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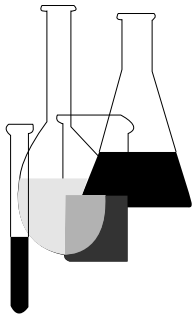
Health Effects Test Guidelines

OPPTS 870.5275 Sex-Linked Recessive Lethal Test in *Drosophila melanogaster*



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 870.5275 Sex-linked recessive lethal test in Drosophila melanogaster.

(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 798.5275 Sex-linked recessive lethal test in Drosophila melanogaster and OECD 477 Genetic Toxicology: Sex-Linked Recessive Lethal Test in Drosophila melanogaster.

(b) **Purpose.** The sex-linked recessive lethal (SLRL) test using *Drosophila melanogaster* (*D. melanogaster*) detects the occurrence of mutations, both point mutations and small deletions, in the germ line of the insect. This test is a forward mutation assay capable of screening for mutations at about 800 loci on the X-chromosome. This represents about 80 percent of all X-chromosome loci. The X-chromosome represents approximately one-fifth of the entire haploid genome.

(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.

Lethal mutation is a change in the genome which, when expressed, causes death to the carrier.

Recessive mutation is a change in the genome which is expressed in the homozygous or hemizygous condition.

Sex-linked genes are present on the sex (X or Y) chromosomes. Sex-linked genes in the context of this guideline refer only to those located on the X-chromosome.

(d) **Reference substances.** These may include, but need not be limited to, ethyl methanesulfonate or *N*-nitrosodimethylamine.

(e) **Test method**—(1) **Principle.** Mutations in the X-chromosome of *D. melanogaster* are phenotypically expressed in males carrying the mutant gene. When the mutation is lethal in the hemizygous condition, its presence is inferred from the absence of one class of male offspring out of the two that are normally produced by a heterozygous female. The SLRL test takes advantage of these facts by means of specially marked and arranged chromosomes.

(2) **Description.** Wild-type males are treated and mated to appropriate females. Female offspring are mated individually to their brothers, and in the next generation the progeny from each separate dose are scored for phenotypically wild-type males. Absence of these males indicates that

a sex-linked recessive lethal mutation has occurred in a germ cell of the P_1 male.

(3) **Drosophila stocks.** Males of a well-defined wild type stock and females of the Muller-5 stock may be used. Other appropriately marked female stocks with multiple inverted X-chromosomes may also be used.

(4) **Control groups**—(i) **Concurrent controls.** Concurrent positive and negative (vehicle) controls should be included in each experiment.

(ii) **Positive controls.** Examples of positive controls include ethyl methanesulfonate and *N*-nitrosodimethylamine.

(iii) **Other positive controls.** Other positive control reference substances may be used.

(iv) **Negative controls.** Negative (vehicle) controls should be included. The size of the negative (vehicle) control group should be determined by the availability of appropriate laboratory historical control data.

(5) **Test chemicals**—(i) **Vehicle.** Test chemicals should be dissolved in water. Compounds which are insoluble in water may be dissolved or suspended in appropriate vehicles (e.g., a mixture of ethanol and Tween-60 or 80) and then diluted in water or saline prior to administration. The use of dimethylsulfoxide as a vehicle should be avoided.

(ii) **Dose levels.** For the initial assessment of mutagenicity, it is sufficient to test a single dose of the test substance for screening purposes. This dose should be the maximum tolerated dose, or that which produces some indication of toxicity, or should be the highest dose attainable. For dose-response purposes, at least three additional dose levels should be used.

(iii) **Route of administration.** Exposure may be oral, by injection or by exposure to gases or vapors. Feeding of the test compound may be done in sugar solution. When necessary, substances may be dissolved in 0.7 percent NaCl solution and injected into the thorax or abdomen.

(f) **Test performance**—(1) **Treatment and mating.** Wild-type males (3 to 5 days old) should be treated with the test substance and mated individually to an appropriate number of virgin females from the Muller-5 stock or females from another appropriately marked (with multiply-inverted X-chromosomes) stock. The females should be replaced with fresh virgins every 2 to 3 days to cover the entire germ cell cycle. The offspring of these females are scored for lethal effects corresponding to the effects on mature sperm, mid or late stage spermatids, early spermatids, spermatocytes and spermatogonia at the time of treatment.

(2) **F_1 matings.** Heterozygous F_1 females from the above crosses should be allowed to mate individually (i.e., one female per vial) with

their brothers. In the F_2 generation, each culture should be scored for the absence of wild-type males. If a culture appears to have arisen from an F_1 female carrying a lethal in the parental X-chromosome (i.e., no males with the treated chromosome are observed), daughters of that female with the same genotype should be tested to ascertain if the lethality is repeated in the next generation.

(3) **Number of matings.** (i) The test should be designed with a predetermined sensitivity and power. The number of flies in each group should reflect these defined parameters. The spontaneous mutant frequency observed in the appropriate control group will strongly influence the number of treated chromosomes that must be analysed to detect substances which show mutation rates close to those of the controls.

(ii) Test results should be confirmed in a separate experiment.

(g) **Data and report**—(1) **Treatment of results.** Data should be tabulated to show the number of chromosomes tested, the number of nonfertile males and the number of lethal chromosomes at each exposure concentration and for each mating period for each male treated. Numbers of clusters of different size per male should be reported.

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

(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 sex-lined recessive lethals. 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 sex-linked recessive lethals or a statistically significant and reproducible positive response at any one of the test points is considered non-mutagenic in this system.

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

(4) **Test evaluation**. (i) Positive results in the SLRL test in *D. melanogaster* indicate that under the test conditions the test agent causes mutations in germ cells of this insect.

(ii) Negative results indicate that under the test conditions the test substance is not mutagenic in *D. melanogaster*.

(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) *Drosophila* stock used in the assay, age of insects, number of males treated, number of sterile males, number of F_2 cultures established, number of F_2 cultures without progeny.

(ii) Test chemical vehicle, treatment and sampling schedule, exposure levels, toxicity data, negative (vehicle) and positive controls, if appropriate.

(iii) Criteria for scoring lethals.

(iv) Number of chromosomes tested, number of chromosomes scored, number of chromosomes carrying a lethal mutation.

(v) Historical control data, if available.

(vi) Dose-response relationship, if applicable.

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

(1) Sobels, F.H. and Vogel, E. The capacity of *Drosophila* for detecting relevant genetic damage. *Mutation Research* 41:95–106 (1976).

(2) Wurgler F.E. et al. *Drosophila* as assay system for detecting genetic changes. Handbook of mutagenicity test procedures. Eds. Kilbey, B.J., Legator, M., Nichols, W., Ramel, C. Elsevier/North Holland Biomedical, Amsterdam (1977) pp.335–373.