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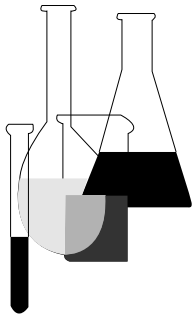
Ecological Effects Test Guidelines

OPPTS 850.1925 Site-Specific Aquatic 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 (703) 305–5805 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 850.1925 Site-specific aquatic 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 797.3100 Site-Specific Aquatic Microcosm Test (proposed in the FEDERAL REGISTER of September 28, 1987 (52 FR 36344)).

(b) **Purpose.** This guideline is intended for use in developing site-specific data on the chemical fate and ecological effects of chemical substances and mixtures (''test substances'') subject to environmental effects test regulations. This guideline prescribes methodologies to predict the potential fate and/or effects of either organic or inorganic substances in a natural aquatic ecosystem using a microcosm made of an indigenous water column and sediment core. This test system is capable of evaluating organic chemical substances, either soluble or insoluble, which may form either air-water surface films or aggregates which sink to bottom sediments. The EPA will use data from this test in assessing the potential hazard of a chemical substance to a particular natural aquatic system (natural system).

(c) **Definitions**. The definitions in section 3 of TSCA and 40 CFR part 792—Good Laboratory Practice Standards apply to this test guideline. The following definitions also apply to this guideline:

Benthic community or benthos means numbers, species composition, size range, and feeding types of organisms present in the sediment of the natural system.

Benthic subsystem means an undisturbed core collected from the natural system and placed in the microcosm.

Bioaccumulation factor or *bioconcentration factor* means the ratio of the concentration of the test substance in an aquatic organism (i.e., biota) to the associated exposure concentration of the test substance from the food particles and the surrounding exposure medium (i.e., water or sediments).

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

Chemical residues means the test substance and its transformation products retained in the water, sediment, surface film, biota, and glass surfaces of the microcosm during the experiment period.

Exposure concentration means the concentration of test substance in the water or the sediment in which the aquatic organisms live.

Natural aquatic system or *natural system* means a particular geographic location consisting of a water column and its associated benthic component.

Radioactivity budget or *radioactivity mass balance* means a quantitative relationship among the input, retention, and export of radioactivity in a microcosm after applying a radiolabeled test substance into the microcosm. The amount of radioactivity added to the microcosm during the test usually is equal to the sum of the radioactivity remaining in the microcosm compartments and the radioactivity exported from the microcosm with the departed water, surface film, and exhaust air.

Ratio of benthic surface area to water volume means the ratio obtained by dividing the calculated benthic surface area of the natural system by the best estimate of water volume of the system.

Sediment means the bottom substrate existing at the mean water depth within the natural system during the period of the test.

Site-specific aquatic microcosm means a miniaturized mimic of a specific natural aquatic system.

Slick protector means a partially submerged glass cylinder within which surface film is removed.

Water column means the water within the natural system or the microcosm tank.

Water flow rates over the sediment surface means the rate of average water flow over the surface of the sediment as measured in the natural system or in the microcosm tank.

Water replacement or *replacement water* means the natural water added to the microcosm at specific intervals to simulate water turnover rate.

Water turbulence means the average water motion in the water column of the natural system or the microcosm tank during the test.

Water turnover rate or *residence time* means the time required for one complete water replacement or exchange within the natural system.

(d) **Test procedures**—(1) **Summary of the test**. A site-specific microcosm is constructed with an indigenous water column and the intact sediment core associated with it. The water and sediment retain their associated organisms in the pelagic and benthic components, respectively, of the natural aquatic system. Environmental variables such as temperature, water turbulence, and water turnover rate are manipulated to be similar to the conditions found in the natural aquatic system. After the test substance is initially introduced into the microcosm, the fate of the test sub-

stance as well as properties indicative of the structure and function of the microcosm are monitored for at least 30 days. Effects of the test substance on the abundance and diversity of aquatic life, and on elemental cycling in the microcosm are determined by comparisons with microcosms that do not contain the test substance.

(2) **Administration of test substance.** (i) Only test substances that are resistant to photolysis (i.e., those having a half-life greater than or equal to 30 days) should be tested in this microcosm system.

(ii) All the test substance added to the microcosms during the study should be accounted for by mass balance. If the test substance is degradable (not persistent), it is recommended that the test substance be radiolabeled.

