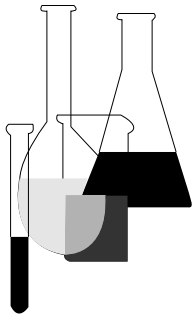
United States
Environmental Protection
Agency

Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–96–336
February 1996

Microbial Pesticide Test Guidelines

OPPTS 885.4340 Nontarget Insect 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) under the heading ‘‘Environmental Test Methods and Guidelines.’’

OPPTS 885.4340 Nontarget insect 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–23.

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

(1) **Test substance.** The actual form of the material to be regarded as the test substance is discussed in OPPTS 885.0001. In addition, any substances used to enhance virulence should be tested along with the test substance. Whenever feasible, dosage shall be in suitable increments up to 100× the LD50 or LC50 of the pathogen in its natural host, or 10–100× the recommended field dosage.

(2) **Test species.** (i) Testing should be performed on three species of insects, representing at least two of the following groups—parasitic dipterans, predaceous hemipterans, predaceous coleopterans, predaceous mites, predaceous neuropterans, parasitic hymenopterans.

(ii) Selection of test species is the responsibility of the researcher. Rationale for selection must be provided. More specifically, the following points apply:

(A) **Viruses.** Testing shall be performed on three species of insects representative of those parasites and predators known or suspected to attack the target host or to share the same ecological habitat.

(B) **Protozoa.** In selecting the three species of nontarget arthropods, at least one should be a major (i.e. important) parasite of the target host. Many protozoans have a wide host range. Accordingly, if possible, more than the minimum (three) species of nontarget organisms should be tested.

(C) **Fungi.** Assuming testing is justified, appropriate organism test species should be the major predacious and parasitic regulatory agents common to the ecosystem where the MPCA will be applied. In addition, testing could include parasites and/or predators specific to the host of the fungal agent.

(D) **Bacteria**. Testing shall be performed on three species of insects representative of those parasites and predators known or suspected to attack the target host or to share the same ecological habitat.

(3) **Controls**. A concurrent control group is recommended and should be treated with microbe-free (or nonviable microbe) material from the culture system used for propagation of the microbial pest control agent.

(4) **Routes of exposure**—(i) **Viruses.** The best routes of exposure will depend on the developmental location of the nontarget organism. Internal parasites may be tested with virus-infected hosts, or if they can be cultured in vitro, the virus can be added to the diet. External stages of parasites and predators, if they are obligatory, may be fed virus-infected hosts, and others may be fed virus-contaminated media or virus suspended in sugar or honey solutions.

(ii) **Protozoa.** (A) In addition to feeding adult predators and parasites of the target insect with the resistant stage of the protozoan, immature stages of the predator or parasite should be exposed. Predators can be fed hosts infected with the protozoan over a period of time. The predator, at a prescribed time, should be checked for protozoan infection.

(B) The protocol for parasites is more complex. Protozoan-infected hosts can be parasitized or parasitized hosts can be fed protozoans. Parasites from protozoan-infected hosts should be examined for protozoan infection. The immature stages as well as adults should be examined. If possible, a primary hymenopterous and dipterous parasite should be examined.

(C) The best route of infection for adult *Hymenoptera* or *Diptera* is oral acquisition of protozoan spores. Predaceous insects could also be fed in this manner, but feeding them infected (live) hosts of known age is more appropriate. In either case, the actual amount of spores consumed cannot be accurately determined. If infection of the parasite or predator adult occurs, the possibility for transovarial or transovum transmission should be examined.

(iii) **Fungi.** Routes of exposure should simulate field conditions as much as possible. In the case of entomopathogenic fungi, environmental conditions (> 90 percent relative humidity) are critical at the time of exposure.

(iv) **Bacteria.** Best routes of exposure will depend on developmental location of the nontarget organism. Internal parasites may be tested with bacteria-infected hosts, or if they can be cultured in vitro, the bacteria can be added to the diet. External stages may be fed bacteria-infected hosts, bacteria-contaminated media, or bacteria suspended in sugar or honey solutions.

