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

This standard is issued under the fixed designation E 1022; the number immediately following the designation indicates the yea original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

ε[1] NOTE—Sections 3, 4, and 5, were editorially renumbered in August 1988.

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 t reported.

1.5 This guide is arranged as follows:

1.6 *This standard does not purport to address*

[1] This guide is under the jurisdiction of ASTM Committee E-47 on Biological Effects of Environmental Fate and is the direct responsibility of Subcommittee E47.01 on Aquatic Assessment and Toxicology.

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...for 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. Specific precautionary statements are given in Section 7.

2. Referenced Documents

2.1 *ASTM Standards:*

D 1129 Terminology Relating to Water[2]

E 380 Practice for Use of the International System of Units (SI) (the Modernized Metric System)[3]

E 729 Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians[4]

E 943 Terminology Relating to Biological Effects and Environmental Fate[4]

E 1023 Guide for Assessing the Hazard of a Material to Aquatic Organisms and Their Uses[4]

E 1191 Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids[4]

E 1193 Guide for Conducting Renewal Life-Cycle Toxicity Tests with Saltwater Mysids[4]

E 1241 Guide for Conducting Early Life-Stage Toxicity Tests with Fishes[4]

E 1295 Guide for Conducting Three Broad, Renewal Toxicity Tests with Ceriodaphnia Dubia[4]

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 D 1129 and E 943 and Guide E 729. For an explanation of units and symbols, refer to Practice E 380.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *apparent steady-state bioconcentration factor*—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 (bioconcentration (net accumulation) is zero for two to four

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

3.2.3 *bioconcentration*—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)*—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.)

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

3.2.6 *depuration curve*—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*—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*—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*—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*—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*—acquisition of a substance from the environment by an organism as a result of any active or passive process.

3.2.12 *uptake curve*—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*—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.)

...ual Book of ASTM Standards, Vol 11.01.

...ual Book of ASTM Standards, Vol 14.02; excerpts in gray pages of Vol

...ual Book of ASTM Standards, Vol 11.05.

3.2.14 *uptake rate constant*—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**).[5] 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
concentrations likely to occur in aquatic org
situations as a result of exposure under compar
except that mobile organisms might avoid
possible. Under the experimental conditions, pa
is deliberately minimized compared to natural
Exposure conditions for the tests may therefore
rable for an organic chemical that has a higl
partition coefficient or for an inorganic chem
substantially onto particulate matter. The amc
substance in solution is thereby reduced in t
therefore the material is less available to m
However, sorption might increase bioaccumula
species that ingest particulate matter (**2**), or
more important source of residues in fish than
stable neutral organic chemicals that have a Lc
4 and 6 (**3**).

5.6 Results of bioconcentration tests can be u
the propensity of different materials to be acc
ionizable organic chemicals can also be ranked
tration using correlations that have been rep
steady-state BCFs and physical–chemical proj
the octanol–water partition coefficient and solu
(**4**). However, when such predictions are imp
the demonstrated limits of the correlation, or i
wise questionable (**1, 5**), a bioconcentration
necessary.

5.7 Results of bioconcentration tests can a
compare the abilities of different species to acc
rials. At steady-state the concentration of
organic chemical in individual organisms, a
tissues within an organism, will probably be
concentration of lipids in the organisms and tis

5.8 Results of bioconcentration tests might b
consideration when assessing hazard (see Gui
deriving water-quality criteria because cons
might be adversely affected by ingesting aquatic
contain toxic materials. However, assessment
consumer organisms must take into account
quantity of material accumulated in tissues of
isms, but also the toxicity of the material to
Further, humans eat only certain portions of
organisms, whereas other predators often cons
tissues.

5.9 Bioconcentration tests might be usefu
structure–activity relationships between test mat
cal availability, metabolism of materials in aqu
and effects of various environmental factors on
tests.

5.10 Uptake and depuration rate constants n
for predicting environmental fate using compart
(**7**).

6. Apparatus

6.1 *Facilities*—Flow-through tanks should b
culturing, holding, and acclimating test organism
dilution-water tank or headbox, or both, might b
dilution water can be gravity-fed into holding a
tanks and the metering system (see 6.3), which p

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

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. A16-h light and 8-h dark photoperiod controlled by a timing device is often convenient, but a 12–12 or 14–10 photoperiod might be desirable to delay maturation of some species. A15 to 30-min transition period (12) 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.

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 (13). 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 E 729). 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 E 729) 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 E 729), 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 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 water. (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, or dilution 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 (**14**) 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.) 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 te materials and solutions of them should be minimized b wearing appropriate protective gloves (especially when wash ing equipment or putting hands in test solutions), laborato coats, aprons, and glasses, and by using dip nets or forceps remove organisms from test solutions.

7.1.1 Special precautions, such as covering test chamber ventilating the area surrounding the chambers, and use of fum hoods, should be taken when conducting tests on volati materials. Information concerning toxicity to humans (8 recommended handling procedures (**9**), and chemical an physical properties of the test material should be studied befo a test is begun. Special procedures may be necessary wit radiolabeled test materials (**10**) and with materials that are, are suspected of being, carcinogenic (**11**).

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

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

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

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

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

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

8. Dilution Water

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

8.1.1 The dilution water should not adversely affect the organisms. For bioconcentration tests, the minimal criterion acceptability of dilution water to test organisms is that healt test organisms will survive in it during acclimation and test without showing signs of stress, such as discoloration unusual behavior. In addition, the water should not affect the ability of organisms to sorb and depurate test material. Ther fore, a better criterion for acceptability of dilution water to organisms is that at least one aquatic animal species survive, grow, and reproduce satisfactorily in it. Unless accep ability of the dilution water has been demonstrated during previous year, it should be demonstrated during the test showing that either (*a*) at least one species will survive, gro

and reproduce acceptably in a laboratory culture or a life-cycle toxicity test (see Guides E 1191, E 1193, and E 1295) or (*b*) at least one species of fish will perform acceptably in a partial life-cycle or early life-stage toxicity test (see Guide E 1241).

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 fresh water 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 E 729) may be used, its use generally is not practical for bioconcentration tests because large volumes are necessary. In addition, it may 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 and to maximize the concentrations of sulfide and iron.

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 (**14**). 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 fresh water or chlorine-produced oxidants in salt water is less than 8 µg/L (**14**).

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,[6] 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

[6] *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*.

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 *Fresh Water*—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[6] 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).

9.1.6 Precision and bias of the analytical methods at planned water concentration(s), the expected steady-state tissue concentration, and one-tenth the expected steady-state tissue concentration.

