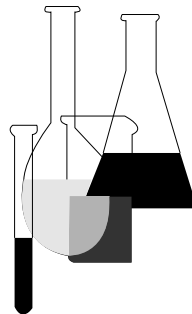




Microbial Pesticide Test Guidelines

OPPTS 885.4600

Avian Chronic Pathogenicity and Reproduction Test, Tier III



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 885.4600 Avian chronic pathogenicity and reproduction test, Tier III.

(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–26.

(b) **Purpose.** The test in this guideline is intended to determine if chronic effects will occur when animals are exposed to low levels of the microbial pest control agent (MPCA) over an extended period of time.

(c) **Test standards.** Data must satisfy the general test standards in OPPTS 885.0001, and the following:

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

(2) **Species.** Testing shall be performed on one avian species (preferably the bobwhite quail or mallard duck). The species selected shall be the same as that used for the avian inhalation pathogenicity test in OPPTS 885.4100.

(3) **Age.** Birds approaching their first breeding season shall be used.

(4) **Controls.** A concurrent control group is required and shall be treated with inactivated MPCA.

(5) **Treatment levels.** At least two treatment levels shall be used. The test concentrations should include an actual or expected field residue exposure level and a multiple of that level such as 5× or 10×.

(6) **Dosing.** The MPCA should be incorporated into the diet whenever possible. The diet should be analyzed for MPCA viability and a new diet should be prepared often enough to ensure that the test animals receive a constant dose throughout the study. Alternate routes of dosing, along with the rationale, should be discussed with the Agency. The registrant should discuss with the Agency before starting this testing.

(7) **Number of birds per treatment group.** Each treatment group should be replicated. For bobwhite quail and mallard ducks, a minimum of 12 pen replicates for each treatment level should be used.

(8) **Duration of exposure.** Birds shall be exposed to treated diets beginning not less than 10 weeks before egg laying is expected, and extending throughout the laying season.

(9) **Duration of test.** The test shall not end before 14 days after the last hatchling leaves the shell.

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

(1) **Testing protocols.** All methods used in performing these tests should be fully referenced or described in detail.

(2) **Test results.** The following information shall be reported for all test groups:

(i) Any observed abnormal behavior.

(ii) All results of tests used to detect the presence of the MPCA.

(iii) Time and date of mortalities.

(iv) Results of gross necropsy tests conducted on all birds dying or manifesting clinical symptomatology at test termination. Necropsies should also be performed on at least 50 percent of the nonaffected birds.

(v) Reisolation of microbes from selected body tissues that would normally be affected by an infection including the liver, kidney, lungs, spleen, cerebrospinal system, testes ovaries, oviducts, and gastrointestinal tract and from all dead embryos, and an assessment of the clinical significance of such isolations.

(vi) Morbidity.

(vii) Accidental deaths or injuries.

(viii) Observed clinical signs and symptoms.

(3) **Test conditions.** The following information shall be reported for treated and untreated test groups:

(i) Species.

(ii) Age.

(iii) Body weight.

(iv) Number and sex of birds in each treatment group.

(v) Individual identification of birds.

(vi) Diet composition and additives (especially antibiotics).

(vii) Diet storage conditions and results of periodic assays for MPCA content.

(viii) Feed consumption (grams per day).

(ix) Observation on palatability or repellency.

- (x) Housing conditions of test birds:
 - (A) Space allocations for mating and nesting.
 - (B) Measures taken to ensure that the birds were protected from injuries.
 - (C) Lighting program, including hours per day and wattage or foot-candles at bird level.

- (xi) Diagram of test layout.

- (xii) Temperature.

- (xiii) Water source and its microbiological and chemical characteristics if sterilized distilled water is not used.

- (xiv) Pretest and test history of the test organisms with respect to medical and chemical treatments.

(4) **Egg and hatching data.** The following information shall be reported for each treatment group:

- (i) Eggs laid (number of eggs per bird per day and per season).

- (ii) Hatching egg storage data.

- (A) Temperature.

- (B) Humidity.

- (C) Number of eggs placed in incubator.

- (D) Egg-turning frequency.

- (iii) Number of embryonated eggs.

- (iv) Number of embryos that hatch.

- (v) Ratio of hatched embryos to the number placed in the incubator.

- (vi) Number of dead embryos.

- (vii) Physical abnormalities in hatchlings.

- (viii) All observed clinical signs and symptoms.

- (ix) Post-hatchling mortality.

- (x) Weights of 14-day-old survivors.

(5) **Pesticide test data.** The concentration of the MPCA in the feed must be monitored weekly, more often if MPCA viability is short, and

the results tabulated. A statistically-sound sampling method should be employed for large quantities of treated feed.

(e) **Tier progression.** (1) If pathogenic and/or reproduction effects are observed at actual or expected exposure levels:

(i) The applicant should reconsider the proposed registration of the product.

(ii) The Agency will, at this time, review all the data and determine if a decision regarding acceptability for registration should be made. Testing at Tier IV, simulated or actual field testing (OPPTS 885.4900) may not be feasible if adequate containment or quarantine methods are not possible. If they are, testing at Tier IV is required as specified by 40 CFR 158.740(e).

(2) If no pathogenic effects are observed at actual or expected field residues exposure levels, no additional testing is ordinarily required.

(f) **References.** The following references are provided for use in the development of test protocols for conducting chronic avian pathogenicity and reproduction tests with microbial pest control agents:

(1) Heinz, G.H. Methylmercury: Second year feeding effects on mallard reproduction and duckling behavior. *Journal of Wildlife Management* 40:82–90 (1976).

(2) Heinz, G.H. and L.N. Locks. Brain lesions in mallard ducklings from parents fed methylmercury. *Avian Diseases* 20:9-17 (1976).

(3) Pesticide Assessment Guidelines Subdivision E—Hazard Evaluation: Wildlife and Aquatic Organisms, EPS–540/9–82–024, October 1982.