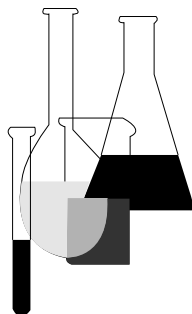




Ecological Effects Test Guidelines

OPPTS 850.1900 Generic Freshwater Microcosm Test, Laboratory



“Public Draft”

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading “Environmental Test Methods and Guidelines” or in paper by contacting the OPP Public Docket at 805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board*. By modem dial 202-512-1387, telnet and ftp: fedbbs.access.gpo.gov (IP 162.140.64.19), or call 202-512-0135 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from the EPA Public Access Gopher (gopher.epa.gov) under the heading “Environmental Test Methods and Guidelines.”

OPPTS 850.1900 Generic freshwater microcosm test, laboratory.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is 40 CFR 797.3050 Generic freshwater microcosm test (proposed in the FEDERAL REGISTER of September 28, 1987 (52 FR 36344)). This guideline may be used with OPPTS 850.7100.

(b) **Purpose.** This guideline is intended for use in developing data on the chemical fate and/or ecological effects of chemical substances and mixtures (“test substances”) subject to environmental effects testing regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94–469, 90 Stat. 2003, 15 U.S.C. 2601 *et seq.*) This guideline prescribes methodologies to predict the potential fate and/or effects of a chemical substance in freshwater ecosystems using various types of microcosms, i.e., standardized aquatic microcosm, naturally derived mixed-flask culture microcosm, or naturally derived pond microcosm, with and without sediment. The microcosms contain freshwater algae and zooplankton with an assortment of unidentified bacteria and fungi. The United States Environmental Protection Agency (EPA) will use data from this test in assessing the potential hazard of a chemical substance to freshwater ecosystems.

(c) **Definitions.** The definitions in second 3 of TSCA and the definitions in Part 792—Good Laboratory Practice Standards apply to this guideline. The following definitions also apply to this guideline:

Aseptic means free from contaminating organisms, e.g., aseptic transfer of an algal culture into a sterilized tube via a sterile inoculating loop.

Axenic means free from other living organisms. An axenic culture (pure culture) of algae contains only one species of algae, no bacteria, and no fungi.

Batch culture means a culture of organisms that use only the initial supply of nutrients in the culture medium. Without replenishment of nutrients, concentrations of nutrients decline and waste products accumulate in the culture medium with the increase in numbers of organisms.

Bioconcentration factor (BCF) means the ratio of the concentration of the test substance in an organism or tissue (i.e., the biota) to the concentration in microcosm water or sediment, as specified.

Carrier means the organic solvent, solubilizer and/or other substance used to disperse the test substance into microcosm water.

Detritivore means an organism (e.g., ostracod) that feeds on detritus, i.e., dead organic matter.

Ecosystem means a community of organisms and its interrelated physical and chemical environment functioning as a unit.

ECX means the experimentally derived test substance concentration, in the aqueous phase, that is calculated to affect X percent of the test species.

Generic microcosm means a general representation of an aquatic ecosystem in which a microcosm is maintained under constant laboratory conditions and no attempt is made to simulate the physical/chemical environment of the natural system.

Gnotobiotic means a culture or community containing only known species or organisms.

Grazer means an animal that grazes or feeds on growing plants, e.g., daphnids, rotifers, and some protozoa.

Herbivore means an animal that feeds on plants, synonymous with grazer.

Linear contrast means the statistical comparison of the means of two treatment groups, e.g., the control and another treatment group.

Medium means the chemically-defined culture solution used in the microcosms.

Microcosm means a miniaturized model of a natural ecosystem.

Naturally-derived means using an assortment of organisms and/or water and sediment collected from one or more natural freshwater ecosystems.

Net daytime production means the increase in dissolved oxygen (DO) concentration in microcosm water during the light phase of the photoperiod.

Nighttime respiration means the decline in DO concentration during the dark phase.

Semicontinuous culture means an algae culture that is periodically harvested by partial draining and replenished with an equal volume of fresh nutrient solution.

Standardized aquatic microcosm (SAM) means a culture of a community containing known species of algae and aquatic invertebrates, but containing uncharacterized species of protozoa and microorganisms.

Treatment group means the replicate microcosms that receive the same amount (if any) of the test substance; controls are treatment groups that receive none of the test substance.

Unialgal culture means the cultivation or growth of a single species of algae; each species of algae is established and maintained in a separate culture.

Xenic means a culture or community containing one or more kinds of unidentified organisms.

(d) **Test procedures**—(1) **Summary of the test.** (i) In preparation for the test, a sufficient number of containers for the test plus an appropriate number of extra containers should be filled with appropriate volumes of nutrient medium or natural water, numbers and types of organisms, and, in some cases, natural or artificial sediment. Microcosm components should be allowed to interact and adjust to one another for a specified period of time. After culling microcosms which deviate most from the group as a whole, microcosms should be randomly assigned to treatment groups and to specific locations in the test area.

(ii) The test should be started by applying the test substance to the microcosms. Appropriate control groups should be established. Microcosms should be sampled and/or monitored for changes in one or more attributes at specified intervals during the exposure period or the recovery period or both. The means of the attributes should be compared using suitable statistical methods to assess the fate or effects of the test substance. Dose-response curves should be plotted for appropriate attributes.

(iii) Microcosms should be monitored for at least 6 weeks after the test substance is applied. Monitoring may be terminated earlier if all test parameters in the treatment microcosms treated with the test substance remain the same as the control microcosms for 2 weeks after the application of test substance (the last application in the case of multiple applications).

(2) **Administration of test substance.** (i) When possible, it is preferred that a test substance be radiolabeled so that its residues can be rapidly and accurately measured by radioassay.

(ii) A test substance that is soluble in water should be dissolved in distilled water to make a stock solution of known concentration; a nominal concentration of test substance could be established in the microcosm by adding a measured volume of stock solution and thoroughly dispersing it by adequate stirring.

(iii) A test substance that is insoluble in water, but that is soluble in relatively non-toxic, water-miscible solvents, such as acetone, should be dissolved in the minimum volume of carrier solvent required to form a homogenous stock solution of known concentration. At the beginning of the test, a measured portion of stock solution should be added to microcosm water and dispersed to form a homogeneous suspension. Carrier controls should be included in the experimental design and monitored simultaneously with microcosms treated with test substance.

