolved versus particulate pathways of exposure.

5.15 Results of bivalve field bioassays might be an important consideration when assessing the hazards of materials to aquatic organisms (see Guide E 1023) or when deriving water or sediment quality guidelines for aquatic organisms (Salazar 1997, Environment Canada 1995). They might also be useful for determining tissue residue criteria. 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 (NOAA and EVS Consultants 1998, Matta et al. 1998).

6.0 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 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 with bioaccumulation and growth and data interpretation. Generally, chemicals are lost during spawning which may underestimate chemical bioavailability. 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 of the test environment could affect both bioaccumulation and biological effects. Water temperatures 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 1. 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.

Table 1. Temperature and salinity tolerance limits for selected bivalve species. Months when potential interferences from spawning may occur are also shown.

	Temperature Limits (°C)		Salinity Limits (‰)		
Species	**Lower**	**Upper**	**Lower**	**Upper**	**Potential Interferences from Spawning**
Macoma sp					
Mytilus edulis	0	29	4	33	January to September
Mytilus trossulus	0	29	4	33	
Crassostrea virginica	-2	36	0	33	May to October
Mya arenaria	0	27	10	35	April to August
Mercenaria mercenaria	-6	35	10	18	
Corbicula fluminea	2	25-27	0	15	
Rangia cuneata	8	32	0	20	February to September
Elliptio complanata					
Dreissena polymorpha					

6.5 Lack of acclimation to deployment water quality conditions could be an interference. If collection and deployment water quality conditions differ, particularly near the tolerance limits , it may be necessary to acclimate the test organisms gradually to the deployment conditions. This transition may be accomplished using serial water dilutions until the proper temperature, salinity, and pH are reached. Acclimation for temperature should proceed no faster than 3°C in 72 h (Guides E1022, E1688). Once acclimated, bivalves can be maintained at the deployment conditions for at least two days before the commencement of a study. However, 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.

6.6 Food supply is extremely important because it obviously 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 (i.e., beginning, middle, end of test). The measurements made (i.e., chlorophyll-a, particulate or total organic carbon, suspended solids) will depend on the feeding strategy of the test species.

6.7 Current speed is extremely 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 using an in-situ current meter or taken 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.

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

6.10 Depending on the environment under assessment, it is possible for the bivalve cages, including the external predator mesh 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 with a soft 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. Some traditional standard methods, such as those advocated by the US EPA, may not be entirely appropriate. 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 (Short and Babcock 1996, Short and Heintz 1997, Page et al. 1995, Bence and Burns 1995).

6.12 Natural variability in the concentrations of chemicals of concern 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 (Salazar and Salazar 1996, 1998). 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. However, sediments may also be highly variable on a small spatial scale (Brumbaugh et al. 1994).

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 endpoints. The processes involved could be better characterized and understood by using various biomarkers in addition to the bioaccumulation and effects endpoints.

7.0 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, particularly when washing equipment or placing hands into test water, effluents, sediment or cleaning solutions, laboratory coats, aprons, and glasses. 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 the sediments.

7.3 Use of ground fault systems is strongly recommended during measurements at

the beginning and end of the tests where electronic equipment such as portable computers are used to record data electronically to help prevent electrical shocks because water is a good conductor of electricity.

8.0 Experimental Design

8.1 Field bioassays with caged bivalves can be used to test for different water and sediment quality parameters, such as chemical bioavailability or bioaccumulation potential, and to assess the associated biological effects after exposure to those parameters. Field bioassays can be designed to provide either a *qualitative* reconnaissance or a *quantitative* assessment involving statistical comparisons of measured endpoints (i.e., chemical concentration in tissues and effects endpoints) 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 Decisions concerning such aspects of experimental design as station location, number of stations per site, number of cages per station, number of bivalves per cage should be based on the purpose of the test and the type of procedure used to calculate the results. Various experimental designs can be applied, with the most common used to:

- compare bivalve tissue chemistry and growth at one or more stations to reference, background, or pre-test conditions.
- compare bivalve tissue chemistry and growth among multiple stations to characterize patterns, trends, or gradients.

8.3 Comparisons can be made in both space and time. Spatial comparisons of parameters of concern can be made by distributing the caged bivalves along physical and chemical gradients. For example, station locations might be distributed along a known physical or chemical gradient in relation to the boundary of a disposal site (Koepp et al. 1987, Gentile et al. 1987, Paul et al. 1989, Phelps et al. 1989, Nelson and Hansen 1991), sewage outfall (Young 1982, Applied Biomonitoring 1999b), or effluent pipe (EVS Consultants 1996, 1997; Applied Biomonitoring 1998, 1999a) or at stations identified as containing elevated concentrations of chemicals in water or sediment as identified in a reconnaissance survey (NOAA and EVS Consultants 1998, Matta et al. 1998, Salazar et al. 1996). 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.

8.4 Caged bivalve bioassay surveys are often part of more comprehensive analyses of biological, chemical, geological, and hydrographic conditions. A useful summary of field sampling design is presented by Green (1984) and Green et al.

(1985). The significance of the statistical correlation can be increased (i.e., as *n* increases the critical r-value required for a given significance level decreases), and relative costs are reduced if additional samples are collected or additional *in-situ* monitors are deployed at the same time as the caged bivalves.

8.5 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, on a limited basis, chemicals and other additives could be pumped over the caged bivalves in much the same way as they are added to laboratory test tanks; chemicals could also be added as coated pellets or rods situated adjacent to the cages to administer controlled exposure. 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.

8.6 *Reference Stations*—The use of one or more reference stations is recommended for field bioassays with caged bivalves. However, 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. For field bioassays with caged bivalves, the reference station(s) should be in proximity to the area of investigation without overlapping the area of investigation. The physical and natural factors (i.e., temperature, salinity, dissolved oxygen, vegetation, currents) at the reference station(s) should be as similar as possible to those conditions at the area under investigation. If available, historical physical and chemical data may be used in identifying potential reference station(s). It is suggested that additional samples be collected and the physical and chemical conditions further assessed at the potential reference station(s) before making a final selection. There is a trend toward using multiple reference stations to account for natural differences and variability among uncontaminated areas that may be most similar to the area under investigation in other ways and would be applicable to caged bivalve studies. It may also be easier to interpret or 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.

8.7 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 (Salazar and Salazar 1996, Luoma 1995, Luoma and Fisher 1997). It would be useful if growth rates of natural populations and caged bivalves were compared, if practical.

8.8 *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 and/or water chemistry (if measured), and measured effects:

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

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

Null Hypothesis #3: There is no relationship between effects endpoints in bivalves and tissues, water, or sediments containing chemicals of concern among stations.

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 assess these gradients in both horizontal and vertical planes in the water column or in bottom sediments.

8.9 *Replication*—For field bioassays with caged bivalves, replication in the exposure assessment is different than replication in the effects assessment because of the common requirement to composite tissues to create sufficient mass for desired chemical analyses. The distance between stations, or replicated 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.

8.9.1 For the exposure assessment, a chemical replicate is formed by combining the tissues of all living bivalves from one cage (See Section 9.3). Compositing is necessary because, in most cases, individual bivalves do not contain sufficient tissue for chemical analysis. The cage provides a convenient way to identify the bivalves to be combined for a chemical replicate. The number of chemical replicates prepared for each station, and hence the number of cages deployed at 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 (i.e., 1 composited tissue sample prepared from each cage) 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. 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 (i.e., 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.

8.9.2 For the effects assessment, each individual bivalve is considered a replicate. The bivalves within a predetermined size range are assigned to cages (See Section 11), and the cages are randomly assigned to stations. The cages are not the sampling unit, they are a convenient way to arrange the individual bivalve replicates. The "treatment" is a location in the open environment with a free exchange and mixing of the water medium. It is assumed that the medium is independently and uniformly applied to all individual bivalve replicates within each treatment. Independence among bivalves within each cage is assumed. This is another advantage of using compartmentalized cages; i.e., to provide a more even exposure system for each individual. This is particularly helpful for some bivalves like marine mussels that tend to move into clumps in uncompartmentalized cages. In addition to the tissue chemistry biomass requirements, the minimum number of bivalves per cage should also consider the following: (a) the expected variance within cages, (b) the expected variance between cages, and (c) 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. As the number of cages (i.e.,experimental units) per station increases, the number of degrees of freedom increases, and therefore, the width of the confidence interval on a point estimate decreases, and the power of a significance test increases.

8.10 *Statistical Analyses and Data Interpretation*—All parameters measured at the end of the test (i.e., whole-animal wet-weight, shell length, tissue weight, shell weight, and chemical concentrations in tissues) can be statistically analyzed. Summary statistics (e.g., mean and standard deviation) can be calculated for each of these parameters on a station-by-station basis. All data to be used in hypothesis testing should be assessed for normality and homogeneity of variances. The null hypotheses should be assessed by conducting a t-test, an ANOVA (See Sections 8.9 and 8.10 for definitions of replicates), or their non-parametric counterparts as identified during the experimental design stage. 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 endpoints along suspected chemical gradients and to establish dose-response relationships between bioaccumulation and growth. If statistical differences are found, a multiple range test, or its non-parametric counterpart, can be used to determine which stations are different from the others. A text book on statistical analyses of biological data can be referenced for appropriate tests and procedures (Zar 1974, Gilbert 1987,

Krebs 1989).

