Designation: E1193 − 97 (Reapproved 2012)

Standard Guide for Conducting *Daphnia magna* Life-Cycle Toxicity Tests[1]

This standard is issued under the fixed designation E1193; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide covers procedures for obtaining laboratory data concerning the adverse effects of a test material (added to dilution water, but not to food) on *Daphnia magna* Straus, 1820, during continuous exposure throughout a life-cycle using the renewal or flow-through techniques. These procedures also should be useful for conducting life-cycle toxicity tests with other invertebrate species, although modifications might be necessary.

1.2 These procedures are applicable to most chemicals, either individually or in formulations, commercial products, or known mixtures. With appropriate modifications, these procedures can be used to conduct tests on temperature, dissolved oxygen, pH, and on such materials as aqueous effluents (also see Guide E1192), leachates, oils, particulate matter, sediments, and surface waters. The technique, (renewal or flow-through), will be selected based on the chemical characteristics of the test material such as high oxygen demand, volatility, susceptibility to transformation (biologically or chemically), or sorption to glass.

1.3 Modification of these procedures might be justified by special needs or circumstances. Although using appropriate procedures is more important than following prescribed procedures, results of tests conducted using unusual procedures are not likely to be comparable to results of standard test procedures. Comparison of results obtained using modified and unmodified versions of these procedures might provide useful information on new concepts and procedures for conducting life-cycle toxicity tests with *D. magna*.

1.4 This guide is arranged as follows:

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

Current edition approved Dec. 1, 2012. Published December 2012. Originally approved in 1987. Last previous edition approved in 2004 as E1193 – 97 (2004). DOI: 10.1520/E1193-97R12.

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

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* Specific hazard statements are given in Section 8.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

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

E943 Terminology Relating to Biological Effects and Environmental Fate

E1023 Guide for Assessing the Hazard of a Material to Aquatic Organisms and Their Uses

E1192 Guide for Conducting Acute Toxicity Tests on Aqueous Ambient Samples and Effluents with Fishes, Macroinvertebrates, and Amphibians

IEEE/ASTM SI 10 American National Standard for Use of the International System of Units (SI): The Modern Metric System

3. Terminology

3.1 The words "must," "should,"" may," "can," and "might" have very specific meanings in this guide.

3.2 *must*—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 directly relate to the acceptability of the test (see 14.1).

3.3 *should*—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," "is often desirable," and "might be desirable" are used in connection with less important factors.

3.4 *may*—used to mean "is (are) allowed to," "can" is used to mean "is (are) able to," and "might" is used to mean "could possibly." Therefore the classic distinction between "may" and "can" is preserved, and "might" is never used as a synonym for either" may" or "can."

3.5 For definitions of other terms used in this guide, refer to Guide E729 and Terminology E943. For an explanation of units and symbols, refer to IEEE/ASTM SI 10.

4. Summary of Guide

4.1 A 21-day life-cycle toxicity test for *Daphnia magna* is described. The test design allows for the test organisms to be exposed to a toxicant using either the renewal technique (with exchange of the total volume of test water and toxicant at least three times a week) or the flow-through technique (with continual water and toxicant addition, usually at least four volume additions per day). At least five concentrations of a test material, a control, and a solvent control (if applicable) replicated at least four times are recommended. Each test concentration has at least ten *Daphnia* per treatment. The technique (renewal or flow-through) which uses a minimum of ten daphnids per treatment has only one daphnid per replicate, whereas the typical technique (renewal or flow-through) utilizes four replicates with at least five daphnids per replicate (≥20 daphnids per treatment). A control consists of maintaining daphnids in dilution water to which no test material has been added to provide (*1*) 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, and so forth, and (*2*) the basis for interpreting data obtained from the other treatments. In each of the other treatments, the daphnids are maintained in dilution water, to which a selected concentration of test material has been intentionally added. Measurement end points obtained during the test include the concentration of the test material and final number alive, final weight, and number of progeny per daphnid. Then data are analyzed to determine the effect of the test material on survival, growth, and reproduction of *D. magna*.

5. Significance and Use

5.1 Protection of an aquatic species requires prevention of unacceptable effects on populations in natural habitats. Toxicity tests are conducted to provide data that may be used to predict what changes in numbers and weights of individuals might result from similar exposure to the test material in the natural aquatic environment. Information might also be obtained on the effects of the material on the health of the species.

