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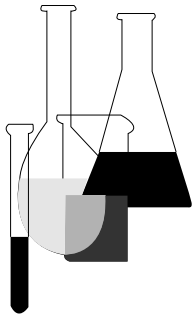
Microbial Pesticide Test Guidelines

OPPTS 885.4100 Avian Inhalation Test, 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 885.4100 Avian inhalation test, 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–17. The Agency recognizes that this test protocol has not yet been evaluated in the laboratory. An inhalation exposure may be acceptable in lieu of instillation and, for some microorganisms, the Agency may accept a request for waiver of this test with appropriate justification. It would be advisable to contact the Agency before performing the respiratory testing.

(b) **Test standards.** Data on avian respiratory pathogenicity of microbial pest control agents (MPCAs) must be derived from tests which satisfy the purposes of the general test standards in OPPTS 885.0001, and all the following test standards:

(1) **Test substance.** The actual form of the material to be used as the test substance is described in OPPTS 885.4000. 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 (preferably bobwhite quail). 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 should be from 14 to 28 days old at the beginning of the testing period. Within a given test, all birds shall be as close to the same age as possible.

(4) **Controls.** (i) A negative, nondosed control group is required.

(ii) A concurrent control group is required and shall be treated with the pure active ingredient that has been inactivated in such a way as to preserve cellular integrity.

(iii) After dosing, two untreated contact control birds are required and shall be placed in with the treatment group receiving the maximum hazard dosage.

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

(6) **Route of exposure.** The test material should be administered by intranasal or intratracheal instillation. Depending on the physical properties of the agent being tested, an alternate route, such as use of an aerosol,

involving the respiratory tissues may be used. However, use of the alternate route must be justified.

(7) **Dosing regimen.** Birds should receive doses daily for 5 days.

(8) **Maximum hazard dosage level.** (i) The maximum hazard dose is defined by the following formula:

maximum daily dose (units) =

[MPCA] in TGAI × 0.2 mL/kg BW × weight of test bird (kg)

where

[MPCA] = concentration of MPCA

TGAI = technical grade of active ingredient

BW = body weight

(ii) For MPCAs that produce a toxin, fractions of this dose should be calculated for lower doses. A reason shall be provided to support any reduction in the highest dosage level.

(9) **Treatment concentrations.** 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)(10) of this guideline.

(10) **Determination of an LD50 or ID50.** (i) The study endpoint must be defined to reflect the pathological activity of the specific agent being tested, 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 must establish that the avian inhalation LC50, defined as the dose required to kill 50 percent of the test organisms, or IC50, defined as the dose necessary to produce overt symptomatology in 50 percent of the test organisms, is greater than the maximum hazard dosage level. If the LC50 or IC50 is less than the maximum hazard dose, a sufficient number of treatment levels should be tested in order to obtain a definitive LC50 or IC50, if possible.

(11) **Duration of test.** Control and treated birds should be observed for at least 30 days after dosing. If symptomatology is manifest at the thirtieth 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) Identification of vehicle or carrier used to disperse the agent and the average amount of vehicle administered to each bird, if a vehicle other than water is used.

(11) Number of birds per treatment level;

(12) Number of controls used;

(13) Time and date of mortalities or symptom onset;

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

(15) Results of gross necropsy and histopathological findings conducted on all birds dying before termination of the test, on a representative sample of those that survived, and on two contact control birds. The necropsy report should include any evidence of respiratory tract involvement and involvement at distant sites including liver, kidney, spleen, cerebrospinal system, gastrointestinal system. Blood samples should also be analyzed for abnormalities. Results should also include any attempts, using appropriate techniques, to reisolate the MPCA from examined tissues.

(16) A clinical assessment of any histopathological findings and lesions noted.

(17) Assessment of the clinical significance of MPCA tissue isolations.

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

(2) If no pathogenic effects are observed in this study, no additional testing at higher tiers ordinarily is 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 test protocols for conducting an avian inhalation pathogenicity test with microbial pest control agents:

(1) Friend, M. and D.O. Trainer. Polychlorinated biphenyl: interaction with duck hepatitis virus. *Science* 170:1314–1316 (1970).

(2) Friend, M. Experimental duck virus hepatitis in the mallard. *Avian Disease* 16:692–699 (1971).

(3) Friend, M.. Duck hepatitis virus interaction with DDT and Dieldrin in adult mallards. *Bulletin of Environmental Contaminant Toxicology* 7:202–206 (1972).

(4) Friend, M. Experimental DDT-Duck hepatitis virus interaction studies. *Journal of Wildlife Management* 38:887–895 (1974).

(5) Friend, M. Experimental Dieldrin-Duck hepatitis virus interaction studies. *Journal of Wildlife Management* 38:896–904 (1974).

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

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