9.2 *Radiolabeled Test Materials*—Radiolabeled test materials have been used occasionally in an effort to simplify the analyses of test solutions and test organisms. Their usefulness is limited greatly by two serious complications: (*a*) Many radiolabeled materials contain more than 1 % radiolabeled impurities; a small amount of an impurity with a high BCF can greatly affect the apparent BCF of a chemical with a much lower BCF; and (*b*) if the radiolabeled chemical is metabolized substantially in the test organisms and one or more of the metabolites are radiolabeled, the apparent BCF of the chemical will be too high. The only way to overcome these two problems is to verify that the radioactivity in the tissue and water is associated with the parent chemical. Such techniques as thin layer chromatography are more useful for demonstrating that the radioactivity is not associated with the parent chemical than verifying that it is associated with the parent chemical. Verification usually requires gas chromatography, which means that the use of radiolabeled test material does not save resources in the determination of the BCF in the long run. Because of these complications, radiolabeled test materials are more useful for demonstrating the presence of metabolites than for measuring BCFs.

9.3 *Stock Solution*—The test material can be added directly to dilution water in the metering system in some cases, bu usually it is dissolved in a solvent to form a stock solution tha is then added to dilution water in the metering system. If stock solution is used, the concentration and stability of the te material in the solvent should be determined before th beginning of the test. If the test material is subject to photoly sis, the stock solution should be shielded from light.

9.3.1 Except possibly for tests on hydrolyzable, oxidizable and reducible materials, the preferred solvent is dilution wate although filtration or sterilization (or both) might be necessary If the hardness or salinity of the dilution water will not b affected, distilled and deionized water may be used. Severa techniques have been developed specifically for preparin aqueous stock solutions of slightly soluble materials (24). Th minimum amount of a strong acid and base may be used in th preparation of an aqueous stock solution, but such reagent might affect the pH of test solutions appreciably. The use of more soluble form of the test material, such as chloride o sulfate salts of organic amines, sodium or potassium salts o phenols and organic acids, and chloride or nitrate (rather tha carbonate or hydroxide) salts of metals, might affect the pH more than the use of the minimum necessary amount of strong acid or base.

9.3.2 If a solvent other than dilution water is used, it concentration in test solutions should be kept to a minimum and should not decrease the survival or growth of the tes species. Triethylene glycol is often a good organic solvent fo preparing stock solutions because of its low toxicity to aquati animals (25), low volatility, and strong ability to dissolve many organic chemicals. Other water-miscible organic solvents such as methanol, ethanol, and acetone may also be used, but they might stimulate undesirable growths of microorganisms, and acetone is also quite volatile. If an organic solvent is used, it should be reagent-grade[6] or better, and its concentration in any test solution should not exceed 0.1 mL/L. Surfactants should not be used in the preparation of a stock solution because they might affect the form of the test material in test solutions. (These limitations do not apply to any ingredient of a mixture, formulation, or commercial product unless an extra amount of solvent is used in preparation of the stock solution.)

9.3.3 If a solvent other than water is used, it might be desirable to conduct simultaneous tests using two chemically unrelated solvents or two different concentrations of the same solvent to obtain information concerning possible effects of the solvent on results of the test.

9.4 *Test Concentration(s)*—Several factors might influence selection of the test concentration(s):

9.4.1 The concentration of test material in a test solution must not stress, irritate, or otherwise adversely affect organisms during the test. The highest acceptable test concentration can often be estimated by dividing, for fish, the 96-h LC50, or for bivalve mollusks, the 48-h EC50 based on survival and development of embryos and larvae, by an appropriate acute-chronic ratio. Appropriate ratios for some materials are about 1, but a few are above 100.

9.4.2 The test concentration must be high enough so that the test material can be accurately measured in the test solution during the uptake phase and in the test organisms after 90

depuration. Therefore, the test concentration should be equal to or higher than the highest of (*a*) three times the background in dilution water; (*b*) three times the detection limit of the analytical method in dilution water; (*c*) thirty times the background in the test organisms divided by the expected steady-state BCF; and (*d*) thirty times the detection limit of the analytical method in tissue divided by the expected steady-state BCF.

9.4.3 The test material should be dissolved in the test solution; it should not be in some other form, such as colloidal, particulate, or emulsified except when these are inherent properties of the test material. Because of problems often encountered with nearly saturated solutions, the test concentration should usually be no more than one half the solubility of the test material in the dilution water.

9.4.4 If an expected concentration in natural water can be estimated, it should be used as the test concentration if it meets the above conditions.

9.4.5 If it is desired to determine whether uptake, depuration, and BCF are independent of the concentration in water, bioconcentration tests should be conducted at test concentrations which cover a range of at least a factor of 10.

10. Test Organisms

10.1 *Species*—Whenever possible and appropriate, tests should be conducted with a species listed in Table 1. These species were selected on the basis of availability; commercial, recreational, and ecological importance; past successful use; and ease of handling in the laboratory. Their use is encouraged to increase comparability of results and availability of much information about a few species rather than a little information about many species. If a desired species is unavailable, a species from a listed genus should be used. A specific strain should be used only when it is of special concern. The scientific name of the species used should be verified using an appropriate taxonomic key. If results are available for a material with a freshwater fish and results are desired with a saltwater animal, a test with a saltwater bivalve mollusk is usually preferable to a test with a saltwater fish, because BCFs will probably be more similar between freshwater and saltwater fish than between bivalve mollusks and either kind of fish.

TABLE 1 Species and Test Temperatures

Species[A]	Test temperature,° C
Freshwater:	
Rainbow trout, *Salmo gairdneri* Richardson	12
Fathead minnow, *Pimephales promelas* Rafinesque	17, 22
Channel catfish, *Ictalurus punctatus* (Rafinesque)	17, 22
Bluegill, *Lepomis macrochirus* Rafinesque	17, 22
Saltwater:	
Blue mussel, *Mytilus edulis* Linnaeus	ambient
Scallop, *Pecten* spp.	ambient
Oyster, *Crassostrea gigas* Thunberg *C. virginica* Gmelin	ambient
Sheepshead minnow, *Cyprinodon variegatus* Lacepede	22
Mummichog, *Fundulus heteroclitus* (Linnaeus)	22
California killifish, *Fundulus parvipinnis* Girard	17
Threespine stickleback, *Gasterosteus aculeatus* Linnaeus	17
Finfish, *Lagodon rhomboides* (Linnaeus)	22
Spot, *Leiostomus xanthurus* Lacepede	22
Shiner perch, *Cymatogaster aggregata* Gibbons	12

[A] The scientific name should be verified using an appropriate taxonomic key.

10.2 *Size*—All organisms in a test should be uniform in size and age.

10.2.1 *Fish*—Unless data on another life stage are specifically desired, tests should be conducted with juvenile fish, that is, post-larval or older and actively feeding, but not sexually mature, spawning, or recently spent. In any single test all fish should be from the same year class, and the standard length (tip of snout to end of caudal peduncle) of the longest fish should be no more than twice that of the shortest fish. It is advantageous to use relatively small fish (less than 10 g each) in order to accommodate the numbers required in usual-sized test chambers. Minimum acceptable size of individual test organisms is determined by the ability to measure test material in tissue, because fish should be large enough to allow for measurement of test material in each organism and its muscle tissue if necessary.

