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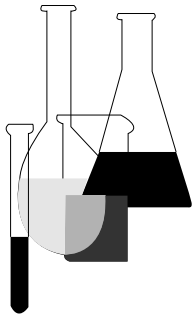
Health Effects Test Guidelines

OPPTS 870.5460
Rodent Heritable Translocation Assays



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 disks or paper copies: call (202) 512–0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (http://www.epa.gov/epahome/research.htm) under the heading ''Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines.''

OPPTS 870.5460 Rodent heritable translocation assays.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are OPPT 40 CFR 798.5460 Rodent heritable translocation assays and OECD guideline 485 Genetic Toxicology: Mouse Heritable Translocation Assay.

(b) **Purpose.** This test detects transmitted chromosomal damage which manifests as balanced reciprocal translocations in progeny descended from parental males treated with chemical mutagens.

(c) **Definitions.** The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this test guideline. The following definitions also apply to this test guideline.

Diakinesis and *metaphase* I are stages of meiotic prophase scored cytologically for the presence of multivalent chromosome association characteristic of translocation carriers.

Heritable translocation is one in which distal segments of non-homologous chromosomes are involved in a reciprocal exchange.

(d) **Test method**—(1) **Principle.** When a balanced reciprocal translocation is induced in a parental male germ cell, the resulting progeny is translocation heterozygote.

(i) **Basis for fertility screening.** Male translocation heterozygotes may be completely sterile. This class consists of two types of translocations:

(A) Translocations between non-homologous chromosomes in which at least one of the breaks occurs close to one end of a chromosome.

(B) Those that carry multiple translocations. The majority of male translocation heterozygotes are semisterile—they carry one or (rarely) two translocations. The degree of semisterility is dependent upon the proportions of balanced and unbalanced (duplication-deficiency) gametes produced in the ejaculate as a function of meiotic segregation. Balanced and unbalanced sperm are equally capable of fertilizing an egg. Balanced sperm lead to viable progeny. Unbalanced sperm result in early embryonic lethality.

(ii) **Basis for cytological screening.** The great majority of male translocation heterozygotes can be identified cytologically through analysis of diakinesis metaphase I spermatocytes. Translocation heterozygotes are characterized by the presence of multivalent chromosome association such

as a ring or chain of four chromosomes held together by chiasmata in paired homologous regions. Some translocation carriers can be identified by the presence of extra long and/or extra short chromosomes in spermatogonial and somatic cell metaphase preparations.

(2) **Description.** Essentially, two methods have been used to screen for translocation heterozygosity—one method uses a mating sequence to identify sterile and semisterile males followed by cytological examination of suspect male individuals; the other method deletes the mating sequence altogether and all F_1 male progeny are examined cytologically for presence of translocation. In the former approach, the mating sequence serves as a screen which eliminates most fully fertile animals for cytological confirmation as translocation heterozygotes.

(3) **Animal selection**—(i) **Species.** The mouse is the species generally used and is recommended.

(ii) **Age.** Healthy sexually mature animals should be used.

(iii) **Number.** The number of male animals necessary is determined by the following factors:

(A) The use of either historical or concurrent controls.

(B) The power of the test.

(C) The minimal rate of induction required.

(D) Whether positive controls are used.

(E) The level of significance desired.

(iv) **Assignment to groups.** Animals should be randomized and assigned to treatment and control groups.

(4) **Control groups**—(i) **Concurrent controls.** No concurrent positive or negative (vehicle) controls are recommended as routine parts of the heritable translocation assay. However, investigators not experienced in performing translocation testing should include a substance known to produce translocations in the assay as a positive control reference chemical.

(ii) **Historical controls.** At the present time, historical control data must be used in tests for significance. When statistically reliable historical controls are not available, negative (vehicle) controls should be used.

(5) **Test chemicals**—(i) **Vehicle.** When appropriate for the route of administration, solid and liquid test substances should be dissolved or suspended in distilled water or isotonic saline. Water-insoluble chemicals may be dissolved or suspended in appropriate vehicles. The vehicle used should

neither interfere with the test chemical nor produce toxic effects. Fresh preparations of the test chemical should be employed.