(iv) A test substance that is insoluble in both water and water-miscible solvents should be dissolved in more than one carrier, for example, consisting of a lipophilic solvent and an emulsifier, and a measured portion of stock solution should be dispersed into microcosm water to form a homogeneous suspension.

(v) In the pond microcosm, where stirring is hampered by the macrophyte vegetation and the potential siltation of natural sediment, the stock solution of test substance may be mixed thoroughly with 1 or 2 L of water taken from the microcosm, and poured slowly back into the microcosm while the microcosm water is gently stirred.

(vi) Sufficient quantities of stock solution should be made as needed to minimize storage time and disposal volume.

(vii) If the test substance is a formulated preparation, the strength of the active ingredient (AI) in the preparation and the concentration of the test substance in microcosm water should be specified in terms of percent AI.

(viii) The nominal concentration of test substance in both stock solution and microcosm water should be confirmed by chemical analyses at the beginning of the exposure period.

(3) **Range-finding test.** (i) A range-finding test may be conducted to establish if definitive testing is necessary and, if it is necessary, to establish concentrations of the test substance for the definitive test.

(ii) Culled, old control, or newly established microcosms should be exposed for 2 weeks to a series of test substance concentrations (e.g., 0.1, 1.0, 10, and 100 mg/L). Controls should also be used. The exposure period may be shortened if sufficient data are gathered in a shorter time.

(iii) The lowest test substance concentration in a test series, exclusive of controls, should be the lowest concentration which can be analytically quantified. The highest concentration should be 100 mg/L or the maximum water solubility of the test substance at ambient temperature. Replicates are not needed, and nominal concentrations of the test substance are acceptable for range-finding. If all calculated EC50s for all species are greater than 100 mg/L or less than the analytical detection limit, definitive testing is not necessary. However, replicates and measured concentrations of the appropriate dose are needed to substantiate this result.

(iv) A range-finding test is not necessary if data on environmental concentrations of the test substance are available from monitoring studies, or environmental releases of the test substance are known or can be predicted from models, and the objective of the test is to bracket environmental concentrations which result from the releases. Otherwise, a range-

finding test is advisable since microcosm response can differ significantly from single species tests.

(4) **Definitive test**—(i) **Purpose.** The purpose of the definitive test is to determine the potential ecological effects and/or fate of a test substance released into the freshwater environment.

(ii) **Concentration.** At least three concentrations of test substance, exclusive of controls, should be tested. The concentration range selected should define the dose-response curves for major microcosm species between EC10 and EC90, unless a known environmental or release concentration is being bracketed. A minimum of six replicate microcosms should be used for each concentration.

(iii) **Controls**—(A) **General requirements.** Each test should include controls consisting of the same nutrient medium or natural water, types of biological groups, kind and amount of sediment (if present), and otherwise should be treated the same as exposed groups, except that none of the test substance is added. If a carrier is used to dissolve or suspend the test substance, additional controls containing the carrier should also be included in the test to determine any effect of the carrier on the microcosms.

(B) **Standardized aquatic microcosm.** To demonstrate the health of standardized microcosms in use, untreated controls should meet the criteria specified below; otherwise, test data may be rejected by EPA, unless adequately justified.

(I) One day 28, the following criteria should be met in the static microcosms:

(i) At least 90 percent reduction in nitrate (NO₃) concentration.

(ii) Algal biomass in each mL of medium has exceeded $2,000 \times 10^4$ (μm)³.

(iii) Oxygen gain has exceeded 4 mg/L (ppm).

(iv) Population density of daphnids, including members of all size groups, has exceeded 85 *Daphnia* per 100 mL.

(v) Coefficient of variation for each microcosm attribute within ± 0.5 more than 50 percent of the time except as noted; coefficient of variation should not be calculated for any nitrate concentration below 2 μM or for oxygen gain below 1 mg/L (ppm).

(vi) pH values in late-afternoon between 6 and 10; coefficient of variation among replicate microcosms within ± 0.05 more than 50 percent of the time.

(2) From day 28 to the conclusion of the test, the performance of control microcosms should always meet the following criteria:

(i) Algal biomass exceeds 100×10^4 (μm)³/mL per mL.

(ii) Positive oxygen gain in daytime.

(iii) Daphnid population density exceeds 15 Daphnia/100 mL.

(iv) More than 50 percent of the time, the coefficient of variation is within ± 0.5 among replicates of control microcosms for algal biomass, daphnid population density, and for oxygen gain above 1.00 mg/L (ppm).

(v) pH values in late-afternoon between 6 and 9, and coefficient of variation for pH values among control replicates within ± 0.05 more than 50 percent of the time.

(3) When control microcosms fail to meet the above criteria, adequate statistical justification is required for EPA acceptance of test data.

(iv) **Initiation and maintenance of microcosms—(A) Standardized aquatic microcosm.** The standardized microcosm should be initiated and maintained as follows:

(1) At least 36 glass jars (or more if extra controls are needed) should be filled with 3 L of culture medium, 200 g of acid-washed silica sand, 0.5 g of rinsed chitin, and 0.5 g of cellulose powder, and sterilized in an autoclave as specified in paragraph (e)(2)(ii)(A)(2) of this guideline.

(2) On day 0, at least 30 of the 36 autoclaved jars containing sterilized culture media should be inoculated with 10 species of algae at 10^3 cells/mL for each species. Algal cultures are covered and incubated on a white table under adequate illumination.

(3) On day 4, algae cultures should be examined for algal abundance, pH, oxygen gain, and other variables and each jar of algal culture should be stocked with five species of animals, which include both grazers and detritivores. The numbers of microinvertebrates to be added to each liter of algal culture are 110 *Hypotrich* protozoans and 30 *Philodina* rotifers. The volume of media with protozoa and rotifers should not exceed 5 mL. The macroinvertebrates to be stocked into each microcosm include:

(i) Sixteen daphnids consisting of 3 adults with embryos, 3 adults without embryos, and 10 juveniles.

(ii) Six ostracods.

(iii) Twelve amphipods consisting of three mating pairs (if possible) and six juveniles.

(4) On day 7, the 30 microcosms should be reexamined and any outliers should be culled. At least 24 microcosms should be selected for the test. The following attributes of microcosms should be used in the selection of the 24 microcosms:

(i) Dissolved oxygen gain in the daytime.

(ii) pH value (pre-light).

