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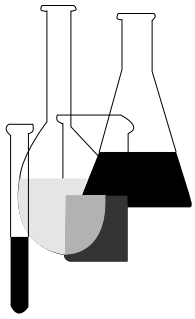
Ecological Effects Test Guidelines

OPPTS 850.1950
Field Testing for Aquatic Organisms



“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 850.1950 Field testing for aquatic organisms.

(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 are OPP 72–7 Simulated or Actual Field Testing for Aquatic Organisms (Pesticide Assessment Guidelines, Subdivision E—Hazard Evaluation; Wildlife and Aquatic Organisms) EPA report 540/09-82-024, 1982 and subsequent guidance on aquatic mesocosm tests under paragraph (e)(3) of this guideline.

(b) **Test standards**—(1) **Test substance.** Unless specified otherwise, data should be derived from testing conducted with an end-use product. An end-use product may be the applicant's own product or a typical end-use product.

(2) **Concentration analysis.** The concentration of the test substance in the water should be determined at the start of the study and samples should be collected periodically for analysis to verify concentrations.

(3) **Test conditions.** The test conditions for conducting field tests should resemble the conditions likely to be encountered under actual use. Specifically, the pesticide should be applied according to the rate, frequency, and method specified on the label.

(4) **Endangered species.** Studies should not be conducted in critical habitats or areas containing, or suspected to contain, endangered or threatened plants or animals which may be threatened by the tests to be conducted.

(5) **Residue levels.** When the test substance is applied under simulated or actual field condition testing, residues should be determined in appropriate vegetation, soil, water, sediments, and other environmental components, and in selected tissues of test organisms.

(6) **Other standards.** Any additional standards for conducting these tests will be provided by the Agency in writing following consultation between the applicant and the Agency, and will take into account the mechanisms by which a pesticide may enter the environment, and the food sources and habitats that may be affected.

(c) **Simulated field studies (mesocosm)**—(1) **Physical description**—(i) **Experimental design.** (A) One acceptable design is a minimum of four experimental treatments consisting of a control which receives no test compound, an *X* treatment level representing expected exposures, an *X*+ treatment level representing an upper bound, and an *X*– treatment level representing a lower bound. At least three replicates per treatment level

are needed to provide the requisite resolution of effects and probability of their occurrence. However, it is recommended that the number of replicates be dictated as a function of the parameters of interest and the sensitivity of their analysis.

(B) Alternative designs which emphasize regression analysis and utilize more treatment levels with fewer or no replicates may also be appropriate. Regression designs are most useful for determining maximum exposure conditions which provide no significant impacts or a specified level of effect in test systems.

(ii) **Mesocosm number.** A minimum of 12 mesocosms is required, with additional mesocosms added as replicates or treatments when needed to increase the sensitivity of analysis for specific parameters.

(iii) **Mesocosm size.** Dimensions of a mesocosm must be large enough to accommodate a viable finfish population. Depth should be sufficient to provide a representative open water area, and sloped sides should provide a littoral area for macrophyte growth and finfish reproduction. An acceptable design would occupy approximately 0.1 acre surface area with a volume of at least 300 m^3 and a maximum depth of 2 m. Sides of the mesocosm should be sloped approximately 1 unit of drop for every 2–3 units of linear distance.

(iv) **Mesocosm features.** (A) Mesocosms can be constructed as dug-out ponds or enclosures of existing impoundments. The mesocosms should be lined with an impervious material of known adsorption for the test compound. The sediment used should be well-defined and representative in composition (percent clay, silt and sand, organic carbon, and organic nitrogen and ion exchange capacity) to pond sediments in the intended use area of the pesticide. The sediment depth at the bottom of the systems should be a minimum of 15 cm. Sediments may consist of natural pond sediment or top soil. If top soil is used, the complete mesocosm should be seasoned for 1 year prior to experimental use. This time is necessary to develop benthic biota. If pond sediments are used, a shorter seasoning period (e.g. 6 mon) is adequate. Organic content of the top soil should be at least 2 percent.

(B) A means of interchange (circulation, fill-drain-refill, etc.) of the water between the systems during initial establishment is desirable to ensure even distribution of biota among the mesocosms. Once the systems have become established or at initiation of a test the circulation should be stopped and each system kept separate from all other systems. The required precautions to ensure no cross contamination from pond overflow during rainstorms, leakage in the circulation system, etc., should be taken from the outset.

(v) **Mesocosm biota.** (A) The mesocosms must contain a representative pond biota. It is recommended that an established pond with diverse

biota act as a parent pond. The water in the mesocosm should be equivalent to the water of the parent pond and biota collected from the parent pond should be evenly distributed to each mesocosm to act as a starter base. Biota from other sources may be used to augment a natural assemblage to ensure adequate representation of important taxa.

(B) Phytoplankton are expected to reach a concentration consistent with the nutrient levels of the system prior to introduction of macroinvertebrates. Nutrient levels should be within a mesotrophic classification. The macroinvertebrate fauna should include representatives of the rotifers, annelids, copepods, cladocerans, amphipods, aquatic insects, and gastropods. Introduced macroinvertebrates, if necessary to augment naturally colonized populations, should not exceed 10 g wet-weight/m^3 and finfish should not be introduced at more than 2 g wet-weight/m^3. Fish species used in the test must be of known sensitivity to the test compound (determined from acute toxicity tests) and appropriate to small pond enclosures. Finfish species used must be native North American species (bluegill sunfish alone or in combination with largemouth bass are recommended).