5.2 Results of life-cycle tests with *D. magna* are used to predict chronic effects likely to occur on daphnids in field situations as a result of exposure under comparable conditions.

5.2.1 Life-cycle tests with *D. magna* are used to compare the chronic sensitivities of different species, the chronic toxicities of different materials, and study the effects of various environmental factors on the results of such tests.

5.2.2 Life-cycle tests with *D. magna* are used to assess the risk of materials to aquatic organisms (see Guide E1023) or derive water quality criteria for aquatic organisms (**1**).[3]

5.2.3 Life-cycle tests with *D. magna* are used to predict the results of chronic toxicity tests on the same test material with the same species in another water or with another species in the same or a different water. Most such predictions take into account the results of acute toxicity tests, and so the usefulness of the results of a life-cycle test with *D. magna* is greatly increased by also reporting the results of an acute toxicity test (see Guide E729) conducted under the same conditions. In addition to conducting an acute toxicity test with unfed *D.*

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

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

magna, it may be desirable to conduct an acute test in which the daphnids are fed the same as in the life-cycle test to see if the presence of that concentration of that food affects the results of the acute test and the acute-chronic ratio (ACR) (see 10.3.1).

5.2.4 Life-cycle tests are used to evaluate the biological availability of, and structure-activity relationships between, test materials and test organisms.

5.3 Results of life-cycle tests with *D. magna* might be influenced by temperature (**2**), quality of food, composition of dilution water, condition of test organisms, and other factors.

6. Apparatus

6.1 *Facilities*—Culture and test chambers are often kept in a room maintained at about 20°C but at separate locations. Alternatively, culture and test chambers may be placed in a temperature-controlled water bath or environmental chamber or incubator. The water-supply system should provide an adequate supply of dilution water to the culture tanks and test chambers. The water-supply system should be equipped for temperature control and aeration, and strainers and air traps should be included in the water-supply system. 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 (**3**). During culturing and testing, daphnids should be shielded from disturbances to prevent unnecessary stress. The test facility should be well-ventilated and free of fumes. A timing device should be used to provide a 16-h light and 8-h dark photoperiod (**4**). A 15 to 30-min transition period when lights go on might be desirable to reduce the possibility of daphnids being stressed by instantaneous illumination; a transition period when lights go off may also be desirable.

6.1.1 When *D. magna* are fed algae, a high-light intensity might cause sufficient photosynthesis to result in an increase of pH high enough to kill daphnids (**5**). Therefore, the maximum acceptable intensity is dependent on the buffer capacity of the dilution water, species, and density of algae, and the kind of test chamber and cover. Light intensities up to 600 lx or a fluence rate of 1 w/m^2 will usually be acceptable, but higher intensities might result in an unacceptably high pH in the culture water.

6.2 *Construction Materials*—Equipment and facilities that contact stock solutions, test solutions, or any water into which daphnids will be placed should not contain substances that can be leached or dissolved by aqueous solutions in amounts that can adversely affect daphnids. In addition, equipment and facilities that contact stock solutions or test solutions should be chosen to minimize sorption of test materials from water. Glass, Type 316 stainless steel, nylon, fiberglass, silicon, and fluorocarbon plastics should be used whenever possible to minimize leaching, dissolution, and sorption. Concrete and rigid (unplasticized) plastics may be used for culture tanks and in the water-supply system, but they should be soaked, preferably in flowing dilution water, for several days before use (**6**). Cast-iron pipe may be used in supply systems, but colloidal iron probably will be added to the dilution water and strainers will be needed to remove rust particles. 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 previously mentioned should not be used unless it has been shown that their use will not adversely affect survival, growth, and reproduction of *D. magna* (see Section 14).

6.3 *Test Chambers:*

6.3.1 *Flow-through tests,* 500-mL to 2-L glass beakers (or equivalent) with a notch (approximately 4 by 13 cm) cut in the lip may be used to expose the *Daphnia* to the test material. The notch should be covered with 0.33-mm opening (U.S. standard sieve size No. 50) stainless steel or polyethylene screening small enough to retain first instar *Daphnia*. The screen can be attached to the beaker with silicone adhesive. The chambers should provide at least 30 mL of solution for each of the initial test daphnid(s).

