



"Genetic Toxicology: Mouse Heritable Translocation Assay"**1. I N T R O D U C T O R Y I N F O R M A T I O N****• P r e r e q u i s i t e s**

- 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)

• S t a n d a r d d o c u m e n t s

There are no relevant international standards.

2. M E T H O D**A. I N T R O D U C T I O N**

The mouse heritable translocation test detects structural and numerical chromosome changes in mammalian germ cells as recovered in first generation progeny.

• P r i n c i p l e o f t h e m e t h o d

The types of chromosome changes detected in this test system are reciprocal translocations and, if female progeny are included, X-chromosome loss. Carriers of translocations and XO females show reduced fertility which is used to select F₁ progeny for cytogenetic analysis. Complete sterility is caused by certain types of translocations (X-autosome and c/t type). Translocations are cytogenetically observed in meiotic cells at diakinesis-metaphase I of male individuals, either F₁ males or male offspring of F₁ females. The XO-females are cytogenetically identified by the presence of only 39 chromosomes in bone marrow mitoses.

B. D E S C R I P T I O N O F T H E T E S T P R O C E D U R E**• P r e p a r a t i o n s*****Test substance***

When possible, test substances are dissolved or suspended in isotonic saline. Chemicals insoluble in water are dissolved or suspended in appropriate vehicles. The vehicle used should

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

Experimental animals

These experiments are performed using mice, in view of their convenience with regard to both ease of breeding and of cytological verification. No specific mouse strain is required. However, the average litter-size of the strain should be greater than 8 and be relatively constant. Sexually mature animals are used.

Number of animals

The number of animals necessary depends upon the spontaneous translocation frequency, and the minimal rate of induction required for a positive result. The test is usually performed by analysis of male F₁ progeny. Large numbers of animals, of the order of 500 F₁ males per dose level, are required.

- Test conditions

Controls

Adequate control data, derived from concurrent and historical controls, should be available. When acceptable positive control results are available from experiments conducted recently in the same laboratory, these results can be used instead of a concurrent positive control.

Dose levels

One dose level is tested, usually the highest dose associated with the production of minimal toxic effects, but without affecting reproductive behaviour or survival. To establish a dose-response relationship, two additional lower doses are required. Non-toxic substances should be tested up to 5 g/kg when using a single dose regimen or up to 1 g/kg/day when using a repeated dose regimen or, if these dosages are not practicable, at the highest dose attainable.

Route of administration

Routes of administration are usually oral intubation or intraperitoneal injection. Other routes of administration may be appropriate. Maximum utility for risk assessment is obtained when the route of administration is relevant to human exposure.

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• Performance of the test

Treatment and mating

Two treatment schedules are available. Single administration of the test substance is most widely used. Administration of the test substance on 7 days/week for 35 days may also be used. The number of matings following treatment is governed by the treatment schedule and should ensure that all treated germ cell stages are sampled. At the end of the mating period females are caged individually. When females give birth, the date, litter size and sex of progeny are recorded. All male progeny are weaned and all female progeny are discarded unless they are included in the experiment.

Testing for translocation heterozygosity

One of two possible methods is used:

- Fertility testing of F_1 progeny and subsequent verification of possible translocation carriers by cytogenetic analysis;
- Cytogenetic analysis of all male F_1 progeny without prior selection by fertility testing.

a) Fertility testing

Reduced fertility of an F_1 individual can be established by litter size observation and/or analysis of uterine contents of female mates.

Criteria for determining normal and reduced fertility must be established for the mouse strain used.

Litter size observation

F_1 males to be tested are caged individually with females either from the same experiment or from the colony. Cages are inspected daily beginning 18 days after mating. Litter size and sex of the F_2 progeny are recorded at birth and litters are discarded thereafter. If female F_1 progeny are tested, the F_2 progeny of small litters are kept for further testing. Female translocation carriers are verified by cytogenetic analysis of a translocation in any of their male offspring. XO-Females are recognized by the change in sex ratio among their progeny from 1:1 to 1:2 males vs. females. In a sequential procedure, normal F_1 animals are eliminated from

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further testing if the test F₂ litter reaches or exceeds a predetermined normal value, otherwise a second or third F₂ litter is observed. F₁ animals that cannot be classified as normal after observation of up to three F₂ litters are either tested further by analysis of uterine contents of female mates or directly subjected to cytogenetic analysis.

Analysis of uterine contents

The reduction in litter size of translocation carriers is due to embryonic death so that a high number of dead implants is indicative of the presence of a translocation in the animal under test. F₁ males to be tested are mated to 2-3 females each. Conception is established by daily inspection for vaginal plugs in the morning. Females are sacrificed 14-16 days later, and living and dead implants in their uteri are recorded.

b) Cytogenetic analysis

Testes preparations are made by the air-drying technique. Translocation carriers are identified by the presence of multivalent configurations at diakinesis-metaphase I in primary spermatocytes. Observation of at least 2 cells with multivalent association constitutes the required evidence that the tested animal is a translocation carrier.

If no breeding selection has been performed, all F₁ males are inspected cytogenetically. A minimum of 25 diakinesis-metaphase I cells per male must be scored microscopically. Examination of mitotic metaphases, spermatogonia or bone-marrow, is required in F₁ males with small testes and meiotic breakdown before diakinesis or from F₁ female XO suspects. The presence of an unusually long and/or short chromosome in each of 10 cells is evidence for a particular male sterile translocation (c/t type). Some X-autosome translocations that cause male sterility may only be identified by banding analysis of mitotic chromosomes. The presence of 39 chromosomes in all of 10 mitoses is evidence for an XO condition in a female.

3. DATA AND REPORTING

• Treatment of results

Data are presented in tabular form.

The mean litter size and sex ratio from parental matings at birth and weaning are reported for each mating interval.

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For fertility assessment of F₁ animals, the mean litter size of all normal matings and the individual litter sizes of F₁ translocation carriers are presented. For analysis of uterine contents, the mean number of living and dead implants of normal matings and the individual numbers of living and dead implants for each mating of F₁ translocation carriers are reported.

For cytogenetic analysis of diakinesis-metaphase I, the number and types of multivalent configurations and the total number of cells are listed for each translocation carrier.

For sterile F₁ individuals, the total number of matings and the duration of the mating period are reported. Testes weights and cytogenetic analysis details are given.

For XO females, the mean litter size, sex ratio of F₂ progeny and cytogenetic analysis results are reported. Where possible, F₁ translocation carriers are preselected by fertility tests. The tables have to include information on how many of these were confirmed translocation heterozygotes.

The data are evaluated by appropriate statistical methods.

• E v a l u a t i o n o f r e s u l t s

There are several criteria for determining a positive result, one of which is a statistically significant increase in the number of translocations observed for at least one test point. Another criterion may be based on the detection of a statistically significant dose-related increase in the number of translocations observed.

A test substance producing neither a statistically significant increase in the number of translocations observed for at least one test point nor a statistically significant dose-related increase in the number of translocations observed is considered non-mutagenic in this system.

• T e s t r e p o r t

The test report should include the following information:

- strain of mice, age of animals, weight of treated animals;
- numbers of parental animals of each sex in experimental and control groups;
- concurrent or historical controls if used and/or available;
- test conditions, detailed description of treatment, dose levels, solvents;
- mating schedule;

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- number and sex of offspring per female, number and sex of offspring raised for translocation analysis;
- time and criteria of translocation analysis;
- number and detailed description of translocation carriers including breeding data and uterine contents data, if applicable;
- cytogenetic procedures and details of microscopic analysis, preferably with pictures;
- statistical evaluation;
- discussion of results;
- interpretation of results.

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

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