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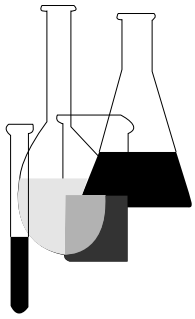
Microbial Pesticide Test Guidelines

OPPTS 885.3650 Reproductive/Fertility Effects



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 885.3650 Reproductive/fertility effects.

(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–30.

(b) **Purpose.** The reproductive/fertility effects study is designed to provide and estimate of potential human hazard from an MPCA if:

(1) Significant infectivity of the MPCA is observed in test animals in the subchronic Tier II study (OPPTS 885.3600), and in which no signs of toxicity or pathogenicity were observed.

(2) The MPCA is a virus which can persist or replicate in mammalian cell culture lines (OPPTS 885.3500).

(3) The MPCA is not amenable to thorough taxonomic classification, but is related to organisms known to be parasitic for mammalian cells.

(4) The MPCA preparation is not sufficiently well purified, but it is indicated that the preparation may contain contaminants which are parasitic for mammals.

(c) **General design.** This guideline for a one-generation reproductive/fertility study is designed to provide information on the effects of an MPCA on fertility and on embryo/fetal development of test animals. Effects of the MPCA on fertility and fetal development to be evaluated include the number of nonpregnant females, numbers that gave normal birth, number of resorptions, litter size, delayed birth, embryolethality, and offspring body weight. Transmittal of the MPCA from parent to offspring also is evaluated.

(d) **Principle of the test method.** The MPCA is administered to male and female parents prior to their mating, and to maternal parents during the resultant pregnancies.

(e) **Substance to be tested.** Testing shall be performed with the technical grade of each active ingredient.

(f) **Test procedures**—(1) **Animal selection**—(i) **Species and strain.** The mouse or the rat are the preferred species. Commonly used laboratory strains should be employed. If another species is used, justification/reasoning for the alternate selection should be provided. All test animals should be free of parasites or pathogens. Strains with low fecundity should not be used.

(ii) **Age.** Test animals should be between 6 and 8 weeks old prior to the first dosing.

(iii) **Sex.** (A) Both males and females are to be studied for an adequate assessment of the MPCA on fertility.

(iv) **Numbers.** Each test and control group should contain at least 20 males and a sufficient number of females to yield at least 20 pregnant females at or near term.

(2) **Control groups.** (i) A concurrent untreated control group is required.

(ii) A separate vehicle control group is not required except when the toxicity of the vehicle is unknown.

(iii) A control group dosed with inactivated MPCA is recommended.

(3) **Dosing**—(i) **Dose level.** One dose level of at least 10^8 units of the MPCA per test animal should be used. Justification/reasoning must be provided if a dose level of at least 10^8 units per test animal is not used. Quantification of the units of the MPCA should be done concurrently with dosing.

(ii) **Dose route.** Administration of the MPCA usually will be by the oral route. If persistence or infectivity of the MPCA in the Tier I studies was observed only after same other route of dosing (e.g. intravenous), this route must be used.

(iii) **Dose frequency.** The frequency of dosing with at least 10^8 units per test animal should be such that a significant level of MPCA is maintained in the parents prior to and during the mating period, and in the female parents during pregnancy.

(4) **Observation period.** Duration of observation should be from the initial dosing with the MPCA to sacrifice of the offspring.

(5) **Observation of animals.** (i) A careful clinical examination should be made on each test animal 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 observations 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 sacrifice.

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

(6) **Mating procedure**—(i) **Parental.** (A) For each mating, each female should be placed with a single, randomly selected male until pregnancy occurs or 3 weeks have elapsed. Mixed matings with other males should be avoided.

(B) Those pairs that fail to mate successfully should be evaluated to determine the cause of the apparent infertility. This may involve such procedures as additional opportunities to mate with other sires or dams, examination of the reproductive organs, and examination of the estrus or spermatogenic cycles.

(C) Each morning, the female should be examined for presence of sperm or vaginal plugs. Day–0 of pregnancy is defined as the day vaginal plugs or sperm are found.

(ii) **Special housing.** Near parturition, pregnant animals should be caged separately in delivery or maternity cages and provided with nesting materials. The cages and materials should be free from contamination by the MPCA. Dosing should cease prior to isolation of the pregnant females.

(7) **Observation of pregnant females.** (i) Food consumption and prolonged parturition should be recorded, in addition to the above clinical observations (paragraphs (f)(5)(i) through (v) of this guideline).

(ii) The duration of gestation should be calculated from day–0 of pregnancy.

(iii) Each litter should be examined as soon as possible after delivery for the number of pups, stillbirths, live births, and the presence of physical and behavioral abnormalities.

(iv) Litters should be weighed at birth or soon thereafter.

(8) **MPCA enumeration in parents and offspring.** (i) Infectivity or persistence should be assessed by using sensitive techniques to deter-

mine, as quantitatively as possible, the presence of the MPCA in test animals.

(ii) Organs, tissues, and body fluids of each male parent should be assayed at the time when it is confirmed that the female of the mated pair is determined to be pregnant.

(iii) Organs, tissues, and body fluids of each female parent should be assayed as soon as possible after birth of the litter.

(iv) Quantitative determinations of the MPCA in the pups from each litter should be made on postpartum day l.

(g) **Data and reporting**—(1) **Evaluation of study results**. (i) An evaluation of test results shall include a reporting of any and all effects of the MPCA on the test animals, all observations made, statistical analyses, MPCA quantification in the dosing preparations and in the parents and offspring, evidence that a significant level of the MPCA was maintained in the parents, dosing schedule, and animal weights. This should include an evaluation of the relationship, or lack thereof, between carriage of the MPCA by parents and abnormal effects an reproduction and fertility.

(2) **Test report.** In addition to the information recommended by OPPTS 885.0001, the test report shall include the following information:

(i) Fertility indices and length of gestation.

(ii) Species and strain.

(iii) Time of death during the study or whether animals survived to termination.

(iv) Effects on reproduction and on offspring.

(v) Time of observation of each abnormal sign, including pathogenicity, and its subsequent course.

(vi) Body weight data for parents and offspring.

(vii) Necropsy findings.

(viii) MPCA enumerations.

(ix) Statistical treatment of results, where appropriate.

(h) **Tier progression.** Any further testing that is to be required will be determined after consultation with the Agency.