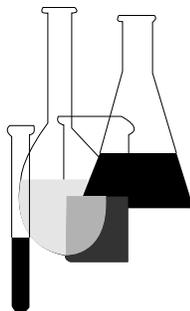




# Microbial Pesticide Test Guidelines

## OPPTS 885.4700 Fish Life Cycle Studies, Tier III



## 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.*).

**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), internet: <http://fedbbs.access.gpo.gov>, or call 202-512-0132 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](http://gopher.epa.gov)) under the heading "Environmental Test Methods and Guidelines."

**OPPTS 885.4700 Fish life cycle studies, Tier III.**

(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.*).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is OPP guideline 154A–28.

(b) **Test standards.** Data must be derived from tests that satisfy the general test standards in OPPTS 885.0001, and the following:

(1) **Test substance.** The actual form of the test material to be used is described in OPPTS 885.0001. In addition, any substances used to enhance virulence should be tested along with the test substance.

(2) **Duration of test.** The fish life-cycle tests require that the test animals be cultured in the presence of the microbial pest control agent (MPCA) during one complete life cycle, i.e. one stage of the life cycle to the same stage of the next generation.

(3) **Test organisms.** (i) Choose the species most likely to be affected by the MPCA being tested. If a microorganism that is closely related (within the same family) to the MPCA causes disease in a certain fish species, that species should be chosen as the test organism.

(ii) Microbial pest control agents that are expected to enter freshwater ecosystems should be tested on freshwater fish. If an estuary is likely to be impacted, marine fish should be used.

(iii) In the event that a species cannot be identified using the foregoing criteria, suggested species are as follows:

(A) Freshwater species—Brook trout (*Salvelinus fontinalis*); fathead minnow (*Pimephales promelas*); bluegill sunfish (*Lepomis macrochirus*); channel catfish (*Ictalurus punctatus*).

(B) Marine/estuarine species—sheepshead minnow (*Cyprinodon variegatus*); Atlantic silverside (*Menidia menidia*).

(4) **Dose levels.** The dose levels shall be equal to that expected to be found in the aquatic environment calculated from application rates with appropriate adjustments to take into account the environmental survival and multiplication characteristics of the MPCA as determined by Tier II testing.

(5) **Test procedure.** Refer to the references under paragraph (e) of this guideline for general guidance in design of fish life cycle tests. The applicant should consult with the Agency for testing details.

(6) **Test duration.** For test species with life cycles of 1 year or less, the test should begin with newly-hatched larvae and continue until 30 days

after progeny hatch. When fish with life cycles that are greater than 1 year, begin the test with fish that are about to commence their first spawn and continue until 90 days after the progeny hatch.

(c) **Reporting and evaluation of data.** In addition to the information specified in OPPTS 885.0001, the test report shall contain the following information on the nontarget test organism:

- (1) Reproductive effects.
- (2) Effects on the growth of larvae and juveniles.
- (3) Detailed records of spawning, egg numbers, fertility, and fecundity.
- (4) Estimated no observed effect level.
- (5) Mortality data.
- (6) Statistical evaluation of effects.
- (7) Locomotion, behavioral, physiological, and pathological effects.
- (8) Definition of the criteria used to determine effects.
- (9) Summary of observed signs of pathogenicity or other effects.
- (10) Isolation, identification, and enumeration of microorganisms responsible for any observed pathogenic effects.
- (11) Stage of life cycle in which effects occurred.

(d) **Tier progression.** (1) Further testing at OPPTS 885.4900 may be required if pathogenic effects are observed.

(2) Additional testing at higher tiers ordinarily is required if pathogenic effects are observed.

(e) **References.** The following may contain useful information for developing acceptable protocols:

(1) American Public Health Association. *Standard Methods for Examination of Water and Wastewater*. 16th edition. American Public Health Assoc., Washington, D.C. (1985), pp. 1268.

(2) Biesinger, K.E. Procedure for *Daphnia magna* tests in standing system. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN (1974).

(3) Biesinger, K.E. Procedure for *Daphnia magna* chronic tests in flowing system. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN (1974).

(4) Biesinger, K.E. Culturing methods for *Daphnia* and certain other cladocerans. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN (1974).

(5) Hansen, D.J. et al. Life-cycle toxicity test using sheepshead minnows (*Cyprinodon variegatus*). Pp. 109–116 in Bioassay Procedures for the Ocean Disposal Permit Program. Environmental Protection Agency, Office of Research and Development EPA–600/9–78–010 (1978).

(6) Nimmo, D.E. et al. Entire life-cycle toxicity test using mysids (*Mysidopsis bahia*) in flowing water. Pp. 64–68 in Bioassay Procedures for the Ocean Disposal Permit Program. Environmental Protection Agency, Office of Research and Development EPA–600/9–78–010 (1978).

(7) Schimmel, S.C. and D.J. Hansen. Sheepshead minnow *Cyprinodon variegatus*: an estuarine fish suitable for chronic (entire life-cycle) bioassays. Proceedings of the 28th Annual Congress of the Southeastern Association of Game-Fish Comm. Pp. 392–398 (1974).

(8) National Water Quality Laboratory Committee on Aquatic Bioassays. Recommended bioassay procedure for fathead minnow *Pimephales promelas* (Rafinesqui) chronic tests. National Water Quality Laboratory, Duluth, MN, 13 pp. (1971) (Revised January 1972.)