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Designation: E1022 – 22

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

This standard is issued under the fixed designation E1022; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

## 1. Scope

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

1.2 Other modifications of these procedures might be justified by special needs or circumstances. Although using appropriate procedures is more important than following prescribed procedures, the results of tests conducted using unusual procedures are not likely to be comparable to those of many other tests. The comparison of results obtained using modified and unmodified versions of these procedures might provide useful information concerning new concepts and procedures for conducting bioconcentration tests.

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

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

1.5 This guide is arranged as follows:


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[1] This guide is under the jurisdiction of ASTM Committee E50 on Environmental Assessment, Risk Management and Corrective Action and is the direct responsibility of Subcommittee E50.47 on Biological Effects and Environmental Fate.

Current edition approved April 1, 2022. Published June 2022. Originally approved in 1984. Last previous edition approved in 2013 as E1022 – 94(2013) which was withdrawn January 2022 and reinstated in April 2022. DOI: 10.1520/E1022-22.

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

1.7 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*Specific precautionary statements are given in Section 7.*This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

1.8 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

## 2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1129 Terminology Relating to Water
D1193 Specification for Reagent Water
D4447 Guide for Disposal of Laboratory Chemicals and Samples
E729 Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians
E943 Terminology Relating to Biological Effects and Environmental Fate
E1023 Guide for Assessing the Hazard of a Material to Aquatic Organisms and Their Uses
E1191 Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids
E1192 Guide for Conducting Acute Toxicity Tests on Aqueous Ambient Samples and Effluents with Fishes, Macroinvertebrates, and Amphibians
E1193 Guide for Conducting *Daphnia magna* Life-Cycle Toxicity Tests
E1241 Guide for Conducting Early Life-Stage Toxicity Tests with Fishes
E1295 Guide for Conducting Three-Brood, Renewal Toxicity Tests with *Ceriodaphnia dubia*
E1688 Guide for Determination of the Bioaccumulation of Sediment-Associated Contaminants by Benthic Invertebrates
E1706 Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates
E1733 Guide for Use of Lighting in Laboratory Testing
E1847 Practice for Statistical Analysis of Toxicity Tests Conducted Under ASTM Guidelines (Withdrawn 2022)[3]
E2122 Guide for Conducting In-situ Field Bioassays With Caged Bivalves
E2455 Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels
SI 10 IEEE/ASTM SI 10 Standard for Use of the International System of Units (SI) (the Modernized Metric System)

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## 3. Terminology

3.1 *Definitions:*

3.1.1 The words "must," "should," "may," "can," and "might" have very specific meanings in this guide. "Must" is used to express an absolute requirement, that is, to state that the test ought to be designed to satisfy the specified condition, unless the purpose of the test requires a different design. "Must" is used only in connection with factors that relate directly to the acceptability of the test (see 13.1). "Should" is used to state that the specified condition is recommended and ought to be met if possible. Although violation of one "should" is rarely a serious matter, violation of several will often render the results questionable. Terms such as "is desirable" are used in connection with less important factors." May" is used to mean "is (are) allowed to," "can" is used to mean "is (are) able to," and "might" is used to mean "could possibly." Thus the classic distinction between" may" and "can" is preserved, and "might" is never used as a synonym for either "may" or "can."

3.1.2 For definitions of other terms used in this guide, refer to Terminologies D1129 and E943 and Guide E729. For an explanation of units and symbols, refer to Standard SI 10.

3.2 *Definitions of Terms Specific to This Standard:*

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

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

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

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

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

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

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

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

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

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

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

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

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

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

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

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

## 4. Summary of Guide

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

## 5. Significance and Use

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

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

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

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

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

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

However, sorption might increase bioaccumulation by aquatic species that ingest particulate matter (**2**), or food may be a more important source of residues in fish than water per se for stable neutral organic chemicals that have a Log $K_{ow}$ between 4 and 6 (**3**).

5.6 Results of bioconcentration tests can be used to compare the propensity of different materials to be accumulated. Nonionizable organic chemicals can also be ranked for bioconcentration using correlations that have been reported between steady-state BCFs and physical–chemical properties, such as the octanol–water partition coefficient and solubility in water (**4**). However, when such predictions are impossible, exceed the demonstrated limits of the correlation, or might be otherwise questionable (**1**, **5**), a bioconcentration test may be necessary.

