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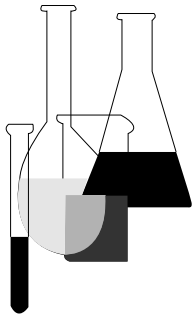
Microbial Pesticide Test Guidelines

OPPTS 885.4050
Avian Oral, Tier I



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 885.4050 Avian oral, Tier I.

(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 154A–16.

(b) **Test standards.** Data must be derived from tests that satisfy the general test standards in OPPTS 885.0001 and the following:

(1) **Test substance.** The actual form of the material to be regarded as the test substance is described in OPPTS 885.0001. In addition, any substances used to enhance virulence or toxicity should be tested along with the test substance.

(2) **Species.** Testing shall be performed on one avian species, one insectivorous and one herbivorous (preferably bobwhite quail). The use of two species of birds with differing diets is recommended in order to take into account possible differences in gastrointestinal physiology. Other species may be used but a justification must be supplied based on increased susceptibility to the MPCA or ecological considerations which preclude the use of recommended species.

(3) **Age.** Birds used in this test shall be from 14 to 24 days old at the beginning of the test period. Within a given test, all birds shall be as near the same age as possible.

(4) **Controls.** (i) A negative, nondosed control group should be performed.

(ii) An infectivity control group should be performed and should be treated with the MPCA inactivated in such a way as to retain the structural integrity of the cell.

(iii) A control group in which the birds are dosed with sterile filtrate from production cultures should be performed concurrently with the test groups.

(5) **Number of birds per dosage level.** Each treatment and control group shall contain at least 10 birds. When only one treatment group is tested, at least 30 birds shall be tested at that level.

(6) **Maximum hazard dosage level.** (i) The highest oral dosage level tested is defined by the following formula:

maximum daily dose (units) =

[MPCA] in TGAI × 5 mL/kg BW × weight of test bird (kg)

where

[MPCA] = concentration of MPCA

TGAI = technical grade of active ingredient

BW = body weight

(ii) When using injection routes, use 0.5 mL/kg BW for intravenous, and 2 mL/kg BW for intraperitoneal.

(iii) For MPCAs that produce a toxin, fractions of this dose should be calculated for lower doses. This dose is administered daily for 5 days. A justification shall be provided to support any reduction in the highest dosage level.

(7) **Treatment conditions.** A single group of birds may be tested at the maximum hazard dose. If deleterious effects, due either to toxicity or pathogenicity are observed, sequentially lower doses should be tested as described in paragraph (b)(9) of this guideline.

(8) **Dosing regimen.** Birds will receive oral doses daily for 5 days.

(9) **Determination of an LD50 or ID50.** (i) The study endpoint must be chosen to reflect the activity of the specific microorganism under test, i.e. if an MPCA is expected to produce a toxin and has little or no infectivity, the appropriate endpoint would be death of the test organism. If, however, the major mechanism is pathogenicity, a more appropriate endpoint would be overt symptomatology.

(ii) The test data should establish that the avian oral LD50, defined as the dose required to kill 50 percent of the test organisms, or ID50, defined as the dose necessary to cause overt symptomatology in 50 percent of the test organisms, are greater than the hazard dosage level. If the LD50 or ID50 is lower than the maximum hazard dose, a definitive LD50 or ID50 with confidence limits should be established.

(10) **Duration of test.** Control and treated groups should be observed for at least 30 days after dosing initiation. If symptomatology or toxic signs are manifest at the 30th day, observation should continue until recovery, mortality, or unequivocal moribundity is established.

(c) **Reporting and evaluation of data.** In addition to the information specified in OPPTS 885.0001, the test report shall contain the following information:

(1) Species.

(2) Age of the birds tested.

(3) Mean body weights for each test and control group at test initiation and weekly thereafter.

(4) Diet analysis (especially antibiotics).

(5) Pen dimensions.

(6) Ambient temperature and humidity.

(7) Photoperiod and lighting.

(8) Total feed consumption for each test and control group at weekly intervals.

(9) Method of test material preparation, concentration of the MPCA and total dose.

(10) Amount of test material dosed per bird.

(11) Amount of vehicle dosed per bird, if a vehicle other than water is used.

(12) Number of birds per group.

(13) LD50 or ID50 in appropriate units with 95 percent confidence limits if obtained.

(14) Methods used for calculation of LD50 or ID50.

(15) Slope of the dose response line, if obtained.

(16) Time and date of mortalities.

(17) Any signs of intoxication, abnormal behavior, and regurgitation (if any occurs).

(18) Reports of any pathogenic symptomatology or pathological changes.

(19) Results of gross necropsies and histopathological findings conducted on enough birds to characterize any gross lesions including attempts, using appropriate techniques, to reisolate the MPCA from examined tissues.

(d) **Tier progression.** (1) If any pathogenic symptoms or toxic signs are observed at any dose level in this study, testing at Tier II, environmental expression testing (OPPTS guidelines 885.5000, 885.5200, 885.5300, and 885.5400), is required as specified in 40 CFR 158.740. In some cases, a subchronic test may serve to better the understanding of effects observed at the Tier I level and alleviate the need for Tier II testing.

(2) If toxic or pathogenic effects are not observed in this Tier I study, additional testing at higher tiers ordinarily is not required. The Agency may require additional testing, however, if it determines that there is a potential risk to birds despite the negative Tier I results.

(e) **References.** The following references are provided for use in the development of acceptable test protocols for conducting an avian oral pathogenicity/toxicity test with a microbial pest control agent.

(1) Friend, M. and D.O. Trainer. Experimental duck virus hepatitis in the mallard. *Avian Disease* 16:692–699 (1971).

(2) Hartmann, G.C. and S.S. Wasti. Avian safety of three species of entomogenous fungi. *Comparative Physiological Ecology* 5:242–245 (1980).

(3) Ignoffo, C.M. Effects of entomopathogens on vertebrates. *Annals New York Academy of Science* 217:141–164 (1973).

(4) Lautenschlager, R.A. and J.D. Podgwaite. Passage of nucleopolyhedrosis virus by avian and mammalian predators of the gypsy moth, *Lymantria dispar*. *Environmental Entomology*. 8:210–214 (1979).

(5) Narayanan, K. et al. Lack of susceptibility of poultry birds to nuclear polyhedrosis virus of groundnut red-hairy caterpillar, *Amsacta albistriga* (W.). *Indian Journal of Experimental Biology*. 16:1322–1324 (1978).

(6) Podgwaite, J.D. and R.R. Galipeau. Effects of nucleopolyhodrosis virus an two avian predators of the gypsy moth. *USDA Forest Service Research Notes*, NE–251, 2 pp. (1978).

(7) Summers, M. et al., R. Engler, L.A. Falcon, and P. Vail, eds. Guidance for Safety Testing of Baculoviruses, pp. 179–184 in: *Baculoviruses for Insect Post Control. Safety Considerations.* American Society for Microbiology, Washington, DC (1975).

(8) Wolf, K. Evaluation of the exposure of fish and wildlife to nuclear polyhedrosis and granulosis viruses. pp. 109–111 in: *Baculoviruses for Insect Pest Control. Safety Considerations.* American Society for Microbiology, Washington, DC (1975).