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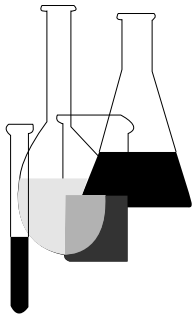
Microbial Pesticide Test Guidelines

OPPTS 885.3000 Background—Mammalian Toxicity/ Pathogenicity/Infectivity



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 885.3000 Background—Mammalian toxicity/pathogenicity/infectivity.

(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 152A–1.

(b) **Toxicology requirements.** OPPTS Series 885, Group C presents protocols for testing to determine the potential for detrimental effects to humans and domestic animals caused by microbial pest control agents (MPCAs). The testing of MPCAs for possible effects on humans and domestic animals is performed in a sequence of three tiers. The general tier sequence and studies involved for MPCAs are outlined in OPPTS 885.0001.

(c) **Purpose.** The purpose of this guideline is to consider the toxicological concerns that the Agency has for MPCA preparations, and to discuss these concerns in relation to the tiered testing scheme for evaluating pathogenic and toxic effects. Major concerns of the Agency for MPCA preparations that relate to toxicology are the following:

(1) Pathogenicity of the MPCA and of microbial contaminants.

(2) Infectivity/unusual persistence of the MPCA and of microbial contaminants.

(3) Toxicity of the MPCA, of microbial contaminants, and of preparation by-products.

(d) **Tier I testing.** The overall purpose of the Tier I studies is to provide a toxicological evaluation of a MPCA preparation with respect to the above three endpoints. The Agency recognizes that pathogenicity, infectivity, and toxicity comprise a complex set of microorganism—host interactions which may not be easily resolved as independent endpoints in all studies. However, it is believed that the data from the Tier I battery of tests will provide a fairly clear evaluation of the potential risks in most cases.

(1) The following Tier I studies are considered appropriate for testing of MPCA preparations:

(i) Acute oral toxicity/pathogenicity study (OPPTS 885.3050).

(ii) Acute pulmonary toxicity/pathogenicity study (OPPTS 885.3150).

(iii) Acute injection toxicity/pathogenicity study (OPPTS 885.3200).

(2) The following Tier I studies are considered appropriate primarily to evaluate the toxicity of chemical components of an MPCA preparation, but are nevertheless performed on the complete MPCA preparation:

(i) Acute dermal toxicity study (OPPTS 885.3100).

(ii) Primary eye irritation study (OPPTS 885.3300).

(iii) Reporting of hypersensitivity incidents (OPPTS 885.3400).

(3) The final Tier I study is specific to viruses and is designed to evaluate adverse effects of any viral pesticide control agent to mammalian cell lines:

(i) Cell culture tests with viral pest control agents (OPPTS 885.3500).

(ii) [Reserved]

(e) **Tier II testing.** Tier II testing is designed to examine situations in which Tier I testing indicates that the MPCA exhibits infectivity or toxicity without any evidence of pathogenicity.

(1) **Infectivity/unusual persistence.** Data from acute toxicity/pathogen-icity studies may indicate that the MPCA is able to infect test animals without eliciting definite signs of pathogenicity or toxicity, or the MPCA may be observed to persist in test animals for periods longer than would normally be expected. The Agency considers that these observations are sufficient to warrant subchronic testing, Tier II (OPPTS 885.3600), in order to determine whether repeated exposure to the MPCA preparation is sufficient to cause toxic or pathogenic effects. Subchronic studies also may be required if an MPCA preparation cannot be sufficiently freed of contaminating microorganisms; or contaminating microorganisms are insufficiently characterized; or there is sufficient reason to believe that microbially-produced toxins exist in the preparation, and these toxins would not exert effects in test animals after acute exposure in the Tier I studies.

(i) The Agency does not choose to set statistical limits to delineate the meaning of ‘‘significant infectivity’’ or to define ‘‘unusual persistence’’ by selecting minimum periods of time for clearance of a microorganism from a test animal. It is believed that these terms are best defined in the context of data that are obtained from the battery of Tier I studies, and in consideration of the type of microorganism and the route of exposure. In general, the MPCA can be considered as ‘‘infective’’ if evidence is obtained that shows that the microorganism can cross or evade natural host barriers to infection. Evidence of replication of the microorganism in the host also bears on the interpretation of whether it is infective for the test animal.

