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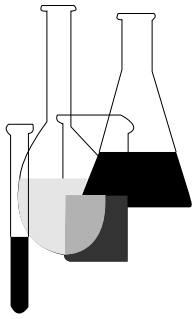
Microbial Pesticide Test Guidelines

OPPTS 885.3600 Subchronic Toxicity/ Pathogenicity



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 885.3600 Subchronic toxicity/pathogenicity.

(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 the OPP guideline 152A–21.

(b) **Purpose.** Subchronic toxicity/pathogenicity data provide information on health hazards likely to arise from subchronic (90–day) exposure to an MPCA preparation.

(c) **Principle of the test method.** The test substance is administered daily to experimental animals at a single high dose level for a time period of at least 90 consecutive days. During this period, animals are observed daily to detect signs of toxicity and pathogenicity. Animals which die during the test period are necropsied, and the MPCA is enumerated from appropriate tissues, organs, and body fluids. At the conclusion of the test, surviving animals are sacrificed and necropsied, and appropriate tissues, organs, and body fluids are analyzed for the quantitative presence of the MPCA.

(d) **Substance to be tested.** (1) The manufacturing-use product (MP) and, if different, the technical grade of each active ingredient (TGAI) shall be tested to support the registration of a manufacturing-use product.

(2) The end-use product (EP) shall be tested to support the registration of an end-use product.

(3) Usually, the form of the MPCA to be tested will be equivalent to the form used in the acute toxicity/pathogenicity Tier I studies (OPPTS 885.3050), and which resulted in significant signs of infectivity or unusual persistence without accompanying signs of pathogenicity or toxicity.

(e) **Test procedures**—(1) **Animal selection**—(i) **Species and strain.** The species and strains of test animal to be used are those in which infectivity/unusual persistence of the MPCA was observed in the acute toxicity/pathogenicity Tier I studies, and in which no significant signs of pathogenicity or toxicity were observed. All test animals should be free of parasites or pathogens. Females should be nulliparous and nonpregnant.

(ii) **Age.** Young adult animals should be used. The weight variation of animals used should not exceed ±20 percent of the mean weight for each sex.

(iii) **Sex and numbers.** At least 20 animals (10 animals of each sex) should be treated with the MPCA.

(2) **Control groups.** (i) A concurrent untreated control group is required.

(ii) A separate vehicle control group is not required except when the toxicity of the vehicle is unknown.

(iii) A control group dosed with inactivated MPCA (i.e. rendered incapable of reproduction or germination or excystment) may prove useful to evaluate toxic properties of the MPCA. Inactivation should be done by a means that allows for reasonable maintenance of the structural integrity of the MPCA.

(3) **Dosing**—(i) **Dose level.** A dose level of « 10^8 units of viable MPCA per test animal is to be administered daily to each test animal. If a dose level of at least 10^8 units of MPCA per test animal is not used, a justification/explanation must be provided.

(ii) **Vehicle.** The recommended vehicle for the technical grade for each active ingredient is one that allows for maintenance of viability, or germination capability, or excystment capability, or, for intracellular parasites, infection capability in a suitable host. The recommended vehicle for the MP or EP is the same material in which the MPCA will be distributed, mixed, suspended, or diluted for application.

(iii) **Route of exposure.** The oral route is to be used if significant infectivity/unusual persistence of the MPCA was observed in test animals in the acute oral toxicity/pathogenicity Tier I study (OPPTS 885.3050). The inhalation route (usually intranasal instillation) is to be used if significant infectivity/unusual persistence of the MPCA was observed in test animals in the acute pulmonary toxicity/pathogenicity Tier I study (OPPTS 885.3150).

(4) **Observation of animals.** (i) A careful cageside examination of each test animal should be made at least once per day.

(ii) Additional observations should be made daily with appropriate actions taken to minimize loss of animals to the study, e.g. necropsy of, and MPCA enumeration from those animals found dead, and isolation of weak or moribund animals.

(iii) Cageside observations should include, but not be limited to, changes in:

(A) The skin and fur.

(B) Eyes and mucous membranes.

(C) Respiratory system.

(D) Circulatory system.

(E) Autonomic and central nervous system.

(F) Somatomotor activity.

(G) Behavior pattern.

(H) Particular attention should be directed to observation of tremors, convulsions, diarrhea, lethargy, salivation, sleep, and coma.

(iv) Individual weights of animals should be determined shortly before the test material is administered, weekly thereafter, and at death or at sacrifice.

(v) Food and water consumption should be determined weekly during the study.

(vi) The time of death should be recorded as precisely as possible.

(5) **Gross pathology.** A gross necropsy of all animals should be performed at the time of death or at sacrifice. All gross pathological changes should be recorded.

(6) **MPCA enumeration in tissues, organs, and body fluids**—(i) **Techniques.** The presence of the MPCA in tissues, organs, and body fluids in those animals that die during the study, and in all animals that survive at termination of the dosing period, should be assessed using sensitive, quantitative techniques. Recovery values and detection and sensitivity limits should be determined and reported for each quantitative enumeration technique used.

(ii) **Tissues, organs, and body fluids.** The MPCA should be enumerated from the kidney, brain, liver, lung, spleen, blood, and representative lymph nodes. Other tissues, organs, and body fluids may have to be examined as indicated by the nature of any toxic and pathogenic effects observed.

(f) **Data and reporting**—(1) **Treatment of results.** In addition to the information provided recommended by OPPTS 885.0001, the test report should include the following information:

(i) The number of animals at the start of the test.

(ii) Time of death of individual animals.

(iii) Number of animals displaying other signs of toxicity and pathogenicity.

(iv) Description of toxic and pathogenic effects.

(v) Definition for one unit of the MPCA used, and the units/test animal in the dosing suspension.

(vi) Body weights, and food and water consumption.

(vii) Necropsy findings.

(viii) Pathology findings.

(ix) MPCA enumeration from tissues, organs, and body fluids, and methods used, and sensitivities and limits of detection,

(x) Verification that each enumeration method is sufficiently sensitive to serve as a useful quantitative assay for the MPCA in tissues, organs, and body fluids.

(2) **Evaluation of results.** An evaluation should include the relationship, if any, between exposure to the test substance and incidence and severity of all abnormalities, including:

(i) Behavioral abnormalities.

(ii) Clinical abnormalities.

(iii) Gross lesions.

(iv) Body weight changes.

(v) Food and water consumption.

(vi) Mortality.

(vii) Toxicity.

(viii) Pathogenicity.

(g) **Tier progression.** (1) If significant or persistent signs of pathogenicity are observed in test animals, consultation with the Agency is required for determination of further testing requirements. The ability of the MPCA to overcome natural host barriers to infection may be considered as a pathogenic trait, even when overt signs of disease are not apparent.

(2) If toxicity effects are observed, in the absence of significant pathogenic effects:

(i) Toxic components of the dosing material are to be identified, and to a practical extent, isolated.

(ii) An acute toxicity study (OPPTS 885.3550) is to be conducted with the toxic components.

(3) If signs of infectivity, pathogenicity, and toxicity are not observed, no further testing is required. However, the registrant is required to provide an in-depth evaluation of possible consequences of unusual persistence of the MPCA in host organisms.

(4) If significant infectivity is observed in the absence of pathogenicity and toxicity, a reproductive and fertility effects study (OPPTS 885.3650) is required.