(iii) Test substances can be either gases, liquids, or solids and may or may not be soluble in water.

(A) If the test substance is soluble in water, it should be dissolved in distilled water to make a stock solution of known concentration. Measured portions of the stock solution should be added to the water in the microcosms and thoroughly dispersed by adequate stirring.

(B) If the test substance is insoluble in water but soluble in a relatively nontoxic, water-miscible solvent such as acetone, it should be dissolved in the minimum volume of carrier solvent required to form a homogeneous stock solution of known concentration. A measured portion of the stock solution should be dispersed into the microcosm water at the beginning of the test to form a homogeneous suspension. Carrier controls should be included in the experimental design and monitored simultaneously with the microcosms treated with the test substance.

(C) If the test substance is a solid and is insoluble in either water or a suitable carrier, it should be ground to a fine powder, weighed to achieve the mass required, and added to a 1–L aliquot of the test water contained in a 2–L separatory funnel. The separatory funnel should be shaken vigorously to achieve as homogeneous a suspension as possible and the suspension should be added to the microcosm water.

(D) If the test substance is a liquid, the measured portion should be added to 1 L of the microcosm water contained in a 2–L separatory funnel, and shaken to achieve as homogenous a suspension as possible. The suspension should be mixed and added to the microcosm tanks.

(E) The amount of test substance remaining in the separatory funnel must be determined by suitable solvent extraction and analyses to accurately determine the amount added to the microcosms.

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

(v) A test substance that is insoluble in both water and water-miscible carriers 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 the microcosm water to form a homogeneous suspension.

(vi) The method and pattern of applying a test substance to microcosms should reasonably reflect the release pattern expected in the natural system. If the input of the test substance to the natural system is other than a one dose application (i.e., multiple application, runoff), the test substance must be added to the microcosm tank in the same manner as the initial dose and each time there is a microcosm water replacement, but only in quantities sufficient to achieve the desired test concentrations in the replacement water.

(3) **Selection of treatment concentration**. (i) Range-finding tests are not recommended, but may be needed to determine treatment concentrations.

(ii) Initially, the microcosms should be treated with concentrations of the test substance that are 0.1, 1, and 10× as high as the average ambient concentration of the test substance observed or predicted in the natural system.

(iii) The test substance should be tested in concentrations of 1, 10, and 100 μg/L, if reliable data on observed or predicted average ambient concentrations are not available.

(4) **Definitive test.** (i) The purpose of the definitive test is to determine the potential fate and ecological effects of a test substance in a specific aquatic ecosystem.

(ii) At least three concentrations of the test substance, exclusive of controls, should be tested for at least 30 days. A minimum of five replicate microcosms should be used for each concentration. All tanks within a given airtight compartment should be treated with the same concentration of the test substance.

(iii) A minimum of five control microcosms should be used in the test for each water-soluble test substance. For those test substances that require a carrier, two of the five control microcosms should be designated carrier controls and treated with the carrier leaving the remaining microcosms as carrier-free controls.

(iv) Two tests are recommended for each test substance. One should be performed in the summer and another in the winter if the fate and ecological effects of the test substance are expected to vary significantly with seasons.

(v) Microcosms should be installed and maintained in the following manner:

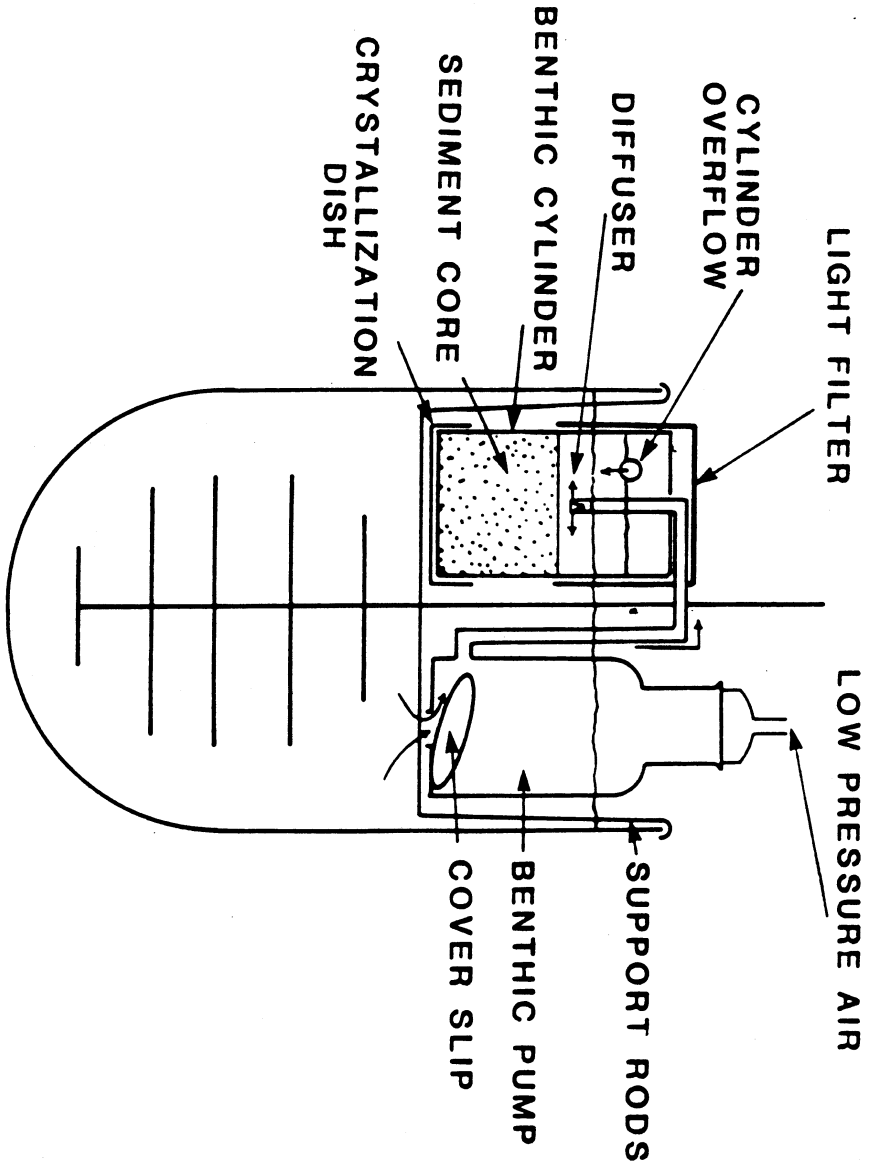
(A) All microcosm tanks should be placed in a water bath maintained within ± 1 °C of the ambient water temperature in the natural system. Water may be pumped from the natural system into the water bath to regulate the temperature in the microcosms if the test laboratory is nearby.

(B) Water for the microcosm should be collected from the natural system, at mid-tide for estuaries, by hand bucketing or nondestructive pumping, e.g., diaphragm pump. If the natural water column in the natural system is stratified, the microcosm water should contain subsamples taken from various depths.

(C) Water samples should be transported to the test facility in glass containers. On arrival at the test facility, water in each container should be distributed equally among microcosms to a prescribed volume of approximately 140 L. Plankton samples must be collected from each microcosm tank and analyzed to ensure homogeneous distribution.

(D) Each sediment core should be collected undisturbed from the natural system by inserting a glass cylinder into the sediment and extracting the core from a prescribed location. The bottom of the core is sealed by seating it in a crystallization dish slightly larger than the cylinder in the following Figure 1. It is desirable to use scuba divers to inspect the uniformity of the benthic component in the natural system, to select representative cores of appropriate length to preserve intact habitats, and to collect the cores with as little disturbance as possible.

FIGURE 1.—EXPERIMENTAL MICROCOSM (NOT DRAWN TO SCALE)



(E) The ratio of benthic surface area to water volume in the microcosm should be made equal to that ratio in the natural system being simulated. Because the water volume in the microcosm is fixed, the desired ratio is obtained by selecting benthic cylinders with the appropriate inner diameter.

(F) The benthic cylinder housing the sediment core should be mounted in the microcosm tank so that the overflow port of the box is 5 cm above the water level in the tank (see Figure 1. in paragraph (d)(3)(v)(D) of this guideline). Any disturbed sediment should be allowed to settle for at least 30 minutes before starting water circulation in the benthic box and water turbulence in the microcosm tank.