10.2.1.1 Mature male and female fish of the same species sometimes contain different concentrations of lipids. Therefore, in tests with organic chemicals either (*a*) the fish should not be sexually mature at any time during the test or (*b*) at the time of analysis, the sex of each fish should be determined and recorded and the concentration of lipids (see 11.9.7) in both males and females should be measured separately. Maturation usually can be prevented by employing a low-temperature or a short day-length photoperiod (see 6.1), or both.

10.2.2 *Bivalve Mollusks*—Use of relatively large mollusks (distance from the tip of the umbo to the distal valve edge greater than 60 mm) makes it difficult to accommodate the large number required in usual-sized test chambers. However, mollusks should be large enough (distance from the tip of the umbo to the distal valve edge more than 40 mm) that test material can be measured in individual organisms and in adductor muscle of scallops if necessary. In any single test, all mollusks should be from the same year class, and the distance from the tip of the umbo to the distal valve edge of the largest mollusk should be no more than 1.5 times that of the smallest mollusk.

NOTE 1—The distance from the tip of the umbo to the distal valve edge is the most objectively defined, easily performed measurement that usefully characterizes the size of live bivalve mollusks of species that are commonly used in bioconcentration tests. All three-dimensional measurements may be desirable for some species. The weight of all soft tissue is the most desirable measurement, but it cannot be measured before a test; this measurement should be performed at the end of a test (see 11.9.5).

10.2.2.1 Spawning of bivalve mollusks should be inhibited during tests. Use of sexually immature animals and a test temperature (see 11.3.2) that is known to inhibit spawning are acceptable preventative measures. Bottoms of test chambers should be checked daily for evidence of spawning, such as a white film of embryos.

10.3 *Source*—All organisms used in a test should be from the same source. Laboratory cultures of such species as the fathead minnow and sheepshead minnow usually can provide organisms whose history, age, size, and quality are known and are similar at all times of the year in all laboratories. Usual sources of other freshwater fish are private, state, and federal hatcheries. Whenever salmon or trout are to be used, they should be obtained from a hatchery that has been certified

disease-free, for example, free of infectious pancreatic necrosis, *Furunculosis*, kidney diseases, enteric redmouth, and whirling disease. Requirements for certification vary from state to state and from species to species. Other suggested species usually are obtained directly from wild populations in relatively unpolluted areas. Importing and collecting permits may be required by local and state agencies.

10.4 *Care and Handling*—Organisms should be cared for and handled properly (**26**) so that they are not unnecessarily stressed.

10.4.1 Whenever aquatic animals are brought into a facility, they should be quarantined for 14 days or until they appear to be disease-free, whichever is longer. No dip nets, cleaning supplies, other equipment, organisms, or water should be transferred from a quarantined tank to any other tank.

10.4.2 To maintain aquatic animals in good condition and avoid unnecessary stress, they should not be crowded or subjected to rapid changes in temperature or water quality. In general, organisms should not be subjected to more than a 3°C change in water temperature in any 12-h period and preferably not more than 3°C within 72 h. The concentration of dissolved oxygen should be maintained between 60 and 100 % saturation (**20**) and continuous gentle aeration is usually desirable. The concentration of total dissolved gas should be less than 105 % saturation (**19, 21**). Except possibly for bivalve mollusks (see 10.5.3), 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 mm or less. The un-ionized ammonia concentration in holding and acclimation tanks should be less than 20 µg/L.

10.4.3 Holding and acclimation tanks should be scraped or brushed as needed. Between use with different groups of test organisms, tanks should be sterilized with an iodophor (**27**) or with 200 mg of hypochlorite/L for 1 h, brushed well once during the hour, and then rinsed well (see 6.5).

10.4.4 Organisms should be handled as little as possible. When handling is necessary, it should be done gently, carefully, and quickly so that organisms are not unnecessary stressed. Organisms that are injured or dropped during handling and fish that touch dry surfaces should be discarded. Dip nets are best for handling fish over 0.5 g each. Such nets are commercially available, or can be made from small-mesh nylon netting, nylon or silk bolting cloth, plankton netting, or similar knotless material. Nets coated with urethane resin are best for handling catfish. Equipment used to handle fish should be sterilized between uses by autoclaving or by treatment with an iodophor (**27**) or 200 mg hypochlorite/L (see 6.5). Hands should be washed before such handling or feeding fish.

10.4.5 Organisms should be carefully observed daily during quarantine, holding, and acclimation for signs of stress, physical damage, mortality, disease, and external parasites. Damaged, dead, and abnormal individuals should be discarded. Open bivalve mollusks that do not close when touched with a probe should be discarded. Bivalve mollusks that never open or do not deposit feces or pseudofeces also should be discarded. If visual examination of the behavior and external appearance of fish indicates that they are not eating or are flipping, flashing, swimming erratically, emaciated, gasping at the surface, hyperventilating, hemorrhaging, producing excessive mucus, or showing abnormal color, the cause should be eliminated. If organisms show any sign of disease or external parasites, appropriate action should be taken (see 10.6).

10.5 *Feeding*:

10.5.1 The concentration of test material in the food should be determined.

10.5.2 At least once a day, fish should be fed a food that will support survival, growth, and reproduction. A batch of food should not be used unless it has been shown that (*a*) the batch will support survival, growth, and reproduction of at least one species of aquatic animal, or (*b*) the concentration of organic chlorine does not exceed 0.15 µg/g (wet weight), or the total concentration of organochlorine pesticides plus PCBs does not exceed 0.3 µg/g (wet weight).

10.5.3 Bivalve mollusks should be provided enough water containing sufficient food to support survival and growth. If unsterilized and unfiltered natural salt water is used without adding algae, at least one litre per hour per individual is usually a minimum for mollusks for which the distance from the tip of the umbo to the distal valve edge is 40 to 60 mm. If the flow rate or the concentration of food, or both, is too low, a saltwater chlorophyte, such as *Monochrysis lutheri* or *Isochrysis galbana*, or a diatom, such as *Thalasiosira*, may be added to the dilution water.

10.6 *Disease Treatment*—Fish may be chemically treated to cure or prevent some diseases using appropriate treatments (see (**28**) and Guide E 729). If they are severely diseased, however, it is often better to destroy the entire lot immediately. Fish with other diseases and all other diseased animals should be discarded immediately, because systemic bacterial infections usually cannot be treated efficiently, internal parasites cannot be removed without extensive treatment, viral diseases cannot be treated, and invertebrates can rarely be treated effectively. Tests must not be begun with treated organisms for at least 15 days after treatment, and organisms must not be treated during a test. Generally, organisms should not be treated during the first 16 h after arrival at a facility because of possible stress or drug treatment during collection or transportation. However, immediate prophylaxis is necessary in some situations, such as treatment of bluegills for columnaris disease during hot weather.