5.7 Results of bioconcentration tests can also be used to compare the abilities of different species to accumulate materials. At steady-state the concentration of a nonionizable organic chemical in individual organisms, and in various tissues within an organism, will probably be related to the concentration of lipids in the organisms and tissues (**6**).

5.8 Results of bioconcentration tests might be an important consideration when assessing hazard (see Guide E1023) or deriving water-quality criteria because consumer animals might be adversely affected by ingesting aquatic organisms that contain toxic materials. However, assessment of hazard to consumer organisms must take into account not only the quantity of material accumulated in tissues of aquatic organisms, but also the toxicity of the material to the consumer. Further, humans eat only certain portions of most aquatic organisms, whereas other predators often consume additional tissues.

5.9 Bioconcentration tests might be useful for studying structure–activity relationships between test materials, biological availability, metabolism of materials in aquatic organisms, and effects of various environmental factors on results of such tests.

5.10 Uptake and depuration rate constants might be useful for predicting environmental fate using compartmental models (**7**).

5.11 Tissues collected after organism exposures based on other testing methods might also be considered for determining bioconcentration according to this guide (see Guides E1241, E1688, E2122, E2455, and Test Method E1706).

## 6. Apparatus

6.1 *Facilities*—Flow-through tanks should be available for culturing, holding, and acclimating test organisms. An elevated dilution-water tank or headbox, or both, might be desirable so dilution water can be gravity-fed into holding and acclimation tanks and the metering system (see 6.3), which prepares the test solutions and delivers them to the test chambers. Strainers and air traps should be included in the water supply system. The test chambers should be in a constant temperature area or recirculating water bath. Headboxes and holding, acclimation, and dilution-water tanks should be equipped for temperature control and aeration (see 8.3). Air used for aeration should be free of fumes, oil, and water; filters to remove oil and water are desirable. Filtration of air through a 0.22-µm bacterial filter might be desirable. During holding, acclimation, and testing, organisms should be shielded from disturbances with curtains or partitions to prevent unnecessary stress. The test facility should be well ventilated and free of fumes. To further reduce the possibility of the contamination of test organisms by test materials and other substances, especially volatile ones, culture, holding, and acclimation tanks should not be in a room in which bioconcentration or toxicity tests are conducted, stock solutions or test solutions are prepared, or equipment is cleaned.

6.1.1 A 16-h light and 8-h dark photoperiod should be provided to reflect natural cycles as is done for other invertebrate toxicity tests (see Guide E1192). This can be controlled by a timing device. Alternatively, a 12–12 or 14–10 photoperiod might be desirable to delay maturation of some species. A 15 to 30-min transition period (**8**) when the lights go on might be desirable to reduce the possibility of organisms being stressed by instantaneous illumination; a transition period when the lights go off may also be desirable (see Guide E1733).

6.2 *Construction Materials*—Equipment and facilities that might contact stock solutions, test solutions, or any water into which test organisms will be placed should not contain substances that can be leached or dissolved by aqueous solutions in amounts that can adversely affect aquatic organisms. In addition, equipment and facilities that contact stock solutions or test solutions should be chosen to minimize the sorption of test materials from water. Glass, Type 316 stainless steel, nylon, and fluorocarbon plastics should be used whenever possible to minimize leaching, dilution, and sorption, except that stainless steel should not be used for tests on metals in salt water. Concrete and rigid (unplasticized) plastics may be used for culture, holding, and acclimation tanks and in the water-supply system, but they should be soaked, preferably in flowing dilution water, for several days before use (**9**). Cast-iron pipe should not be used with salt water and probably should not be used in freshwater supply systems, because colloidal iron will be added to the dilution water, and strainers will be needed to remove rust particles. Specially designed systems are usually necessary to obtain salt water from a natural water source (see Guide E729). Copper, brass, lead, galvanized metal, and natural rubber should not contact dilution water, stock solutions, or test solutions before or during the test. Items made of neoprene rubber and other materials not mentioned above should not be used unless a sensitive aquatic species (see 8.2.3) can survive for 48 or 96 h (see Guide E729) in static water in which the items are soaking.