(G) The benthic pump should be mounted beside the benthic cylinder with the outlet diffuser of the pump submerged below the surface of the water (overflow port of the cylinder) but above the sediment surface (see Figure 1. in paragraph (d)(3)(v)(D) of this guideline). The rate of water flow over the sediment surface in the microcosm tank should be adjusted to be equivalent to the average water flow rate over the sediment surface in the natural system.

(H) The light intensity over the microcosms should be adjusted to produce an abundance of phytoplankton statistically equivalent to that in the natural system. Preliminary tests should be performed to establish the proper light intensity over the microcosms and should be done with all the microcosm equipment and facilities (i.e., water bath, tank paddle, benthic cylinder and pump) in place. The preliminary tests should be performed at several light intensities for at least 14 days. The photoperiod in both preliminary and definitive tests should be set once every 7 days to match the actual photoperiod within 0.5 h in the location of the natural system.

(I) The light intensity on the surface of the sediment core in the microcosms should be adjusted to the level that is equivalent to the average light intensity on the sediment surface in the natural system. Light intensity can be adjusted by covering the upper portion of the benthic cylinder with a screen, such as a nylon net, or other spectrally-neutral light filters.

(J) The speed of the stirring paddle installed in the microcosm tanks should be adjusted to generate a water turbulence level statistically equivalent to that in the natural system, as measured in the gypsum dissolution method. This method measures the turbulence level by the average dissolution of pure gypsum. Weight loss should be at least 5 to 10 percent. This may take several hours depending on temperature and turbulence. Dissolution rates should be measured and water turbulence adjusted in the microcosms before each test.

(K) Any resuspended sediment that settles on the bottom of a microcosm tank should be collected with a tubing pump and returned to the benthic cylinders when water turnover is simulated.

(L) Water turnover in the natural system should be simulated in the microcosm as follows:

(*1*) A measured portion of the water in each microcosm tank should be replaced at least three times every 7 days with water newly collected from the natural system.

(*2*) The water replacement should match the water turnover rate observed in the natural system.

(*3*) Water replacement should be scheduled immediately after sampling of microcosm water and should occur on the same day.

(*4*) The volume of microcosm water to be removed each time should be the difference between the calculated volume to be replaced and the total volume of water samples removed to keep the water volume at 140 L.

(M) If the test substance accumulates in a thin film on the surface of water in the microcosm tank, a portion of the film should be removed with a filter pad or other absorbent material prior to removal of the volume of water to be replaced. This simulates the surface film advective transport from the natural system. The area (in squarecentimeters) of surface film to be removed should be equal to the product of the ratio of the replacement water volume to total tank volume ratio and the surface area of the tank water, minus the area displaced by the benthic pump and cylinder.

$$\text{Film area removed} = \frac{\text{Replacement water}}{\text{Total tank volume}} \times [\text{Tank water surface area} - (\text{Benthic pump area} + \text{Benthic cylinder area})]$$

(vi) Sampling procedures for the study of chemical fate should be performed as follows:

(A) Water samples should be taken at approximately 0, 1, 2, 3, 6, 12, and 24 h after the initial application of the test substance. Therefore, samples should be taken before each water replacement. Water samples should be collected through a slick protector within which the surface film has been removed. Samples may be taken more frequently to follow the fate of a chemical substance that is disappearing from the system at a relatively rapid rate. The samples should be collected at a location at least

3 cm from the side of the tank and 10 cm below the water surface while both the stirring paddle and the benthic pump are in operation.

(B) If the test substance accumulates in a thin film on the water surface, it should be sampled with a filter pad before each water replacement. The quantity of a radiolabeled test substance absorbed onto the filter membrane can be easily determined with liquid scintillation counting assuming all radioactivity represents the original form of the test substance. If the test substance has degraded, the percentage of the total radioactivity that is the test substance should be determined.

(C) Samples of selected zooplankton species in the microcosm should be collected once every 7 days to be analyzed for the test substance and, if practical, for its transformation products.

(D) Air samples should be collected once every seven days with a suitable sampler. For example, and inverted crystallization dish equipped with inlet and outlet tubes on the side may be placed above the water surface to collect air samples for chemical analysis; fresh air could be drawn by a vacuum pump at the end of the sampling train, entering the modified dish through the inlet tube, sweeping over the water surface, and carrying any volatilized forms of the test substance through the outlet tube to a suitable trap for subsequent quantification. Under the inverted dish, air flow over the water surface should be adjusted to match the flow rate over the rest of the water surface in the microcosm. The duration for each sample collection should be kept as short as possible.