6.3.2 *Renewal tests,* beaker ranging in size from 100 to 1000 mL. A notched chamber is not required for a renewal test. Each chamber should provide at least 40 mL of solution for each of the initial test daphnid(s).

6.3.3 Any container made of glass, Type 316 stainless steel, or a fluorocarbon plastic may be used if (*1*) each chamber is separate with no interconnections, (*2*) each chamber contains at least 30 mL of test solution (see 12.4) per first-generation daphnid for flow-through tests and at least 40 mL for renewal tests, (*3*) there is at least 1000 mm^2 of air to water interface per daphnid, and (*4*) the test solution is at least 30 mm deep. Static test chambers should be covered with glass, stainless steel, nylon, or fluorocarbon plastic covers to keep out extraneous contaminants and to reduce evaporation of test solution. All chambers and covers in a test must be identical. Covers are not required for flow-through studies.

6.4 *Cleaning*—Test chambers and equipment used to prepare and store dilution water, stock solutions, and test solutions should be cleaned before use. New equipment should be washed with detergent and rinsed with water, a water-miscible organic solvent, water, acid (such as 5 % concentrated nitric acid), and washed at least twice with distilled, deionized, or dilution water. Some lots of some organic solvents might leave a film that is insoluble in water. Also, stronger nitric acid, for example, 10 %, might cause deterioration of silicone adhesive; an initial rinse with 10 % concentrated hydrochloric acid might prevent such deterioration. A dichromate-sulfuric acid cleaning solution can generally be used in place of both the organic solvent and the acid, but it might attack silicone adhesives. At the end of every test, all items that are to be used again should be immediately (*1*) emptied, (*2*) rinsed with water, (*3*) 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 (*4*) rinsed at least twice with distilled, deionized, or dilution water. Acid is useful for removing mineral deposits. Test chambers should be rinsed with dilution water just before use.

6.5 *Acceptability*—Before a toxicity test is conducted in new test facilities, it is desirable to conduct a "non-toxicant" test, in which all test chambers contain dilution water with no added test material. This test will reveal (*1*) whether *D. magna* will survive, grow, and reproduce acceptably (see Section 14) in the new facilities, (*2*) whether there are any location effects on survival, growth, or reproduction, and (*3*) the magnitude of the within-chamber and between-chamber variance.

7. Reagents

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where such specifications are available.[4] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the test.

8. Hazards

8.1 Many materials can affect humans adversely if precautions are inadequate. Therefore, skin contact with all test materials and solutions should be minimized by wearing appropriate protective gloves (especially when washing equipment or putting hands in test solutions), laboratory coats, aprons, and glasses, and by using dip nets or tubes to remove daphnids from test solutions. Special precautions, such as covering test chambers and ventilating the area surrounding the chambers, should be taken when conducting tests on volatile materials. Information on toxicity to humans (**7**), recommended handling procedures (**8**), and chemical and physical properties of the test material should be studied before a test is begun. Special procedures will be necessary with radiolabeled test materials (**9**) and with materials that are, or are suspected of being, carcinogenic (**10**).

8.2 Disposal of stock solutions, test solutions, and test organisms might pose special problems in some cases; therefore, health and safety precautions and applicable regulations should be considered before beginning a test. Removal or degradation of test material might be desirable before disposal of stock and test solutions.

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

8.4 Acidic solutions and hypochlorite solutions should not be mixed together because hazardous fumes might be produced.

8.5 Because dilution water and test solutions are usually good conductors of electricity, use of ground fault systems and leak detectors should be considered to help prevent electrical shocks.

8.6 To prepare dilute acid solutions, concentrated acid should be added to water, not vice versa. Opening a bottle of concentrated acid and mixing concentrated acid with water should be performed only in a well-ventilated area.

9. Dilution Water

9.1 *Requirements*—The dilution water should (*1*) be acceptable to *D. magna*, (*2*) be of uniform quality, and (*3*), except as stated in 9.1.4, not unnecessarily affect results of the test.

9.1.1 The dilution water must allow satisfactory survival, growth, and reproduction of *D. magna* (see Section 14).

9.1.2 The quality of the dilution water should be uniform, allowing the brood stock to be cultured and the test conducted in water of the same quality. In particular, during culture or testing, or both, the range of hardness should be ±10 % of the average.

