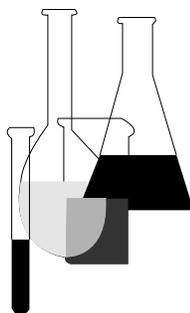




# Microbial Pesticide Test Guidelines

## OPPTS 885.4300

### Nontarget Plant Studies, 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 885.4300 Nontarget plant studies, 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–22.

(b) **Test standards.** In addition to satisfying the applicable general test standards outlined in OPPTS 885.0001, this study should meet the following standards:

(1) **Test substance.** The actual form of the material to be regarded as the test substance is discussed in section OPPTS 885.0001. In addition, any substances used to enhance virulence should be tested along with the test substance.

(2) **Dose levels.** One concentration level equal to no less than the maximum label rate shall be tested. The phrase “the maximum label rate” means the amount of active ingredient in the recommended quantity of carrier, such as water to be used per land area or applied directly to the surface of a 15–cm or 6–in column of water.

(3) **Test species.** The number of species tested depends on the similarity of the microbial pest control agent (MPCA) to known plant pathogens. A rationale for selection of the species to be tested must be provided to the EPA.

(i) **Animal-controlling MPCAs.** (A) When the pesticide is intended to control animals, including insects, the plants to be tested should include six species of Dicotyledoneae of at least four families and four species of Monocotyledoneae of at least two families. These species should be selected from the plants of most important commercial value (Table 1. under paragraph (e) of this guideline).

(B) For MPCAs that have aquatic uses or may be expected to disseminate to, and survive in, aquatic ecosystems, additional aquatic plants must be tested to include: *Selenastrum capricornutum* (a freshwater green alga), *Lemna gibba* (duckweed), *Skeletonema costatum* (a marine diatom), *Anabaena flos-aquae* (a blue-green bacterium), and a freshwater diatom.

(C) EPA will consider requests for waiver of part or all of this requirement if it can be shown that the MPCA occurs naturally in the area of intended usage and the level used does not exceed the naturally occurring concentration.

(ii) **Plant-controlling MPCAs and MPCAs similar to known plant pathogens.** When the pesticide is intended to control plant growth and development (microbial herbicides), or is otherwise closely related to a plant pathogen, in addition to testing the range of plants identified for

animal-controlling MPCAs, testing must be performed on all plants of economic importance (horticultural/agronomic) or known to be beneficial to maintenance of the ecosystem that have any reasonable likelihood of serving as hosts. This selection of additional plant species should be based upon a survey of plants closely related (same genus or, if not available, same family) to the target plant and a survey of known hosts of pathogens closely related to the microbial herbicide.

(4) **Controls.** Both positive and negative controls should be included in the test protocols.

(i) Negative (untreated) controls should be as pest-free as reasonably possible. In addition, in the case of MPCAs that are readily disseminated (wind, insects, etc.), it may be necessary to conduct tests such that negative controls and treated plants are grown in separate geographic locations or in separate contained greenhouses under identical environmental conditions so that reliable negative controls can be maintained. Alternatively, the negative control may be treated with a nonphytotoxic chemical pesticide known to provide effective control of the microbial pesticide. Since it is sometimes difficult to detect adverse effects, such as delayed maturation or loss in vigor, growth, quality, yield, or stand, it is important to analyze untreated controls using a sensitive, specific analytical method to determine whether or not MPCA infection has occurred.

(ii) Positive controls are required for microbial herbicides, or for MPCAs similar to known plant pathogens, in order to ascertain that environmental conditions are such that penetration, infection, and disease development are likely to occur in a susceptible host. The positive control should be selected to closely resemble the subject MPCA in terms of taxonomy and optimal conditions for infection and disease development, if known. In the case of a MPCA not intended for herbicidal use, the positive control may consist of a known plant pathogen, with taxonomic characteristics similar to the MPCA and its susceptible host. In the case of a microbial herbicide, however, the positive control should consist of the target pest weed and the microbial herbicide.

(5) **Environmental test conditions.** When the optimum conditions for penetration, infection, and disease development are known or suspected, it is important, particularly for microbial herbicides, to simulate these conditions rather than those known to be optimum for plant growth and development. In many cases, however, the optimum environment may be similar.

(6) **Application of MPCA.** The test plants should be exposed to the MPCA by whatever route of exposure would be expected by the proposed use pattern. This route of exposure should be supplemented by other routes of exposure if indicated by the mode of transmission of typical pathogens of the test plant or, for microbial herbicides, if indicated by the mode

of transmission of similar plant pathogens. In some cases, wounding of plants or simulation of (or actual) insect vectors-might be appropriate. In other cases, seed treatment, root (soil) application, or foliar spray might be the most appropriate method.

(7) **Timing of application.** Plant test species should be treated at the time of most likely susceptibility (this may be known for microbial herbicides) or at the normal stage of maturity when application to target areas is initiated.

(8) **Observations.** Plants should be observed weekly or more frequently until normal harvest or death, or, in the case of perennials, at regular intervals for at least 2 years. If no obvious adverse effects are evident after these observation periods, the roots, foliage, fruit, vascular tissues, etc. should be analyzed for the presence of the organism using sensitive, specific methods. It is important to complete such analyses because obvious disease development in perennials may take several years and asymptomatic plants may serve as sites for proliferation and survival of the organism, thus providing a reservoir of the organism in the environment.

(c) **Reporting.** In addition to the information specified in OPPTS 885.0001, the test report shall contain the following information.

(1) Rationale for selection of the species tested.

(2) Description of the growth chambers, greenhouse, or other type of test facility including containment provisions and monitoring devices for maintaining proper environmental conditions.

(3) Temperature and humidity ranges, including any significant deviations encountered in course of the experiment.

(4) Photoperiod and lighting.

(5) Any abnormal adverse or beneficial effects in treatment and/or control groups, including dates and times the effects were observed.

(6) Methods for any statistics used for analysis of results.

(d) **Tier progression.** If any adverse effects resulting from infection occur or analyses indicate that asymptomatic infection has occurred, testing at Tier II (Environmental Expression, OPPTS 885.5000, 885.5200, 885.5300, and 885.5400) is required as specified in 40 CFR 158.740. In some cases, a subchronic test may serve to better understand the effects observed at the Tier I level and might alleviate the need for Tier II testing.

(e) **Plant species for testing.** The following Table 1. lists plants of commercial value (see paragraph (b)(3)(i) of this guideline).

**Table 1.—25 Major Agricultural Crops**

Listed by Monetary Value	Listed by Production Weight
corn	corn
soybeans	alfalfa/hay
wheat	wheat
cotton	soybeans
tobacco	sorghum
sorghum	sugarcane
potatoes	sugarbeets
oranges	potatoes
barley	oats
rice	oranges
grapes	grapes
peanuts	apples
apples	cotton
sugarbeets	grapefruit
sugarcane	peanuts
tomatoes	plums
lettuce	sunflower
oats	barley
strawberries	peaches
beans	tobacco
onions	beans
peaches	cucumbers
almonds	rice
sunflower	rye
broccoli	peas

NOTE: Depending upon the predicted use pattern, certain forest tree species, ornamental trees and shrubs, and weed species may need testing.