(E) The quantity of test substance adsorbed onto the glass surfaces of the microcosm above and below the water surface should be sampled and estimated as follows:

(*1*) For estimates of the test substance adsorbed onto the glass of the microcosm tanks below the surface, glass rods of known surface area should be suspended in the water column, and removed periodically from the water and placed in a scintillation counting vial for radioassay. If a surface film is present, glass rods should be removed through a slick protector. If possible, the estimated quantity of the radiolabeled chemical substance on the glass surfaces using the glass rod method should be verified with extraction of the test substance from all subsurface glass surfaces whenever a microcosm is sacrificed during the test.

(*2*) A portion of the interior microcosm tank wall extending from the water surface to the lip of the tank should have an appropriate absorbent material attached to it. This material should be removed and extracted at the conclusion of the test to provide an estimate of the amount of the test substance adsorbed to the tank walls above the water.

(*3*) Any unlabeled test substance on the glass surface should be thoroughly extracted and quantified after the water and sediment are removed from the microcosm.

(F) The quantities of the test substance in the benthic component should be determined as follows:

(*1*) One of the five replicate microcosm tanks for each of the three treatments should be randomly selected for sampling and samples of the core contents should be collected on day 10; another of the remaining replicate microcosm tanks should be selected for sampling and samples should be collected on day 20. The three remaining treated replicates and the controls should be sampled at the end of the test on day 30.

(*2*) Three sediment subcores, at least 25 cm in diameter by 7 cm in depth, should be collected from each benthic component to determine the vertical distribution of the test substance in the benthic component, i.e., concentration of test substance in each centimeter of the sediment core.

(*3*) Before triplicate sediment subcores are taken, the surface film (if present) on both the microcosm tank and the benthic cylinder should be removed with suitable tools such as a suction skimmer or a sheet of absorbent material, and the water in both the tank and the benthic cylinder should be drained.

(*4*) Samples of each of the major animal species in the benthic component should be analyzed for the test substance and its transformation products, if possible.

(vii) Sampling procedures for ecological effects study should be performed as follows:

(A) Water samples from microcosms should be taken as described in paragraphs (d)(4)(vi)(A) and (d)(4)(vi)(B) of this guideline.

(B) When water replacement and ecological effects sampling occur on the same day, biological samples should be taken first.

(C) Samples of at least 2 mL of water should be collected daily from the microcosms and such samples should be analyzed for enumeration and identification of phytoplankton.

(D) Samples of at least 2 L of water should be collected from the microcosms at least twice each week and such samples should be analyzed for enumeration and identification of zooplankton and transient larval forms. The water samples should be collected at a rate sufficient to overcome the zooplankters' avoidance reaction and should be screened through a 20–μM plankton net. The retained organisms should be rinsed into a

Petri dish and preserved for subsequent determination of population density and species composition.

(E) The ammonium-nitrogen concentration in the water column of the microcosms and the natural system should be determined once every 7 days.

(F) Population densities of phytoplankton and zooplankton in the natural system should be determined at least twice each week, and ammonium-nitrogen concentration in natural water should be measured at least once every 7 days. This can be done conveniently at the time for water replacement.

(G) The flux rate of ammonium-nitrogen between the benthic component and its associated water column should be determined weekly by stopping the benthic pump for a period of 1 to 3 h. Ammonia concentrations in water above the benthic component should be measured at the beginning and end of this period. The flux rate should be expressed as the weight of ammonium-nitrogen produced by each square meter of sediment surface area per hour.

(H) The abundance and diversity of benthos should be determined. Benthic animals should be captured by sieving the wet sediment through a 0.5 mm screen. All animals retained on the screen should be identified and counted. Similar characterization of the benthic community of the natural system should be established at the time of the experiment.

(5) **Analytical measurements**—(i) **Instrumental methods**. Atomic absorption and gas chromatography are preferable to colorimetric methods for quantitative analyses of metals and organic compounds, respectively. Liquid scintillation counting is recommended for quantitative analysis of radiolabeled test substances, and high-pressure liquid chromatography is recommended in conjunction with liquid scintillation counting for separation and quantification of the test substance and its transformation products.

