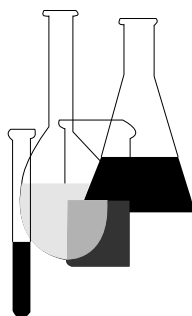




Microbial Pesticide Test Guidelines

OPPTS 885.4200 Freshwater Fish Testing, Tier I



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.*).

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OPPTS 885.4200 Freshwater fish testing, Tier I.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of 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–19.

(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 material to be considered as the test substance is described in OPPTS 885.0001. In addition, any substance used to enhance virulence or toxicity should be tested along with the test substance.

(2) **Test organisms.** (i) Testing shall be performed on one fish species, preferable the rainbow trout if the microbial pest control agent (MPCA) has only a terrestrial use and direct aquatic exposure is not expected, or two fish species, preferably the bluegill sunfish and rainbow trout, when direct aquatic exposure is anticipated. Other species of fish may be used, but a justification must be supplied based on an increased susceptibility to the MPCA or ecological considerations that preclude the use of recommended species.

(ii) The following characteristics should guide species selection:

(A) Fish species likely to prey upon or scavenge the target host organisms should be tested, when applicable.

(B) Testing of young fish is preferable. Very young (not yet actively feeding), spawning, or recently spent fish should not be used.

(C) Fish should weigh between 0.5 and 5.0 g and be from the same year class. The length of the longest fish should be no more than twice that of the shortest fish.

(iii) Ten fish per group should be used in multiple group testing, 30 fish in single group testing.

(3) **Route of exposure.** (i) The test substance shall be administered as a suspension directly into the water (i.e. aqueous exposure).

(ii) Additionally, the MPCA should be administered through the oral route of exposure, preferably through incorporation in standard fish food or through the use of infected insects.

(4) **Maximum hazard dose.** (i) At a minimum, the concentration in the test water (for aqueous exposure) should, whenever possible, be at least 10^6 units/mL or at least 1,000× the maximum calculated pesticide concentration in a 6-in layer of water immediately following a direct appli-

cation to a 6-in layer of water, whichever is greater and attainable. Measures should be taken to ensure that the initial concentration of the MPCA is maintained throughout the test should be described.

(ii) Feed used in the dietary exposure should be supplemented with the test substance to achieve a microbial concentration per gram of food of at least 100× the calculated cell density per milliliter in a 6-in layer of water immediately following a direct application to a 6-in layer of water.

(5) **Controls.** (i) A negative, nondosed control group should be run concurrently the test groups.

(ii) A control group in which the fish are exposed to sterile filtrate from production cultures should be performed concurrently with the test groups.

(6) **Test duration.** The fish should be observed for a minimum of 30 days after dosing. If symptomatology is present at the 30th day, observation should be continued until recovery, mortality, or unequivocal moribundity is established.

(7) **Treatment concentrations.** A single, group of fish may be tested at the maximum hazard dose. If deleterious effects, due either to toxicity or pathogenicity are observed, sequentially lower doses should be tested as described in paragraph (b)(8) of this guideline.

(8) **Determination of LC50 or ID50.** (i) The study endpoint must be chosen to reflect the activity of the specific microorganism under test, i.e. if an MPCA is expected to produce a toxin and has little or no infectivity, the appropriate endpoint would be mortality. If, however, the major mechanism is pathogenicity, a more appropriate endpoint would be overt symptomatology.

(ii) The data should establish that the freshwater fish LC50, defined as the dose required to kill 50 percent of the test organisms, or IC50, defined as the dose necessary to produce overt symptomatology in 50 percent of the test organisms, is greater than the maximum hazard dosage level. If the LC50 or IC50 is lower than the hazard dose, an LC50 or IC50 with confidence intervals should be established.

(c) **Reporting and evaluation of data.** In addition to information meeting the general reporting requirements of OPPTS 885.0001, a report of the results of a fish toxicity and infectivity test must include the following:

(1) LC50 or IC50 determination, including all associated parameters, e.g. slope, goodness of fit, etc., along with the raw mortality data.

(2) Detailed description of the steps taken to determine microorganism dissemination, replication, or survival in any test animals tissues, organs, or fluids.

(3) Detailed description of dilution water, including:

(i) Sterilization method.

(ii) Source.

(iii) Chemical characteristics (e.g. dissolved oxygen content, pH, chlorine content, dissolved salts).

(iv) Pretreatments (if any).

(4) Detailed description of the test, including:

(i) Design.

(ii) Container size.

(iii) Medium (e.g. depth and volume).

(iv) Prophylactic treatments.

(v) Number of organisms per treatment level.

(vi) Loading (weight of organisms per unit volume of medium).

(vii) Lighting, acclimation, and test temperatures (averages and range).

(viii) Amount of test substance administered by each route of exposure.

(ix) Any unusual feature of the test method.

(5) Detailed descriptions of methods (or references to established methods) used for all chemical analyses of water for chemical content and MPCA concentrations.

(6) Detailed description of methods used for all microbial analyses of water, transport hosts and test organisms, and results of such analyses.

(7) Detailed description of the effects of exposure to the test substance including:

(i) The criteria used to determine the effects.

(ii) Percentages of test animals that died or showed symptomology.

(iii) A summary of these observations.

(8) Any additional relevant information about the test or its results that would assist in the determination of hazard potential.

(d) **Tier progression.** (1) If toxic or pathogenic effects are observed, testing at Tier II (environmental expression testing (OPPTS 885.5000, 885.5200, 885.5300, 885.5400,)) is required as specified in 40 CFR 158.740(e). In some cases, a subchronic test may serve to better understanding of the effects observed at the Tier I level and alleviate the need for Tier II testing.

(2) Further testing generally is not required if results of this study do not indicate toxic or pathogenic effects. The Agency may require additional testing if it determines that there is a potential risk to fish despite negative Tier I results.

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

(1) Standard Methods for Examination of Water and Wastewater. 14th Ed. American Public Health Association, Washington, DC (1975).

(2) ASTM-Standard E 729–80, Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

(3) Committee on Methods for Toxicity Tests with Aquatic Organisms. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. USEPA Ecological Research Series, EPA 660/3–75–009. 61 pp. (1975).

(4) Hetrick, F.M. et al. Increased susceptibility of rainbow trout to infectious hematopoietic necrosis virus after exposure to copper. *Applied and Environmental Microbiology* 37:198–201 (1979).

(5) Huang, E. and J.S. Pagano. Nucleic acid hybridization technology and detection of proviral genomes. Chapter 13 in: *The Atlas of Insect and Plant Viruses*, K. Maramorosch, Ed. Academic Press, NY (1977).

(6) Ignoffo, C.M. et al. Susceptibility of aquatic vertebrates and invertebrates to the infective stage of the mosquito nematode *Reesimermis nielsenii*. *Mosquito News* 33:599–602 (1973).

(7) Tatner, M.F. et al. The tissue localization of *Aeromonas salmonicida* in rainbow trout, *Salmo gairdneri* Richardson, following three methods of administration. *Journal of Fish Biology* 25: 95–108 (1984).