10.7 *Holding*—Test organisms should be held in uncontaminated, aerated water of constant temperature and quality in a flow-through system with a flow rate of at least two volume additions per day for fish and 1 L/h per organism for bivalve mollusks for which the distance from the tip of the umbo to the distal valve edge is 40 to 60 mm; higher flow rates are often desirable. Organisms should be held in the dilution water and at the temperature at which they will be tested. Temperatures listed in Table 1 are generally good temperatures at which to hold the respective species. For long holding periods, however, it is generally easier and safer to hold fish at temperatures lower than those listed in Table 1 because metabolic rate and the number and severity of disease outbreaks are reduced.

10.8 *Acclimation*—To prevent test organisms from being stressed by an instantaneous change in water quality

temperature when placed in the test chambers, several days before the beginning of the test an appropriate number of similar-sized individuals should be transferred from a holding tank to an acclimation tank with a flow rate of at least two volume additions per day for fish and 1 L/h per organism for bivalve mollusks for which the distance from the tip of the umbo to the distal valve edge is 40 to 60 mm. The water in the acclimation tank should be gradually changed from 100 % holding water to 100 % dilution water over a period of two or more days. Similarly, the water temperature should be changed at a rate not to exceed 3°C within 72 h until the test temperature is reached. All organisms must be maintained in dilution water at the test temperature for at least the last 48 h before they are placed in test chambers. Complete acclimation, which has not been adequately experimentally defined, may take considerably longer; therefore, acclimation times longer than the minimum specified should be used when possible.

10.9 *Quality*—All organisms used in a test should be of acceptable quality.

10.9.1 Damaged, dead, abnormal, and diseased organisms should be dealt with in accordance with 10.4.5 and 10.6.

10.9.2 Organisms should have been either (*a*) reared using water and food that support acceptable survival, growth, and reproduction of at least one aquatic species and meet the specifications of 8.1 and 10.5, respectively; (*b*) obtained from a body of water that does not receive a discharge or chemical treatment and maintained using water and food as above; or (*c*) analyzed to show that (*1*) they do not contain elevated concentrations of chemicals to which they are probably exposed and (*2*) either the concentration of organic chlorine does not exceed 0.15 µg/g (wet weight) or the total concentration of organochlorine pesticides plus PCBs does not exceed 0.3 µg/g (wet weight). For (*c*), analysis of a few representatives from a batch is sufficient; analysis of organisms from each shipment or before each test is not necessary.

10.9.3 A few representative organisms should be analyzed for test material. If the concentration of test material is more than 10 % of the expected steady-state concentration (see 9.1), it might be desirable to hold the organisms in dilution water for a while or obtain other test organisms.

10.9.4 Mollusks should not be used for a test for at least 4 days, and fish for at least 14 days, after arrival at the test facility.

10.9.5 A group of organisms should not be used for a test if the individuals appear to be diseased or otherwise stressed or if more than 3 % die during the 48 h immediately preceding the test. If a group fails to meet these criteria, all individuals should be either discarded or treated, held an additional 10 days, and reacclimated if necessary.

11. Procedure

11.1 *Experimental Design*—The important aspects of the experimental design of bioconcentration tests are the number of test chambers per treatment, the durations of the uptake and depuration phases, the number and spacing of sampling points during each phase, and the number of samples taken and analyzed at each sampling point. The most desirable experimental design would provide good estimates (see sections 5.2, 5.3, and 11.1.2.2) of the apparent and projected steady-state BCFs, the uptake and depuration rate constants, and confidence limits on all four for the least cost. The most desirable experimental design usually can only be devised after a preliminary test, but the optimal design is rarely necessary to make practical decisions concerning bioconcentration of a material. Cost-effective experimental designs, using appropriate sampling schedules or lengths of the phases, usually provide adequate data and can be devised before testing for many materials (see the following sections and Table 2). However, it is usually wise to anticipate the possibility that either or both phases might have to be extended and that more than the minimum number of sampling points might be necessary. In addition, data obtained during a test might suggest revision of initial estimates if samples are analyzed quickly enough.

11.1.1 The primary focus of the physical and experimental design of the test and statistical analysis of the data is the experimental unit, which is defined as the smallest physical entity to which treatments can be assigned independently. Because test solution can flow from one compartment to another, but not from one test chamber to another, the test chamber is the experimental unit. As the number of test chambers (that is, experimental units) per treatment increases, the width of the confidence interval on a point estimate decreases and the power of a hypothesis test increases. With respect to factors that might affect the results within test chambers and, therefore, the results of the test, all chambers in the test should be treated as similarly as possible. For example, the test temperature in all test chambers should be as similar as possible unless the purpose of the test is to study the effect of temperature on the BCF. Test chambers are usually arranged in one or more rows. Treatments must be assigned randomly to individual test chamber locations. A randomized block design (with each treatment being represented in each block, which

TABLE 2 Minimum Organism Sampling Schedule

Material	1	2	3	4
Log K_{ow}[A]	3.13	5.01	5.73	5.89[B]
S[C] (days)	2	12	24	28[B]
Uptake phase[D]				
Initial	0	0	0	0
$S/16$	0.13	0.75	1.5	1.8
$S/8$	0.25	1.5	3	3.5
$S/4$	0.5	3	6	7
$S/2$	1	6	12	14
Additional[E]	...	...	18	21
S	2	12	24	28
$S + 1$[F] or $S + S/12$[G]	3	13	26	...[H]
$U = S + 2$[F] or $S + S/6$[G]	4	14	28	...[H]
Depuration phase[I]				
$U + D/4$	4.5	17	34	35[J]
$U + D/2$	5	20	40	42[J]
$U + 3D/4$	5.5	23	46	49[J]
$U + D$	6	26	52	56[J]

[A] K_{ow} = octanol-water partition coefficient.
[B] Or higher.
[C] S = estimated number of days to apparent steady-state (in these examples, S is estimated from Log K_{ow}).
[D] U = length of uptake phase.
[E] Additional midterm sampling point so that no two consecutive points are more than 7 days apart.
[F] When S is less than 12 days.
[G] When S is between 12 and 24 days.
[H] Uptake phase need not last longer than 28 days.
[I] D = length of depuration phase = S.
[J] $D = S = U = 28$ days.

may be a row or a rectangle) is preferable to a completely randomized design.

11.1.2 *Length of Uptake Phase*—The uptake phase must continue until either apparent steady-state or 28 days is reached (see 11.1.2.2). The criterion for attainment of apparent steady-state is that three sets of BCFs based on samples (whole body for fish and total soft tissue for bivalve mollusks) taken at appropriate intervals are not significantly ($\alpha = 0.05$) different when tested, using such techniques as analysis of variance or orthogonal polynomial coefficients (**29**). If S, the estimated number of days to apparent steady-state, is 12 days or less, the intervals between the three sets of samples should be 24 h or longer. If S is between 12 and 24 days, samples should be taken at intervals of $S/12$ days or longer. Thus, the minimum length of the uptake phase, U, would be $U = S + 2$ days if S is less than 12 days, and $U = S + S/6$ if S is between 12 and 14 days.

