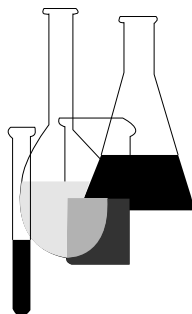




# Microbial Pesticide Test Guidelines

## OPPTS 885.4280 Estuarine and Marine Animal 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), 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 885.4280 Estuarine and marine animal testing.**

(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–21.

(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 material is described in OPPTS 885.0001. In addition, any substances used to enhance virulence or toxicity should be included.

(2) **Test organisms.** (i) Toxicity and pathogenicity shall be determined for one species of shrimp, preferably *Palaemonetes vulgaris* and one estuarine or marine fish species.

(ii) Testing of additional estuarine or marine animal species may be required in Tier I as specified in paragraph (d)(3)(i) of this guideline.

(iii) Estuarine or marine animals likely to prey upon or scavenge the diseased target host organisms should be tested, when applicable.

(iv) Tests should be conducted using young fish, from the same year class, weighing between 0.5 and 5.0 gm. Very young (not yet actively feeding), spawning, or recently spawned fish should not be tested. The length of the largest fish should be no more than twice that of the shortest fish.

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

(ii) A control group in which the animals are exposed to the sterile filtrate from the manufacturing-use product should be performed concurrently with the test groups.

(4) **Method of pesticide administration.** (i) For fish, the microbial pest control agent (MPCA) will be administered as a suspension directly into the water and, separately, incorporated into the food.

(ii) For shrimp, the MPCA will be incorporated into food pellets which are fed to the test organisms.

(5) **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 cell density in a 6-in layer of water immediately following a direct application to a 6-in layer of water, whichever is greater and attainable.

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

(6) **Test duration.** The test should last at least 30 days. If pathogenicity and/or toxicity is apparent at the 30th day, observations should continue until recovery, mortality, or unequivocal moribundity is established.

(7) **Number of organisms per concentration.** The number of test organisms per group should be 10 for fish and 30 for shrimp.

(8) **Treatment concentrations.** A single group 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)(9) of this guideline.

(9) **Determination of LC50 or ID50.** (i) Satisfactory data must establish an IC50 with 95 percent confidence limits or, that the IC50 is greater than the highest dose.

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

(A) A precise LC50 or value with 95 percent confidence intervals, or

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

(c) **Reporting and evaluation of data.** In addition to information meeting the general requirements of OPPTS 885.0001, a report of the results of estuarine or marine animal toxicity and pathogenicity tests must include the following:

(1) LC50 data (if the test substance produces a toxin).

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

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

(4) Other pertinent details, 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 (e.g. placing test substance in water which contains organisms or placing organisms in water which contains the test substance).

(vi) Number of organisms per treatment.

(vii) Loading (weight of organisms per unit volume of medium or unit of surface).

(viii) Lighting.

(ix) Acclimation and test temperatures (average and range).

(x) Salinities.

(xi) Amount of test substance administered.

(xii) Any unusual feature of the test.

(5) Detailed description 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 in 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) A statement of the percentage of organisms that died or showed effects from the treatment.

(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 (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) If no toxic or pathogenic effects are observed, no further testing at higher tiers is ordinarily required, except as noted in paragraph (d)(3) of this guideline.

(3) If efficacy or beneficial insect tests indicate a broad host spectrum such that susceptibility of estuarine or marine invertebrates is indicated, either:

(i) Additional estuarine or marine invertebrate species must be tested following the guidelines in paragraphs (b) through (d) of this guideline, or

(ii) Testing at Tier II environmental expression (OPPTS 885.5000, 885.5200, 885.5300, 885.5400) is required as specified in 40 CFR 158.740(e).

(4) If toxic or pathogenic effects are observed in tests conducted in accordance with the requirements of this section, testing at Tier II environmental expression (OPPTS 885.5000, 885.5200, 885.5300, 885.5400) is required. Otherwise, no further tier testing is required.

(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). pp. 1193.

(2) ASTM Standard E 729–80, Practice for Conducting Static Acute Toxicity Tests with Larvae of Four Species of Bivalve Molluscs. American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

(3) Bioassay Procedures for the Ocean Disposal Permit Program. USEPA, Office of Research and Development. EPA–600/9–78–010;pp. 121 (1978).

(4) Bahner, L.H. et al. A salt-water flow-through bioassay method with controlled temperature and salinity, *Progress in Fish–Culture* 37:126–129 (1975).

(5) Clark, J.R. and R.L. Clark, eds. Seawater systems for experimental aquariums. US Interior Department, Fish and Wildlife Service, Bureau of Sport. Fish. Wild. Research Report No. 63, 192 pp. (1964).

(6) 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. (Marine and estuarine species listed in this publication are acceptable.) (1975).

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- (17) Strickland, J.D.H., and T.R. Parsons. 1968. A practical handbook of seawater analysis. Fish Res. Board Can. Bull. No. 167., 311 pp.
- (18) Summers, M., R. Engler, L.A. Falcon, and P. Vail, eds. 1975. Baculoviruses for Insect Pest Control: Safety Considerations. Selected papers from EPA–USDA Working Symposium, American Society for Microbiology Washington, D.C.
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(20) 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).

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

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