(iii) Abundance of daphnids and the presence of ostracods and amphipods.

(iv) Abundance of *Selenastrum* and *Chlamydomonas*.

(5) Selected microcosms should be randomly assigned to one of the treatment groups including the controls, and located on the support table in a six-block design as follows:

(i) Each of the 24 selected microcosms (the number of microcosms for a typical test) should be randomly assigned to one of the four treatment groups (including the control), appropriately labeled, and treated with appropriate concentrations of the test substance except that the control microcosm does not receive the test substance.

(ii) Each of the six microcosms in each of the four treatment groups should be randomly assigned to one of the six block groups on the table; therefore, each block group has four microcosms, one from each treatment group.

(iii) Finally, each of the four microcosms in each block group should be randomly assigned to one of the four specific locations within that block on the table.

(iv) To facilitate the handling of microcosms during the test, a series of new numbers should be assigned to the microcosms according to their ordered locations on the table.

(6) The test substance should be added after sampling on experiment day 7 (see paragraph (c)(4)(iv)(A)(4) of this guideline).

(7) The standardized microcosm should be sampled and examined at least once every 7 days after the test substance is added and reinoculated as follows:

(i) After sampling and enumeration on each Friday, any microcosm that is underpopulated (less than three individuals) with mature macroinvertebrates should be reinoculated with reproductive age adults so that each microcosm contains at least three individual amphipods, daphnids, and ostracods.

(ii) About 0.05 mL (1 drop) of dense *Hypotrich* protozoan culture and the same volume of dense *Philodina* rotifer culture should be added to each microcosm after each examination.

(iii) Each microcosm should be reinoculated every 7 days with about 0.05 mL of an algal mixture that is prepared by pooling equal volumes of monoculture from each of the 10 algal species.

(B) Naturally-derived mixed-flask microcosm. The mixed-flask microcosm should be initiated and maintained as follows:

(1) A culture medium should be prepared from fresh refrigerated stock solution (warmed to ambient temperature before measuring) in sufficient volume to fill each container with 950 mL of culture medium from the same stock solution.

(2) Stock cultures, which are derived from biotic samples collected from a variety of ecosystems, should be at least 3 months old before they are inoculated into the microcosms.

(3) Each microcosm should contain 50 mL of inoculum, 950 mL of culture medium, and 50 mL of acid-washed sand, and should be randomly assigned to one of the four treatment groups, including controls.

(4) Inoculum in each 50-mL beaker should be placed under microcosm water with the beaker and decanted into the microcosm water to avoid exposing the zooplankton to the air during inoculation and cross-seeding.

(5) Microcosms are placed in the environmental chamber according to a randomized block design.

(6) All microcosms should be cross-seeded at least twice per week for 3 weeks following inoculation. Cross-seeding should be performed by collecting a 50-mL aliquot of a homogeneous suspension from each microcosm, carefully pooling and mixing them together and returning a 50-mL aliquot of the mixture to each microcosm.

(7) Each microcosm should be reinoculated weekly with a 50-mL inoculum.

(8) Following weekly reinoculation, distilled water should be added to each microcosm to return the volume to 1 L to compensate for loss of water through evaporation.

(9) The test substance (and carrier, if needed) should be introduced into appropriate microcosms 6 weeks following initial inoculation of the system.

(C) Naturally-derived pond microcosm. The pond microcosms should be initiated and maintained as follows:

(1) All microcosm components, including water, sediment and biota, should be collected from a single ecosystem, preferably on the same day they are to be used. A shallow pond is the best source of material for pond microcosms, but littoral zones of lakes, or slow-moving rivers, may be acceptable alternatives.

(2) Water should be collected before sediment. At least 60 L of water should be collected from the pond for each microcosm.

(3) Sediment should be collected from the upper 26 cm of the pond bottom and placed in appropriate containers for transportation. Stones, twigs, and other large debris should be removed before the sediment is placed in microcosm containers. At least 12 L of sediment are required for each microcosm.

(4) If a macrophyte community is present in the pond, a portion should be collected from the bottom and placed in an appropriate container. All organisms naturally associated with the macrophyte community may be included in the samples except crayfish. At least 100 gm of the macrophytes is needed for each microcosm. If macrophyte communities are unavailable in the pond, filamentous algae communities may be collected instead if present.

(5) Water, sediment, and biota should be protected from bright sunlight and extreme temperatures, and placed, as soon as possible, in an environmental chamber that is set at a temperature equal to that of the pond water.

(6) The glass aquaria should be positioned in the environmental chamber before filling.

(7) Approximately 12 L of sieved sediment should be placed in each aquarium, resulting in a layer of sediment about 6.7 cm thick. Sediment in each transportation container should be equally divided among all microcosms.

(8) If interstitial water sampling is planned, two suitable water collectors, such as a glass diffuser, should be embedded in the sediment of each microcosm. The fritted-glass disk of the air diffusers should be positioned 4 cm below the sediment surface which is leveled and smoothed.

(9) Approximately 55 L of pond water should be added slowly to each aquarium. Pond water in each transportation container should be equally divided among all microcosms. To minimize resuspension of sediment during water filling, a plastic film may be used to cover the sediment layer and a simple diffuser should be used to dissipate the kinetic force of the water flow. The diffuser may be made of the bottom half of a 4-L polyethylene jug with holes punched around the perimeter.

(10) One hundred grams of drained macrophytes or filamentous algae from the source, such as *Elodea canadensis*, should be planted in the sediment in each microcosm.

(11) After macrophytes are planted, 1 to 2 L of water remaining in the macrophyte collection container should be added to each microcosm as an additional source of biota.

(12) The microcosm should be incubated in the environmental chamber for at least 4 weeks before the test substance is applied.

(13) Distilled water should be added to the microcosms periodically to compensate for the loss of water through evaporation. If a significant volume of microcosm water is removed in sampling, it should be replaced with an equal volume of dechlorinated tap water or well water.

