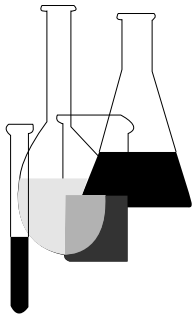
United States
Environmental Protection
Agency

Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–96–316
February 1996

Microbial Pesticide Test Guidelines

OPPTS 885.3100
Acute Dermal Toxicity/ Pathology



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 885.3100 Acute dermal toxicity/pathology.

(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–11.

(b) **Purpose.** Acute dermal toxicity data provide information on health hazards likely to arise from a single dermal application of soluble or particulate chemicals associated with a preparation of the MPCA, and/or associated with other ingredients in formulations of the MPCA, and/or associated with products from genetic material intentionally introduced into the MPCA.

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

Acute dermal toxicity is the adverse effect occurring during or following a 24–hour dermal exposure to a single dose of a test substance.

(d) **Principles of the test method.** The MPCA in each formulation to be tested is applied in a single high dose to the skin of experimental animals. Subsequently, observations of effects and deaths are made. Animals that die during the test are necropsied, and at the conclusion of the test, the surviving animals are sacrificed and necropsied as indicated by the nature of the toxic effects observed.

(e) **Substance to be tested.** (1) The manufacturing-use product shall be tested to support the registration of each manufacturing-use product.

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

(f) **Test procedures**—(1) **Animal selection**—(i) **Species and strain.** Although several mammalian test species may be used, the albino rabbit is the preferred species. Commonly used laboratory strains should be employed. If another species is used, the investigator should provide justification/reasoning for the alternative selection. 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 with healthy intact skin are recommended.

(iv) **Numbers.** At least 10 animals (5 animals of each sex) should be used.

(2) **Control groups.** Neither a concurrent untreated nor vehicle control group are required except when the toxicity of the vehicle is unknown.

(3) **Dosing**—(i) **Dose level.** The test substance should be applied at 2 g/kg BW. If a dose level of less than 2 g/kg BW is used, a justification/ explanation must be provided.

(ii) **Vehicle.** Where necessary, the formulation to be tested is suspended in a suitable vehicle. An aqueous solution should be used. The recommended vehicle for the end-use product usually is the same material in which the MPCA will be mixed, suspended, or diluted for application.

(iii) **Volume.** The moisture content of the test material should not be excessive, but should be sufficient to prevent significant drying of the test material during the exposure period, and to ensure good contact with the skin.

(4) **Exposure duration.** The exposure duration should be for approximately 24 h.

(5) **Observation period.** The observation period should be at least for 14 days, but should not be fixed rigidly. It should be determined by the toxic reactions, rate of onset, and length of recovery period and may be extended when considered necessary. The time at which toxicity signs appear and disappear, and their duration are important.

(6) **Preparation of animal skin.** (i) Approximately 24 h before the test, fur should be removed from the dorsal and ventral area of the trunk of each test animal by clipping or shaving.

(ii) Not less than 10 percent of the body surface area should be cleared for application of the test substance. The weight of the animal should be taken into account when deciding on the area to be cleared and on the dimensions of the covering.

(7) **Application of the test substance** (i) The test substance should be applied uniformly over an area which is approximately 10 percent of the total body surface area.

(ii) The test substance should be held in contact with the skin with porous gauze and a nonirritating tape throughout a 24–h exposure period. The test site further should be covered in a suitable manner to retain the gauze dressing and test substance and ensure that the animals cannot ingest the test substance. Restrainers may be used to prevent the ingestion of the test substance, but complete immobilization is not recommended.

(iii) At the end of the exposure period, residual test substance should be removed, where practical, using water.

(8) **Observation of animals.** (i) A careful clinical examination should be made at least once each day.

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

(A) The skin (including signs of irritation) 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.

(iii) Individual weights of animals should be determined shortly before the test material is administered, weekly thereafter, and at death or at final sacrifice. Changes in weight should be calculated and recorded when survival exceeds 1 day.

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

(v) At the end of the 24–h exposure period, and daily thereafter, any signs of skin irritation should be recorded and scored.

(9) **Gross pathology.** Consideration should be given to performing a gross necropsy of all animals if indicated by the appearance of toxic effects. If done, all gross pathological changes should be recorded.

(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.

(iv) Description of toxic effects.

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

(vi) Dry weight and net weight determinations of the test material applied per kilogram body weight of the test animal.

(vii) Body weights and time taken.

(viii) Necropsy findings, when performed;and,

(ix) Pathology findings, when performed.

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

(i) Behavioral abnormalities.

(ii) Clinical abnormalities.

(iii) Skin lesions and skin irritation.

(iv) Body weight changes.

(v) Mortality.

(vi) Toxicity.

(i) **Tier progression** (1) If evidence of significant and/or persistent toxicity are observed, toxic components of the dosing material are to be identified, and to a practical extent, isolated.

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

(ii) [Reserved]

(2) If no toxic effects are observed, no further testing is required.