6.3 *Metering System:*

6.3.1 The metering system must be designed to accommodate the type and concentration(s) of test material and the necessary flow rates of test solutions. The system must reproducibly (see 11.10.3.2) supply the selected concentration(s) of test material (see 9.4). A variety of metering systems, using various combinations of syringes, "dipping birds," siphons, pumps, solenoids, valves, etc. (see Guide E729), has

been used successfully. Because a bioconcentration test usually consists of a control treatment and one concentration of test material, the metering system usually consists of one device for metering a solution of the test material, two devices for metering dilution water, and two small chambers for mixing (and splitting, if replicate test chambers are used) the individual test solutions before they enter test chambers.

6.3.2 The metering system should be calibrated before each test by determining the flow rate through each test chamber and measuring either the concentration of test material in each test chamber or the volume of solution used in each portion of the metering system. The general operation of the metering system should be visually checked each morning and afternoon throughout the test. The metering system should be adjusted during the test if necessary.

6.3.3 The flow rate through each test chamber should be at least five volume additions per 24 h, but might need to be greater depending on the loading (see 11.4). In tests with bivalve mollusks, the minimum necessary flow rate might also depend on the amount of food available in the dilution water (see 11.4.3). It is usually desirable to use a flow rate of at least ten volume additions per 24 h, especially at the beginning of the test when uptake is greatest, but a higher flow rate will increase the amount of dilution water and test material used. A higher flow rate is also desirable if there is rapid loss of test material due to microbial degradation, hydrolysis, oxidation, photolysis, reduction, sorption, or volatilization. At any particular time during a test, the flow rates through any two test chambers should not differ by more than 10 %. If comparable numbers of test organisms are removed from all chambers, the depth of solution or the flow rate, or both, in all test chambers may be equally reduced, as long as the flow rate remains at least five volume additions per 24 h and the loading (see 11.4) and temperature (see 11.3) remain acceptable.

6.4 *Test Chambers:*

6.4.1 In a toxicity test with aquatic organisms, a test chamber is defined as the smallest physical unit between which there are no water connections. However, screens and cups may be used to create two or more compartments within each chamber. Test solution can therefore flow from one compartment to another within a test chamber but, by definition, cannot flow from one chamber to another. Because solution can flow from one compartment to another in the same test chamber, the temperature, concentration of test materials, and levels of pathogens and extraneous contaminants are likely to be more similar between compartments in the same test chamber than between compartments in different test chambers in the same treatment. Chambers should be covered to keep out extraneous contaminants and to reduce the evaporation of test solution and test material. All of the chambers and compartments in a test must be identical.

6.4.2 Test chambers are usually constructed by welding (not soldering) stainless steel or by gluing double-strength or stronger window glass with clear silicone adhesive. Stoppers and silicone adhesive sorbs some organochlorine and organophosphorus pesticides that are then difficult to remove. Therefore, as few stoppers and as little adhesive as possible should be in contact with the test solution. If extra beads of adhesive are needed for strength, they should be on the outside of chambers rather than on the inside.

6.4.3 The minimum dimensions of test chambers and the minimum depth of test solution depend on the size of the individual test organisms (see 10.2) and the loading (see 11.4). The smallest horizontal dimension of test chambers should be at least 1.5 times the largest horizontal dimension of the largest test organism. For fish the depth of test solution should be at least 3 times the height of the largest test organism; in addition, the test solution should be at least 150 mm deep for fish over 0.5 g (wet weight) each, and at least 50 mm deep for smaller fish. Chambers filled to within 150 mm of the top sometimes need to be covered to prevent fish from jumping out. With bivalve mollusks, the test solution should completely submerge the organisms throughout the test. Tests with bivalve mollusks for which the distance from the tip of the umbo to the distal valve edge is less than 60 mm and tests with small fish, for example, less than 10 g, are often conducted in 300 by 600 mm by 300 mm deep all-glass test chambers containing 30 L of solution. Use of excessively large volumes of solution in test chambers will unnecessarily increase the amount of dilution water and test material used or the average retention time, or both.