(v) **Sampling procedures**—(A) **Ecological effects.** Sampling of microcosms for routine monitoring and final sampling can be performed as follows:

(1) Each species of macroinvertebrates, including daphnids, ostracods, and amphipods, in the microcosm can be counted visually if the numbers of animals are less than 20 and the water is clear enough for counting. When a dense population or turbid water hampers direct counting of all macroinvertebrates in the microcosm, a series of 100-mL subsamples should be taken out of the standardized microcosm for enumeration of each macroinvertebrate species until 20 of each invertebrate are counted or 6 subsamples are removed, whichever occurs first. Water samples should be quickly captured and confined in a wide-mouth sampler before removal. Periphyton should be scraped from the glass surface and thoroughly dispersed into the culture media preceding sampling of the water column. Zooplankton should be counted in the mixed-flask microcosm by removing a series of 25 mL subsamples. Four such samples are usually sufficient. In the pond microcosm, zooplankton population should be measured twice per week. They are captured with a 2-L beaker that is submerged rapidly into the microcosm water, concentrated on a 80- μ m mesh plankton bucket, stained, and preserved. Population density for three groups of zooplankton, (i.e., cladocera, copepod, and rotifers) should be counted in the pond microcosm: major groups of zooplankton should be identified according to genus, or species if possible.

(2) The population density of protozoa and rotifers should be determined in standardized aquatic microcosms, a water sample of up to 2 mL should be dispersed in a 0.01-, 0.1- or 0.2-mL aliquot on counting plates (e.g., Palmer cell with water depth of 4 mm) at 12 \times magnification under a stereomicroscope. The total volume of aliquots examined should contain at least 50 individuals per species.

(3) The population density of each algal species can be counted twice per week. In the standardized aquatic microcosm, at least 50 cells should be counted for each known algal species from a series of up to 35 fields on the counting chamber under the microscope. If species cannot be identified, the major genus of the phytoplankton and periphyton should be identified for the following groups of algae: diatoms, green algae, euglenoid, and blue-green algae.

(4) Filamentous algae in the algal mat should be examined every 7 days with a microscope to detect the potential extinction of any inoculated algae and the possible presence of contaminant algal species.

(5) The biomass of primary producers should be estimated twice per week with *in vivo* fluorescence or optical density of chlorophyll *a* in acetone solution.

(6) The rate of uptake of dissolved inorganic carbon-14 by phytoplankton should be measured every 7 days as follows:

(i) Primary productivity in each microcosm should be measured in duplicate bottles under the same light intensity as that intensity over the microcosm, with a set of two duplicate bottles placed in the dark as controls.

(ii) Dissolved inorganic ^{14}C should be kept sterile before the test. For example, it may be kept in a sealed ampule and autoclaved.

(iii) About 100 mL of water should be taken from each microcosm, sieved through a 440- μm nylon screen and placed in a 125-mL conical flask.

(iv) The sieved phytoplankton suspension in each flask should be shaken vigorously and poured into a set of four 16.5 mL test tubes until water rises to the rim of each tube, which are then sealed with a serum stopper.

(v) About 10 μCi of ^{14}C -labeled NaHCO_3 (specific activity about 1.0 $\mu\text{Ci}/1.0 \mu\text{g}$) per milliliter of alkaline aqueous solution should be maintained at pH 9.5, packed in a glass ampule, and sterilized after the ampule is sealed.

(vi) About 1 μCi of $\text{NaH}^{14}\text{CO}_3$ in 0.1 mL aqueous solution should be injected into each of the four 16.5-mL test tubes. Two of the tubes should be immediately placed in the dark inside a light-tight box while the other two should be exposed to the same level of light intensity as that prevailing over the microcosms. All tubes should be vigorously shaken during the 2-h incubation.

(vii) After incubation, the phytoplankton culture in each tube should be filtered through a 0.45 μm filter membrane over a vacuum flask. The

membrane filter and the phytoplankton retained on its surface should be dried and stored in a desiccator over silica gel before the radioassay.

(viii) Immediately before liquid scintillation counting, each filter membrane with the phytoplankton materials should be fumed over concentrated hydrochloric acid for at least 90 seconds to remove remaining inorganic ^{14}C , and placed in a counting vial for radioassay.

(ix) The counting rate for each liquid scintillation counting vial that holds the particulate matter from one of the incubation tubes should be properly calibrated for quenching effects.

(x) If the absolute rate of carbon assimilation (besides the relative ^{14}C uptake) is desired, the total dissolved inorganic carbon should be determined. The total content of dissolved inorganic carbon in the microcosm, which affects the specific activity of ^{14}C (added as NaHCO_3) in the incubation tube, should be measured simultaneously with measurement of ^{14}C uptake rate. Total CO_2 content is usually calculated from measured values of total carbonate alkalinity and pH in the microcosm water. It can also be measured by gas chromatography if the buffering capacity of the microcosm medium interferes with the alkalinity-pH method.

(7) The content of chlorophyll *a* in microcosm water should be measured weekly as follows:

(i) A sample of microcosm water, from 30- to 60-mL depending on the standing crop of algae, should be sieved through a 0.3-mm nylon screen to remove any macroinvertebrates among the phytoplankton.

(ii) Sieved microcosm water should be filtered under suction through a 0.45 μm filter pad, which is covered with a fine powder of MgCO_3 at about 10 mg/cm^2 of filter area. Following filtration, phytoplankton on the filter pad should be immediately extracted for chlorophyll *a* or temporarily stored at $-30\text{ }^\circ\text{C}$.

(iii) Retained on the filter pad, the phytoplankton and magnesium carbonate should be placed in a glass, pestle-type homogenizer with 3 to 5 mL of 90 percent acetone and homogenized at 500 rpm for about 1 min.

(iv) After each homogenate is transferred to a graduated centrifuge tube equipped with a cap, the homogenizer and its pestle should be rinsed 2 to 3 \times with 90 percent acetone before its next use. The final volume of pooled homogenate and washes should be adjusted to 15.0 mL.

(v) After the cap is fastened, the centrifuge tube with its contents should be allowed to stand in a dark, cold (below $10\text{ }^\circ\text{C}$) place for at least 1 h, and centrifuged at 4,000–5,000 g for approximately 10 minutes. Any turbid supernatant should be recentrifuged if its absorbance at 750 nm exceeds 0.005 at 1 cm of light path.

(vi) Without disturbing the precipitate, the supernatant in the centrifuge tube should be poured or pipetted into a tube, capped, placed in a dark place, and warmed to room temperature before quantification of chlorophyll *a* by a fluorometric or spectrophotometric method.

