OECD GUIDELINE FOR TESTING OF CHEMICALS

477

Adopted:
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"Genetic Toxicology: Sex-linked Recessive Lethal Test in *Drosophila melanogaster*"

1. INTRODUCTORY INFORMATION

• Prerequisites

– Solid, liquid, vapour or gaseous test substance
– Chemical identification of test substance
– Purity (impurities) of test substances
– Solubility characteristics
– Melting point/boiling point
– pH (where appropriate)
– Vapour pressure data (if available)

• Standard documents

There are no relevant international standards.

2. METHOD

A. INTRODUCTION, PURPOSE, SCOPE, RELEVANCE, APPLICATION AND LIMITS OF TEST

The sex-linked recessive lethal (SLRL) test using *Drosophila melanogaster* detects the occurrence of mutations, both point mutations and small deletions, in the germ line of an 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 per cent of all X-chromosomal loci. The X-chromosome represents approximately one-fifth of the entire genome (1).

• Definitions

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 Test Guideline refer only to those located on the X-chromosome.

• Reference substances

The following are examples of substances which might be used as a positive control:

– ethyl methanesulphonate
– N-nitroso-dimethylamine

• Principle of the test method

Mutations in the X-chromosome of *Drosophila 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.

B. DESCRIPTION OF THE TEST PROCEDURE

Methods for conducting the SLRL test in Drosophila are described in references (1) and (2). 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 cross 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.

• Preparations

Stocks

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

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Test substances

Test substances should be dissolved in water. Substances which are insoluble in water may be dissolved or suspended in appropriate vehicles (e.g. a mixture of ethanol and Tween-60 or 80), then diluted in water or saline prior to administration. Dimethylsulfoxide should be avoided as a vehicle.

- Test conditions

Route of administration

Administration may be oral, by injection or by exposure to gases or vapours. Feeding of the test substance may be done in sugar solution. When necessary, substances may be dissolved in a 0.7 per cent NaCl solution and injected into the thorax or abdomen.

Exposure levels

For the initial assessment of mutagenicity, one exposure of the test substance is used, that exposure being the maximum-tolerated concentration or that producing some indication of toxicity, where possible. If the test is being used as a method for verification, at least two additional exposure levels should be used.

Controls

Negative (vehicle) controls should be included. However, if appropriate laboratory historical control data are available, no concurrent controls are needed.

- Performance of the test

Wild-type males (3 to 5 days old) are treated with the test substance and mated individually to an excess of virgin females from the Muller-5 stock or females from another appropriately marked (with multiply inverted X-chromosomes) stock. The females are replaced with fresh virgins every two to three 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.

Heterozygous F_1 females from the above crosses are allowed to mate individually (i.e. one female per vial) with their brothers. In the F_2 generation, each culture is 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), a daughter of that female with the same genotype should be tested to ascertain whether the lethality is repeated at the next generation.

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

Test results should be confirmed in a separate experiment.

3. DATA AND REPORTING

- Treatment of results

Data should be tabulated to show the number of chromosomes tested, the number of non-fertile 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 sizes per male should be reported.

Several statistical techniques are acceptable in evaluating sex-linked recessive lethal tests. Clustering of recessive lethals originating from one male should be considered and evaluated in an appropriate statistical manner.

- Evaluation of results

There are several criteria for determining a positive response, one of which is a statistically significant dose-related increase in the frequency of sex-linked recessive lethal

mutations. Another criterion may be a reproducible and statistically significant positive response for at least one of the test points.

A test substance producing neither a statistically significant dose-related increase in the frequency of sex-linked recessive lethal mutations nor a statistically significant and reproducible positive response at any one of the test points is considered non-mutagenic in this system.

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

• Test report

The test report should also include the following information:

– stock: Drosophila stock or strains used, age of insects, number of males treated, number of sterile males, number of F_2 cultures established, number of F_2 cultures without progeny, number of chromosomes tested, number of chromosomes carrying a lethal mutation detected at each germ cell stage

– test conditions: detailed description of treatment and sampling schedule, exposure levels, toxicity data, negative (solvent) and positive controls, if appropriate

– criteria for scoring of lethal mutations

– exposure/effect relationship, where possible

– statistical evaluation

• Interpretation of results

Positive results from the SLRL-test in Drosophila indicate that a substance induces mutations in the germ line of the insect. Negative results indicate that, under the test conditions, the test substance does not induce mutations in the germ line of the insect.

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4. L I T E R A T U R E

1. F.H. Sobels and E. Vogel, *Mutation Res. 41*, 95-106 (1976).

2. F.E. Würgler, H. Sobels and E. Vogel, in *Handbook of Mutagenicity Test Procedures* (edited by B.J. Kilbey, *et al.*), pp. 335-373, Elsevier, Amsterdam (1977).