B.20. SEX-LINKED RECESSIVE LETHAL TEST IN *DROSOPHILA MELANOGASTER*

1. METHOD

1.1. Introduction

See General Introduction Part B.

1.2. Definition

See General Introduction Part B.

1.3. Reference substances

None.

1.4. Principles of the test method

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 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% of all X-chromosal loci. The X-chromosome represents approximately one-fifth of the entire haploid genome.

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.

1.5. Quality criteria

None.

1.6. Description of the test method

Preparations

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.

Test substance

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. Dimethylsulphoxide (DMSO) should be avoided as a vehicle.

Number of animals

The test should be designed with a predetermined sensitivity and power. The spontaneous mutant frequency observed in the appropriate control will influence strongly the number of treated chromosomes that must be analysed.

Route of administration

Exposure 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% NaCl solution and injected into the thorax or abdomen.

Use of negative and positive controls

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

Exposure levels

Three exposure levels should be used. For a preliminary assessment one exposure level of the test substance may be used, that exposure level being either the maximum tolerated concentration or that producing some indication of toxicity. For non-toxic substances exposure to the maximum practicable concentration should be used.

Procedure

Wild-type males (three to five days old) are treated with the test substance and mated individually to an excess of virgin females from the Muller-5 stock or from another appropriately marked (with multiple 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) daughters of that female with the same genotype should be tested to ascertain whether the lethality is repeated in the next generation.

2. DATA

Data should be tabulated to show the number of X-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. These results should be confirmed in a separate experiment.

Appropriate statistical methods should be used in evaluation sex-linked recessive lethal tests. Clustering of recessive lethals originating from one male should be considered and evaluated in an appropriate statistical manner.

3. REPORTING

3.1. Test report

The test report shall, if possible, contain the following information;

-stock: *Drosophila* stocks 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 carrying a lethal detected at each germ cell stage,
-criteria for establishing the size of treated groups,
-test conditions. detailed description of treatment and sampling schedule, exposure levels, toxicity data, negative (solvent) and positive controls, if appropriate,
-criteria for scoring lethal mutations,
-exposure/effect relationship where possible,
-evaluation of data,
-discussion of results,
-interpretation of results.

3.2. Evaluation and interpretation

See General Introduction Part B.

4. REFERENCES

See General Introduction Part B.