(vii) In spectrophotometry, the band-width of each monochromatic light should be 3 nm or less. The absorbance (A) of the acetone extract should be measured at 750, 663, 645, and 630 nm against a 90 percent acetone blank. The concentration of chlorophyll *a* (x) in the acetone extract (in micrograms per milliliter) should be calculated from the length of the optical path (in centimeters) and the absorbance at each of the four wave lengths using the formula:

$$[x] = m11.64(A_{663} - A_{750}) - 2.16(A_{645} - A_{750}) + 0.10(A_{630} - A_{750}) \%$$

(light path).

(viii) The concentration of chlorophyll *a* in a water sample (in micrograms per milliliter) is calculated by multiplying the concentration in the extract by the volume of the extract (in milliliters), and dividing the product by the total volume of the water sample (in liters).

(8) At least twice each week, the peak and troughs on the diel curve of DO in microcosm water can be measured to estimate oxygen gain and loss resulting from daytime photosynthesis and nighttime respiration, respectively. The morning measurement of DO should be taken immediately before the light is turned on, while the afternoon measurement should be taken in the late afternoon or evening after the DO concentration in each microcosm has reached the peak in its diel cycle. At least once during the early part of the study, DO readings should be taken hourly during the light cycle to determine when the peak occurs. The net daytime community production, which is the gain in DO during the 12-hour photophase, should be calculated as the difference between the DO concentration at the end of the photophase and the DO concentration at the end of the preceding dark phase. The net nighttime community respiration, which is the loss of DO in the microcosm during the dark phase, should be calculated as the difference between the DO concentration at the end of the photophase and the DO concentration at the end of the following dark phase.

(9) The pH values of microcosm water should be read to 0.01 unit after the pH meter is calibrated with standard buffers of pH 7 and pH 10, and the pH probe should be rinsed very thoroughly between readings. The pH value should be taken at the same time day on scheduled sampling dates after addition of the test substance to the microcosm as, for example, 0, 1, 2, 3, 5, 7, 10, 14, 21, 28, 35, and 42 days after addition of the test substance. It is preferable to take the pH reading at the end of the dark phase to reflect community respiration or at the end of the photophase to reflect photosynthetic activity.

(10) Dissolved nutrients in the microcosms should be monitored at least twice each week for the first 4 weeks and at least once every 7 days thereafter; the samples should be filtered through a 0.45 μm membrane and kept frozen before they are analyzed by standard analytical methods for soluble reactive phosphorus, ammonia, nitrite, and nitrate.

(11) Net daytime community production and net nighttime community respiration should be measured on scheduled sampling dates as, for example, days 0, 1, 2, 3, 5, 7, 10, 14, 21, 28, 35, and 42 after addition of the test substance.

(12) Biomass decomposition rate, represented by the decomposition rate of ^{14}C -glucose in 15 mL of microcosm suspension, can be measured on scheduled sampling dates as, for example, days 0, 1, 2, 3, 5, 7, 10, 14, 21, 28, and 35, after addition of the test substance to the microcosms. Sampling for biomass decomposition (^{14}C -glucose decomposition) should precede reinoculation if both occur on the same day. The ^{14}C -glucose decomposition should be performed as follows:

(i) A 15 mL water sample should be collected in a 50-mL flask.

(ii) A glucose solution that contains 0.15 μCi radioactivity in 0.3 mL of distilled water should be added to the flask.

(iii) The flask should be immediately sealed with a specially designed serum stopper, fitted with a plastic center well containing a 2×5 cm strip of chromatographic paper, and shaken gently for approximately 15 min in the dark.

(iv) The heterotrophic activity should be stopped by injecting 1.0 mL of 2N H_2SO_4 into the flask. A CO_2 trapping agent, such as carbosorb, should be immediately injected onto each filter paper under the stopper after the acidification to collect the evolving CO_2 .

(v) The flask should be gently shaken for at least 2 h, and the ^{14}C activity in the filter paper should be counted with a liquid scintillation counter.

(vi) The percentage deviation in the counts per minute (CPM) of the treatment from the control should be calculated.

(13) Total alkalinity, dissolved organic carbon, and specific conductivity of microcosm water can be measured weekly.

(14) Interstitial water in the sediment, if present, can be collected weekly to be analyzed for ammonium-nitrogen content. The first 5-mL water sample from the embedded gas diffuser, as specified in the pond microcosm, should be discarded, and the second sample of 10 mL should be filtered before chemical analysis.

(15) Any extinction of macrophytes, such as *Elodea canadensis* in the pond microcosm, in treated microcosms can be noted during the test, and biomass of macrophytes should be determined at the end of the test.

(16) The extinction and reappearance of benthic fauna, such as insects, snails, and oligochaetes, can be observed weekly in those microcosms containing natural sediments.

(17) Water-borne bacteria can be counted weekly.

(B) **Chemical fate.** Sampling should be performed according to the following procedures:

(1) The initial concentration of test substance in microcosm water should be determined by chemical analysis of samples that are taken immediately after the test substance is thoroughly dispersed in microcosm water.

(2) The dissolved test substance, its total residue, or both should be measured in the filtrate of microcosm water semiweekly immediately after the test substance is applied and at least once more during the first week, measured at least once during the second week, and measured biweekly until the end of the test. The filtrate may be substituted with unfiltered microcosm water if the test substance is partitioned into the particulate fraction in such a high proportion that the chemical concentration in the filtrate fraction falls below the analytical detection limit for the test substance using the most practical analytical method.

(3) The concentration of test substance in macrophyte shoots, if present, can be measured biweekly if the sample is less than 5 percent of biomass.

(4) Distribution of the test substance among compartments of microcosms can be determined at the end of the test; the components may include:

(i) Macrophytes, subdivided into roots, shoots, and leaves.

(ii) Phytoplankton.

(iii) Zooplankton.

(iv) Benthic fauna.

(v) Sediment core, sectioned into 1-cm subcores.

(vi) Periphyton, if any.

(5) **Analytical measurements**—(i) **Chemical.** Standard analytical methods should be used in performing analyses. The analytical method used to measure the amount of test substance in a sample should be vali-

dated by appropriate laboratory practices before beginning the test. An analytical method is not acceptable if likely degradation products of the test substance, such as hydrolysis or oxidation products, give positive or negative interference which cannot be systematically identified and mathematically corrected.

(ii) **Numerical.** (A) The following data should be obtained from the standardized microcosm test:

(1) Abundance of each species of alga and macroinvertebrate.

(2) Abundance of each type of microscopic animal (i.e., protozoa and rotifers).

(3) Net daytime production.