(ii) **Dose levels.** At least two dose levels should be used. The highest dose level should result in toxic effects (which should not produce an incidence of fatalities which would prevent a meaningful evaluation) or should be the highest dose attainable or 5 g/kg body weight.

(iii) **Route of administration.** Acceptable routes of administration include oral, inhalation, admixture with food or water, and IP or IV injection.

(e) **Test performance**—(1) **Treatment and mating.** The animals should be dosed with the test substances 7 days per week over a period of 35 days. After treatment, each male should be caged with two untreated females for a period of 1 week. At the end of 1 week, females should be separated from males and caged individually. When females give birth, the day of birth, litter size, and sex of progeny should be recorded. All male progeny should be weaned, and all female progeny should be discarded.

(2) **Testing for translocation heterozygosity.** When males are sexually mature, testing for translocation heterozygosity should begin. One of two methods should be used; the first method involves mating, determining those F_1 progeny which are sterile or semisterile and subsequent cytological analysis of suspect progeny; the other method does not involve mating and determining sterility or semisterility; all progeny are examined cytologically.

(i) **Determination of sterility or semisterility**—(A) **Conventional method.** Females are mated, usually three females for each male, and each female is killed at midpregnancy. Living and dead implantations are counted. Criteria for determining normal and semisterile males are usually established for each new strain because the number of dead implantations varies considerably among strains.

(B) **Sequential method.** Males to be tested are caged individually with females and the majority of the presumably normal males are identified on the basis of a predetermined size of one or two litters. Breeding pens are examined daily on weekdays beginning 18 days after pairing. Young are discarded immediately after they are scored. Males that sire a litter whose size is the same as or greater than the minimum set for a translocation-free condition are discarded with their litter. If the litter size is smaller than the predetermined number, a second litter is produced with the same rule applying. Males that cannot be classified as normal after production of a second litter are tested further by the conventional method or by cytological confirmation of translocation.

(ii) **Cytological analysis.** For cytological analysis of suspected semisteriles, the air-drying technique is used. Observation of at least two diakinesis-metaphase 1 cells with mutivalent association constitutes the required evidence for the presence of a translocation. Sterile males are examined by one of two methods, those with testes of normal size and sperm in the epididymis are examined by the same techniques used for semisteriles. Animals with small testes are examined by squash preparations or, alternatively, by examination of mitotic metaphase preparations. If squash preparations do not yield diakinesis-metaphase 1 cells, analysis of spermatogonia or bone marrow for the presence of unusually long or short chromosomes should be performed.

(f) **Data and report**—(1) **Treatment of results.** (i) Data should be presented in tabular form and should include the number of animals at risk, the germ cell stage treated, the number of partial steriles and semisteriles (if the fertility test is used), the number of cytogenetically confirmed translocation heterozygotes (if the fertility test is used, report the number of confirmed steriles and confirmed partial steriles), the translocation rate, and either the standard error of the rate or the upper 95 percent confidence limit on the rate.

(ii) These data should be presented for both treated and control groups. Historical or concurrent controls should be specified, as well as the randomization procedure used for concurrent controls.

(2) **Statistical evaluation.** Data should be evaluated by appropriate statistical methods.

(3) **Interpretation of results.** (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of heritable translocations. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of heritable translocations or a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) **Test evaluation.** (i) Positive results in the heritable translocation assay indicate that under the test conditions the test substance causes heritable chromosomal damage in the test species.

(ii) Negative results indicate that under the test conditions the test substance does not cause heritable chromosomal damage in the test species.

(5) **Test report.** In addition to the reporting recommendations as specified under 40 CFR part 792, subpart J, the following specific information should be reported:

(i) Species, strain, age, weight, and number of animals of each sex in each group.

(ii) Test chemical vehicle, route and schedule of administration, and toxicity data.

(iii) Dosing regimen, doses tested, and rationale for dosage selection.

(iv) Mating schedule and number of females mated to each male.

(v) The use of historical or concurrent controls.

(vi) Screening procedure including the decision criteria used and the method by which they were determined.

(vii) Dose-response relationship, if applicable.

(g) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Generoso, W.M. et al. Heritable translocation test in mice. *Mutation Research* 76:191–215 (1980).

(2) [Reserved]