6.4.4 *Cleaning*—The metering system, test chambers, and equipment used to prepare and store dilution water, stock solutions, and test solutions should be cleaned before use. New items should be washed with detergent and rinsed with water, a water-miscible organic solvent, water, acid (such as 10 % concentrated hydrochloric acid), and at least twice with distilled, deionized, or dilution waterr, or ASTM Type II water (Specification D1193). (Some lots of some organic solvents may leave a film that is insoluble in water.) A dichromate-sulfuric acid cleaning solution may be used in place of both the organic solvent and the acid, but it might attack silicone adhesive and requires special disposal techniques. At the end of every test, all items that are to be used again should be immediately (*a*) emptied, (*b*) rinsed with water, (*c*) cleaned by a procedure appropriate for removing the test material (for example, acid to remove metals and bases; detergent, organic solvent, or activated carbon to remove organic chemicals), and (*d*) rinsed at least twice with distilled, deionized, dilution water, or ASTM Type II water. Acid is useful for removing mineral deposits, and 200 mg of hypochlorite/L is often useful for removing organic matter and for disinfection. A solution containing about 200 mg of $OCl^-$ /L is conveniently prepared by adding 6 mL of liquid household chlorine bleach to 1 L of water. Hypochlorite is quite toxic to most aquatic animals (**10**) and is difficult to rinse from some construction materials. It is often removed by reaction with sodium thiosulfate, sodium sulfite, or sodium bisulfite or by autoclaving in distilled water for 20 min or by drying the item and letting it sit for at least 24 h before use. An item cleaned or disinfected with hypochlorite should not be used unless it has been demonstrated at least once that unfed test organisms held for at least 48 h in static dilution water in which the cleaned item is soaking do not show more signs of stress, such as discoloration, unusual behavior, or death, than do unfed test organisms held in static dilution water

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containing a similar item that was not treated with hypochlorite. Alternatively, peracetic acid is a less noxious antimicrobial than hypochlorite that avoids these issues. The metering system and test chambers should be rinsed with dilution water just before use.

6.5 *Acceptability*—New holding, acclimation, and testing facilities should be tested for toxicity before use (see 6.2 and 8.1.1).

## 7. Safety Precautions

7.1 Many materials can affect humans adversely if precautions are inadequate. Therefore, skin contact with all test materials and solutions of them should be minimized by wearing appropriate protective gloves (especially when washing equipment or putting hands in test solutions), laboratory coats, aprons, and glasses, and by using dip nets or forceps to remove organisms from test solutions.

7.1.1 Special precautions, such as covering test chambers, ventilating the area surrounding the chambers, and use of fume hoods, should be taken when conducting tests on volatile materials. Information concerning toxicity to humans **(11)**, recommended handling procedures **(12)**, and chemical and physical properties of the test material should be studied before a test is begun. Special procedures may be necessary with radiolabeled test materials **(13)** and with materials that are, or are suspected of being, carcinogenic **(14)**.

7.2 Although disposal of stock solutions, test solutions, and test organisms poses no special problems in most cases, health and safety precautions and applicable regulations should be considered before beginning a test. Removal or degradation of test material might be desirable before disposal of stock and test solutions.

7.3 Cleaning equipment with a volatile solvent such as acetone should be performed only in a well-ventilated area in which no smoking is allowed and no open flame, such as a pilot light, is present.

7.4 An acidic solution should not be mixed with a hypochlorite solution because hazardous fumes might be produced.

7.5 To protect hands from being cut by sharp edges of shells, cotton work gloves should be worn (over appropriate protective gloves (see 7.1) if necessary) when bivalve mollusks are handled.

7.6 Because dilution water and test solutions are usually good conductors of electricity, use of ground fault systems and leak detectors should be considered to help avoid electrical shocks. Salt water is such a good conductor that protective devices are strongly recommended.

7.7 To prepare dilute acid solutions, concentrated acid should be added to water, not vice versa. Opening a bottle of concentrated acid and adding concentrated acid to water should be performed only in a fume hood.

