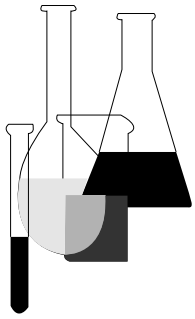
United States
Environmental Protection
Agency

Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–96–315
February 1996

Microbial Pesticide Test Guidelines

OPPTS 885.3050 Acute Oral 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 885.3050 Acute oral 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 OPP guideline 152A–10.

(b) **Purpose.** In the assessment and evaluation of the toxic or pathogenic characteristics of an MPCA, determination of acute oral toxicity/pathology usually is an initial step. It provides information on health hazards likely to arise from a single exposure by the oral route. The purpose of the acute oral study is to provide initial information on the toxicity, infectivity, and pathology of a MPCA using a single high dose exposure and an adequate post-exposure observation period.

(c) **Definitions.** The following definitions apply to this test guideline.

Acute oral toxicity and *acute oral pathogenicity* are the adverse effects occurring from the oral administration of a single dose of a MPCA. Acute oral toxicity also may be the adverse effects occurring from the oral administration of a microbially produced substance, or from other ingredients in any test substance.

Dose level is the amount of MPCA administered. It is expressed as units of the microorganism administered per test animal.

Units of MPCAs One unit of representative MPCA groups usually would be defined as follows: (Due to the wide diversity of forms among microorganisms, other definitions of a unit of a MPCA may be equally appropriate.)

(i) *Bacterial or fungal spore, bacterial or protozoan cyst:* An intact individual spore or cyst as determined microscopically, and usually the entity that produces a single CFU on appropriate germination medium.

(ii) *Fungal mycelium:* 10^{-9} gram dry weight or, after standardized preparatory procedures, a mycelium-producing entity on semisolid growth medium.

(iii) *Protozoa:* An intact vegetative organism, spore, or cyst of the members in the various classes of this phylum.

(iv) *Vegetative bacterium:* A single, viable organism, and usually the entity that produces a single colony forming unit (CFU) on an appropriate semisolid growth medium.

(v) *Virus:* An intact, complete virion or a polyhedral body as determined by microscopy, and, usually the entity that produces an infective unit (IU) on appropriate host cells or tissues.

(d) **Principles of the test method.** The test MPCA is administered orally by gavage in a single high dose to experimental animals. Subsequent observations of effects and deaths are made and rate of clearance of the MPCA is estimated. Animals that die during the test are necropsied, and at the conclusion of the test the surviving animals are sacrificed and necropsied. Infectivity of the MPCA is evaluated periodically during the test, and at the conclusion of the test.

(e) **Substance to be tested.** (1) The technical grade of each active ingredient shall be tested to support the registration of each manufacturing-use product and each end-use product.

(2) The form (e.g. vegetative cell, spore, cyst, virion) of the MPCA used in testing should be equivalent to the form that is intended for registration or application. To the extent possible, the test MPCA also should be equivalent to the MPCA intended for registration or application with respect to stage of growth, possession of organelles and appendages and expression of phenotypic traits (including products from intentionally introduced genes). If significant exposure to other forms of the MPCA are expected, or if changes in form of the MPCA occur in the host, these forms also may have to be tested.

(f) **Characteristics of the test MPCA.** The test MPCA should be thoroughly characterized as required in Group A of this series of guidelines—Product Analysis Test Guidelines.

(g) **Test procedures**—(1) **Animal selection**—(i) **Species and strain.** Although several mammalian test species may be used, the mouse or the rat are the preferred rodent species. Commonly used laboratory strains should be employed. If another species is used, justification/reasoning for the alternative selection should be provided. 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 in a test should not exceed ± 20 percent of the mean weight for each sex.

(iii) **Sex.** Equal numbers of animals of each sex are required.

(iv) **Numbers.** At least six animals (three animals of each sex) should be used. A sufficient number of additional animals should be used to allow for interim sacrifice for infectivity determinations, and also, for control groups.

(2) **Control groups.** (i) A concurrent untreated control group of four animals per sex is required. Half of the animals in the control group (i.e., two animals per sex) should be housed separately from the test group of

animals dosed with MPCA, and the remainder of the control animals (the ''shelf control'' group) should be housed with the dosed animals.

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

(iii) Control groups 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.** One dose level of at least 10^8 units of the MPCA per test animal should be used. If a dose level of at least 10^8 units per test animal is not used, a justification/explanation must be provided. The test material should not be diluted to reach the limit dose at 10^8 units per test animal.

(ii) **Vehicle.** The recommended vehicle for the technical grade of 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.