8.10.1 Power analyses performed on data from caged bivalve studies in Alaska (EVS Consultants 1996, 1997) 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 percent absolute difference between the test and reference/control station(s) (Salazar and Salazar 1995; Applied Biomonitoring 1999a).

8.10.2 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 (a) the advantages and disadvantages of various alternative procedures, and (b) appropriate preliminary tests such as those for outliers and heterogeneity. Preprocessing of data might be required to meet the assumptions of the analyses.

8.11 *Test Duration*—The field bioassay begins when the caged bivalves are deployed at the test and reference stations. 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, particularly if metals are among the chemicals of concern. Chemical equilibrium for most chemicals is generally achieved in marine and freshwater bivalves within a period of approximately 60 to 90 days (Clark and Finley 1975; Pittinger et al. 1985; Meador et al. 1995; Salazar and Salazar 1996, 1997; Luoma 1995; Luoma and Fisher 1997; Naes et al. 1995a,b; Young 1982; Calambokidis et al.1979). However, if both exposure and effects endpoints are being measured, it may be advantageous to continue the test for 60 to 90 days to facilitate chemical equilibrium and allow adverse effects like growth an opportunity to manifest themselves. Extending the exposure period may also increase the ability to detect statistically significant differences among effects endpoints. If effects endpoints are not being measured and the only low molecular weight organic compounds, such as some PAHs, are of concern, the exposure period may be reduced substantially; e.g., hours or days (Salazar and Salazar 1997).

9.0 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 such as a lean-to. Making weight measurements aboard boats or floating piers is not recommended. Length measurements made with calipers are not affected by the instability associated with boats or floating piers.

9.2 *Construction Materials*—Equipment and facilities 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 add to the accumulation in their tissues. In addition, equipment and facilities that contact 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 and rigid plastics (i.e., PVC) may be used for weights and for frames, but they should be soaked, preferably in flowing fresh or seawater, for several days to a week or more before use. 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/or lines. Plastic cable ties should not contain metal stops as these can easily corrode and/or break upon exposure to aquatic conditions. Loss of the stop can result in detachment or equipment loss. 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*

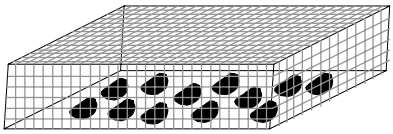
9.3.1 Cages with individual compartments are recommended for field studies with caged bivalves. The basic concept behind the deployment configuration is to maximize mesh size to maximize water flow to the test animals yet maintain a mesh size small enough to contain the test animals; the separation of individuals into individual compartments allows equal exposure to each bivalve. 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 endpoints 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 endpoints, 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 Figure 1A (Weber 1988). 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 (Dauble et al. 1985, Negus 1966;), but this may be prohibitively time

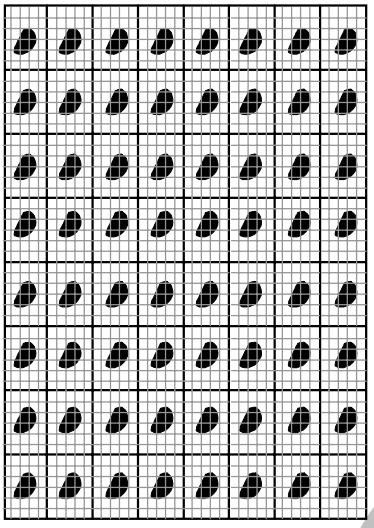
consuming if large numbers of animals are being caged. Numbering with different glues and epoxies could also introduce other unwanted variables. Cages can also be rigid with fixed compartments (Figure 1B), as in plastic trays and wire baskets, and have been used in freshwater (Foe and Knight 1985, 1987) and marine (Salazar and Salazar 1996) environments. Cages can also be a combination of flexible material with compartments attached to a rigid frame (Figures 1C), as with mesh bags attached to a PVC frame. This approach has been used in freshwater (Salazar et al. 1996, Applied Biomonitoring 1999b), estuarine (Matta et al. 1998), and marine habitats (EVS Consultants 1996, 1997; Salazar and Salazar 1997, Applied Biomonitoring 1998, 1999a). The mesh bags are created from mesh tubing similar to that used in bivalve aquaculture (i.e., oyster culch net). The mesh bags are used to hold the bivalves; the bivalves are separated within the mesh bags by placing a plastic cable tie, or other restricting device between individuals. The cable ties or other restricting devices should not be so tight that the mesh is constricted to the point that it does not allow the shell to open during respiration. If mesh bags are used, they should be securely fastened to the PVC frame with knots and/or plastic cable ties. Different cage designs have been 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 (Muncaster et al. 1990).

9.3.3 The final dimensions of the deployment cages depend on the size of the individual test organisms. Another advantage of using the flexible mesh bags and PVC pipe to create the cages is that the size of the individual compartments and the overall cage frame size can be easily adjusted. Sufficient space should be provided in each compartment to allow test animals to 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, the individual compartments should be at least twice the actual shell length, width, and height of the test animals. A 6" (ca. 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 (i.e., the distance between constricting cable ties) should approximate the expected end-of-test shell length plus 5 to 10 mm. The mesh bag should be long enough to accommodate the desired number of bivalves per bag plus sufficient 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 3/4" (ca. 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.

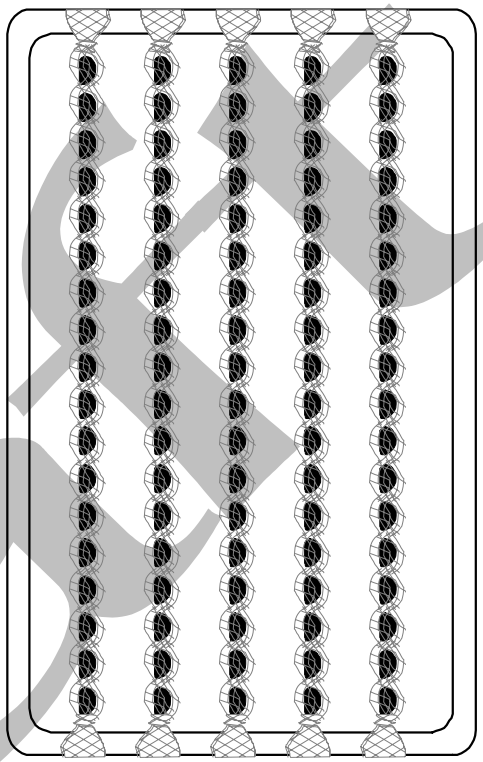
9.3.4 Cages should be constructed at least one week prior to initiation of the field study to allow sufficient time for soaking and leaching of volatile compounds associated with their construction. The appropriate primer and glue should be used with PVC . After construction, the cages should be soaked in clean fresh, estuarine, or seawater to leach the volatile compounds. 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 1/4" (ca. 0.64 mm) hole to allow water to enter the pipe and remove trapped air. The corners of the frame should not be drilled to eliminate the potential for weakening 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.



A. Mesh basket (front view)



B. Compartmentalized mesh tray (top view)

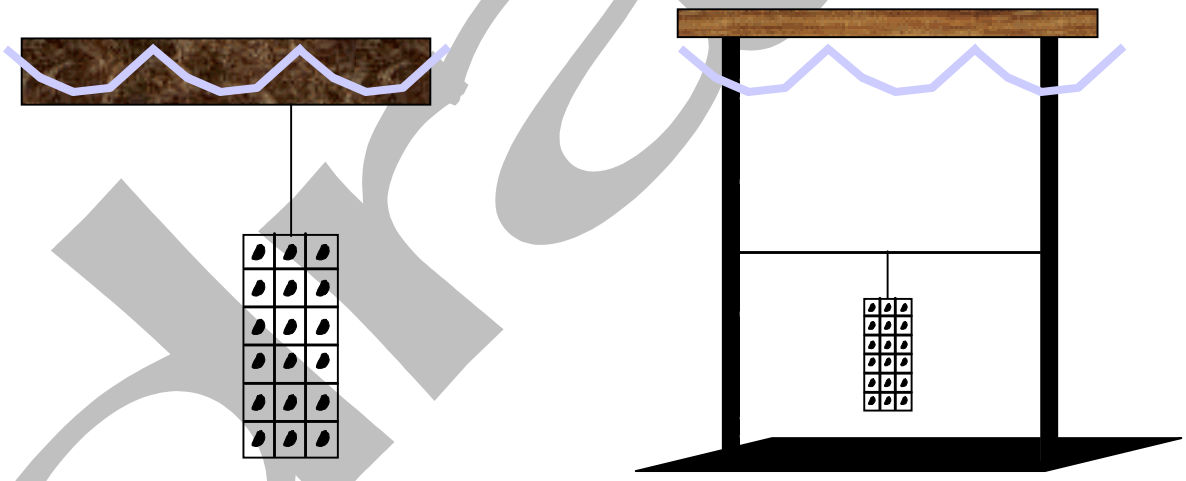


C. PVC frame supporting mesh bags (side view)

Figure 1. Possible cage types for in-situ field tests with bivalves.