11.1.2.1 Although the time to apparent steady-state, S, depends on the species, size, physiological condition, and test conditions, it seems to be reasonably similar for most juvenile fishes under the conditions described herein. However, S seems to be smaller for mollusks than for fishes under comparable conditions. Thus, a reasonable estimate of S can sometimes be obtained from the results of a bioconcentration test on the same or similar material with a different species. In addition, for materials whose uptake and depuration follow a two-compartment, two-parameter model, a useful estimate of S can often be derived from the solubility in water or the octanol–water partition coefficient. Persistent, nonionizable organic chemicals often seem to fit such a model within experimental error. Other materials might fit such a model well enough for the predictions to be useful in designing a bioconcentration test. For the two-compartment, two-parameter model, the time to reach 95 % of steady-state in the uptake phase is about equal to the time to reach 95 % of depuration in the depuration phase.

Therefore, $S = (ln\ (1/(1.00–0.95)))/k_2 = 3.0/k_2$,

where:

S = number of days,
ln = logarithm to base e, and
k_2 = first-order depuration rate constant in day^{-1}.

For fishes, k_2 can be estimated (**1**) as follows: antilog $(1.47–0.414 \log K_{ow})$,

where:

K_{ow} = octanol–water partition coefficient, and
log = logarithm to base 10.

The octanol–water partition coefficient can be measured directly or estimated from solubility in water (**30**) or by means of high-pressure liquid chromatography (**31**). Alternatively, organisms can be statically exposed with no measurement of the concentration of the test material, followed by an experimental determination of k_2 in clean water and calculation of S. S may also be estimated directly by conducting a preliminary bioconcentration test.

11.1.2.2 Rarely is there a practical necessity for S to be longer than 28 days. If apparent steady-state is not reached in 28 days, a longer uptake phase usually will not provide sufficient additional information to justify the additional cost because the apparent steady-state BCF will usually be no more than a factor of 2 higher than the 28-day BCF. In addition, a *projected steady-state* BCF can be calculated, but will usually be no more than a factor of 4 higher than the 28-day BCF (**32**). Unless a better estimate of the steady-state BCF is desired, if the estimated time to steady-state is more than 24 days, the test may be designed with $S = 18$ days, and no samples need to be taken between 21 and 28 days or after 28 days during the uptake phase to determine whether or not apparent steady-state is reached (Table 2).

11.1.2.3 Regardless of the estimate of S at the beginning of the test, if samples are analyzed quickly enough during the test, for example, within 24 to 48 h, it may be possible to calculate a better estimate of S or it might be found that apparent steady-state has been reached, allowing the termination of the uptake phase and the beginning of the depuration phase.

11.1.2.4 If real-time verification of the attainment of apparent steady-state is impossible or unlikely, the test must be designed and conducted based on available information, and no adjustments based on data obtained during the test will be possible. In this situation, the best option probably is to design the test as described, but plan to extend the uptake and depuration phases past the minimum times and take additional samples. Data analysis will indicate whether an apparent steady-state BCF can be reported. Whether or not an apparent steady-state BCF can be reported, a projected steady-state BCF should be calculated and reported if possible.

11.1.3 *Organism Sampling Schedule During Uptake Phase*—Except for the controls, a minimum of five sampling points should be distributed in a geometric series, with the fifth at time S. The five sampling points should be close to $S/16$, $S/8$, $S/4$, $S/2$, and S. No sampling interval should be greater than seven days, so an additional sampling point might have to be inserted when the uptake phase lasts longer than 14 days (Table 2). Further, if S is less than 24 days, two more evenly spaced intervals must follow S so that attainment of apparent steady-state can be evaluated (see 11.1.2). This assumes that the uptake phase ends and the depuration phase begins at time U. If not, additional sampling would have to occur at appropriately spaced intervals to the end of the uptake phase. When U is estimated to be less than 28 days, it is usually desirable to plan for two to four additional sampling points in case S has been underestimated. These additional sampling points are most likely to be needed if the uptake curve does not approach steady-state in an asymptotic manner.

11.1.4 *Length of Depuration Phase*—The depuration phase should continue until the concentration of test material in the organisms is less than 10 % of the steady-state concentration or is below the detection limit in tissue. Because the time to reach 10 % of steady-state in the depuration phase is usually slightly less than the time to reach 95 % of steady-state in the uptake phase, the length, D, of the depuration phase past 28 days is rarely justified by the additional information gained. If samples are analyzed quickly enough, data collected during the uptake phase can be used to calculate a better estimate of D. More importantly, if samples are analyzed quickly enough during the depuration phase, a very good estimate of the appropriate length of the depuration phase might be available at or soon after the beginning of the depuration phase.

11.1.5 *Organism Sampling Schedule During Depuration Phase*—Except for the controls, a minimum of four sampling points should be distributed in an arithmetic series during the depuration phase, with the fourth being at time D after the beginning of the depuration phase. The four sampling points should be close to $D/4$, $D/2$, $3D/4$, and D. It is usually desirable to allow for the possibility of sampling points at $5D/4$ and $6D/4$, in case D has been underestimated or in case depuration is not linear. Extension of the depuration phase is often desirable because information concerning the last portion of depuration is often useful and can be obtained at minimal cost because test material is not added to, nor frequently measured in, test solution during the depuration phase.

11.1.6 At a minimum, control organisms should be sampled at the beginning and end of the uptake phase and at the end of the depuration phase.

11.1.7 *Number of Samples*—At each sampling point sufficient organisms should be collected to allow for at least four analyses. If the organisms are large enough for individual analyses, only four organisms need be sampled. Because samples obtained close to $1.6/k_2$ during the uptake phase provide more information than other samples (**33**), a better estimate of the uptake rate constant will result from obtaining twice as many samples at $S/2$ than at other sampling points during the uptake phase.

11.1.8 *Water Sampling Schedule*—At least two samples of the test solution must be collected for measurement of test material whenever samples of the organisms are collected during the uptake phase. Samples of the test solution should also be collected both 24 and 48 h before the beginning of the uptake phase (see 11.5) and at least every third day during the uptake phase. In addition, samples should be taken about one third and two thirds of the way through the depuration phase. Samples of the test solution in the control treatment should be taken at the beginning and end of the uptake phase and at the end of the depuration phase.

11.1.9 It might be desirable to sample test solutions and organisms from two or more test chambers so that variation in BCFs and rate constants within and between test chambers can be compared (see 14.6).

