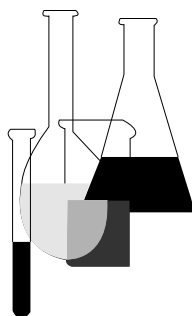




Microbial Pesticides Test Guidelines OPPTS 885.5000 Background for Microbial Pesticides Testing



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) under the heading "Environmental Test Methods and Guidelines."

OPPTS 885.5000 Background for microbial pesticides 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 155A-1.

(b) **Purpose.** Tier II tests are intended to demonstrate whether a microbial pest control agent (MPCA) is able to survive or replicate in the environment, thereby indicating which nontarget organism(s) may be exposed to the microbial agent, as well as provide an indication of the level of exposure. A determination of the environmental expression of an MPCA includes an evaluation of the growth of the agent when introduced into a new environment—the way the MPCA may alter its growth habits, take advantage of new environmental conditions or take advantage of changes in the existing equilibrium among microbial species (e.g. in a commensal association, an association in which one species benefits and the other is unaffected). The expression of a microorganism's presence may result in its insertion into a new habitat and continued propagation in the new habitat. Terrestrial and aquatic (freshwater and marine) environments are to be considered, as indicated by results from Tier I, the product type and the product usage. Advance consultation with Agency personnel is recommended since requirements may be modified on a case-by-case basis. The Tier II guidelines consist of descriptions of tests to determine the environmental expression of an MPCA in a terrestrial environment (OPPTS 885.5200), in a freshwater environment (OPPTS 885.5300), and in a marine or estuarine environment (OPPTS 885.5400). If Tier II data is negative (no adverse effects), no further testing is needed. Tier progression is fully explained in OPPTS 885.0001.

(c) **Definitions.** The following definitions apply to OPPTS 885.5200, 885.5300, and 885.5400.

Contained environment. Refers to the use of natural materials from the proposed use-environment (sediment, soil, plants, and marine/estuarine liquids) arranged as naturally as possible, and held within a plastic, glass, or other container to prevent the escape of the microbial agent.

Substance to be tested. A typical end-use product (EP) or the technical grade of the active ingredient (TGAI) shall be tested.

Observation period. This should be of sufficient duration to determine survival, growth, or decline (to the limit of detection) of the MPCA in the appropriate environment unequivocally. Decline of the MPCA should be followed for a sufficient period of time to detect regrowth of the agent after a prolonged adaptation period.

Regrowth. The number of microorganisms present may decrease initially (perhaps to an undetectable level) and then increase (regrowth) to or above the initial cell density. Observations should continue for a period of time sufficient to assure lack of regrowth. In addition, efforts to resuscitate the MPCA (such as enrichment techniques and/or passage in susceptible hosts) should be attempted to support the conclusion that the microbial agent no longer persists in the environment.

(d) **Approach.** (1) Environmental expression testing consists of simulated terrestrial and aquatic applications of the MPCA. Terrestrial applications are conducted in a contained environment (greenhouse) to assess expression in soil and vegetation. Aquatic applications are conducted in aquariums to assess expression in water and sediment. The need for terrestrial, freshwater, or marine environmental expression testing under specific conditions depends on:

- (i) The Tier I tests in which adverse effects were observed.
- (ii) The intended use patterns for the MPCA.

(2) Testing is only required to assess environmental expression when susceptible nontarget species may be exposed. Tier I tests define the need and the environment to be examined. In some cases, no tests may be needed. For example, if adverse effects are noted with terrestrial plants and the intended use pattern calls for use in freshwater only, probably no additional tests would be required. If additional tests are required (e.g. adverse effects with terrestrial insects and a terrestrial use pattern), the timing (season of use, time of day for release) and location (climate, soil type) of usage, as well as doses shown to be effective in Tier I, must be considered in selecting Tier II conditions for establishing environmental expression of the test microbe. This simulation of the particular natural environment reflecting the area of use of the product is necessary in order to achieve the overall objective of Tier II testing—determining whether the introduced microorganism will survive, multiply, and potentially threaten the nontarget species. The intent is to define potential exposure of susceptible nontarget species to the test product.