(5) **Duration of test/endpoints**—(i) **Viruses.** (A) Control and treated insects should be observed for a duration of at least 30 days after dosing, or in cases where an insect species cannot be cultured for 30 days, until control mortality rises above 20 percent. In cases where signs, symptoms and pathologies are detected, the treated insects should be examined in detail at late stages of infection, at moribund, and at death. Such tests need not be prolonged to 30 days, if death of treated insects occurs prior

to 30 days. In all cases of pathologies in the treated nontarget insects, it is essential that the etiology of the infection be established.

(B) The end-points should be based on the frank development of pathologies and on the early mortality of the treated as compared to the untreated (control) nontarget organisms.

(ii) **Protozoa.** (A) Test duration should be determined on a case-by-case basis. The most appropriate end-point for protozoan diseases for determining pathogenic effects is the presence of the vegetative stages (shizonts or meronts) in the tissues of nontarget insects. The schizonts within suspected tissues can be detected by making a smear, staining with Giemsa stain, and examining the slide with oil immersion using a compound microscope. The nontarget insect should be alive when the tissues are removed for the smear because the shizonts are fragile and are usually destroyed by other microbes or are distorted upon death of the host.

(B) Protozoan spores can be used as an indicator of infection. However, if the infection is light, the few spores could have come from the inoculum. If spores are abundant (a relative term) and occur in the tissues of the nontarget insect, it is likely that it is infected.

(C) Another way to confirm infection is to conduct histological studies of the tissues using standard methods and looking for spores and other pathological effects. The end-point would be just before death of the organism or a prescribed period of time.

(D) Death of the nontarget insect is a good end-point if the protozoan is virulent. However, since most protozoans have chronic effects on their host, changes in behavior, size, or color could be used as an end-point. In each case, a microscopic examination to find schizonts or spores is essential to confirm the presence of the protozoan. Koch's postulates to confirm the virulence of the isolate should be run.

(iii) **Fungi.** (A) With entomopathogenic fungi, test duration can be limited to 8–10 days.

(B) Mortality time, expressed as LT50 (time course of population mortality), is considered the most reliable parameter for bioassaying fungi of insects in the laboratory. Pathogenicity should be confirmed by identifying the fungal agent as the original inoculum.

(iv) **Bacteria.** Control and treated insects should be observed for 21 to 30 days after dosing, if this is possible. In cases where the insect species cannot be cultured for 21 to 30 days, observation should continue until control mortality rises above 20 percent.

(c) **Reporting and evaluation of data.** The reporting provisions are the same as those specified in OPPTS 885.0001.

(d) **Tier progression.** (1) Data derived from Tier I testing will be used in conjunction with available information on use pattern, host range, and other similar factors, to assess potential for adverse effects. If data indicate potential for adverse effects, Tier II testing will be 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.

(2) If no toxic or pathogenic effects are observed in this study, additional testing is ordinarily not necessary.

(e) **References.** The following references are provided for use in the development of acceptable test protocols for conducting toxicity/pathogenicity tests with microbial pesticides:

(1) Andreadis, T.G. *Nosema pyrausta* infection in *Macrocentrus grandii*, a braconid parasite of the European corn borer, *Ostrinia nubilalis*. *Journal of Invertebrate Pathology* 35:229–233 (1980).

(2) Brooks, W.M. Protozoa: Host—parasite—pathogen interrelationships. *Miscellaneous Publication of the Entomological Society of America* 9:105–111 (1973).

(3) Gardner, W.A. et al. Susceptibility of the two-spotted spider mite, *Tetranychus urticae* Koch, to the fungal pathogen *Hirsutella thompsonii* Fisher, *Florida Entomology* 65:458–465 (1982).

(4) 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).