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. If floating or fixed piers are available in the assessment area, they could provide a potentially effective substrate of opportunity for attaching bivalve cages. Figures 2 through 5 provide various deployment configurations. For simplicity, rigid cages are used in these examples. PVC frames supporting bivalves in mesh bags can also be used in the same deployment schemes. Figure 2 shows caged bivalves attached to floating (Salazar and Salazar 1996) and fixed piers (Salazar et al. 1995). Under most circumstances structures such as piers may not be available and open-water, non-structural

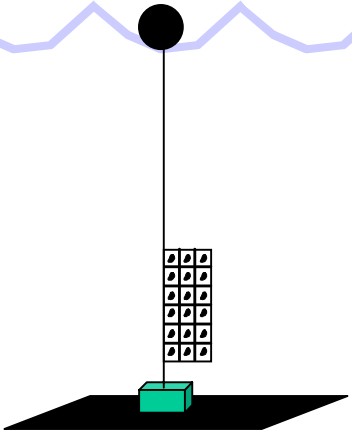
deployments should be used as shown in Figure 3A (Salazar and Salazar 1996) and Figure 3B (EVS Consultants 1996, 1997). Cages can be deployed to evaluate both water column and bottom sediments. This configuration also helps avoid potential contamination from the substrate such as piers, where creosote or other chemical could confound the results of the assessment. A more direct assessment of bottom sediment is possible with fixed bottom deployments as shown in Figures 4A (Salazar et al. 1996, NOAA and EVS Consultants 1998) and Figure 4B (Salazar and Salazar 1997, Salazar and Salazar, in prep). 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 energy areas such as the intertidal zone. The most sophisticated assessments include a gradient design with cages placed at multiple depths and distances from suspected sources, as shown in Figure 5 (Applied Biomonitoring 1998, 1999a). Each of the preceding deployment configurations uses rigid or flexible compartmentalized cages. Similar deployment configurations have been used with rigid and flexible uncompartmentalized cages (Stephenson et al. 1980a,b; Young et al. 1976; Widdows et al. 1981; Koepp et al. 1987). Placing cages along suspected chemical gradients in three dimensional space helps to identify not only potential sources of chemicals, but the relative contribution of chemicals in the water column and sediments based on chemical concentrations measured in bivalve tissues.



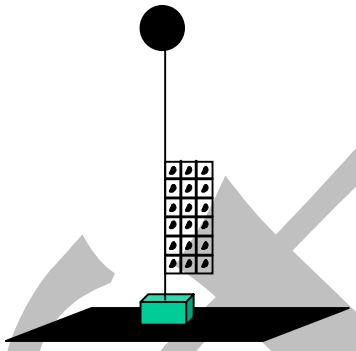
A. Compartmentalized cage suspended from floating pier.

B. Compartmentalized cage suspended from line attached between two pier pilings.

Figure 2. Pier deployments: floating or fixed.

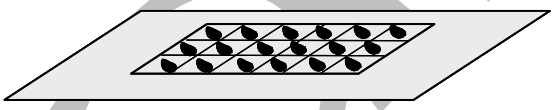


A. Compartmentalized cage attached to line with surface float and bottom anchor.

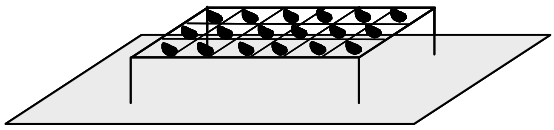


B. Compartmentalized cage attached to line with subsurface float and bottom anchor.

Figure 3. Open-water, Non-structural Deployments.



A. Compartmentalized cage placed directly on bottom sediment.



B. Compartmentalized cage on legs used to raise cage above bottom sediment or intertidal zone.

Figure 4. Fixed Bottom Deployments.

9.4.1 *Water Column Assessment*—To evaluate the bioavailability and potential effects of chemicals within the water column, the cages can be suspended from a fixed mooring (i.e., floating pier, piling, or other fixed structure), suspended within the water column by attaching it to a line that has an anchor or weight on one end and a surface- or subsurface buoy attached to the other end, or fixed in the water column by attaching legs to the cage and pushing the legs into the sediments to hold the cage in place. Depending on the species of bivalve used, bivalves in cages deployed directly on top of the sediments can be used to assess chemicals within the water column as well. Factors that should be considered during the deployment of cages for surface water assessments include change in tidal height (i.e., to ensure the cages are at the desired depth during both low and high tides), slope of bottom material (i.e., to ensure the cages do not slide down a steep slope during the exposure period), and boating activity and recreational activity in the vicinity of the cages (i.e., to avoid cages being removed by or tangled within propellers). A thorough reconnaissance of the deployment area should be conducted prior to setting out the cages to allow identification of potential deployment impediments and potential interference from the public. Subsurface floats should be appropriate to accommodate the weight of the line plus cages at the depth of deployment. This type of surface water monitoring has been used to evaluate PAHs (Applied Biomonitoring 1999a), dioxins and methylmercury (EVS Consultants 1996, 1997), tributyltin (Salazar and Salazar 1991, 1998), pulp mill effluents (Applied Biomonitoring 1998) in marine environments. Similar methods have been used to assess freshwater sites (Applied Biomonitoring 1999b, Muncaster et al. 1990).

Gradient Design: Multiple Depths and Distances

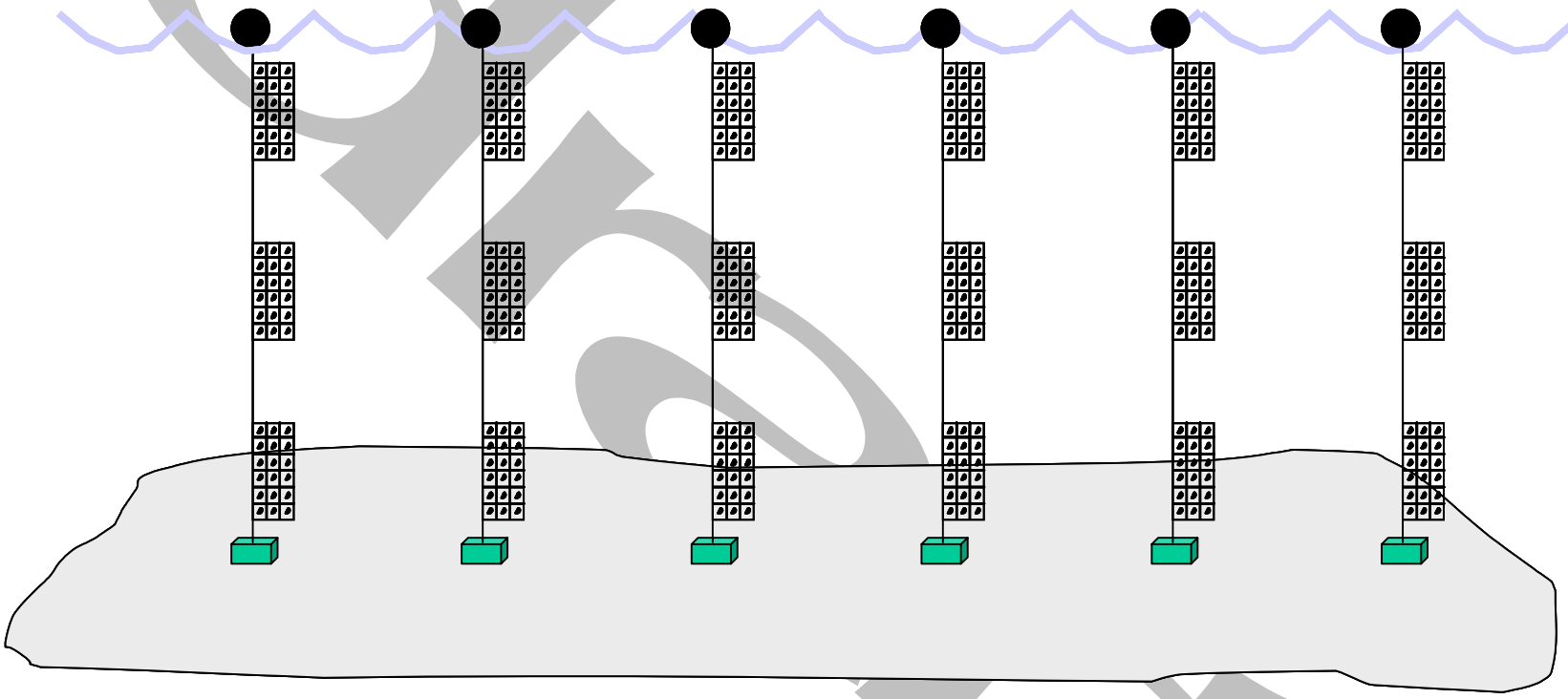


Figure 5. Compartmentalized cages attached to line at three depths near surface (with surface float and bottom anchor) and at multiple distances from a suspected contaminant source.