(ii) The Agency recognizes that certain forms of microorganisms (e.g. spores) may be cleared from the host animal at a rate slower than vegetative forms of the same microorganism. In addition, in the Tier I tests, it is expected that certain MPCAs may be isolated from the test animal, even at the end of the recommended observation periods. Such isolations should not automatically trigger Tier II testing. The data will be interpreted in light of the decline curves generated, and also in light of any evidence that the MPCA replicates in the host animal.

(2) **Toxicity.** (i) Toxicity of a MPCA preparation to test animals may be caused by substances produced by, or which are constituents of, the MPCA or any contaminating microorganisms; or by substances that are present as nonbiological components of the preparation. The Tier I toxicity/infectivity studies are designed to detect acute toxic effects of biological or nonbiological components of a MPCA preparation, in the absence of signs of pathogenicity or infectivity. The acute dermal toxicity study is designed primarily to evaluate toxic effects due to chemical components of the MPCA or to nonbiological components of a MPCA preparation. The Agency believes that only rarely would it be expected that a microorganism could bypass the skin barrier or infect epithelial cells of a healthy host animal solely by virtue of being placed in contact with the skin. An acute injection toxicity/pathogenicity study has been placed in Tier I, to evaluate possible adverse effects of a MPCA preparation once the skin barrier is deliberately bypassed.

(ii) If toxic effects are observed in the Tier I studies, in the absence of signs of infectivity or pathogenicity, a Tier II acute toxicity study normally is required, with the toxic components of the MPCA preparation being used as the dosing material. Additional studies designed solely for evaluating toxicity effects of MPCA preparations are not included in the Tier II or Tier III testing scheme for microbial pesticides because appropriate test protocols already exist in OPPTS Series 870 guidelines—Health Effects Test Guidelines for testing of conventional chemical pesticides. These guidelines can be consulted, if required, to provide a full evaluation of toxicity due to components of a MPCA preparation.

(f) **Tier III testing.** In general, if it is determined from Tier I test results that the MPCA is pathogenic for the test animals, the Agency is to be consulted to determine the next appropriate course of action. It has been the Agency's experience that recognized mammalian pathogens have not been considered as likely candidates for registration as pesticides. However, it is not inconceivable that recognized pathogens of mammals may be considered for development as MPCAs, for instance, as rodenticides. In such cases, careful consideration and thorough evaluation for pathogenic effects of the MPCA for nontarget mammals is to be provided. Also, the Agency realizes that certain recognized mammalian pathogens that have been rendered nonpathogenic by recombinant DNA or equivalent techniques, may be considered for use as microbial pesticides.

In these cases, the effectiveness of the disarming procedure will have to be clearly established.

(1) **Viruses and protozoa.** These species require special attention for the following reasons:

(i) Viruses are obligate intracellular parasites, and protozoa may be obligate or facultative intracellular parasites.

(ii) These intracellular parasites may be less amenable to sufficient taxonomic identification.

(iii) Preparations of these MPCAs that are free of contaminating organisms or cellular debris may be difficult to obtain.

(iv) They may be present as contaminants in preparations of other MPCAs.

(2) **Agency concern.** (i) The studies that comprise Tier III primarily are designed for evaluating the potential of MPCAs, or microbial contaminants of MPCA preparations, that are recognized to be intracellular parasites of mammalian cells, or are shown to be, by data resulting from conducting the lower tier studies. It is expected that registrants would not ordinarily pursue registration of such MPCAs. The Tier III studies also may be appropriate for evaluating known mammalian parasites that have been disarmed of pathogenic traits by use of recombinant DNA or equivalent techniques.

(ii) The Agency is concerned about proper characterization and identification of microorganisms that are present as contaminants in MPCA preparations, including formulations and newly-produced batches of any MPCA. Appropriate quality control procedures and appropriate biological analyses shall be employed to ensure that adverse human and mammalian health effects will not occur from use of MPCA products contaminated with other microorganisms. Also, records of biological analyses shall be maintained and made available to the Agency if required.