(4) Net nighttime respiration.

(5) Chlorophyll *a* concentration.

(6) Water pH.

(7) Nutrients (at least nitrate) in water.

(B) The following data should be obtained from the mixed-flask, microcosm test:

(1) Abundance of phytoplankton and zooplankton.

(2) Net daytime production (DO gain).

(3) Net nighttime respiration (DO loss).

(4) Chlorophyll *a* concentration.

(5) Water pH.

(6) ¹⁴C glucose decomposition rate.

(C) The following data should be obtained for the pond microcosm:

(1) Abundance of phytoplankton and zooplankton.

(2) Abundance of each type of benthic fauna.

(3) Net daytime production.

(4) Net nighttime respiration.

(5) Chlorophyll *a* concentration.

(6) Water pH, alkalinity, conductivity, and dissolved oxygen.

(7) Concentrations of the test substance in each compartment of the microcosm.

(8) Bioconcentration factor.

(D) Means and standard deviations of each chemical and biological attribute specified in this test rule should be calculated for the replicates of each treatment and control groups.

(E) EC50 values and their 95-percent confidence limits should be calculated for each of the appropriate attributes for the time between application of the test substance and recovery from test substance treatment, if data are adequate for statistical analysis. Otherwise, ECX should be calculated as the percent deviation of an attribute in a treatment group from that in the control.

(F) Appropriate statistical analyses (e.g., Dunnett's procedure) should be performed to determine whether significant differences in attributes exist between the carrier (if appropriate) and carrier-free controls and between the control and treated groups, and between microcosms receiving different concentrations of test substance.

(G) For the pond microcosm, appropriate statistical analyses should be performed to determine whether significant differences in the amount and in the bioconcentration factor of the test substance exist between treated different compartments within treated microcosms and between treated microcosms receiving different treatments.

(e) **Test conditions**—(1) **Test species**—(i) **Selection.** (A) The organisms inoculated into the standardized microcosm should include 10 algal species; 1 each of protozoa, rotifer, daphnid, ostracod, and amphipod species; and an uninvited assortment of unidentified heterotrophs, such as bacteria and fungi.

(1) The following 10 species of algae should be included: *Anabaena cylindrica*, *Ankistrodesmus* sp., *Chlamydomonas reinhardi*, *Chlorella vulgaris*, *Lyngbya* sp., *Nitzschia kutzigiana*, *Scenedesmus obliquus*, *Selenastrum capricornutum*, *Stigeoclonium* sp., *Udotheric* sp.,

(2) *Daphnia magna* should be included. Species identity of the test daphnids should be verified using appropriate systematic keys.

(3) Amphipods, *Hyaletia azteca*, also named *H. knickerbockeri*, should be used in the test. Mating pairs and the young are inoculated into the microcosm.

(4) Ostracods chosen should be either *Cypridopsis* or *Cyprinotus* sp. Only adults should be used.

(5) Protozoa should belong to the order *Hypotrichida*, and the culture should be 72–h–old when it is inoculated into the microcosm.

(6) Rotifers should belong to the *Philodina* sp.

(B) Inoculum for the mixed-flask microcosm test should at least contain the following:

(1) Two species of single-celled green algae or diatoms.

(2) One species of filamentous green alga.

(3) One species of nitrogen-fixing blue-green alga.

(4) One species of grazing macroinvertebrate.

(5) One species of benthic, detritus-feeding macroinvertebrate.

(6) Bacteria and protozoa.

(C) The following broad groups of biota should be included in the pond microcosm: Macrophyte, phytoplankton, periphyton, zooplankton, and benthic animals.

(ii) **Source.** (A) Each unialgal culture that is a part of the 10-species composite inoculum for all standardized microcosms in a test should be of the same batch that in turn is subcultured to the exponential growth phase from a single source. Before the test, at least two successive subcultures outside the microcosm are required to acclimate the algal monoculture from agar slant to microcosm medium. A semicontinuous culture system is recommended for culture of unicellular algae. *Anabaena*, *Ankistrodesmus*, *Selenastrum*, and *Lyngbya* should be cultured in batch culture before they are inoculated into microcosms. Recommended procedures for culturing algae as well as the other organisms used in this test are described by Taub and Read under paragraph (g)(2) of this guideline.

(B) The original stock culture for the mixed-flask microcosm should be collected from a variety of natural ecosystems. New stock culture should be added to the old stock cultures at least twice each year. To prepare the inoculum for microcosms, samples from several different aged stock cultures should be mixed together. Stock cultures should be at least 3 mon old to be used as a source of microcosm inoculum. Distilled water should be added to the stock cultures in the open aquaria as needed to replace losses by evaporation. Aquatic organisms collected from a variety of natural ecosystems should be inoculated into culture medium to start stock cultures.

(C) Organisms for the pond microcosm should be obtained from the same natural ponds that supply the water and sediment used in the microcosm.

(2) **Facilities**—(i) **Apparatus.** (A) The environmental chambers or room housing the microcosms should provide adequate environmental controls to meet temperature, irradiation, photoperiod, and air circulation requirements. Chambers should be designed to prevent escape of contaminated internal air into the external environment by using appropriate filtering devices to prevent contamination of the external environment with the test substance.

(B) Laboratory facilities where the test substance is handled should have nonporous floor covering, absorbent bench covering with impermeable backing, and adequate disposal facilities to accommodate liquid waste (e.g., test and waste solutions containing the test substance at the end of each test), and solid wastes (e.g., bench covering, lab clothing, disposable glassware, or other contaminated materials).

(C) The test substance should be stored in a room separate from stock cultures and microcosms.

(D) A large autoclave capable of sterilizing several 1-gal microcosm containers should be used. An autoclave large enough for sterilizing culture medium in a 20-L (5-gal) carboy is desirable.

(E) The dimensions of the bench space for the gnotobiotic microcosms should be at least 2.6×0.85 m and should have a white top or white covering.

(F) Standard laboratory equipment and, if the test substance is radiolabeled, a liquid scintillation counter for radioassays is required.

(G) For the standardized and mixed-flask microcosm tests, a special sampler should be used to capture macroinvertebrates from the microcosm. The sampler should be taller than the microcosm to reach the bottom of the jar, have a large diameter for free passage of water into the sampler, and a rubber stopper attached to a long glass rod to stir the water before sampling and to seal the bottom of the sampler for transferring water out of the microcosm after the sample is taken.