9.4.2 *Sediment Assessment*—One way to evaluate chemicals associated with surficial sediments is to position the cages directly on top of the sediments, and allowing the bivalves to bury themselves. This facilitates positioning the test species in the upper layers of sediment where most benthic organisms are commonly found and the sediments that are most commonly evaluated for chemical analysis (Guides E1391 and E1525) as well as use in laboratory bioaccumulation tests (Guide E1688) and laboratory toxicity tests (Guides E1367 and E1706). Allowing the bivalves to bury themselves is generally less stressful than forced burial, and allows the cages to be positioned without divers in water deeper than 2 m. Placing cages directly on top of the sediments and allowing the bivalves to reposition themselves has been used successfully in freshwater (Salazar et al. 1996, NOAA and EVS Consultants 1998) and marine (Matta et al. 1998) environments. For sediments composed primarily of sand and fine material, the bivalves gain exposure to the sediments as the sediments infiltrate the mesh material. Although confined within the mesh material, bivalves can work themselves into the sediment if sufficient room within their compartments is provided. In some cases it may be necessary to push the cage into the sediment to ensure exposure to chemicals associated with those sediments. This approach has been used before (Cain and Luoma 1985), although without compartmentalized cages. However, forcing the cages and bivalves into the sediments may induce severe stress resulting in high mortalities. Forcing the cages into the sediments, or digging out sediments to bury the cages can disturb the integrity of the sediments and alter the natural biogeochemical processes occurring. Anchors, rebar, or cages with legs can be used to ensure the cages remain at the desired position. Anchors or cement blocks can be attached to the sides of the cage with line. Rebar can be used as a weight and strapped to the side of the cage or it can be bent into a “U”, and pushed over the cage into the sediment to secure the cage in place. However, rebar should be coated with rubberized coatings or covered by plastic bags to prevent potential metal exposure. The cage can be constructed with legs (i.e., like a table) and the legs pushed into the sediment to secure the cage in position. Factors that should be considered when deploying cages on top of the sediments include presence of natural vegetation, type of substrate, boating traffic, and recreational activity. A thorough reconnaissance of the deployment area should be conducted prior to setting out the cages to allow identification of potential deployment impediments.

10.0 Test Organisms

10.1 *Species*—Many bivalve species have been used for assessing bioaccumulation and/or effects in marine, estuarine, and freshwater environments (Table 2). In most cases, the most widely used approach has been monitoring resident populations. The existing information on species life history, ability to accumulate chemicals of concern, and physiological effects can be applied to in-situ field bioassays. Species selection should be made carefully, considering conditions at the natural habitat of the species and factors such as physical conditions (i.e., temperature, salinity, dissolved oxygen, pH) at the area under investigation, presence/absence in area under evaluation, documented ability to accumulate chemical(s) of concern,

documented sensitivity to chemical(s) of concern, life history (i.e., spawning cycle, life-stage requirements, and threatened/endangered status), availability, and ease of handling in the field. Choice of test species may have to be modified to accommodate conditions at different test sites and the question that is being asked in the experiment. Ideally, species or genera with wide geographic distributions should be selected, so that test results can be compared among different sites and different test conditions. Depending on the particular question that is being asked, it may be most important to select species that are found, have been found, or could be found in the assessment area. Species used should be identified with an appropriate taxonomic key, and identifications should be verified by a taxonomic authority wherever possible. It may be necessary to conduct a pilot study to determine if the test animals can survive under the particular environmental conditions at a particular site. The US Fish and Wildlife Service (Koch, 1999) has identified the following mussels as possible surrogate species for in-situ field testing in lieu of threatened and endangered species in the Virginia area:

Rainbow mussel (*villosa iris*)
Snuffbox mussel (*Epioblasma triquetra*)
Pimpleback mussel (*Quadrula pustulosa*)
Tennessee pigtoe mussel (*Fusconaia barnesiana*)

Table 2. Partial list of bivalves used in monitoring marine and freshwater environments. Many species can be used in estuarine environments due to their ability to tolerate a wide range of salinities. "*" indicates species used in studies with compartmentalized cages; all other species have been deployed in non-compartmentalized cages.

Marine Species			
Mussels	Oysters	Clams	Scallops
Arca zebra *Mytilopsis sallei* *Mytilus edulis ** *Mytilus galloprovincialis** *Mytilus trossulus** *Mytilus californianus** *Perna perna* *Perna viridis **	*Crassostrea gigas* *Crassostrea angulata* *Crassostrea virginica** *Crassostrea rhizophorae* *Ostrea angasi* *Ostrea edulis* *Ostrea lurida* *Pteria sterna* *Saccostrea commercialis*	*Anadara granosa* *Cardium edule* *Chione stutuchburyi* *Circenita callipyga* *Gomphina melanaegis* *Macoma nasuta** *Macoma balthica* *Mercenaria mercenaria* *Meretrix lamarchi* *Mya arenaria* *Panopea abrupta* *Scrobicularia plana* *Spisula solidissima* *Venerupis japonica*	*Aequipecten opercularis* *Amusium japonicum* *Argopecten irradians* *Argopecten purpuratus* *Chlamys islandica* *Chlamys varia* *Crassodoma gigantea* *Hinnites multirugosus* *Lima hians* *Pecten maximus* *Placopecten magellanicus*

Freshwater Species			
Mussels		Clams/Cockles	
Actinonaias ligamentina *Actinonaias pectorosa* *Amblema plicata* *Amblema perplicata* *Anodonta cygnea* *Anodonta grandis* *Anodonta piscinalis* *Anodontites trapesialis* *Dreissena polymorpha** *Elliptio complanata** *Fusconia subrotunda* *Hydridella menziesi* *Lampsilis radiata* *Margaritifera falcata* *Medionidus conradicus* *Pyganodon grandis** *Quadrula quadrula* *Unio pictorum* *Villosa nebulosa* *Villosa vanuxemensis* *Westralunio carteri*		*Anadara trapezium* *Corbicula fluminea** *Corbicula manilensis* *Musculium transversum* *Rangia cuneata* *Sphaerium striatinum* *Sphaerium simile**	

10.2 *Commonly Used Taxa*—The environmental requirements and sensitivity of new bivalve test species should be established before they are widely used in field tests. The sensitivity and bioaccumulation potential of a prospective new test species could be compared with a more commonly used species to establish its relative utility. This is most commonly established in side-by-side transplants. Monitoring variations in water quality parameters and sediment characteristics (i.e., particle size, organic enrichment, sulfides) can help distinguish the effects of these parameters from the effects of chemical exposure. The taxa most commonly used in in-situ field bioassays are described below.

10.2.1 *Mytilus sp.* is an intertidal bivalve that has been successfully used in transplant studies since the late 1970's (Martin and Severeid 1984). The sensitivity of this species to salinities less than 10 g/kg limits its use to testing marine/estuarine areas, but the large data base that has been developed for the response of *Mytilus sp.* to a variety of habitats and chemicals establishes its usefulness as a test species as well as a reference species for comparing the sensitivity of other species. Species of the genus *Mytilus* are widely distributed on the west coasts of North America (Gosling 1992).

10.2.2 *Corbicula fluminea* is a freshwater clam that has been used extensively in field transplants and laboratory studies (Belanger et al. 1986a,b, 1987, 1990; Foe and Knight 1985, 1987). Numerous laboratory studies have also been conducted on this species and several symposia have been conducted on its biology and ecology.

10.2.3 *Elliptio complanata* and *Pyganodon grandis* (formerly *Anodonta grandis*) are

freshwater unionid mussels that have been used extensively for monitoring water column and sediment exposures in northern parts of the US and in Canada (Salazar et al. 1996, Malley et al. 1996, Couillard 1995a,b). Numerous laboratory studies have also been conducted on these species and several symposia have been conducted on its biology and ecology.

10.2.4 *Dreissena polymorpha*, a freshwater mussel, is a relative newcomer to bivalve field bioassays. However, the rapid proliferation of this nuisance species has rapidly increased the number of laboratory and field studies that have been conducted on bioaccumulation and growth (Mersch and Pihan 1993, Mersch et al. 1996, Morrison et al. 1995, Reincke 1992). It is particularly important to limit unwanted introductions of nuisance species into areas where they are not already found.

10.2.5 *Macoma* sp. is a marine/estuarine clam that has been used extensively in laboratory and field studies to assess bioaccumulation and growth (Luoma 1995, Luoma and Fisher 1997). It is commonly found in many environments on several coasts. It has been successfully transplanted in many different areas, and there are a number of supporting laboratory studies.