11.2 *Dissolved Oxygen*—The dissolved oxygen concentration in each test chamber should be between 60 and 100 % saturation (**20**) at all times during the test. Because results of bioconcentration tests are based on measured rather than calculated concentrations of test material, some loss of material by aeration is not detrimental and test solutions may be aerated gently. Vigorous aeration, however, should be avoided because it can stress test organisms, resuspend fecal matter, and greatly increase volatilization. Because aeration readily occurs at the surface, efficient aeration can be achieved with minimum turbulence by using an air lift to transfer solution from the bottom to the surface. Aeration should be the same in all test chambers, including the controls, at all times during the test.

11.3 *Temperature*:

11.3.1 *Fish*—Although any temperature that is suitable for the test species is an acceptable test temperature, investigators are encouraged to select temperatures from the series 7, 12, 17, 22, 27, and 32°C to facilitate interlaboratory comparisons. The temperatures listed in Table 1 should be used as the selected test temperatures for the listed species whenever possible. Other temperatures from the series can be used for studying the effect of temperature on the results of bioconcentration tests. The difference between the highest and lowest measured temperatures during a test should not exceed 2°C and must not exceed 6°C.

11.3.2 *Bivalve Mollusks*—Because adjustment of the temperature of large volumes of unfiltered natural salt water (see 10.5.3) is especially difficult, bioconcentration tests with saltwater bivalve mollusks are often conducted at ambient temperature. The temperature should always be between 8 and 28°C and should be low enough to inhibit spawning (see 10.2.2.1). The difference between the highest and lowest measured temperatures during a test must not exceed 10°C.

11.4 *Loading*:

11.4.1 The grams of organism (whole body of fish, total soft tissue of bivalve mollusks; wet weight, blotted dry) per litre of solution in the test chambers should not be so high that it affects the results of the test. Therefore, the loading should be limited to ensure that (*a*) the concentrations of dissolved oxygen and test material do not fall below acceptable levels, (*b*) the concentrations of metabolic products do not exceed acceptable levels, and (*c*) the organisms are not stressed because of crowding or lack of food.

11.4.2 For fishes listed in Table 1, loading in the test chambers should not exceed 1 g/L of solution passing through the test chamber in 24 h and should not exceed 10 g/L of test solution in the chamber at any time at or below the lower of 17°C and the temperature(s) listed in Table 1. At higher temperatures, loading should not exceed 0.5 g/(L/day) or 5 g/L. At all temperatures, a lower loading should be used if aggression occurs.

11.4.3 In bioconcentration tests with bivalve mollusks, the maximum loading might depend mostly on the amount of food available in the dilution water (see 10.5.3). If supplementary saltwater algae are not added to the dilution water for bivalve mollusks for which the distance from the tip of the umbo to the distal valve edge is 40 to 60 mm, the loading in the test chambers should not exceed one organism per litre of solution passing through the chamber in one hour; a lower loading is often desirable.

11.4.4 For both fish and bivalve mollusks, a lower loading or higher flow rate, or both, should be used, if necessary, to meet the following three criteria at all times during the test in each test chamber: (*a*) the concentration of dissolved oxygen should be at least 60 % saturation (see also 11.2); (*b*) the concentration of un-ionized ammonia should not exceed 20 µg/L; and (*c*) after time $S/4$, the concentration of test material should not be lowered more than 20 % because of uptake by test organisms.

11.4.5 Comparable loadings should be used for other species.

11.5 *Beginning the Test*:

11.5.1 After test solutions have been flowing through the chambers long enough that the concentration(s) of test material have probably reached steady-state, two sets of water samples should be taken at least 24 h apart. The analyses should verify

that the concentration(s) of test material have reached steady-state before organisms are placed in the test chambers.

11.5.2 The measured concentration of test material in each treatment should be no more than 30 % higher or lower than the nominal concentration. The cause should be identified if the difference is more than 30 %. Measurement of the concentration of test material in the solution flowing into the test chamber will indicate whether the cause is in the metering system or the test chamber. If the concentration entering the test chamber is too high, the stock solution might have been prepared incorrectly or the metering system might have been calibrated incorrectly. If the concentration is too low, additional possible causes are microbial degradation, hydrolysis, oxidation, photolysis, reduction, sorption, and volatilization, and a faster flow rate is probably desirable.

11.5.3 The test begins when the test organisms are first placed in the test solution.

11.5.4 A representative sample of the test organisms must be either (*a*) impartially distributed among the test chambers by adding to each test chamber no more than 20 % of the number of test organisms to be placed in the chamber and repeating the process until each test chamber contains the desired number of test organisms or (*b*) assigned either by random assignment of one organism to each test chamber, random assignment of a second organism to each test chamber, etc., or by total randomization. It might be convenient to assign organisms to other containers and then to add them to the test chambers all at once.

11.6 *Care of Organisms*—The environment of the test animals can have an immediate and profound effect on their metabolism and respiration and, therefore, on uptake and depuration. Thus, disturbance and handling should be minimized and the animals should be provided uniform suitable conditions. Handling and feeding of organisms and cleaning of chambers should be done gently, carefully, and quickly.

11.7 *Feeding*—At least once a day fish should be fed a food that will support survival, growth, and reproduction (see 10.5.2). Feeding and sampling schedules should be coordinated (see 11.9.4) and excess food should be removed about 30 min after feeding. Food for bivalve mollusks should be supplied in the dilution water (see 10.5.3).

11.8 *Cleaning*—Test chambers should be siphoned once a day or as often as necessary to remove fecal matter, excess food, algae, and bacterial growth.

11.9 *Biological Data*:

11.9.1 The organisms in each treatment should be observed daily during the test for signs of disease, stress, irritation, and other adverse effects.

11.9.2 The sampling schedule and number of organisms to be removed should be determined as described in 11.1.

11.9.3 The results of all tissue analyses should be based on wet tissue weight. If results are based on dry tissue weight, the ratio of wet to dry weight should be determined.

11.9.4 Fish should be removed in an impartial manner with as little disturbance as possible. A different net should be used for each treatment. Feeding and sampling schedules should be coordinated so that fish are obtained at least 4 h, and longer if possible, after they are fed. After removal, fish should be rinsed with dilution water if accompanied by extraneous matter, blotted dry, and killed by pithing the brain with a dissecting needle or by severing the spinal cord above the opercular region with scissors. The weight (wet weight, blotted dry) and standard length (tip of snout to end of caudal peduncle) of each fish should be determined within 15 min of sampling. If fish are sexually mature, the gender should be determined. The whole body should be either analyzed for test material within 8 h or preserved in a manner appropriate for the test material.

11.9.5 Bivalve mollusks should be removed in an impartial manner and the distance from the tip of the umbo to the distal valve edge measured. The shell should be opened by severing the adductor muscle without piercing the animal, shaking the mollusk three times to remove excess water, and removing the top shell. The remaining adductor muscle should be severed where it attaches to the lower shell and all soft tissue removed intact. The total soft tissue should be weighed and either analyzed within 8 h or preserved in a manner appropriate for the test material.