(iii) **Volume.** The maximum volume of liquid that can be administered at one time depends on the size of the test animal. In rodents, the volume should not exceed 2 mL/100 g body weight. Variability in test volume should be minimized.

(iv) **Dose quantification.** Techniques used to quantify the units of MPCA in any dose will depend on the group of microorganisms to which the MPCA belongs. Where possible, determinations of viable, or potentially viable, or infective units in each dose should be made. A measurement of metabolism associated with a defined biomass may be the preferred technique for quantification of mycelial forms of MPCAs. Quantification should be done concurrently with testing.

(4) **Exposure.** (i) The test substance should be administered in a single dose by gavage, using a stomach tube or suitable intubation cannula.

(ii) Animals should be fasted overnight prior to test substance administration. After the substance has been administered, food may be withheld for a further 3–4 h.

(iii) If a single dose is not possible, the dose may be given in smaller portions over a period not exceeding 24 h. Where a dose is administered in fractions over a period, it may be necessary to provide the animals with food and water, depending on the length of the period.

(5) **Observation period.** The observation period should be at least for 21 days after dosing. However, the duration of observation should not

be fixed rigidly. It should be determined by the type of MPCA administered and its rate of clearance from the test animals. Duration of the observation period also would depend on the time at which signs of toxicity and pathology appear and disappear, and the time to death of the animals.

(6) **Observation of animals.** (i) A careful clinical examination of all animals should be made at least once each 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, change 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) Weights of individual animals should be determined shortly before the test material is administered, weekly thereafter, and at death or at interim or final sacrifice. Changes in weight should be calculated and recorded when survival exceeds 1 day.

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

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

(8) **Clearance of MPCA.** Feces from test animals should be collected soon after dosing and frequently during the study and examined for the presence of the MPCA to estimate clearance of the MPCA after oral administration. Methods (e.g. immunological assays, DNA probes) other than those used for quantification of MPCAs in each dose may prove useful. Recovery values and detection and sensitivity limits should be determined and reported for each technique used.

(9) **MPCA enumeration in tissues, organs, and body fluids.** Infectivity or persistence should be assessed by using sensitive techniques to determine the presence of the MPCA in tissues, organs, and body fluids. Methods other than those used for quantification of MPCAs in each dose may prove useful. Recovery values and detection and sensitivity limits should be determined and reported for each technique used. Methods selected for MPCA enumeration should, if possible, allow for detection of microbial replication.

(10) **Interim sacrifice.** For evaluating infectivity and clearance, the MPCA should be enumerated from tissues, organs, and body fluids of three treated animals per sex, sacrificed at 3 days after, and at one week intervals after dosing. The number of interim sacrifice periods required will depend on the nature of the test microorganism, and should be sufficient to establish a pattern of clearance adequately. The MPCA also should be enumerated from the tissues, organs, and body fluids of the ''shelf control'' group at final sacrifice.

(11) **Tissues, organs, and body fluids.** (i) For infectivity and persistence determinations, the MPCA should be enumerated from the kidney, brain, liver, lung, spleen, blood, representative lymph nodes, and, where appropriate, from lesions and from the injection site.

(ii) Other tissues, organs, and body fluids may have to be examined as indicated by the nature of any toxic and pathogenic effects observed.

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

(i) 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 pathology.

(iv) Description of toxic and pathogenic effects.

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

(vi) Body weights and time taken.

(vii) Necropsy findings.

(viii) Pathology findings, when performed.

(ix) Infectivity/persistence findings.

(x) Estimate of rate of MPCA clearance.

(xi) Description of all enumeration methods used for MPCA detection and quantification.

(xii) 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 the incidence and severity of all abnormalities, including:

(i) Behavioral abnormalities.

(ii) Clinical abnormalities.

(iii) Gross lesions.

(iv) Body weight changes.

(v) Mortality.

(vi) Toxicity.

(vii) Infectivity.

(viii) Pathology.

(i) **Tier progression.** (1) If persistent or significant signs of pathology of the MPCA for the test animals is observed in Tier I, acute oral toxicity/pathology tests may be required in nonrodent animal species. Consultation with the Agency to discuss further testing requirements is recommended.

(2) If toxin production by the MPCA is suspected, or if toxin production is indicated by significant or persistent signs of toxicity in the test animals, in the absence of signs of infectivity or pathology;

(i) The toxic component(s) of the dosing material is (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 component(s).

(3) If significant infectivity or unusual persistence of the MPCA is observed in the absence of signs of toxicity or pathogenicity, a subchronic (90-day) study (OPPTS 885.3600) is required.