(ii) **Chemical.** (A) A stock solution of the test substance should be prepared just before use, and its nominal concentration and purity should be confirmed by chemical analysis. Standard analytical methods, if available, should be used to determine the chemical concentration in microcosm samples and stock solution. The analytical methods used to measure all environmental samples should be validated before the beginning of the test.

(B) Concentrations of the test substance, and its transformation products, if possible, should be measured for the following components of the microcosm:

(*1*) Air.

(*2*) Surface film, if present.

(*3*) Water column, both particulate and dissolved fractions.

(*4*) Various layers of the benthic component.

(*5*) Representative species of zooplankton.

(*6*) Representative benthic organisms.

(*7*) Glass surfaces above and below the water surface.

(C) If a radiolabeled test substance is used, a complete budget of all radioactivity should be calculated, including the amount of radioactivity added to the microcosm, removed by gas transport and water replacement, and remaining among the compartments of the microcosm.

(iii) **Numerical**. (A) Mean and standard deviations of biological attributes should be calculated for each treatment and control. The following information should be determined: Abundance of phytoplankton, zooplankton, and each type of benthic fauna. If the species of plankton can be identified, abundance should be calculated for each one.

(B) Statistical analyses should be performed to determine:

(*1*) Whether significant differences exist in biological attributes between:

(*i*) The control microcosms and the natural system.

(*ii*) The carrier control and the carrier-free control.

(*iii*) The control and the microcosms treated with the test substance.

(*2*) Whether significant differences exist in the amount, export, and bioconcentration of the test substance among:

(*i*) Different compartments of the microcosms receiving the same treatment, and

(*ii*) The microcosms receiving different treatments.

(e) **Test conditions**—(1) **Test species.** (i) The organisms tested should include the indigenous fauna and flora representing both the pelagic and benthic communities of the natural system, except the macrofauna.

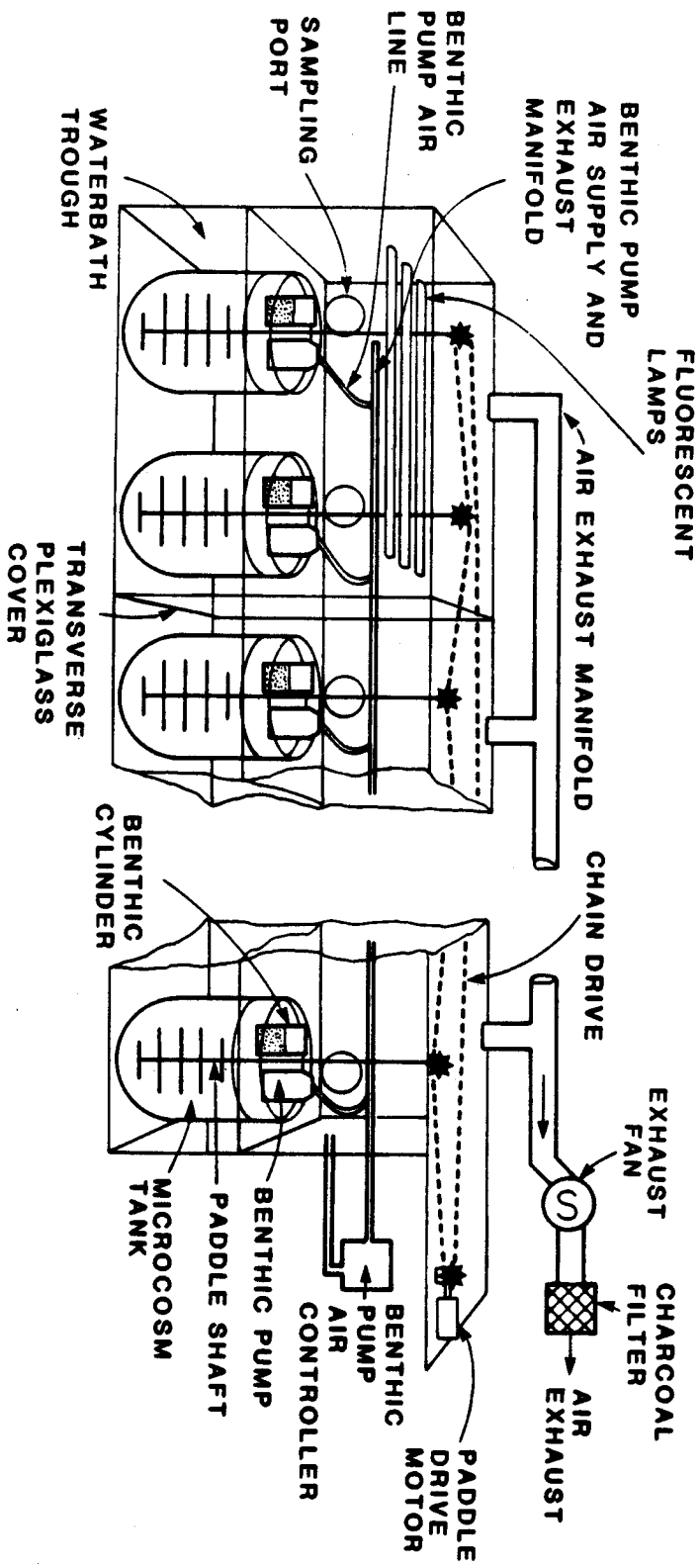
(ii) Neither acclimation nor supplemental food is necessary for the test organisms.