10.2.6 *Rangia cuneata* is an estuarine species that can tolerant freshwater conditions (US DOI 1985, Deleon et al. 1988, Demas and Demcheck 1987, Fucik et al. 1977, Lunsford and Blem 1995). It has been used in a number of field transplants as well as laboratory studies.

10.2.7 *Crassostrea sp.* has been used extensively in transplant studies in marine and estuarine studies (Kennedy et al. 1996, Waldock et al. 1996). Oysters survive and grow better than marine mussels at lower salinities and accumulate many chemicals such as tributyltin and copper by about a factor of two above mussels. The shells of *Crassostrea* and other oyster species are usually more difficult to measure because of the irregular shell shape and protrusions.

10.3 *Size and Age of Test Organisms*—All bivalves used in an in-situ field study should be from the same age class and as uniform as possible in size. Age class is more difficult to determine when obtaining specimens from the wild as opposed to culturing facilities because wild populations are a composite of several age classes, with some individuals growing at different rates. In most bivalve species, size is a function of age, so if individuals from the same age cohort are selected, they will be within a fairly uniform size range. Under adverse conditions (i.e., crowding, exposure to chemicals, or exposure to unfavorable natural factors), some individuals may demonstrate limited growth. The age and/or size class of the prospective species should be chosen so that sensitivity to chemicals or bioaccumulation potential is not affected by state of maturity or reproduction. It is recommended that specimens in a sub-adult age class be used because this age class has the greatest potential for growth of somatic tissue, reproductive tissue, and shell. If adult specimens are used, the study should not be conducted during active spawning. This prevents loss of

accumulated lipophilic chemicals.

10.3.1 Bivalves can be counted and sorted according to size to determine whether sufficient numbers have been collected in the appropriate size range. Shell length or whole-animal wet-weight should be used to select individuals for use in a field bioassay. Although whole-animal wet-weights provide a more accurate measurement of animal size, shell lengths are rapid to measure and are an easy way to sort individuals. Shell length should be determined with vernier calipers with a capacity to measure to 0.1 mm. Whole-animal wet-weights should be determined with an analytical balance with a capacity to measure to 0.01 g. The final size ranged used in the field study should be based on the maximum number of animals in the minimum size range. As a starting point, it is recommended that the size range used in the test be approximately 5 to 10% of the average maximum size of the species (i.e, for *Mytilus* a 5 to 7 mm size range is suggested as the maximum shell length this genus is about 70 mm). By minimizing the size range of individuals in the study, the variability in bioaccumulation and associated biological effects will also be minimized. The absolute size range used for a given species will depend on the size of the species and the availability of specimens. The decision to use juveniles, sub-adults, or adults depends on the experimental design. There is a tendency among many bivalve species for the smallest animals to grow at the greatest rates and accumulate the highest concentrations of chemicals. Various bivalve life stages have been proposed as part of an integrated environmental assessment (Granmo 1995). There are ASTM standard guides for a saltwater bivalve embryo test (Guide E724), bioconcentration tests with adult saltwater bivalves (Guide E1022), and bioaccumulation of sediment-associated chemicals (Guide E1688). A freshwater glochidia test is also being developed (Milam et al. 1998). While bivalve larvae are often assumed the most sensitive life stage, there is a growing body of evidence suggesting they are not necessarily more sensitive than adults (Beaumont et al. 1987, Butler et al. 1990, Luoma 1995, Salazar and Salazar, 1996, Widdows and Donkin 1992).

10.4 *Source*—Bivalves can be obtained from either natural populations or from culturing facilities. All individuals used in a field study should be from the same population, because different populations of the same species might have different sensitivities to or bioaccumulation capacities of the same chemical. Bivalves can be collected from wild populations in an uncontaminated (i.e., chemicals present at concentrations lower than at the area under investigation) area although it may be easier to purchase species field grow-out facilities or laboratory culture facilities. The advantages of using cultured or farmed animals is that the genetic and environmental history of the test animals is well known, and the assurances of being uncontaminated are greater. A sample of the prospective test bivalves should be measured for contamination in their tissues, particularly for the chemicals of concern. Collecting permits for field-collected bivalves might be required by some local and state agencies.

10.5 *Number of Specimens*—The number of bivalves collected should account for dead or dying individuals, individuals injured during handling, and the ability to minimize

the size range as much as practical. Therefore, the number collected should be equal to the total number required for deployment at each of the stations plus the number required for the baseline, beginning of test tissue chemistry measurements, plus approximately 20 to 50 percent.

10.6 *Collection*—Natural populations should be sampled with methods appropriate to their distribution. For intertidal marine species or freshwater species in shallow water, populations can be sampled by hand. For subtidal marine species or freshwater species in deeper water, SCUBA or a small biological dredge can be used for collection. Infaunal bivalves can be separated from sediment by gentle sieving. Sieves and containers used to collect and transport bivalves should be marked "live only" and should never be used for working with formalin or any other toxic materials. Water used for sieving should be at the same temperature and salinity as bottom water at the collection site. Some species of marine and freshwater mussels produce byssal threads as an attachment mechanism. Particular care should be used when removing mussels from substrates to which they have attached to avoid damage to the byssal glands, an internal organ that secrets the byssal threads. Damage or removal of the byssal gland can lead to mortality. A knife or scissors should be used to remove the mass of byssal threads visible on the outside of the mussel shell. This process will reduce the possibility of injury to the mussel. All epiphytic growth should be removed from the exterior of the bivalve shells. Plant or animal growth can usually be removed by hand; a soft brush or scraper may be required to remove barnacles, tube worms, or other tenacious organisms.

10.7 *Handling*—Test organisms should be handled as little as possible. When handling is necessary, it should be done carefully, gently, and quickly so that specimens are not unnecessarily stressed. Every effort should be made to maintain bivalves in well-aerated, flowing water for as long as possible between collection, sorting, and deployment procedures. The water used during the holding period(s) should be from a known source, free of chemical contamination. When transporting bivalves over great distances that require extended periods of time, it is better to keep them moist and cold than to maintain them in water that could become stagnant and low in dissolved oxygen. This is best accomplished by placing the specimens in an ice chest with wet ice on the bottom. Newspaper or paper toweling should be placed between the specimens and ice to prevent direct contact with the ice. Wet paper towels can also be placed over the specimens to provide additional moisture.

10.8 *Holding*—Test organisms should be transplanted as soon as possible and holding times minimized. If necessary, test organisms can be acclimated to water quality conditions at the deployment site, as identified in Section 6.5. In larger studies where it may be difficult to collect a sufficient number of specimens in one day, a laboratory or field site can be used as a holding facility while the remainder are collected.

10.8.1 Some infaunal bivalves may require holding in sediment until initiating the

test. Supplementary feeding for laboratory held specimens should not be necessary if holding is less than one week; specimens held in the field will continue to feed on natural sources of food.

10.9 *Animal Quality*—All bivalves used in a test need to be of acceptable quality. Before initiating a test, a qualified bivalve taxonomist should be consulted to ensure that the animals collected are all of the same species. This is particularly important with some freshwater bivalves where species differences may be extremely difficult to determine based on shell morphology. Even in the genus *Mytilus* there are subtle differences that may not be obvious, particularly in areas where the two species could be found side-by-side (Gardner 1996, Kenchington et al. 1995, Suchanek et al. submitted, McDonald and Koehn 1988, Bates and Innis 1995)

10.10 Although it is extremely difficult to determine healthy animals when the shell is closed, gaping animals that close very slowly or do not close at all should not be used. A putrefied smell emanating from the batch of test bivalves indicates one or more dead specimens. Dead specimens should be removed. Enough bivalves should be collected to provide approximately 20 to 50 percent more individuals than are required for the test to account for possible dead individuals or shell breakage during the test initiation process.

11.0 Field Procedures

11.1 *Test Initiation: Presort*—The first step of test initiation is to sort all bivalves into size groups, with each size group in its own small bucket. As indicated in Section 10.2, sorting can be based on either shell length or whole-animal wet-weight. Shell length should be determined with vernier calipers with a capacity to measure to 0.1 mm. Whole-animal wet-weights should be determined with an analytical balance with a capacity to measure to 0.01 g. Sorting allows the narrowest size range with the maximum number of specimens to be determined. All bivalves in the predetermined size class should be initially retained. After the presort, the number of bivalves per each size category should be determined. The test should be started using the minimum size range with a target range of 5 to 10 mm (i.e., 20 to 25 mm, 33 to 38 mm, 36 to 46 mm) that contains the maximum number of individuals.

11.1.1 During the presort, test organisms should also be kept out of water, but they should be kept cool and moist by providing shade (i.e., prevent exposure to direct sunlight) and/or ice packs. If air temperatures are excessively warm, it may be necessary to hold specimens in an ice chest with wet ice. Once the specimens are sorted into size groups, clean water can be added to the buckets. Since the density of animals has been reduced by this time and the shells have been cleaned of epiphytic growth, it is easier to maintain them in clean water. Nevertheless, water temperature should be checked regularly and can be maintained within a desired range by placing plastic bags filled with ice in each bucket. This is particularly critical when working in a laboratory or other facility where room temperatures can exceed temperatures at the

deployment site. A rapid rise in temperature of adult organisms which have ripe gametes could induce spawning, which would add another unwanted variable to the test. If in-situ monitors are used during the deployment period, they can also be used during the sorting and holding period to document temperatures during pre-deployment.