(ii) **Containers and media**—(A) **Standardized microcosm.** (1) The containers used in each standardized microcosm test should be new glass jars with the capacity of at least 1 gal (3.8 L). The jars should be at least 25 cm in height and 16.0 cm in diameter, with an opening 10.6 cm in diameter. The new jars should be washed with diluted (1:10) HCl, flushed with tap water, and rinsed with distilled water before use.

(2) Each standardized microcosm should contain at least 3 L of a medium, such as Taub's T82MV, in addition to an artificial sediment made of silica sand (200 g) enriched with chitin (0.5 g) and cellulose (0.5 g). Before use, the sand should be washed with diluted (1:10) HCl for 2 h, repeatedly rinsed with clean water until the pH rises to 7, and dried in

an oven at 120 °C. The crude chitin from commercial sources should be rinsed with distilled water, air-dried, ground in a blender, and sifted through a 0.4 mm sieve. The cellulose powder, which is also packing material for chromatographic columns, is commercially available.

(B) **Naturally derived mixed-flask microcosm.** Hard-glass containers (e.g., 1-L Pyrex beakers), should be selected for testing organic substances in mixed-flask microcosms. Polypropylene beakers may be used for testing inorganic substances.

(C) **Naturally derived pond microcosm.** For the pond microcosm test, 72-L glass aquaria (60 cm long by 30 cm wide by 40 cm deep) should be used as containers. About 12 L of sieved sediment and 55 L of pond water should be added to each aquarium.

(D) **Materials and equipment.** Materials and equipment that contact test solutions should be selected to minimize sorption of test substances from the microcosm and should not contain substances that can be leached into aqueous solution in quantities that can affect test results.

(iii) **Cleaning and sterilization.** Microcosm containers, stock culture containers, nutrient storage containers, and all other glassware should be cleaned before use. All glassware and equipment should be washed according to good standard laboratory procedures to remove any residues remaining from manufacturing or previous use. Dichromate solution should not be used for cleaning glassware. In the standardized microcosm, all glass containers and equipment for culturing and testing organisms should be sterilized by autoclave where possible. DO and pH probes may be cleaned with ethanol and thoroughly rinsed with distilled water before use. All sampling devices should be sterilized before each test; sampling devices in contact with lake water or sediment should be sterilized after each use.

(iv) **Nutrient media.** (A) Taub's T82MV (see paragraph (g)(2) of this guideline) medium is recommended for use in the standardized microcosm. Its composition is given in the following Table 1.

Table 1.—Nutrient Medium, Taub T82MV

[pH adjusted to 7.0 with dilute HCl (1:10)]

Compound	Molecular weight	Concentration		
		Units	Element of concern	mg/L
		mM		
NaNO ₃	85.0	0.5	N	7.0
MgSO ₄ ·7H ₂ O	246.5	0.1	Mg	2.43
KH ₂ PO ₄	136.0	0.04	P	1.23
NaOH	40.0	0.099	Na	2.27
CaCl ₂ ·2H ₂ O	147.0	1.0	Ca	40.0
NaCl	58.5	1.5	Na	34.5
Al ₂ (SO ₄) ₃ ·18H ₂ O	666.5	0.0048	Al	0.26
Na ₂ SiO ₃ ·9H ₂ O	284.0	0.80	Na	36.8
sand			Si	22.4
Trace Metals:		μM		
FeSO ₄ ·7H ₂ O	278.0	1.12	Fe	0.0625
H ₃ BO ₃	61.8	0.75	B	0.008
ZnSO ₄ ·7H ₂ O	287.5	0.025	Zn	0.0015
MnCl ₂ ·4H ₂ O	197.9	0.25	Mn	0.0135
Na ₂ MoO ₄ ·2H ₂ O	242.0	0.025	Mo	0.0024
CuSO ₄ ·5H ₂ O	249.7	0.005	Cu	0.00032
Co(NO ₃) ₂ ·6H ₂ O	291.0	0.0025	Co	0.00015
EDTA	292.0	1.42	EDTA	0.4145
Vitamins:				
Calcium pantothenate	476.5	1.47	—	0.70
Cyanocobalamin (B ₁₂)	1,355.4	0.000022	—	0.00003
Thiamin (B ₁)	337.3	0.18	—	0.06
Riboflavin (B ₂)	376.4	0.11	—	0.04
Nicotinamide	122.1	1.06	—	0.13
Folic Acid	441.4	0.75	—	0.33
Biotin	244.3	0.12	—	0.03
Putrescine	161.1	0.19	—	0.03
Choline	181.7	2.75	—	0.50
Inositol	216.2	5.09	—	1.10
Pyridoxine monohydrochloride	205.7	2.43	—	0.50

(B) The recommended medium for growth and establishment of stock cultures for the mixed-flask microcosm is Taub's T82, which is the same as T82MV without vitamins. The modified Taub's no. 36 medium (Leffler 1981) under paragraph (g)(1) of this guideline used in the early protocol development is also adequate.

(C) There is no need to add nutrients to pond microcosms.

(3) **Test parameters.** Environmental conditions for the microcosms should be maintained as follows:

(A) Temperature within 21 to 25 °C (23 ± 2 °C).

(B) Photoperiod of 12 h light/12 h darkness.

(C) Standard deviation of light intensities among the microcosms within ± 10 percent of the mean and a light intensity of $150 \mu\text{Em}^{-2}\text{sec}$ for this test.

(e) **Reporting.** (1) The final report should include, but not necessarily be limited to, the following information:

(i) Name and address of the facility performing the study and the dates on which the study was initiated and completed, terminated, or discontinued.

(ii) Objectives and procedures stated in the approved protocol, including any changes in the original protocol.

(iii) Statistical methods used for analyzing the data.

(iv) The test substance identified by name, Chemical Abstract Service (CAS) Registry number or code number, source, lot or batch number, strength, purity, and composition, or other appropriate characteristics.

(v) Stability of the test substance under the conditions of administration.

(vi) A description of the methods used, including the facilities and supporting equipment.

(vii) A description of the test system used, including: Microcosm dimensions and water volume, sediment type and volume if used, temperature, photoperiod, and light intensity over the water surface.

(viii) A description of the organisms included in the microcosms representing various functional groups that are essential for the maintenance of a healthy microcosm.

(ix) A description of the nutrient media, if applicable.