7.8 Some test materials, as well as some materials used to preserve test organisms, may be inherently hazardous. Caution needs to be used when handling these materials. Guidelines for the handling and disposal of hazardous materials should be strictly followed (Guide D4447). When working with any potentially hazardous materials, including those used for analytical measurements (for example, acid used during alkalinity titrations), users need to wear appropriate protective equipment (for example, safety glasses and gloves). Common laboratory protective wear should also be used to reduce exposure to potential biological hazards (for example *Salmonella*, *Vibrio* ssp.). All laboratory-specific health and safety considerations should be followed.

## 8. Dilution Water

8.1 *Requirements*—The dilution water should (*a*) be in adequate supply; (*b*) be acceptable to the test organisms; (*c*) be of uniform quality; and (*d*) except as stated in 8.1.4, not affect the results of the test unnecessarily.

8.1.1 The dilution water should not adversely affect the test organisms. For bioconcentration tests, the minimal criterion for acceptability of dilution water to test organisms is that healthy test organisms will survive in it during acclimation and testing without showing signs of stress, such as discoloration or unusual behavior. In addition, the water should not affect the ability of organisms to sorb and depurate test material. Therefore, a better criterion for acceptability of dilution water to test organisms is that at least one aquatic animal species can survive, grow, and reproduce satisfactorily in it. Unless acceptability of the dilution water has been demonstrated during the previous year, it should be demonstrated during the test by showing that either (*a*) at least one species will survive, grow, and reproduce acceptably in a laboratory culture or a life-cycle toxicity test (see Guides E1191, E1193, and E1295) or (*b*) at least one species of fish will perform acceptably in a partial life-cycle or early life-stage toxicity test (see Guide E1241).

8.1.2 The dilution water should not unnecessarily affect the results of a bioconcentration test because of such things as sorption or complexation of test material. Therefore, except as stated in 8.1.4, concentrations of both total organic carbon (TOC) and particulate matter should be less than 5 mg/L for tests with fish and less than 20 mg/L for tests with saltwater bivalve mollusks (see 10.5.3).

8.1.3 The quality of the dilution water should be uniform during the test. During a test in freshwater the range of hardness should be less than 5 mg/L or 10 % of the average, whichever is higher. During a test in salt water the range of salinity should be less than 2 g/kg or 20 % of the average, whichever is higher.

8.1.4 If it is desired to study the effect of an environmental factor such as TOC, particulate matter, or dissolved oxygen on the results of a bioconcentration test, it will be necessary to use a water that is naturally or artificially high in TOC or particulate matter or low in dissolved oxygen. If such a water is used, it is important that adequate analyses be performed to characterize the water and that a comparable test be available or conducted in a more usual dilution water to facilitate interpretation of the results in the special water.

8.2 *Source:*

8.2.1 Although reconstituted water (see Guide E729) may be used, its use generally is not practical for bioconcentration tests because large volumes are necessary. In addition, it may

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be difficult to provide saltwater bivalve mollusks with adequate amounts of acceptable food (see 10.5.3) when reconstituted water is used.

8.2.2 If a natural water is used, it should be obtained from an uncontaminated, uniform quality source. For fresh water, a well or spring is usually preferable to a surface water. If a surface water is used for fresh or salt waters, the intake should be positioned to minimize fluctuations in quality and the possibility of contamination.

8.2.2.1 For bioconcentration tests with saltwater bivalve mollusks, it might also be desirable to position the intake to maximize the amount of plankton that will support growth and survival (see 8.3.2).

8.2.3 Chlorinated water should not be used as, or in the preparation of, dilution water because total residual chlorine and chlorine-produced oxidants are quite toxic to aquatic animals (**10**). Dechlorinated water should be used only as a last resort because dechlorination is often incomplete. Sodium bisulfite should be better for dechlorinating water than sodium sulfite and both are more reliable than carbon filters, especially for removing chloramines (**15**). Some organic chloramines, however, react slowly with sodium bisulfite (**16**). In addition to residual chlorine, municipal drinking water often contains unacceptably high concentrations of copper, lead, zinc, and fluoride, and quality is often rather variable. When necessary, excessive concentrations of most metals can usually be removed with a chelating resin (**17**). If dechlorinated water is used as dilution water or in its preparation, either (*1*) the acceptability of the dilution water must be demonstrated (see 8.1.1) during the test or (*2*) it must be shown three times each week on nonconsecutive days that in fresh samples of dilution water either (*a*) *Acartia tonsa*, mysids (not more than 30-h post release of a species that can live for 48 h without food), bivalve mollusk larvae, or first-instar daphnids can survive for 48 h without food, or (*b*) the concentration of total residual chlorine in freshwater or chlorine-produced oxidants in salt water is less than 8 µg/L (**10**).

