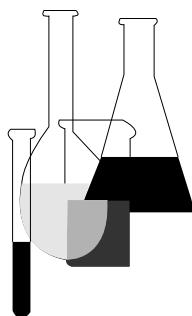




# Microbial Pesticide Test Guidelines

## OPPTS 885.4240 Freshwater Aquatic Invertebrate 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.*).

**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 202-512-1530 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 885.4240 Freshwater aquatic invertebrate 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–20.

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

(2) **Test organisms.** (i) For microbial pest control agents (MPCAs) having terrestrial use patterns, where direct aquatic exposure is not expected, one species of benthic invertebrate should be tested. For MPCAs where direct aquatic exposure is anticipated, testing shall be performed on two aquatic invertebrate species, one of which is planktonic and the other benthic.

(ii) The species of aquatic invertebrate selected should bear as close a taxonomic relationship to the target host as possible.

(iii) Aquatic invertebrate species likely to prey upon or scavenge the diseased target host organisms should be tested, when applicable.

(iv) Larval stages of invertebrates should be used whenever possible.

(v) Twenty invertebrates/group should be used if there are multiple test groups. Fifty invertebrates should be used for single group testing.

(3) **Controls.** (i) A negative, nondosed control group should be performed concurrently with the test groups.

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

(4) **Method of pesticide administration.** (i) The test substance shall be administered as a suspension directly into the water (i.e. aqueous exposure).

(5) **Maximum hazard dose.** 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 application to a 6–in layer of water, whichever is greater and attainable. The initial concentration of MPCA should be maintained throughout the test

and the method used for maintenance of the dose level should be described.

(6) **Test duration.** The test duration should be at least 21 days. If pathogenicity and/or toxicity are apparent at the 21st day, observation should continue until recovery, mortality, or unequivocal moribundity is established.

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

(8) **Determination of LC50 or ID50.** (i) Satisfactory data must establish whether or not the test substance is pathogenic to the test organisms during a sufficiently long period of exposure and observation.

(ii) If the test substance produces a toxin, the data must establish either:

(A) A definitive LC50 value with 95 percent confidence intervals.

(B) That the LC50 is greater than the highest dose.

(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 an aquatic invertebrate toxicity and infectivity test must include:

(1) Raw data and EC50 calculations including 95 percent confidence intervals.

(2) A detailed description of the steps taken to determine microorganism dissemination, replication, or survival in the test animal tissues, organs, or fluids.

(3) A detailed description of dilution water, including source, chemical characteristics (e.g. dissolved oxygen content, pH, dissolved salts), method of sterilization, and pretreatment, (if any).

(4) A detailed description of the test, including:

(i) Design.

(ii) Container size.

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

(iv) Pretreatments, if any.

(v) Method of exposing organisms to the test substance.

- (vi) Number of organisms per treatment.
  - (vii) Lighting, acclimation, and test temperatures (averages and range).
  - (viii) Amount of test substance administered.
  - (ix) Any unusual feature of the test method.
- (5) Detailed descriptions of methods (or references to established methods) used for chemical analyses of water for chemical content and MPCA concentrations.
- (6) Detailed descriptions of methods used for all microbial analyses of water and test organisms, and the 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) Statement of percentages of organisms that died or showed effects of treatment.
  - (iii) A summary of these observations, including changes in life cycle (duration, fecundity, and morphology).
- (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, and 885.5400)) shall be required. 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) If no toxic or pathogenic effects are observed, no further testing at higher tiers ordinarily is required, except as noted in paragraph (d)(3) of this guideline.
- (3) If host spectrum or beneficial insect tests indicate a broad host spectrum such that hazard toward aquatic invertebrates is indicated, either:
- (i) Additional aquatic invertebrate species must be tested as described in paragraphs (b) through (d) of this guideline.
  - (ii) Testing at Tier II, environmental expression (OPPTS 885.5000, 885.5200, 885.5300, and 885.5400)) is required.
- (4) If toxic or pathogenic effects are observed in tests conducted in accordance with paragraph (d)(3)(i) of this guideline, testing at Tier II environmental expression (OPPTS 885.5000, 885.5200, 885.5300, and

885.5400)) is required. If not, no further tier testing is ordinarily required. The Agency may require additional testing, however, if it determines that there is a potential risk to aquatic invertebrates despite negative Tier I results.

(e) **References.** The following references 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). pp. 1193.

(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) Committees on Methods for Toxicity Tests with Aquatic Organisms. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. USEPA Ecological Research Series, EPA 660/375-009. pp. 61.

(4) 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).

(5) 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).

(6) Lightner, D.V. et al. Testing Penaeid shrimp for susceptibility to an insect Nuclear Polyhedrosis virus. *Environmental Entomology* 2:611-613 (1973).

(7) Pagano, J.S. and E. Huang. The application of RNA-DNA cytohybridization to viral diagnostics. In: *Viral Immunodiagnosis*. E. Kurstak and R. Morisset, eds. Academic Press, NY (1974).

(8) Reynolds, G.J. Enzyme labelled antibody in histopathology. *Qualityline* Winter 1978/1979:2-10 (1978).

(9) Summers, M., R. Engler, L.A. Falcon, and P. Vail, eds. *Baculoviruses for Insect Pest Control: Safety Considerations*. Selected papers from EPA-USDA Working Symposium, American Society for Microbiology, Washington, DC (1975).

(10) Undeen A.H. and J.V. Maddox. The infection of nonmosquito hosts by injection with spores of the microsporidan *Nosema algerae*. *Journal of Invertebrate Pathology* 22:258-265 (1973).

(11) Van Essen, F.W. and D.W. Anthony. Susceptibility of nontarget organisms to *Nosema algerae* (Microsporida: Nosematidae), a parasite of mosquitoes. *Journal Invertebrate Pathology* 28:77–85 (1976).

(12) Weber, C.E. (ed.) Biological field laboratory methods for measuring the quality of surface waters and effluents. USEPA Environmental Monitoring Series, EPA–670/473–001 (1973).