9.1.3 The dilution water should not unnecessarily affect results of a life-cycle test with *D. magna* because of such things as sorption or complexation of test material. Therefore, except as stated in 9.1.4, concentrations of both total organic carbon (TOC) and particulate matter should be less than 5 mg/L.

9.1.4 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 life-cycle test with *D. magna*, 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 the laboratory's usual culture dilution water to facilitate interpretation of the results in the special water.

9.2 *Source:*

9.2.1 The use of reconstituted water might increase comparability of test results between laboratories. The hard reconstituted fresh water (160 to 180 mg/L as $CaCO_3$) described in Guide E729 has been used successfully. Addition of 2 µg of selenium(IV) and 1 µg of crystalline vitamin B_{12}/L might be desirable (**11**). Other water sources (natural or reconstituted) may be used if they have been demonstrated to provide adequate daphnid survival, growth, and reproduction.

9.2.2 Natural fresh waters have been used successfully. Natural waters should be obtained from an uncontaminated source of consistent quality. A well or spring is usually preferable to a surface water. If a surface water is used, the intake should be positioned to minimize fluctuations in quality and the possibility of contamination and should maximize the concentration of dissolved oxygen to help ensure low concentrations of sulfide and iron.

9.2.3 Dechlorinated water is not recommended as a dilution water for *Daphnia magna*. Dechlorinated water should be used only as a last resort because dechlorination is often incomplete and residual chlorine is quite toxic to *D. magna* (**12**). Sodium bisulfite is probably better for dechlorinating water than sodium sulfite, and both are more reliable than carbon filtration, especially for removing chloramines (**13**). Some organic chloramines, however, react slowly with sodium bisulfite (**14**). In addition to residual chlorine, municipal drinking water often contains unacceptably high concentrations of copper, lead, zinc, and fluoride, and quality is often rather

[4] *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 *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

variable. When necessary, excessive concentrations of most metals can usually be removed with a chelating resin (**15**).

9.3 *Treatment:*

9.3.1 Dilution water should be aerated intensively by such means as air stones, surface aerators, or column aerators (**16,17**) prior to the addition of test material. Adequate aeration will bring the pH and concentrations of dissolved oxygen and other gases into equilibrium with the air, and minimize oxygen demand and concentrations of volatiles. The concentration of dissolved oxygen in dilution water should be between 90 and 100 % saturation to help ensure that dissolved oxygen concentrations are acceptable in test chambers. Supersaturation of dissolved gases, which might be caused by heating dilution water, should be avoided to prevent gas bubble disease (**18,19**).

9.3.2 Filtration through sand, sock, bag, or depth-type cartridge filters may be used to keep the concentration of particulate matter acceptably low (see 9.1.3).

9.3.3 Dilution water that might be contaminated with undesirable microorganisms may be passed through a properly maintained ultraviolet sterilizer (**20**) equipped with an intensity meter and flow controls or passed through a filter with a pore size of 0.45 µm. Water that might be contaminated with *Aphanomyces daphniae* should be autoclaved (**3**).

9.4 *Characterization:*

9.4.1 The following items should be measured at least twice each year, and more often if, (*1*) such measurements have not been made semiannually for at least two years, or (*2*) surface water is used: hardness, alkalinity, conductivity, pH, particulate matter, TOC, organophosphorus pesticides, polychlorinated biphenyls (PCBs), chlorinated phenoxy herbicides, ammonia, cyanide, sulfide, chloride, bromide, fluoride, iodide, nitrate, phosphate, sulfate, calcium, magnesium, sodium, potassium, aluminum, arsenic, beryllium, boron, cadmium, chromium, cobalt, copper, iron, lead, manganese, mercury, molybdenum, nickel, selenium, silver, and zinc.

9.4.2 For each analytical method used (see 13.3) to measure the parameters listed in 9.4.1, quantification of the limit should be below either (*1*) the concentration in the dilution water or (*2*) the lowest concentration that has been shown to adversely affect the survival, growth, or reproduction of *D. magna* (**21**).