8.3 *Treatment:*

8.3.1 Dilution water should be aerated intensively by using air stones, surface aerators, or column aerators (**18, 19**) before addition of test material. Adequate aeration will bring the pH and concentrations of dissolved oxygen and other gases into equilibrium with air and minimize oxygen demand and concentrations of volatiles. The concentration of dissolved oxygen in dilution water should be between 90 and 100 % saturation (**20**) to help ensure that dissolved oxygen concentrations in the test chambers are acceptable. Supersaturation by dissolved gases, which can occur when dilution water is heated, should be avoided to prevent gas bubble disease (**18, 21**).

8.3.2 For tests with bivalve mollusks, unfiltered, unsterilized natural salt water is often used to provide as much natural planktonic food as possible (see 10.5.3).

8.3.3 Except possibly for tests with bivalve mollusks (see 8.3.2), filtration through sand, sock, bag, or depth-type cartridge filters may be used to keep the concentration of particulate matter acceptably low (see 8.1.2) and as a pretreatment before ultraviolet sterilization or filtration through a finer filter, or both.

8.3.4 Except possibly for tests with bivalve mollusks (see 8.3.2), it might be desirable to pass salt water from a surface water source through a filter effective to 15 µm or less to remove parasites.

8.3.5 Except possibly for tests with bivalve mollusks (see 8.3.2), dilution water that might be contaminated with undesirable microorganisms may be passed through a properly maintained ultraviolet sterilizer (**22**) equipped with an intensity meter and flow controls or passed through a filter with a pore size of 0.22 µm or less.

8.3.6 Hardness, salinity, and pH may be adjusted by the addition of appropriate reagent grade chemicals,[5] sea salt, acid, base, and deionized or distilled water if it has been shown that the addition will not adversely affect the test organisms.

8.4 *Characterization*—The following items should be measured at least twice each year and more often if such measurements have not been made semiannually for at least two years or if a surface water is used:

8.4.1 *All Waters*—Alkalinity, pH, conductivity, particulate matter, TOC, total organophosphorus pesticides, organic chlorine (or total organochlorine pesticides plus PCBs), chlorinated phenoxy herbicides, ammonia, cyanide, sulfide, bromide, fluoride, iodide, nitrate, phosphate, sulfate, calcium, magnesium, potassium, aluminum, arsenic, beryllium, boron, cadmium, chromium, cobalt, copper, iron, lead, manganese, mercury, molybdenum, nickel, selenium, silver, and zinc.

8.4.2 *Freshwater*—Hardness, chloride, and sodium.

8.4.3 *Salt Water*—Salinity.

8.4.4 For each method used (see 12.3), the detection limit should be below either (*a*) the concentration in the dilution water or (*b*) the lowest concentration that has been shown to adversely affect the test organisms (**23**).

## 9. Test Material

9.1 *General*—The test material should be reagent-grade[5] or better, unless a test on a formulation, commercial product, or technical-grade or use-grade material is specifically needed. Before a test is begun, the following should be known about the test material:

9.1.1 Identities and concentrations of major ingredients and major impurities, for example, impurities constituting more than approximately 1 % of the material.

9.1.2 Solubility and stability in dilutions with water.

9.1.3 Expected steady-state BCF. This might be obtained from the results of tests on the same or a similar material with the same or a different species. For organic chemicals, this might be obtained from correlations that have been reported between steady-state BCFs and such physical–chemical properties as the octanol-water partition coefficient and solubility in water (**4**).

9.1.4 Estimated time to apparent steady-state (see 11.1.2.1).

9.1.5 Acute toxicity to the test organisms (a measurement or estimate of chronic toxicity is desirable).

[5] *Reagent Chemicals, American Chemical Society Specifications* , American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Reagent Chemicals and Standards*, by Joseph Rosen, D. Van Nostrand Co., Inc., New York, NY, and the *United States Pharmacopeia*.

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