(3) Data should be developed which would indicate the dynamics of population fluctuation (i.e. growth and/or survival curves) of the microorganism. The experimental design should consider parameters such as inoculum size, potential for blooms or regrowth, and physical parameters such as relative humidity, pH, and temperature.

(4) The following Table 1. summarizes need for testing under this guideline.

Table 1.—Summary of Environmental Expression Testing as Determined by Tier I Test Results and Use Patterns

Tier I Test with Positive Results (Test Species)	Proposed Use Patterns for MPCA		
	Terrestrial	Freshwater	Estuarine/Marine
OPPTS 885.4050 and .4100			
Avian testing—mallard	NA ¹	F ³	EM ⁴
Avian testing—quail	T ²	NA ¹	NA ¹
OPPTS 885.4150			
Mammalian testing	T	F	NA ¹
OPPTS 885.4200, .4240, and .4280			
Fish testing—freshwater spp.	F	F	NA ¹
Fish testing—estuarine/marine spp.	EM	EM	EM
OPPTS 885.4240 and .4280			
Aquatic invertebrate testing—freshwater spp.	F	F	NA ¹
Aquatic invertebrate testing—estuarine/marine spp.	EM	EM	EM
OPPTS 885.4300			
Terrestrial plant testing	T	NA ¹	NA ¹
OPPTS 885.4300			
Aquatic plant testing—freshwater spp.	F	F	NA ¹
Aquatic plant testing—estuarine/marine spp.	EM	EM	EM
OPPTS 885.4340 and .4380			
Terrestrial insect testing	T	NA ¹	NA ¹

¹ NA: Based on results of Tier I tests and the proposed use pattern, adverse effects are not expected. However, the Agency may require such tests on an individual basis.

² T: Tests to determine expression in a terrestrial environment are necessary.

³ F: Tests to determine expression in a freshwater environment are necessary.

⁴ EM: Tests to determine expression in an estuarine or marine environment are necessary.

(e) **Applicability.** This section outlines the general test standards that apply to the terrestrial, freshwater, and aquatic studies. Applicants for registration should also comply with the specific test standards established for the particular test being conducted. In the case of conflict between general and specific test standards, the latter shall govern.

(f) **Test standards.** Data satisfying the provisions of OPPTS Series 885, Group E should meet the general test standards in OPPTS Series 835—Fate, Transport, and Transformation Test Guidelines, and OPPTS Series 840—Fate and Transport Field Studies Test Guidelines, with the following exception: The most specific available methods for the identification and quantification of the MPCA should be used. The methods used should be consistent with those in OPPTS 885.0001.

(g) **Reporting and evaluation of data—(1) Results.** (i) Data collected to determine whether the MPCA is able to survive, persist, or replicate in the environment under Tier II test conditions is best expressed in the form of a population growth or decline curve for the MPCA, although other applicable methods of presenting the data may be used.

(ii) Test reports should also contain the information designated in the following list, modified as necessary to be applicable to the microbial agent and environment being tested. This information should be given in sufficient detail to adequately define potential impact on growth characteristics of the test organism. In general, the amount of information and kind of data that should be reported in Tier II testing are very similar for any of the environments being examined and are, where applicable, as follows:

(A) Type of system and components used.

(B) Source of components.

(C) Equilibrium period before addition of MPCA.

(D) Temperature, light requirements, humidity, or moisture content, pH of the medium, oxygen requirements.

(E) Type of formulation (EP or TGAI).

(F) Application rate or dose level.

(G) Identity of test substance and composition of formulation.

(H) Characteristics of water in aquatic systems.

(I) Method of application.

(J) Sampling amount, schedule, and sensitivity of detection.

(K) Tabulation/graphs of population dynamics.

(L) Discussion of test results.

(2) [Reserved]

(h) **Tier progression.** If the results of Tier II expression testing in simulated, contained environments show that the MPCA will survive and persist in the environment under study, thereby exposing nontarget organisms to the MPCA, further testing on the Tier III level is required. If the results are negative (the microbial agent does not survive or persist), no further testing is required. Unequivocal determination of negative results may be difficult to prove. Diligent efforts using enrichment techniques may be needed to prove the results are negative and that progression to Tier III is not required.