(x) A description of the experimental design, treatment concentrations and media, and pattern of administration.

(xi) The materials, the methods, and the results of any range-finding test.

(xii) For the definitive test, reported results should include:

(A) For the standardized microcosm, a description of the following ecological effects and the fate of the test substance in the biota:

(1) Phytoplankton abundance, in numbers per milliliter, for each species.

(2) Population density of rotifers and protozoans, in numbers per milliliter, for each species.

(3) Abundance of daphnids, in numbers per liter, for each size group (small, medium, and large).

(4) Abundance of amphipods, in numbers per microcosm, for each size group (small and large).

(5) Abundance of ostracods, in numbers per microcosm.

(6) Relative abundance of phytoplankton in microcosms.

(i) Absorbance density at 440 nm, as an index of the particulate materials, including phytoplankton.

(ii) Content of chlorophyll *a*.

(iii) In vivo fluorescence.

(7) Concentrations of major mineral nutrients, such as orthophosphate, ammonia, nitrite, and nitrate in the filtrate of microcosm water.

(8) Primary productivity, as measured by ¹⁴C-uptake methods.

(9) Community production and respiration, measured by the three-point methods (the net gain in dissolved oxygen during the photophase is the photosynthetic production of phytoplankton, while the loss of DO during the dark phase is an indicator of community respiration).

(10) Carrier effects when a carrier is used. Assessed by comparing biological variables in carrier controls to those in plain-water controls.

(11) Chemical effects assessed by comparing biological data in treated microcosms to that in plain-water controls or in combined controls for both the carrier and plain water.

(B) For the mixed-flask microcosm, a description of the following ecological effects and the fate of the test substance in biota:

(1) Phytoplankton abundance for the entire community or standing crop for each of the major species, in number of plants per milliliter.

(2) Zooplankton abundance for the community or standing crop for each life stage of the major species, in numbers of animals per liter.

(3) Type and total number of the benthic organisms, or the standing crop for each species of benthic organism, in numbers of organisms per square meter.

(4) Carrier effects when carrier is used.

(5) Chemical effects assessed by comparing treated microcosms to controls.

(6) EC50 values for the test substance expressed in terms of pH change, net daytime community production, net nighttime community respiration, and decomposition rate of organic matter.

(7) Concentration of test substance residues in aquatic organisms or in specific tissues.

(8) The bioconcentration factors of the test substance or its total residues.

(9) Effect of the initial concentration of the test substance on its bioconcentration factor.

(C) For the pond microcosm, a description of the following ecological effects and fate of the test substance in biota:

(1) Phytoplankton abundance for the entire community or standing crop for each of the major species, number of phytoplankton per milliliter or chlorophyll *a* concentration.

(2) Chlorophyll *a* content of periphyton and the major groups of periphytons, such as diatoms, green algae, blue-green algae, and euglenoid, if possible, genus or species names.

(3) Abundance of macrophytes in the microcosm calculated by estimating the volume of microcosm water occupied by the macrophytes and determining the standing crop of the macrophytes, including tops and roots.

(4) Zooplankton abundance for the community or standing crop for each life stage of the major species, in number of animals per liter.

(5) Type and total number of benthic organisms, or standing crop for each species of benthic organism, in number of organisms per square meter.

(6) Concentration of major dissolved nutrients, such as ammonium-nitrogen, nitrate and nitrite, and orthophosphate, in microcosm water.

(7) Carrier effects when carrier solvent is used.

(8) Chemical effects assessed by comparing treated microcosms to controls.

(9) The median effect concentration (EC50) and its 95-percent confidence limit if the concentration of test substance causes partial reduction in any biological attribute in enough treatment groups. If the partial reduction occurs in only a few treatment groups, indicate the percentage reduction of biological abundance caused by the concentration of test substance (ECX).

(10) Element cycling such as ammonium-nitrogen content, in micrograms per liter.

(11) Maximum and minimum diel DO concentration on sampling date.

(12) Net daytime production and net nighttime respiration, in milligrams per liter of DO change.

(13) Ratio of production to respiration (P/R ratio).

(14) Concentrations of the test substance in both particulate and dissolved fractions of the water column.

(15) Concentration of test substance in representative species of zooplankton and benthos.

(16) Concentration of test substance in periphyton.

(17) Vertical distribution of the test substance in the sediment core.

(18) Concentrations of the test substance in total biota.

(19) Concentrations of the test substance which may include its transformation products, at steady state in the water column and sediment profile, and the amount in the periphyton on the glass surface.

(20) Effect of the test substance concentration applied to the microcosm on the residual concentration of the test substance in each compartment.

(21) Bioconcentration factors of the test substance or its total residues.

(22) Effect of the initial concentration of test substance on its bioconcentration factors.

(D) A description of any circumstance that may have affected the quality or integrity of the data, including reporting and explaining any significant excursions from normal for microcosm parameters during the test.

(xiii) The name of the sponsor, study director, principal investigator, names of other scientists or professionals, and the names of all supervisory personnel involved in the study.

(xiv) A description of the transformations, calculations, or operations performed on the data, and a statement of the conclusion drawn from the analysis.

(xv) The signed and dated reports of each of the individual scientists or other professionals involved in the study, including each person who, at the request or direction of the testing facility or sponsor, conducted

an analysis or evaluation of data or specimens from the study after data generation was completed.

(xvi) The locations where all specimens, raw data, and the final report are stored.

(xvii) The statement prepared and signed by the quality assurance unit.

(g) **References.** The following references should be consulted for additional background information on this guideline, :

(1) Leffler, J.W. *Tentative protocol of an aquatic microcosm screening test for evaluating ecosystem-level effects of chemicals*. Final report, EPA Contract No. 68-01-5043 (Subcontract No. T6411(7197)025 with EPA Office of Toxic Substances, Washington, DC (1981)). Available from J.V. Nabholz, 7403, Environmental Effects Branch, Health and Environmental Review Division, Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, 401 M St., SW., Washington, DC 20460-0001.

(2) Taub, F.B., and Read, P.L. *Standardized aquatic microcosm protocol*. Draft final report, U.S. Food and Drug Administration Contract No. 223-83-7000 with FDA, Washington, DC 20005 (1986). Available from Dr. B.L. Hoffmann, U.S. FDA, HFF-304, Environmental Impact Staff, 1110 Vermont Ave., NW., Suite 710, Washington, DC 20005.