10. Test Material

10.1 *General*—The test material should be reagent grade[4] 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:

10.1.1 Identities and concentrations of major ingredients and major impurities. For example, impurities constituting more than about 1 % of the material.

10.1.2 Solubility and stability in the dilution water and solvents.

10.1.3 Measured acute toxicity to *D. magna*.

10.1.4 Measured or estimated chronic toxicity to *D. magna*.

10.1.5 Precision and bias of the analytical method at the planned concentration(s) of test material.

10.1.6 Estimate of toxicity to humans.

10.1.7 Recommended handling procedures (see 8.1).

10.2 *Stock Solutions:*

10.2.1 Stock solutions are usually prepared prior to dosing the dilution water to obtain the desired test concentrations. Water-soluble test materials can often be added directly to dilution water to prepare a stock solution (or in some cases the test solution). Test materials that are moderately soluble or insoluble in water are often dissolved in a solvent to form a stock solution that is then added to dilution water. If a stock solution is used, the concentration and stability of the test material in the stock solution should be determined before beginning the test. If the test material is subject to photolysis, the stock solution should be shielded from light. If the test material hydrolyzes or biodegrades rapidly, it might be necessary to prepare new stock solutions daily.

10.2.2 The preferred carrier for stock solutions is dilution water except possibly for tests on hydrolyzable, oxidizable, and reducible materials. Filtration or sterilization, or both, of the water might be necessary. If the hardness of the dilution water in the test system will not be affected, distilled and deionized water are also acceptable for stock solution preparation. Several techniques have been specifically developed for preparing aqueous stock solutions of slightly soluble materials (**22**). Minimum necessary amounts of strong acids and bases may be used to prepare aqueous stock solutions, but such reagents might affect the pH of test solutions appreciably. Use of a more soluble form of the test material, such as chloride or sulfate salts of organic amines, sodium or potassium salts of phenols and organic acids, and chloride or nitrate salts of metals, might affect the pH even more than the use of the minimum necessary amount of strong acid or base.

10.2.3 If a solvent other than dilution water is used, its concentration in test solutions should be kept to a minimum and should not affect survival, growth, or reproduction of *D. magna*. Because of their low toxicities to aquatic animals (**23**), low volatilities, and high abilities to dissolve many organic chemicals, dimethylformamide and triethylene glycol are often good organic solvents for preparing stock solutions. Other water-miscible organic solvents, such as methanol, ethanol, and acetone, may also be used as carriers, but they might stimulate undesirable growths of microorganisms, and acetone is quite volatile. If an organic solvent is used, its concentration in any test solution should not exceed 0.1 mL/L. Surfactants should not be used in the preparation of stock solutions because they might affect the form and toxicity of the test material in test solutions. (These limitations do not apply to any ingredients of a mixture, formulation, or commercial product, unless an extra amount of solvent is used in the preparation of the stock solution.)

10.2.4 If a solvent other than water is used as a carrier, at least one solvent control, using solvent from the same batch used to make the stock solution, in addition to the dilution-water control, must be included in the test.

10.2.4.1 If the test contains both a dilution-water control and a solvent control, the survival, growth, and reproduction of *D. magna* in the two controls should be compared (see X1.4). If a statistically significant difference in either survival, growth, or reproduction is detected between the two controls, the solvent control is normally used for meeting the requirements specified in Section 14 and as the basis for the calculation of results. Judgment might be required in the choice of which control data to use to compare with treatments, especially when the solvent concentration is not constant in the treatments. If no statistically significant difference is detected, the data from both controls should be used for meeting the requirements specified in Section 14 and as the basis for calculating the results.

10.2.5 If a solvent other than water is used as a carrier, 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 solvent on results of the test.

10.3 *Test Concentration(s):*

10.3.1 If the test is intended to provide a good estimate of the highest concentration that will not unacceptably affect the survival, growth, or reproduction of *D. magna*, the test concentrations (see 12.1.1.1) should bracket the best prediction of that concentration. Such a prediction is usually based on the results of an acute toxicity test (see Guide E729) with the test material using the same dilution water and *D. magna* neonates (for example, individuals less than 24-h old). Because the food used in the life-cycle test sometimes affects the results of the acute test (**24**,**25**), acute tests should be conducted with and without the food added to the dilution water prior to conducting the chronic study. If an acute-chronic ratio has been determined for the test material with a species of comparable sensitivity, the result of the acute test with *D. magna* can be divided by the acute-chronic ratio. Except for a few materials (**26**), acute-chronic ratios determined with daphnids are typically less than ten. Thus, the highest concentration of test material in a life-cycle test with *D. magna* is typically selected to be equal to the lowest concentration that caused adverse effects in a comparable acute test.