11.2 *Final Measurements and Distribution*— A randomized distribution process (Salazar and Salazar 1995) can be used to ensure an even distribution of bivalves across stations based on size. The even distribution of specimens among stations can be accomplished by filling the cages in a particular sequence. This applies to both compartmentalized mesh bags which are attached to PVC frames to form a cage, and to rigid compartmentalized trays. Once the final size range has been identified, the animals should be remeasured for shell length, weighed for the first time. All animals within a 1-mm grouping should be distributed before using animals from a larger size group. This process is repeated for the remaining size groups until the mesh bags or rigid cages are filled; each station will then have approximately the same number of individuals from the each size group.

11.2.1 *Set-up for Distribution*—If using mesh bags as described in Sections 9.3.2 and 9.3.3, identification tags, made of durable plastic or other inert material, should be attached to the mesh bags, which have been knotted 1 foot from the end. The identification tag should be secured with a plastic cable tie to the bag near the knot. A water indelible, permanent marker should be used to label tags with both the cage number and the bag number: for example, a label of **2–3**, indicates Cage 2, Bag #3. Color coded beads strung through a plastic cable tie and fastened to the mesh bag can also be used for purposes of identification. This is recommended as a backup in case the identification tag is lost during the study; the colored bead can be used to identify bag number on the PVC frame, which is also labeled with an indelible marker. If using an other type of compartmentalized cage, ensure that the cage is clearly identified, and each compartment within the cage is numbered.

11.2.2 Once all mesh bags have been labeled, the bags can be separated into groups according to bag number—with all the bags with a **–1** into one group, **–2** into another group, etc. The distribution process is based on bag number; all bags of a common number are filled at a given time. To initiate the distribution process, gather all bags that have a **"–1"** on the label; there should be one for each cage number. Attach these bags to the PVC distribution frame in cage number sequence (Figure 2). If rigid, compartmentalized cages are used, line up the cages so all cages can be filled simultaneously. Place the cage so the number 1 compartment is in the upper left position.

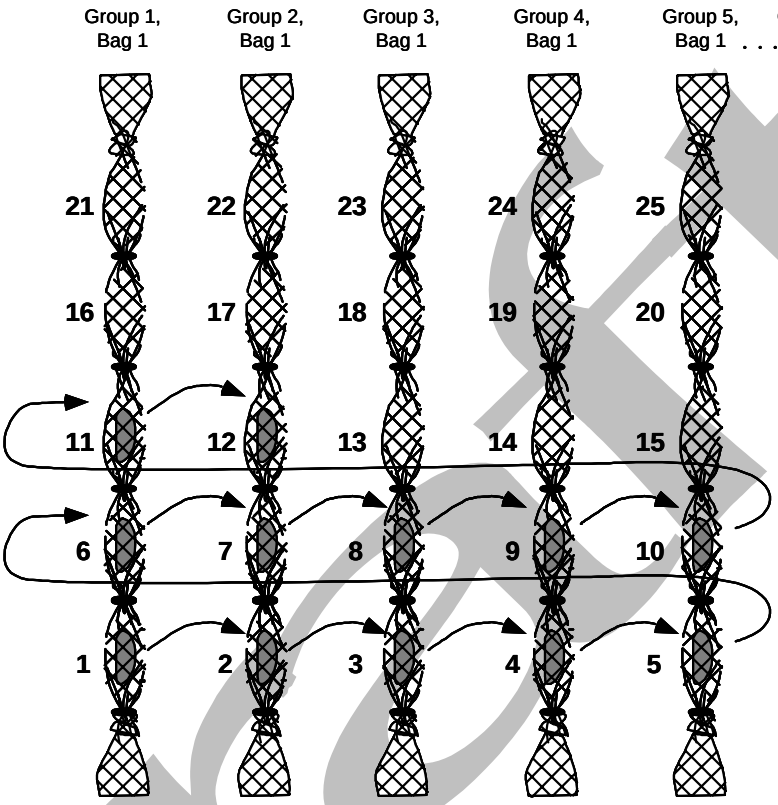
11.2.3 *Prepare Bivalves for Distribution*—Place bivalves from the smallest size group into a tray or tub containing water. The bivalves need to be maintained in water during the measurement and distribution process, and the water temperature should be maintained as close as possible (approximately ±5°C) to temperatures at the deployment site. In most temperate latitudes this can be accomplished by placing

plastic bags containing wet ice in the tub with the bivalves; the temperature can be monitored with a thermometer that remains in the tray or with in-situ monitors. It is essential that the bivalves be completely submerged and flat on the bottom prior to measurement. Bivalves with air between their valves either float on the surface or sit upright on the bottom. These bivalves should not be used because the air will bias the whole-animal wet-weights (water weighs more than air). Do not use individuals that float, are buoyant at one end, or do not close upon stimulation. Bivalves that float contain air which can be released prior to use. Floating individuals can be transferred to a separate container, where, if left undisturbed, they will likely purge the trapped air.

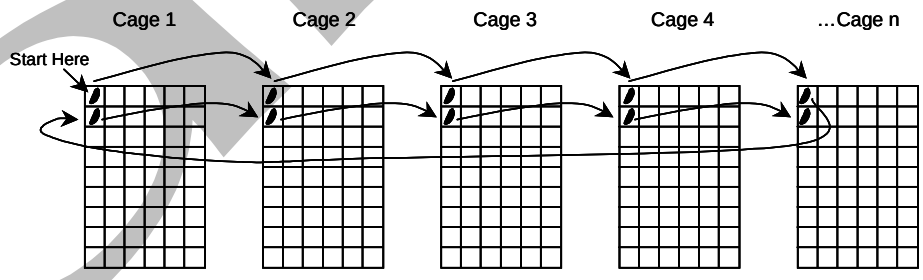
11.2.4 *Initiate Measuring and Distribution*—Under normal conditions (i.e., submerged, respiring), the bivalve shells will be slightly agape (approximately 1 mm). Most species will respond to light physical stimulation by tightly closing their shells. Bivalves that do not completely close their shells upon movement or light physical stimulation (i.e., agitation of the water around the bivalves or lightly tapping the shell) should be considered unhealthy and should not be used. In addition, bivalves that have broken shells or holes in their shells should not be used.

11.2.4.1 Initiate the distribution process by randomly selecting one specimen from the tray, making sure it is alive and shells are tightly closed. Using a paper towel, blot excess water from exterior of the individual and measure its shell length with a caliper and weight with an analytical balance. Record this data to a spreadsheet created for summarizing the data. The data can be recorded either electronically or manually. If the data are entered into an electronic spreadsheet, it is recommended that a manual record also be made as a backup in a case of computer failure. Once the specimen is measured and weighed, drop it into the first mesh bag (Figure 6A) on the distribution rack or into the first cell of the first compartmentalized tray (Figure 6B). For the mesh bags, affix a 10-cm cable tie around the mesh material above this individual. The cable tie should be adjusted so that it is tight enough to prevent the animal from passing through but the cable tie can be moved if necessary. The cable tie should not be fully tightened to allow the mesh to expand, if necessary, to accommodate movement and growth of the individual during the test (See Section 9.3.3). Randomly take another specimen from the tray and measure its shell length and weight, recording the data in the spreadsheet. Drop this individual into the second mesh bag on the distribution rack or into the first cell in the second compartmentalized tray. For the mesh bag, affix a cable tie. Repeat process until one individual has been placed into each mesh bag or each compartmentalized tray. Continue adding bivalves, one at a time to either the mesh bags or the compartmentalized trays (Figure 6A, B). For the mesh bags, completing one “row” before another row is started, until each mesh bag contains the desired number of individuals. When all of the bags on the distribution rack have been filled, remove the bags, knot or cable tie the open end, leaving a tail length of approximately 0.3 m. For the compartmentalized trays, securely affix a mesh cover such that the bivalves can not migrate from one compartment to another. Place the completed bags or compartmentalized trays into a cooler lined with ice and moist paper towels. Repeat the above process until all the mesh bags or compartmentalized trays

1 are filled.



A. Distribution process for mesh bags.



B. Distribution process for rigid compartmentalized cages.

Figure 4. Distribution process for caging bivalves.

11.2.5 If using an electronic spreadsheet, it can be customized to report minimum, maximum and average shell lengths and weights as they are entered. These values can be compared across cages to identify any individuals that outside the pre-determined size range and to check on a close, even distribution. To ensure statistical similarity among stations, an Analysis of Variance (ANOVA) can be run on both shell length and whole-animal wet-weight data. The data can be analyzed by cage and by station (i.e., pooled cages) if more than one cage is to be deployed at each of the test stations. If statistically differences are found, the test animals should be redistributed to eliminate this difference. For mesh bags, redistribution may require cutting a small opening in the mesh to replace the outlier. The opening can be secured with a plastic cable tie after inserting a replacement individual. For rigid compartmentalized trays, redistribution will require removing the mesh top.

