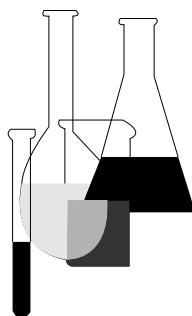




Microbial Pesticide Test Guidelines

OPPTS 885.3200

Acute Injection 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.*).

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 885.3200 Acute injection 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-13.

(b) **Purpose.** Acute injection toxicity/pathogenicity data provide information on health hazards likely to arise from a single exposure where the skin is bypassed as a barrier. The purpose of the acute injection study is to provide initial information on the toxicity, infectivity, and pathogenicity of a MPCA using a single dose exposure and an adequate post-exposure observation period.

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

Acute injection toxicity and *acute injection pathogenicity* are the adverse effects occurring from the intravenous or intraperitoneal administration of MPCA. Acute injection toxicity also may be the adverse effects occurring from the intravenous or intraperitoneal administration of a microbially produced 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 colony forming unit (CFU) on appropriate germination medium.

(ii) *Fungal mycelium:* 10⁻⁹ gm 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 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 by intravenous or intraperitoneal injection in a single high dose to experimental animals. Subsequent observations of effects and deaths are made, and, for the intravenous route only, the 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. In general, smaller microorganisms (e.g. bacteria) should be administered by intravenous injection and larger microorganisms (e.g. fungi, protozoa) should be administered by intraperitoneal injection.

(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 physical nature of some technical grade preparations may render injection testing impractical. In such cases, the test substance may consist only of the purest infective form of the microorganism. However, justification/reasoning must be provided by the investigator to support a request for exempting specific technical grade preparations from testing by injection dosing.

(3) 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 is expected, or if changes in form of the MPCA occur in the host, then these forms also may have to be tested.

(f) **Characteristics of the MPCA.** The test MPCA should be thoroughly characterized as required in OPPTS 885.1000.

(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 used. 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 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 two animals/sex is required. A “shelf control group” is not required.

(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. If included, 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 10^7 units of the MPCA per test animal should be used. If a dose level of at least 10^7 units per test animal is not used, a justification/explanation must be provided.

(ii) **Vehicle.** The recommended vehicle 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 by intravenous or intraperitoneal injection at one time depends on the size of the test animal. 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 via a needle and syringe in a single dose.

(ii) If a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 h.

(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, when determined, 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 pathogenicity 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, 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 interim or final sacrifice. Changes in weight should be calculated and recorded when survival exceeds one 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. All gross pathological changes should be recorded.

(8) **Clearance of MPCA: Intravenous injection.** Blood from test animals should be collected during the study and examined for the presence of the MPCA to estimate clearance of the MPCA after dosing. The first analysis should be done on three animals per sex as soon as reasonably possible after dosing. 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. Determinations of clearance are not required after intraperitoneal injection.

(9) **MPCA enumeration in tissues, organs, and body fluids: Intravenous injection.** Infectivity and clearance 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. MPCA enumeration in tissues, organs, and body fluids is not required after intraperitoneal injection.

(10) **Interim sacrifice: Intravenous injection only.** 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 then at 1 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 adequately establish a pattern of clearance.

(11) **Tissues, organs, and body fluids: Intravenous injection only.**
(i) For infectivity and persistence determinations, the MPCA should be enumerated from the kidneys, brain, liver, lung, spleen, blood, representative lymph nodes, caecum contents, and, where appropriate, from lesions and the inoculation 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 pathogenicity.

(iv) Description of toxic and pathogenic effects.

(v) Definition for one unit of the MPCA used, and the units per test animal in the dose.

(vi) Body weights and time taken.

(vii) Necropsy findings.

(viii) Pathology findings, when performed.

(ix) Infectivity findings (intravenous injection only).

(x) Estimate of rate of MPCA clearance (intravenous injection only).

(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) Pathogenicity.

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

(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 pathogenicity:

(i) Toxic components of the dosing material are to be identified, and where possible, isolated.

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