(vi) **Mesocosm treatment.** Treatment levels of the mesocosms should be based on exposure models and residue monitoring data if available. In a three-replicate by four-treatment design, the three experimental treatments should be separated into a low, intermediate, and high treatment (dosed) and a control treatment (undosed). The intermediate treatment should approximate the estimated environmental concentration determined through modeling and experiential data for the intended pesticide use. It is recommended that the low treatment be 1/10 and the high treatment 10× the intermediate concentration. Regression designs should bracket expected exposures and expected response concentrations. Loading of pesticide into the mesocosms is to be by direct overspray to simulate drift and aerial deposition and with a sediment/water slurry channeled into the system at predetermined points to simulate runoff. Model predictions with available monitoring data will dictate the timing, frequency, and mode of introduction of the test material.

(2) **Measured parameters**—(i) **Chemical/physical properties.** (A) Mesocosm water should be monitored for pH, temperature, transparency (turbidity), dissolved oxygen, alkalinity, total nitrogen, total phosphorus, conductivity (total hardness), and particulate and dissolved organic carbon at appropriate intervals (e.g., biweekly). Observations are to be made at several locations throughout the mesocosm (which will be dictated by the physical design of the mesocosm) and at appropriate depths to allow quantification of vertical and horizontal variations. A complete water analysis should be conducted at test initiation and termination, and at significant periods during the test (i.e., pesticide inputs, substantial changes in other observed parameters, etc.). Temperature, pH, and dissolved oxygen should be monitored on a continuous basis for 24 h on a biweekly schedule and

at significant periods during the test to provide an estimate of gross production and community respiration.

(B) Mesocosm sediment must be analyzed for pesticide content, particle size, cation exchange capacity, organic content, and pH at the initiation of the test.

(ii) **Biological structure.** (A) Biota will be identified to species or lowest taxonomic unit practical. The schedule for sampling and collection of biological samples will depend on the design and composition of the mesocosm and must be determined prior to the initiation of the test. Collections should not be so frequent as to disrupt the system.

(B) Phytoplankton are to be collected from the water column, dominant species identified, and biomass determined by measuring chlorophyll a and phaeophytin. All samples should be preserved for archival reference. Periphyton are to be collected from glass slide substrates placed in the mesocosm and exposed for a minimum of 2 weeks. Periphyton should be analyzed for chlorophyll and ash-free weight. Macrophytes are to be identified to species, biomass determined by dry weight, and percent cover of the mesocosm determined.

(C) Zooplankton will be collected weekly with tube cores of the water column and vertical net tows. All samples are to be archived for future reference. Zooplankton samples will be analyzed biweekly by enumerating and identifying dominant species. Cladocerans should be identified to genus and differentiated by size (e.g., measured for length of muon). Macroinvertebrates, at a minimum, should be collected from emergent insect traps and artificial substrates. Sampling of sediment directly (e.g., Ekman dredge), should be employed cautiously, if necessary for tracking benthic community parameters, to minimize disruption to the benthic community. Samples should be enumerated, identified to lowest practical taxon, and archived.

(D) Finfish will be identified to species, enumerated, sexed (when possible) and measured in length and weight (wet) at introduction into the mesocosms and at test termination. Also at test termination, females will be assessed for fecundity and all collected fish will be examined for gross pathology. Spawning substrates will be placed in the systems and periodically surveyed for number of deposited eggs.

(E) Toxicity testing and bioassays with indigenous fauna on-site and in the laboratory may be used to assist in confirming cause and effect relationships.

(iii) **Residue analysis.** Residues of the test material and major degradates/metabolites will be analyzed at appropriate intervals to the environmental properties of the compound in the water, sediments, and biota at a sensitivity consistent with concentrations of concern.

(iv) **Meteorological conditions.** Continuous monitoring of air temperature, wind velocity, precipitation, evaporation, and solar radiation are required within 1 mile of the mesocosm test facility.

(d) **Actual field studies.** Data from an actual field study are required on a case-by-case basis to support registration of an end-use product intended for outdoor application. Consultation with the Agency is advised before undertaking these tests. Whenever data are required, the determination will be made in writing by the Agency and will state which properties and use patterns of the product were used in the determination.

(e) **References.** The following references can provide useful background information for conducting a simulated or actual field study for aquatic organisms.

(1) Graney, R.L. et al. (Eds.). *Aquatic Mesocosm Studies in Ecological Risk Assessment*, Lewis, Boca Raton, FL (1994).

(2) Hill, I.R. et al. (Eds). *Freshwater Field Tests for Hazard Assessment of Chemicals*, Lewis, Boca Raton, FL (1994).

(3) Touart, L.W. Aquatic Mesocosm Tests to Support Pesticide Registrations. U.S. Environmental Protection Agency, Hazard Evaluation Division; Technical Guidance Document. National Technical Information Service, Springfield, VA) (1988).

(4) Voshell, Jr., J.R. (Ed.). *Using Mesocosms to Assess the Aquatic Ecological Risk of Pesticides: Theory and Practice.* MPPEAL 75 (1989).