11.2.6 *Mesh Bags Post-distribution Activities*—Once the bivalves have been distributed to all mesh bags, sort the bags by Cage Number. If the bags are not to be attached to the PVC cages until a later time, secure these bags together with a large plastic cable tie. Then, transport bivalves to a pre-identified holding area in the lab or field with uncontaminated flowing water. Hold in the water overnight, or until ready to attach bags to the PVC cages.

11.2.7 *Rigid Compartmentalized Trays Post-distribution Activities*—Once the bivalves have been distributed to all the compartmentalized trays, transport bivalves to a pre-identified holding area in the lab or field with uncontaminated flowing water. Hold in the water overnight, or until ready to deploy.

11.2.8 *Distributing the Baseline, Beginning-of-test Tissue Chemistry Individuals*—If mesh bags are used to hold the test specimens rather than a rigid, compartmentalized cage, it is recommended that the bivalves to be used for the baseline tissue chemistry measurements be separated during the distribution process. This can be accomplished by placing individuals identified for baseline tissue chemistry into compartmentalized trays. The use of compartmentalized trays for the tissue chemistry specimens eliminates the need to remove individuals from the mesh bags once the distribution process is completed. The individuals should be placed into the compartmentalized trays in order, i.e., with the first individual measured placed into compartment #1, the second into compartment #2, etc. At the end of the distribution process, the tissues should be removed for chemical analysis according to the procedures in Section 11.11.

11.3 *Attachment of Mesh Bags to PVC Frames*—A set of mesh bags attached to a PVC frame constitute a cage. Attach the mesh bags to the PVC frame by knotting the tail ends of the mesh directly to the PVC. If there is insufficient material to make a secure knot, use 6" (ca. 15 cm) cable ties to firmly attach mesh to the PVC frame. Allow a little slack in the mesh bag during attachment; the mesh should not be stretched so tightly that it restricts bivalve movement. If a temperature recording device is used, it should be attached to the frame at this time. If predators are of concern, wrap the PVC frame with a heavy duty plastic mesh, with a mesh size appropriate to

deter predators of concern (e.g., approximately 1 to 2.5 cm mesh size).

11.3.1 It may be necessary to adjust the space between bivalves in the mesh bags so that the mesh bags can be attached to the PVC frame without being too taught or too loose. During the attachment process, slide the cable ties as necessary to increase or decrease the over space between individuals without compromising the space available for each individual (i.e., do not decrease the space between animals so that there is insufficient space for them to open their valves during respiration).

11.4 *Deployment*—Deploy caged bivalves at stations as per the procedures in Section 9.4.

11.5 *Retrieval and End-of-Test Measurements*—At each station, after retrieving the caged bivalves remove any foreign material, if present, by dipping into the water. The exterior of the shells and the mesh bags should be wiped with paper towels if a sheen or other coating is present. If the bags of bivalves are removed from the PVC frames, a separate ice chest lined with wet ice and moist paper towels should be used to transport the bivalves to the processing site. Otherwise, cover the bivalve cages with a tarp or other protective covering during transportation.

11.5.1 Other ASTM guides (e.g., Guide E1688) traditionally recommend a 24-hour depuration period for bivalves tested under laboratory conditions. In the field where it may take many hours to retrieve the caged bivalves, it may not be convenient or necessary to require a depuration period that long. A number of studies have been conducted where the depuration period ranged from 0 to 24 hours. It may be necessary to use a depuration period in excess of 24 hours for sediment ingesting bivalves. This can be accomplished by suspending the caged bivalves at the reference station(s), or at another location known to be relatively free of contamination when compared to the test stations, for appropriate time periods.

11.5.2 At the processing site, bivalves from all bags constituting a cage or all bivalves in one compartmentalized tray should be processed together. It is critical to retain the order of bivalves during the end-of-test measurements. For mesh bags, it is recommended to remove the bivalves from the mesh bags and place them in a compartmentalized tray to facilitate end-of-test measurements. Starting with Bag–1 from a given cage. Starting at the end of the bag with the plastic label, remove the bivalves and place them, in sequential order, into a compartmentalized plastic tray. The tray should be been drilled with holes to allow water circulation. After all individuals from Bag—1 have been transferred to the compartmentalized tray, repeat the process with the remaining bags, maintaining bag sequence (i.e., process Bag—2, then Bag—3, etc.) If a dead or missing individual is encountered, leave its corresponding compartment in the tray empty; or place a marker (i.e., a bead or other device) in the compartment. This will ensure the order of individuals is maintained. Depending on the number of bivalves used, it may be necessary to use more than one compartmentalized tray to hold all the bivalves from a given cage. For bivalves deployed in rigid

compartmentalized trays, remove the mesh cover.

Approximately 5 minutes before initiating length and weight measurements, set the tray(s) into a tub or larger tray containing clean water. The water can be collected from the reference station, the holding facility, or another source of clean water. Upon placement of the compartmentalized tray containing bivalves into the tub of water, some individuals may "float, " indicating air trapped between their valves. It is essential that the bivalves do not float prior to making the weight measurement. As with beginning-of-test measurements, they should not contain any air between their valves as the presence of air will compromise the whole-animal weight measurements. It may be necessary to leave the bivalves undisturbed for approximately 5 to 10 minutes in the water in order for them to open their valves and releasing trapped air. Once the air has been released, the bivalves can be taken from the tray and measured.

11.5.3 Starting with the bivalve in the number 1 compartment, begin the shell length and weight measurements. Make sure the individual is alive and the shells are tightly closed before removing from the compartmentalized tray. The end-of-test measurement procedures are similar to those in Section 11.2.4: blot the excess water from the exterior of the individual's shells, measure shell length along the longest axes, and obtain a whole-animal, wet-weight measurement. Record the data, either electronically or manually onto spreadsheets. After the individual is measured, place it into a separate compartmentalized tray in the number 1 compartment. Do not put these compartmentalized trays in larger tubs containing water as it is not necessary to keep bivalves in water once the growth measurements are made. Continue the end-of-test measurements, measuring one individual at a time and retaining order of individuals. For dead or missing individuals, transfer the marker from one compartmentalized tray to the other and enter a "M" or "D" into the spreadsheet to indicate "missing" or "dead. After all bivalves in a cage are measured and weighed, begin the tissue removal process as described in Section 11.7.

11.6 *Analysis of Tissues for Background Contamination*—For in-situ field studies with an exposure component, the initial or background concentration of chemicals in tissues of the test organisms should be analyzed for the chemicals of concern. If possible, it is advantageous to perform these analyses prior to deployment at the test stations. Otherwise, use the tissues from individuals identified for baseline, beginning-of-test tissue chemistry (Section 11.2.8). However, exposure studies may be conducted without prior chemical analysis tissues if the bivalves are collected from an area that is monitored for chemical contamination and known to be free of toxicants, or if the tissues of those bivalves have been monitored regularly as in culture facilities. Bivalves collected from unmonitored areas should not be used in field bioassays unless their tissues are analyzed for baseline, beginning-of-test chemical concentrations. Bivalves collected from potentially contaminated areas should not be used in field bioassays unless the experimental design specifically requires use of that population. For in-situ studies that only assess effects, it is not necessary to analyze tissues for baseline, beginning-of-test chemical concentrations. However, as beginning- and end-

of-test tissue chemistry data can aid in the interpretation of effects data, these analyses are recommended.

11.7 *Collection and Preparation of Bivalve Tissues for Chemical Analysis*—All equipment used for tissue extraction/collection should be of corrosion resistant stainless steel, anodized aluminum, or borosilicate glass. If corrosion resistant stainless steel is unavailable, use regular stainless steel products, carefully checking before each use for signs of rust, pitting, or corrosion. Do not use if rust, pitting, or corrosion is evident. Before each use, all instruments (i.e., cutting board, shucking knife, weigh pans) should be cleaned according to the minimum following process: wash with a soap-free cleaning solution, hot tap water rinse, deionized water rinse. If deemed necessary by the investigator, an acetone, hexane or 95% ethanol rinse can follow the last water rinse. Allow the instruments to air dry to remove the potential for adding water to the tissues being collected.

11.7.1 During tissue collection, the order of bivalves must be maintained; tissue weights are recorded by individual and will be paired with whole-animal wet-weights and other size metrics. Use the compartmentalized trays for holding bivalves prior to shucking, and maintain order after tissues are removed.

11.7.2 If using cutting boards made of a material other than corrosion resistant stainless steel, anodized aluminum, or borosilicate glass, cutting boards should be covered with aluminum foil and cleaned as indicated in Section 11.7. If gloves are worn during the shucking process, ensure that they are powder free. Wash hands thoroughly with a soap-free cleaning solution, or replace gloves between processing a cage of bivalves.

