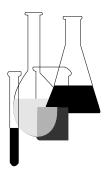


Microbial Pesticide Test Guidelines

OPPTS 885.4650 Aquatic Invertebrate Range Testing, 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.*).

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OPPTS 885.4650 Aquatic invertebrate range testing, Tier III.

- (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 153A–27.
- (b) **Test standards**. Data must be derived from tests that satisfy the general test standards in OPPTS 885.0001 and OPPTS 885.5000 through 885.5400.
- (1) **Test organisms.** Species most likely to be affected by the MPCA should be used in this test. If a microorganism that is closely related (within the same family) to the MPCA causes disease in a certain invertebrate species, that species should be included in this test.
- (2) **Use pattern.** (i) Microbial pest control agents that are expected to enter freshwater ecosystems should be tested on freshwater aquatic invertebrates. Likewise, if an estuary is likely to be impacted, marine invertebrates should be included.
- (ii) Testing should be performed on two members of the following orders:

Freshwater—*Cladocera*, *Copepoda*, *Ephemeroptera*, *Trichoptera*.

Marine—Copepoda, Crustacea.

- (3) **Method of pesticide administration**. The test substance shall be administered either as a suspension in the test water (aqueous exposure) and/or in the diet as determined from results of Tier I tests.
- (4) **Dose levels**. The dose level 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 being tested, as determined by Tier II testing.
- (5) **Test duration**. The test duration shall be the same as Tier I studies.
- (c) **Reporting and evaluation of data.** The requirements in Tier I, OPPTS 885.4050 and 885.4100, apply.
- (d) **Tier progression.** (1) Further testing at Tier IV (OPPTS 885.4750) may be required if pathogenic effects are observed,
- (2) Additional testing at higher tiers ordinarily is not required if pathogenic effects are not observed.

- (e) **References.** The following references should be consulted for additional background material on this test guideline.
- (1) American Public Health Association. *Standard Methods for Examination of Water and Wastewater*. 14th edition, 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) 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).
- (7) Couch, J.A. et al. Environmental significance of baculovirus infections in estuarine and marine shrimp. *Annals N.Y. Academy of Science* 219:528–536 (1975).
- (8) Couch, J.A. Design and Test of a Simple System for the Preliminary Evaluation of Infectivity and Pathogenesis of Insect Virus in a Nontarget Estuarine Shrimp. *Journal of Invertebrate Pathology* 43:351–357 (1984).
- (9) DeBen, E.A. Design and construction of saltwater environment simulator. Federal Water Quality Administration, Pacific N.W. Water Laboratory, Working Paper 71:1–30 (1970).
- (10) Hetrick, F.M. et al. Increased susceptibility of rainbow trout to infectious hematopoetic necrosis virus after exposure to copper. *Applied Environmental Microbiology* 37:198–201 (1979).
- (11) 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).

- (12) Ignoffo, C. M. et al. Susceptibility of aquatic vertebrates and invertebrates to the infective stage of the mosquito nematode, *Reesimermis nielseni*. *Mosquito News* 33:599–602 (1973).
- (13) Lightner, D.V. et al. Testing Penaeid shrimp for susceptibility to an insect Nuclear Polyhedrosis virus. *Environmental Entomology* 2:611–613 (1973).
- (14) 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).
- (15) Reynolds, G.J. Enzyme labelled antibody in histopathology. *Qualityline* Winter 1978/1979:2–10 (1978).
- (16) Shelbourne, HE. 1962. Experimental seawater systems for rearing fish larvae. Pp.81–93 **in** Seawater Systems for Experimental Aquariums. J.R. Clark and R.L. Clark, eds. U.S. Dept. Int., Fish. Wild. Serv., Bur. Sport Fish. Wild. Res. Rep. No.63. 192 pp.
- (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. *Baculoviruses for Insect Pest Control: Safety Considerations*. Selected papers from EPA-USDA Working Symposium, American Society for Microbiology, Washington, DC (1975).
- (19) 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).
- (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 Invertebrate Pathology* 28:77–85 (1976).
- (22) 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).