10.3.2 In some situations (usually regulatory), it is only necessary to determine whether one specific concentration of test material unacceptably affects survival, growth, or reproduction. These situations usually arise when the concentration resulting from the direct application of a material to a body of water is known, or when the material is thought to be nontoxic at its solubility limit in water. When there is only interest in one specific concentration, it is often only necessary to test that specific concentration (see 12.1.2).

11. Test Organisms

11.1 *Species*—*D. magna* has been extensively used for acute and life-cycle toxicity tests because it is one of the largest cladoceran species, is easy to identify, and is available from many laboratories and commercial sources. These procedures might also be suitable for other daphnid species, although modifications might be necessary. The identities of daphnids obtained from laboratories and commercial sources should be verified, regardless of any information that comes with the organisms. *D. magna* should be verified using the scheme of Brooks (**27**). The identification of other daphnids may vary with the taxonomic reference used (**28**,**29**).

11.2 *Age*—Life-cycle tests with *D. magna* should begin with organisms less than 24-h old.

11.3 *Source*—All daphnids used in a test should be from the same brood stock. This brood stock must have been cultured for at least two generations using the same food, water, and temperature as will be used in the life-cycle test. This will not only acclimate the daphnids, but will also demonstrate the acceptability of the food, water, and so forth, before the test.

11.4 *Brood Stock:*

11.4.1 Brood stock can be obtained from another laboratory or a commercial source. When daphnids are brought into the laboratory, they should be acclimated to the dilution water by gradually changing the water in the culture chamber from the water in which they were transported to 100 % dilution water over a period of two or more days. Daphnids should be acclimated to the test temperature by changing the water temperature at a rate not to exceed 3°C within 12 h until the desired temperature is reached. Generally, acclimation to pH should not exceed more than 1.5 pH units per day.

11.4.2 *D. magna* has been cultured in a variety of systems, such as in large groups in aquaria, in groups of one to five in 100 to 250-mL beakers, or in specially designed chambers (**30**).

11.4.3 To maintain *D. magna* in good condition, the brood stock should be cultured so as to avoid unnecessary stress due to crowding, rapid changes in temperature, and water quality. Daphnids should not be subjected to more than a 3°C change in water temperature in any 12-h period and preferably not more than a 3°C change in any 72-h period. Cultures should be regularly fed enough food to support adequate reproduction. Culture chambers should be cleaned periodically to remove feces, debris, and uneaten food. If culture chambers are properly cleaned and the density of daphnids is kept low, for example, no more than 1 daphnid/30 mL, the surface water/air interface should provide adequate dissolved oxygen. Organisms used for testing must produce at least 60 young per adult during a 21-day test.

11.5 *Food*—Various combinations (see Appendix X2) of trout chow, yeast, alfalfa, and algae, such as *Ankistrodesmus convolutus*, *Ankistrodesmus falcatus*, *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, and *Raphidocelis subcapitata* (formerly *Selenastrum capricornutum*) (**31**), have been successfully used for culturing and testing *D. magna*. The concentration of test material (number of cells for algae) in the batch of food used should be determined. The experience gained over the past decade has shown that it is very important to incorporate algae into the diet to maintain consistently healthy daphnids (**32-34**).

11.6 *Handling*—*D. magna* should be handled as little as possible. When handling is necessary, it should be done gently, carefully, and quickly so that the daphnids are not unnecessarily stressed. Daphnids should be introduced into solutions beneath the air-water interface. Daphnids that touch dry surfaces or are dropped or injured during handling should be discarded. Smooth glass tubes with an inside diameter of at least 5 mm should be used for transferring adult *D. magna*, and the amount of solution carryover should be minimized. Equipment used to handle daphnids should be sterilized between use by autoclaving or by treatment with an iodophor (**35**) or with 200 mg of hypochlorite/L for at least 1 h (see 6.4).