11.9.6 In addition to the samples specified in 11.9.4 and 11.9.5, in tests with fish and scallops, four samples of muscle (with or without skin) or adductor muscle, respectively, should be obtained at the end of the uptake phase from additional organisms for measurement of the test material in edible tissue.

11.9.7 To greatly increase in usefulness of results of bioconcentration tests on organic chemicals, the concentration of lipids (see 12.4) in the organisms in the control and each test concentration should be measured at the beginning and end of the uptake phase and at the end of the depuration phase in the same tissues in which test material is measured. If fish are sexually mature or if bivalve mollusks spawned, the concentration of lipids in males and females should be measured separately.

11.9.8 Analysis of tissue samples for likely reaction and degradation products of the test material is desirable, especially if a radiolabeled test material is used (see 9.2).

11.9.9 All organisms used in a test should be destroyed at the end of the test.

11.10 *Measurements on Test Solutions*:

11.10.1 *Water Quality*—During tests conducted in fresh water, hardness, alkalinity, pH, and conductivity should be measured at the beginning, middle, and end of the test and at least weekly in the control treatment. During tests conducted in salt water, salinity should be measured daily and pH should be measured at the beginning, middle, and end of the test and at least weekly in the control treatment. During all tests, alkalinity and pH should also be measured in the highest test concentration to determine whether these are affected by the test material. During all tests, the dissolved oxygen concentration must be measured in at least one test chamber per treatment (*a*) at the beginning and at least weekly, (*b*) whenever there is an interruption in the metering system, and (*c*) whenever the behavior of the test organisms indicates that the dissolved oxygen concentration might be too low. If a measured dissolved oxygen concentration is less than 60 % of saturation, corrective action should be taken immediately, and measurements must be performed at least daily until 60 % is reached. Particulate matter and total organic carbon should be measured

weekly in the test chambers during tests with bivalve mollusks; these measurements are desirable in tests with other organisms. Weekly determination of un-ionized ammonia is desirable. If the test material is a neutral organic chemical whose log K_{ow} is greater than 4, dissolved organic carbon should be measured weekly.

11.10.2 *Temperature*—Throughout acclimation and the test in at least one test chamber, either the temperature must be measured or monitored at least hourly or the maximum and minimum temperatures must be measured daily. In addition, near the beginning, middle, and end of the test, the temperature must be measured concurrently in all test chambers.

11.10.3 *Test Material:*

11.10.3.1 The concentration of test material in each treatment, including the controls, must be measured in test solutions in accordance with the schedule described in 11.1.7. Water samples should be taken by pipetting or siphoning through glass or fluorocarbon plastic tubing from a point midway between the top, bottom, and sides of the test chamber and should not include any surface scum or material stirred up from the bottom or sides. If test material might be lost due to sorption onto the walls of the sample container, the container and siphon or pipette should be rinsed with test solution before collecting the sample. Water samples should be collected directly into appropriate-sized containers from which the test material can be extracted or analyzed directly. A second sample should be taken and analyzed after filtration or centrifugation to determine the percentage of test material associated with particulate matter, especially if the concentration of particulate matter present in the test solution is greater than 5 mg/L (see 8.1.2).

11.10.3.2 For each treatment, the highest of all the measured concentrations obtained during the uptake phase divided by the lowest must be less than 2. If it is not, the metering system should be checked and additional samples from the proper chambers should be analyzed to determine if either the sampling or the analytical methods are inadequate. If the test organisms are probably being exposed to significant concentrations of one or more reaction or biodegradation products, measurement of the product(s) is desirable (see 9.2). A faster flow rate may also be desirable.

12. Analytical Methodology

12.1 The methods used to analyze water and tissue samples for test material might determine the usefulness of the test results because all results are based on measured concentrations. For example, if the analytical method measures any impurities or reaction or biodegradation products along with the parent test material, then results can be calculated only for the whole group of materials, and not for parent material by itself. Measurement of major products, in addition to parent material, is usually desirable in both water and tissue samples (see 9.2, 11.9.8, and 11.10.3.2).

12.2 It is usually advantageous to analyze tissue and water samples within 48 h of collection to prevent degradation or loss of test material and to help establish the lengths of the uptake and depuration phases (see 11.1.2.3 and 11.1.4). If samples cannot be analyzed immediately, they should be handled and stored appropriately (34) to minimize loss of test material by such things as microbial degradation, hydrolysis, oxidation, reduction, photoxidation, volatilization, and sorption.

12.3 Chemical and physical data should be obtained using appropriate ASTM standards whenever possible. For the measurements for which ASTM standards do not exist or are not sensitive enough, methods should be obtained from other reliable sources (35). The concentration of un-ionized ammonia can be calculated from the pH, temperature, and concentration of total ammonia (36).

12.4 A variety of methods are available for measuring lipids (37). In addition, in bioconcentration tests on organic chemicals, "lipids" are sometimes measured by evaporating and weighing a portion of the extract obtained in the extraction of the test material from the tissue, using an organic solvent.

12.5 The precision and bias of each analytical method used should be determined in appropriate matrices, that is, the tissue(s) of concern, samples taken from dilution water containing test organisms, and food. When appropriate, reagent blanks, recoveries, and certified reference standards should be included whenever samples are analyzed.

12.6 *Analysis of Radiolabeled Materials*:

12.6.1 When bioconcentration tests are conducted using a radiolabeled material, the tissue samples can be prepared for counting using a tissue solubilizer. However, it is usually easier to prepare the samples using an apparatus that combusts the sample and traps the resulting radiolabeled carbon dioxide.

12.6.2 When bioconcentration tests are conducted using a radiolabeled organic material, total radioactivity should be measured on all samples. In addition, selected water and tissue samples should be checked to determine the percentage of the radioactivity associated with impurities and reaction and biodegradation products (see 9.2), usually using either gas or liquid chromatography.

13. Acceptability of the Test

13.1 A bioconcentration test should usually be considered unacceptable if one or more of the following occurred, except that if, for example, the temperature was measured numerous times, one difference of more than 6°C (see 13.1.5) might be inconsequential. However, if the temperature was measured only a minimum number of times, one difference of more than 6°C might indicate that more differences would have been detected if it had been measured more often.