(2) **Facilities**—(i) **Supporting equipment**. (A) The capacity of the water bath used to maintain the water temperature and the flow rate of the water through the water bath should be such that the water temperature

in all microcosms will be kept within ± 1 °C of the ambient water temperature in the natural system.

(B) Cool white fluorescent light should uniformly illuminate the water surface of all microcosms. The fluorescent lights should be mounted on a canopy above the microcosm tanks, (see Figure 2). The desired, uniform light intensity is achieved by wrapping the fluorescent lamps with aluminum foil.

FIGURE 2.—EXPERIMENTAL MICROCOSM FACILITY



(C) In the room containing the microcosms, no light source except that specifically for the microcosms should be allowed.

(D) To match the water turbulence in the natural system, the water turbulence level in the microcosms should be controlled by the speed of an electric motor that is mounted with its chain drive and drive shafts above the canopy and controls the speed of all stirring paddles (see Figure 2 in paragraph (e)(2)(i)(B) of this guideline).

(E) The gypsum dissolution method measures the water turbulence level by the average dissolution rate (i.e., weight loss/time) of cubes (2.5 cm × 1.5 cm × 1.0 cm) of pure gypsum ($CaSO_4$) suspended in the microcosm tank or in the natural system. Gypsum cubes from the same source and lot should be used for the entire set of dissolution tests in the microcosms and in the natural system.

(F) The airspace between the canopy and water bath should be enclosed and sealed with acrylic plastic sheets to facilitate containment of the test substance transported into the gas phase (atmosphere) from the water (see Figure 2 in paragraph (e)(2)(i)(B) of this guideline).

(*1*) The enclosed volume under the canopy and above the water bath should be divided into relatively airtight compartments with Plexiglas panels mounted transversely to the module and extending approximately 5 cm below the water surface of the water bath.

(*2*) Each airtight compartment should have its own air outlet to the exhaust, a removable front cover to facilitate setting up and filling the microcosm tanks, and hinged ports in the front cover to provide access to the tanks during testing.

(G) Airflow over the water surface (microcosms and water bath) in each compartment should be maintained by a manifold connected to an exhaust fan which draws the air from all compartments through its outlet tube and vents the exhaust air through a charcoal filter and a stack outside the laboratory building (see Figure 2 in paragraph (e)(2)(i)(B) of this guideline).

(ii) **Microcosm.** Each microcosm is a multitrophic level model that combines pelagic and benthic communities similar to those existing in the natural system.

(A) Hard glass (e.g., Pyrex) containers are preferred to soft glass or plastic ones for the testing of organic chemicals.

(B) For each experiment, at least 20 microcosm tanks should be required. Each tank, about 140 L in capacity should hold enough water and sediment to support the quantity of benthic invertebrates present in the benthic subsystem, such as a medium-sized shellfish, for 30 days or more.

(C) The benthic cylinder, up to 30 cm tall, should have an inner diameter that makes the ratio of the sediment surface area to water volume in the microcosm equal to that in the natural system.

(D) The benthic cylinder, which holds the sediment core, should be sealed at the bottom end with a crystallization dish.

(E) The benthic pump (see Figure 1, in paragraph (d)(3)(v)(D) of this guideline) should be an all-glass, air displacement pump. It should be large enough to provide the appropriate water flow rate over the sediment surface.