11.7.3 Tissues are removed according to the following process. Start with the first individual in the compartmentalized tray, work with one individual at a time, and retain order of individuals. Place bivalve on the cutting board. Slide the knife blade between bivalve shells, severing posterior and anterior adductor muscles. Spread the shells apart to reveal soft tissues. If preparing tissues from clams or oysters, it may be necessary to notch the shell prior to inserting knife blade between their shells. Use a separate knife designated only for the purposes of shell notching. Be sure that none of this shell material is combined with the soft tissue material. Using tip of knife blade, separate tissue from shell, scraping as much of adductor muscle from points of attachment as possible. Holding tissues to shell with blade of shucking knife, tip shell to drain excess liquid (i.e., the seawater that was trapped between the shells during the measurement process).

11.7.4 After complete separation, keep the tissues in the shell and use the shell as a “holding dish” until tissue weights are made. If the two shell halves should become separated, place one half under the other. Place the shell(s) containing the separated tissue on a tray lined with aluminum foil, keeping bivalves in order and sufficient space between individuals to prevent the shell of one individual from touching the soft tissue

of another. Minimize exposure of bivalve tissue to hands, aluminum foil, and any other surface other than the interior of the specimen's original shell. Repeat process until all bivalves constituting a "chemical replicate" are shucked.

11.7.5 Place weigh pan on analytical balance; tare balance. Pick up first specimen, and using shucking knife blade tip, slide tissue onto weigh pan. Allow balance to stabilize. Record weight, either electronically or manually. Tare material on balance. Continue adding tissues, one at a time, recording weights of each individual. Tare after each addition. When all tissues of a "chemical replicate" have been weighed, transfer tissues from weigh pan to prepared sample jar by gently sliding them off the foil. Tightly cap sample jar, affix prepared label, and place the tissue samples in a cool location (i.e., ice chest containing gel packs, wet, or dry ice, or a freezer) depending on specifications of analytical laboratory performing the tissue chemistry analyses. If using aluminum foil to line surfaces, discard foil and clean all sampling equipment as described in Section 11.11 before proceeding to next sample.

11.8 *Quality Assurance/Quality Control Procedures*—Quality assurance is a program designed to provide accurate and precise results. Included are selection of proper technical methods, sample collection, selection of limits, and qualifications and training of personnel. Quality control are specific actions required to provide information for the quality assurance program. Included are standardization, calibration, replicates, and control and check samples suitable for statistical estimates of confidence of the data (Rand 1995).

11.8.1 To ensure that the measuring instruments (i.e., calipers and balance) are providing accurate readings, the instrument can be tested by measuring a standard weight or shell length. For the balance, one or more from a series of standard weights (i.e., 10, 50, 100 and 200 g) can be applied to the balance at intervals throughout the measurement process. For example, after every 100 measurements made on the balance, a standard 100 g weight can be applied to the balance. If the balance is off by more than 1 percent (1 g), the balance should be recalibrated; it may be necessary to reweigh some of the previous individuals depending on the degree of off-calibration. The measurement accuracy of the calipers can be checked by completely closing the device and recording the displayed measurement, which should be 0.000 mm. If the caliper displays a value greater than 0.5 mm, the unit should be re-zeroed. If available, the calipers can be used to measure a standard length. Depending on the degree of off-calibration, it may be necessary to remeasure some of the previous individuals.

11.8.2 Quality assurance/quality control (QA/QC) procedures of bivalve measurements should be used primarily in the development process as practitioners refine their methodology. Once the methods have become routine, it may only be necessary to use the QA/QC procedures on a yearly basis to confirm that no artifacts have inadvertently entered the methodology.

11.8.2.1 One suggested approach for QA/QC procedures for the bivalve

measurements is to remeasure and reweigh 5 percent of the animals These QA/QC measurements can be performed during the initial and end-of-test measurement processes. QA/QC shell length measurements and whole-animal wet-weight measurements outside ±5 percent of the original measurements may be considered unacceptable error measurements. The remeasuring of animal shell length and weight occurs throughout the measurement process as each series of bags is processed to ensure that all measurements are within the limits defined as acceptable. A 1.0 mm (±0.5 mm) variance in shell length and a 0.5 g (±0.25 g) variance in weight are suggested limits. If the results of the remeasurements fall outside of these limits, it is recommended that the previous batch of 100 individuals be remeasured. The hard copy data sheets can contain a separate row for the QA/QC measurements. To facilitate the process, it is suggested that these QA/QC measurements be made on the last "row" of bivalves to be entered into a series of bags.

11.9 *Sample Containers, Handling, and Preservation*—Precleaned sample containers should be purchased from a supplier or provided by the analytical laboratories. Each jar should be sealed, affixed with a completed label, assigned a unique tag number, and stored under appropriate conditions. Sample labels should be made of self-adhering, waterproof material; an indelible pen should be used to fill out each label. Each sample label should contain the project number, sample identification, preservation technique, analyses, date and time of collection, and initials of the person(s) preparing the sample. A completed sample label should be affixed to each sample container. In addition, a unique numbered tag can be affixed to each sample container. Chain-of-custody forms and tamper-proof tape can be used for projects that are litigation sensitive. The preservation of tissue samples is a function of chemical analytes and methods used by the analytical laboratory. The analytical laboratory should provide guidance on proper handling and preservation of tissue samples. For most analyses, samples should be protected from light and refrigerated at 4°C (±2°C) from the time of receipt until they are extracted and analyzed. If other priorities interfere with these requirements, the samples can be frozen at -20°C (Guide E1688).

12.0 Ancillary Measurements

12.1 *Temperature*—Marine and freshwater species should be selected to match the site-specific temperatures in the area of concern. Ideally, if species are naturally found in the area or have been found in the area in the past, it is a good indication that temperature tolerances are appropriate. Since temperature could influence bioaccumulation and growth (Bayne 1976, Widdows and Donkin 1992), it is important to monitor temperature during the course of the test using *in-situ* temperature monitors.

12.2 *Food* —As with temperature, if indigenous populations of the bivalves of choice are found in the area of concern, it is a good indication that there is adequate food to support caged bivalves in the area. Since food could also influence bioaccumulation and growth (Bayne 1976; Widdows and Donkin 1992), it may be helpful to measure parameters such as chlorophyll a, particulate or total organic carbon

and suspended solids during the course of the test.

13.0 Acceptability of Test

13.1 An acceptable test should meet both survival and growth criteria because the data are intended for different purposes. Survival is a generic indication of overall health and test acceptability. Growth may be a more sensitive indicator of health than survival, and tissue weights should be monitored to demonstrate meaningful bioaccumulation potential. Mean survival at the reference stations(s) should be $\geq$45 percent to have confidence in the bioaccumulation and effects results.

13.2 There should not be significant loss in either end-of-test tissue weights or whole-animal wet-weights when compared to measurements made at the beginning of the test, particularly at the reference station(s). If tissue weights decrease by more than 20 percent, this could provide valuable effects information. However, this loss in tissue weight could be accompanied by loss in chemicals and represents a biased estimate of potential chemical bioavailability. Therefore, results are considered unacceptable for interpreting the environmental significance of bioaccumulation if (1) the end-of-test tissue weights are more than 20 percent lower than the beginning-of-test estimates, **and** (2) the end-of-test tissue weights are significantly less ($\alpha = 0.05$) than the beginning-of-test estimates.

14.0 Report

14.1 A record of the results of an acceptable caged bivalve exposure and effects test should include the following information:

14.1.1 Names of test and investigator(s), name and location of laboratory, and dates of initiation and termination of test;

14.1.2 Source of test animals, scientific name and how verified, initial whole-animal wet-weights, shell lengths, and estimates of tissue weights as well as end-of-test percent survival, whole animal wet-weights, shell lengths, and estimates of tissue weights. Means, ranges, and standard deviations of all measurements.

14.1.3 Description of the experimental design and cages, including any attached instrumentation and predator deterring devices, water depth and depth of cages at time of deployment, the number of animals per station, station coordinates, and any other outstanding features of the area to assist in station-finding.

14.1.4 Averages and ranges of the acclimation temperature during the measurement and distribution process as well as the time spent out of water while in transit to the measurement location at the beginning of the test and while in transit to the deployment locations at the beginning and end of the test.

14.1.5 Reproductive state of the test animals including degree of gonad development. Note whether bivalves spawned during the beginning-of-test or end-of-test measurements.

14.1.6 A table of data on concentrations of chemicals (including percent lipids and m water in tissues) in water, sediment, and tissues in sufficient detail to allow independent statistical analyses. Table should also include analytical methods and laboratory qualifiers.

14.1.7 A table of survival, effects, and tissue chemistry data (including percent moisture content of the tissues) in sufficient detail to allow independent statistical analyses.

14.1.8 Anything unusual about the test, any deviation from these procedures, and any other relevant information.

14.1.9 Published reports should contain enough information to clearly identify the methodology used and the quality of the results.

15.0 Keywords

field bioassay *in-situ* exposure effects bivalve bioaccumulation growth

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