



"Genetic Toxicology: Rodent Dominant Lethal Test"**1. INTRODUCTORY INFORMATION****• Prerequisites**

- Solid, liquid, vapour or gaseous test substance
- Chemical identification of test substance
- Purity (impurities) of test substance
- 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**

Dominant lethal (DL) effects cause embryonic or foetal death. Induction of a dominant lethal event after exposure to a test substance indicates that the substance has affected germinal tissue of the test species. Dominant lethals are generally accepted to be the result of chromosomal aberrations (structural and numerical anomalies), but gene mutations and toxic effects cannot be excluded.

• Definitions

A dominant lethal mutation is one occurring in a germ cell which does not cause disfunction of the gamete but which is lethal to the fertilised egg or developing embryo.

• Reference substances

The following are examples of the type of substance which might be used as a positive control:

- triethylenemelamine
- cyclophosphamide
- ethyl methanesulphonate

- Principle of the test method

Generally, male animals are exposed to the test substance and mated to untreated virgin females. The various germ cell stages can be tested separately by the use of sequential mating intervals. The females are sacrificed after an appropriate period of time, and the contents of the uteri are examined to determine the numbers of implants and live and dead embryos. The calculation of the dominant lethal effect is based on comparison of the live implants per female in the treated group to the live implants per female in the control group. The increase of dead implants per female in the treated group over the dead implants per female in the control group reflects the post-implantation loss. The post-implantation loss is calculated by determining the ratio of dead to total implants from the treated group compared to the ratio of dead to total implants from the control group. Pre-implantation loss can be estimated on the basis of corpora lutea counts or by comparing the total implants per female in treated and control groups.

B. DESCRIPTION OF THE TEST PROCEDURE

Several treatment schedules are available. The most widely used requires single administration of the test substance. Other treatment schedules, such as treatment on five consecutive days, may be used if justified by the investigator.

Individual males are mated sequentially to virgin females at appropriate intervals. The number of matings following treatment is governed by the treatment schedule and should ensure that germ cell maturation is adequately covered. Females are sacrificed in the second half of pregnancy, and the uterine contents are examined to determine the total number of implants and the number of live and dead embryos.

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- Preparations

Test substances

Where appropriate, test substances should be dissolved or suspended in water or isotonic saline. Chemicals insoluble in water may be dissolved or suspended in appropriate vehicles. Normally, freshly prepared solutions or suspensions of the test substance should be employed.

- Experimental animals

Selection of species

Rats or mice are recommended as the test species. Healthy, sexually mature animals are randomised and assigned to treatment and control groups. Strains with low background dominant lethality, high pregnancy frequency and high implant numbers are recommended.

Number of animals

An adequate number of animals should be used, taking into account the spontaneous variation of the biological characteristics being evaluated. The number chosen should be based on the predetermined sensitivity of detection and power of significance. For example, in a typical experiment, the number of males in each group should be sufficient to provide between 30 and 50 pregnant females per mating interval.

Housing and feeding conditions

The environmental conditions should meet the needs of the test species in accordance with good animal husbandry.

- Test conditions

Route of administration

The usual routes of administration are oral or by intraperitoneal injection. Other routes may be appropriate.

Dose levels

Normally, three dose levels should be used. The highest dose should produce signs of toxicity (e.g. slightly reduced fertility). However, in an initial assessment of dominant lethality a single high dose may be sufficient. Non-toxic substances should be tested up to 5 g/kg or, if this is not practicable, then at the highest dose attainable.

Controls

Generally concurrent positive and negative (vehicle) controls should be included in each experiment. When acceptable positive control results are available from experiments conducted recently (within the last twelve months) in the same laboratory these results can be used instead of a concurrent positive control.

Positive control substances should be used at a dose which demonstrates the test sensitivity.

- Performance of the test

Individual males are mated sequentially at appropriate predetermined intervals to one or two virgin females. Females should be left with the males for at least the duration of one oestrus cycle or alternatively until mating has occurred as determined by the presence of sperm in the vagina or by the presence of a vaginal plug.

The number of matings following treatment is governed by the treatment schedule and should ensure that germ cell maturation is adequately covered.

Females are sacrificed in the second half of pregnancy and uterine contents are examined to determine the number of implants and live and dead embryos. The ovaries may be examined to determine the number of corpora lutea.

3. DATA AND REPORTING

- Treatment of results

Data should be tabulated to show the number of males, the number of pregnant females, and the number of non-pregnant females.

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Results of each mating, including the identity of each male and female, should be reported individually. The mating interval, dose level for males, and the numbers of live implants and dead implants should be enumerated for each female. The post-implantation loss is calculated by determining the ratio of dead to total implants from the treated group compared to the ratio of dead to total implants from a control group. Re-implantation loss can be calculated as the difference between the number of corpora lutea and the number of implants or as a reduction in the average number of implants per female in comparison with control matings. Where pre-implantation loss is estimated, it should be reported.

Data are evaluated by appropriate statistical methods. Differences among animals within the control and treatment groups should be considered before making comparisons between treated and control groups.

- Evaluation of results

There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of dominant lethals.

A test substance which does not produce a statistically significant dose-related increase in the number of dominant lethals 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:

- species and strain of animals used, age and weights of animals, number of animals of each sex in treated and control groups
- test substance, vehicle used, dose levels and rationale for dosage selection, negative (vehicle) and positive controls

- experimental observations, including signs of toxicity
- route and duration of exposure
- mating schedule
- methods used to determine that mating has occurred (where applicable)
- time of sacrifice
- criteria for scoring dominant lethals
- dose-response relationship, where applicable

- Interpretation of results

A positive dominant lethal assay suggests the possible genotoxicity of a test substance in the germ cells of the treated sex of the test species.

A negative result suggests that, under the conditions of the test, the test substance may not be genotoxic in the germ cells of the treated sex of the test species.

4. LITERATURE

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