(3) **Other issues**—(i) **Identification and characterization of MPCAs.** (A) A sufficient and appropriate taxonomic evaluation of the MPCA is required for an initial assessment of potential adverse health effects to mammals, including humans. In addition to the requirements of OPPTS 885.1100 through 885.1500, data on the effects of temperature range on growth of the MPCA also are useful in determining whether the MPCA may survive or grow at mammalian body temperatures.

(B) If the MPCA is genetically altered, the methods used to derive the product are to be described in detail; and the correct structure of the gene construct in the engineered MPCA product is to be confirmed. The requirements set forth in OPPTS 885.1100 through 885.1500 for genetically altered MPCAs also must be met.

(ii) **Allergy/hypersensitivity.** The Agency believes that to require reporting of observed allergic responses of humans to MPCA preparations is sufficient to adequately address health problems due to types of these reactions. Based on the information provided in such reports, the Agency would recommend adequate precautions in handling the material. In some instances, the MPCA will be a recognized common allergen. In these cases, the Agency may require proper precautionary label statements. The Agency strongly recommends that appropriate respiratory tract coverings be worn when there might be accidental exposure to aerosols of MPCA preparations. The purpose of the recommended coverings is to minimize unnecessary stimulation of the respiratory system.

(iii) **Eye irritation/injury.** Appropriate eye protection is recommended when exposure (including accidental exposure) to aerosols of the MPCA preparation may occur. If it is demonstrated (e.g. by appropriate label precautionary statements) that the eyes of workers/applicators will be protected by appropriate coverings (e.g. goggles), the primary eye irritation study (OPPTS 885.3300) may not be required.

(4) **Special considerations for Tier III testing**—(i) **Data from oncogenicity studies**—(A) **Purpose.** Data from oncogenicity testing are useful in providing an estimate of potential human hazard from an MPCA if the potential for oncogenic effects exists when a component of the MPCA formulation is a virus, and any of the following criteria are met:

(*1*) The virus is an intended or an unintended ingredient in the product and is known to be oncogenic for mammals, or is sufficiently closely related to such viruses.

(*2*) The virus is an intended or unintended ingredient in the product and is demonstrated to transform mammalian cells in cell culture tests (OPPTS 885.3500).

(*3*) Efforts at characterization of a virus component of the product are insufficient to allow for the conclusion that the virus is potentially oncogenic.

(B) **Evaluation of oncogenic potential.** The study is not intended for evaluating the oncogenic potential of any product that may be due to chemical components, whether or not they are produced by an MPCA.

(C) **Test standards.** Since test standards will be developed on a case-by-case basis, consultation with the Agency is advised before performing an oncogenicity study.

(ii) **Data from immunodeficiency testing**—(A) **Purpose**. Data from immunodeficiency testing are useful in providing an estimate of human health hazard from an MPCA if a potential for induction of immunodeficiency in mammals exists when a component of the MPCA is a virus,

and the virus is, or is related to, any virus that is known to interact with components of mammalian immune systems and cause a state of immunodeficiency.

(B) **Test standards.** Since test protocols will be developed on a case-by-case basis, consultation with the Agency is advised before performing immunodeficiency studies.

(iii) **Data from primate testing—(A) Purpose.** Data from primate testing may be useful in providing estimates of potential human hazard from an MPCA if:

(*1*) The potential for causing infectivity, pathogenicity, oncogenicity, or immunodeficiency is indicated by the presence of certain intracellular parasites in the pesticidal product.

(*2*) A potential for adverse effects in primates exists when a component of the MPCA formulation, during at least some stage in its life cycle, can be an intracellular parasite of mammalian cells, and any of the following criteria are met:

(*i*) A component of the MPCA formulation is a virus, and the virus is able to cause cytopathic effects in mammalian host cell lines in cell culture tests (OPPTS 885.3500) and, in addition, is demonstrated to replicate in mammalian host cells.

(*ii*) A component of the MPCA formulation is a known parasite of mammalian host cells.

(*3*) Unresolved positive pathogenic effects from Tier I tests might be specific to test animals used.

(*4*) The taxonomic properties of the MPCA indicate that human pathogenicity might be of significant concern.

(B) **Test standards.** Since test protocols will be developed on a case-by-case basis, consultation with the Agency is advised before performing this study.