13.1.1 The test was begun with organisms within ten days after treatment for a disease or the organisms were treated during the test,

13.1.2 The test organisms were not maintained in dilution water at the test temperature for at least the last 48 h before they were placed in test chambers,

13.1.3 The uptake phase was terminated before either apparent steady-state or 28 days was reached,

13.1.4 More than 10 % of the organisms in any treatment died or showed signs of disease, stress, or other adverse effects,

13.1.5 The highest and lowest measured test temperature differed by more than 6°C during the test with fish or by more than 10°C during a test with bivalve mollusks,

13.1.6 The time-weighted average measured dissolved oxygen concentration was less than 60 % of saturation in any test chamber,

13.1.7 The concentration of test material in the test solutions was not measured as specified in 11.1.8,

13.1.8 During the uptake phase, the highest measured concentration of test material in a test chamber was more than twice the lowest in the same test chamber, or

13.1.9 The percentage of radioactivity associated with impurities in both water and tissue was not determined using gas or liquid chromatography when a radiolabeled test material was used.

13.2 An assessment should be made of the significance of the concentrations of test material in the organisms (see 11.1.7) and water (see 11.1.8) in the control treatment and in the food (see 10.5.1).

14. Calculation of Results

14.1 Sometimes several sets of results can be calculated from one test. Separate results can be calculated for different kinds of tissue samples (whole body, muscle, etc.) if the necessary measurements were performed on each kind of sample. Also, separate results can sometimes be calculated for different kinds of materials (parent test material, impurities, test material plus reaction and biodegradation products) if appropriate measurements are performed. If possible, calculation of separate results for (*a*) parent test material and (*b*) parent test material plus its reaction and biodegradation products that are structurally similar and are not much more soluble in water or which have comparable or greater toxicity are especially desirable. The reported results must reflect the kinds of measurements actually performed.

14.2 The concentration, C_w, of test material in water and the concentration, C_t, in tissue should be corrected for recoveries of less than 90 % and should be expressed in comparable units. For example, if C_w is in µg/L, then C_t should be in ng/g or µg/kg. Unless the test material is man-made, C_t should probably be corrected for the concentration in the control organisms. If the test material was radiolabeled, C_w and C_t should be corrected for radioactive decay and background radioactivity.

14.2.1 For neutral organic chemicals with a log K_{ow} greater than 4, the percent of the total chemical in the water that is dissolved freely should be estimated (3). The first step is to measure the percent of the total chemical in the water that is dissolved; filtration or centrifugation, or both, can usually be used to separate the particulate material from the dissolved material. The percent of the dissolved chemical that is dissolved freely can then be estimated by using the concentration of dissolved organic carbon in the water and the K_{ow} of the chemical.

14.3 If apparent steady-state is reached during the uptake phase (see 11.1.1), the apparent steady-state BCF should be calculated as the geometric mean of the BCFs obtained during apparent steady-state. Calculation of 95 % confidence limits is desirable. If apparent steady-state is not reached, the BCF at the end of uptake phase should be calculated. In either case, if the uptake curve does not approach steady-state in an asymptotic manner, sufficient BCFs should be calculated to indicate the shape of the uptake curve and the highest measured BCF.

14.4 The uptake rate constant(s), depuration rate constant(s), projected steady-state BCF, and 95 % confidence limits for each should be calculated. These can be calculated using an appropriate compartmental model and nonlinear parameter estimation methods (38), but such calculations will usually require a computer program. Alternatively, uptake rate constant(s), depuration rate constant(s), and the projected steady-state BCFs can be derived by simple graphical algebraic methods, but 95 % confidence limits probably cannot be obtained and the results might be questionable (38). These calculations do not require a computer, but are facilitated by a calculator that has the natural log function.

14.5 If BCFs are available for enough appropriate times, calculation of a projected steady-state BCF might be possible by fitting an equation to the data (32).

14.6 The calculation procedure(s) and the interpretation of the confidence limits should be appropriate to the experimental design. For example, if point estimates and confidence limits are calculated from test organisms that were all exposed in the same test chamber, the confidence limits only take into account variation within that batch of test organisms and do not take into account variation between batches, chambers, laboratories, etc.

15. Documentation

15.1 A record of the results of an acceptable bioconcentration test should include the following information either directly or by reference to available documents:

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

15.1.2 Source of the test material, its lot number, composition (identities and concentrations of major ingredients and major impurities), known physical and chemical properties, identity and concentration of any carrier (solvent) used, and, if the test material is radiolabeled, the percentage of radioactivity associated with impurities and how determined;

15.1.3 Source of the dilution water, its chemical characteristics, a description of any pretreatment, and results of any demonstration of the ability of a species to survive, grow, and reproduce in the water;

15.1.4 Source of the test organisms, scientific name and how verified (and strain for salmonids when appropriate), observed diseases, disease treatments, holding and acclimation procedures, food, feeding method, and rate, and (*a*) for fish age (if known), life stage, and means and ranges of the weight (wet, blotted dry), and standard length (tip of snout to end of caudal peduncle) at the beginning and end of the uptake phase and at the end of the depuration phase, or (*b*) for bivalve mollusks, age (if known) and the mean and range of the distance from the tip of the umbo to the distal valve edge at the beginning of the test and the means and ranges of the weight (wet, shaken dry) of the total soft tissue at the beginning and end of the uptake phase and at the end of the depuration phase;

15.1.5 Source of the food, its composition, concentration of the test method and other contaminants, and feeding frequency and ration;

15.1.6 Description of the experimental design and test chambers, the depth and volume of solution in the chambers, the number of organisms per treatment, the loading and lighting, a description of the metering system, and the flow rate as volume additions per 24 h;

15.1.7 Range and time-weighted average measured dissolved oxygen concentration (as % of saturation), and a description of any aeration performed on test solutions before or during the test;

15.1.8 Averages and ranges of the acclimation temperature and test temperature and method of measurement;

15.1.9 Percentage of test organisms in the control treatment and each of the other treatments that died or showed signs of disease, stress, or other adverse effects;

15.1.10 Whether fish were sexually mature or whether bivalve mollusks spawned during the test;

15.1.11 Description of tissue and water samples analyzed, and methods used to obtain, prepare, and store them;

15.1.12 Methods used for, and results (with standard deviations or confidence limits) of, chemical analyses of water quality and concentrations of test material impurities and reaction and biodegradation products in tissue and water, including validation studies and reagent blanks;

15.1.13 Methods used for, and results of, measurements of lipids;

15.1.14 A table of data on concentrations of test material (and lipids if available) in test solutions and tissue in sufficient detail to allow independent statistical analysis;

15.1.15 Either (*a*) the apparent steady-state BCF or the BCF at the end of the uptake phase, or (*b*) the projected steady-state BCF and the uptake and depuration rate constants, or (*c*) both (*a*) and (*b*); the 95 % confidence limits for each, if available, and a description of how all values were calculated, including whether corrections were made for recoveries, background, and radioactive decay;

15.1.16 Ratio of wet to dry tissue weights if results are based on dry tissue weights;

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

15.2 Published reports should contain sufficient information to clearly identify the procedure used and the quality of the results.

16. Keywords

16.1 bioaccumulation; bioconcentration; bioconcentration factor (BCF); bivalve mollusks; depuration phase; fishes; uptake phase

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