(F) To minimize disturbance of the sediment core by the discharge from the benthic pump, a diffuser should be attached to the water outlet tube of the benthic pump to direct the outgoing water into several horizontal streams over the sediment surface.

(G) If the test substance forms a thin film covering the microcosm water surface, a 6–cm length of glass cylinder, or surface film protector, should be partially submerged in the water to provide a sampling port for uncontaminated water samples after the surface film inside the cylinder is removed.

(iii) **Cleaning**. Microcosm tanks, benthic cylinders, crystallization dishes, benthic pumps, support rack, slick protectors, and glass rods should be cleaned before use. All equipment should be washed according to standard laboratory practices to remove any residues remaining from manufacturing or previous use. A dichromate solution should not be used for cleaning glass containers. Solvents and/or high temperature (450 °C for 8 h) combustion may be necessary to ensure the ultimate cleanliness of the microcosms and associated glass components. If cleansing solvents are used, disposal should conform to existing Federal regulations.

(3) **Test parameters**. Environmental conditions in the microcosm should simulate the natural aquatic system as closely as possible.

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

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

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

(3) Statistical methods employed for analyzing data.

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

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

(6) A description of the methods used, including:

(i) Description of microcosm facilities and supporting equipment; and

(ii) Description of natural system being simulated, including boundaries of natural system, pelagic community, benthic community, sediment type, water quality, history of natural system, light regime, ratio of benthic surface area to the water volume, water turbulence rate, water flow rate over sediment surface, water turnover rate, light intensity over sediment surface, seasonal attributes (e.g., water temperature), and ecological attributes (e.g., productivity).

(7) A description of the test system used, including: microcosm tank size, sediment core size, ratio of benthic surface area to water volume, light intensity on water surface, light intensity on sediment surface, water flow rate over sediment surface, and water turbulence.

(8) A description of the experimental design, treatment concentrations, and methods and pattern of administration. The report results should include:

(i) The results of the preliminary tests.

(ii) For the definitive test, various ecological effects and chemical fate parameters may include:

(A) **Ecological effects**. (*1*) Phytoplankton abundance, in numbers per mL, for the community or for each species.

(*2*) Zooplankton and transient larval forms abundances, in numbers per liter, for the community or for each life stage of each species.

(*3*) Number of organisms in the benthic community or, if known, in each species, expressed in numbers per m^3. Indicate the categories of benthic organisms if species identification is not feasible.

(*4*) Concentrations of major nutrients, such as ammonium-nitrogen, in the water column.

(*5*) Carrier effects when a carrier solvent is used.

(*6*) Assessment of microcosm realism by comparing the biological attributes in the natural system to that in the control microcosms.

(*7*) Effects of the test substance are assessed by comparing the treated microcosms to carrier controls.

(B) **Chemical fate**. (*1*) The concentrations of test substance in representative species of zooplankton and benthic organisms.

(*2*) The amount of test substance transported to the atmosphere.

(*3*) The amount of test substance adsorbed onto the glass surface of the microcosm.

(*4*) The vertical distribution of the test substance in the sediment core of the benthic component.

(*5*) The uptake and biotransformation of the test substance in biota.

(*6*) A mass balance consisting of the total quantity of the test substance added to the microcosm, the quantities exported from the microcosm and the quantities remaining in the microcosm.

(*7*) Concentrations of the test substance and its transformation products, at steady state in the water column and sediment core, and the amount on the glass surfaces both above and below the water surface and on the surface film, if present.

(*8*) The effect of treatments on the residual concentrations of the test substance in each ecosystem compartment.

(C) **Transport of test substance and its transformation products**. (*1*) Amount of test substance and transformation products exported from the microcosm through the air, water replacement, and removal of surface film.

(*2*) The effect of the treatments on the export rate of test substance and transformation products from each ecosystem compartment and on the total amount of test substance being exported.

(D) **Bioaccumulation potential of test substance in aquatic organisms**. (*1*) The concentrations of test substance residues in aquatic organisms (mass of test substance per kilogram wet weight).

(*2*) The bioaccumulation factor for selected benthos as well as water column species, such as zooplankton.

(*3*) The effect of the treatments concentration on the bioaccumulation factor.

(*4*) A description of all circumstances that may have affected the quality or integrity of the data.

(*5*) 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.

(*6*) A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis.

(*7*) 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.

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

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