# B.23. MAMMALIAN SPERMATOGONIAL CHROMOSOME ABERRATION TEST

## 1. METHOD

This method is a replicate of the OECD TG 483, Mammalian Spermatogonial Chromosome Aberration Test (1997).

### 1.1 INTRODUCTION

The purpose of the *in vivo* mammalian spermatogonial chromosome aberration test is to identify those substances that cause structural chromosome aberrations in mammalian spermatogonial cells (1)(2)(3)(4)(5). Structural aberrations may be of two types, chromosome or chromatid. With the majority of chemical mutagens, induced aberrations are of the chromatid type, but chromosome-type aberrations also occur. This method is not designed to measure numerical aberrations and is not routinely used for that purpose. Chromosome mutations and related events are the cause of many human genetic diseases.

This test measures chromosome events in spermatogonial germ cells and is, therefore, expected to be predictive of induction of inheritable mutations in germ cells.

Rodents are routinely used in this test. This *in vivo* cytogenetic test detects chromosome aberrations in spermatogonial mitoses. Other target cells are not the subject of this method.

To detect chromatid-type aberrations in spermatogonial cells, the first mitotic cell division following treatment should be examined before these lesions are lost in subsequent cell divisions. Additional information from treated spermatogonial stem cells can be obtained by meiotic chromosome analysis for chromosome-type aberrations at diakinesis-metaphase I when the treated cells become spermatocytes.

This *in vivo* test is designed to investigate whether somatic cell mutagens are also active in germ cells. In addition, the spermatogonial test is relevant to assessing mutagenicity hazard in that it allows consideration of factors of *in vivo* metabolism, pharmacokinetics and DNA-repair processes.

A number of generations of spermatogonia are present in the testis with a spectrum of sensitivity to chemical treatment. Thus, the aberrations detected represent an aggregate response of treated spermatogonial cell populations, with the more numerous differentiated spermatogonial cells predominating. Depending on their position within the testis, different generations of spermatogonia may or may not be exposed to the general circulation, because of the physical and physiological Sertoli cell barrier and the blood-testis barrier.

If there is evidence that the test substance, or a reactive metabolite, will not reach the target tissue, it is not appropriate to use this test.

See also General Introduction Part B.

1.2 DEFINITIONS

**Chromatid-type aberration:** structural chromosome damage expressed as breakage of single chromatids or breakage and reunion between chromatids.

**Chromosome-type aberration:** structural chromosome damage expressed as breakage, or breakage and reunion, of both chromatids at an identical site.

**Gap:** an achromatic lesion smaller than the width of one chromatid, and with minimum misalignment of the chromatids.

**Numerical aberration:** a change in the number of chromosomes from the normal number characteristic of the animals utilised.

**Polyploidy:** a multiple of the haploid chromosome number (*n*) other than the diploid number (i.e. 3*n*, 4*n* and so on).

**Structural aberration:** a change in chromosome structure detectable by microscopic examination of the metaphase stage of cell division, observed as deletions, intrachanges or interchanges.

1.3 PRINCIPLE OF THE TEST METHOD

Animals are exposed to the test substance by an appropriate route of exposure and are sacrificed at appropriate times after treatment. Prior to sacrifice, animals are treated with a metaphase-arresting substance (e.g. colchicine or Colcemid®). Chromosome preparations are then made from germ cells and stained, and metaphase cells are analysed for chromosome aberrations.

1.4 DESCRIPTION OF THE TEST METHOD

1.4.1 **Preparations**

1.4.1.1 *Selection of animal species*

Male Chinese hamsters and mice are commonly used. However, males of other appropriate mammalian species may be used. Commonly used laboratory strains of young healthy adult animals should be employed. At the commencement of the study the weight variation of animals should be minimal and not exceed ± 20% of the mean weight.

1.4.1.2 *Housing and feeding conditions*

General conditions referred in the General Introduction to Part B are applied although the aim for humidity should be 50-60%.

1.4.1.3 *Preparation of the animals*

Healthy young adult males are randomly assigned to the control and treatment groups. Cages should be arranged in such a way that possible effects due to cage placement are minimized. The animals are identified uniquely. The animals are acclimated to the laboratory conditions for at least five days prior to the start of the study.

1.4.1.4 *Preparation of doses*

Solid test substances should be dissolved or suspended in appropriate solvents or vehicles and diluted, if appropriate, prior to dosing of the animals. Liquid test substances may be dosed directly or diluted prior to dosing. Fresh preparations of the test substance should be employed unless stability data demonstrate the acceptability of storage.

1.4.2 **Test conditions**

1.4.2.1 *Solvent/vehicle*

The solvent/vehicle should not produce toxic effects at the dose levels used and should not be suspected of chemical reaction with the test substance. If other than well-known solvents/vehicles are used, their inclusion should be supported by data indicating their compatibility. It is recommended that wherever possible, the use of an aqueous solvent/vehicle should be considered first.

1.4.2.2 *Controls*

Concurrent positive and negative (solvent/vehicle) controls should be included in each test. Except for treatment with the test substance, animals in the control groups should be handled in an identical manner to animals in the treated groups.

Positive controls should produce structural chromosome aberrations *in vivo* in spermatogonial cells when administered at exposure levels expected to give a detectable increase over background.

Positive control doses should be chosen so that the effects are clear but do not immediately reveal the identity of the coded slides to the reader. It is acceptable that the positive control be administered by a route different from the test substance and sampled at only a single time. In addition, the use of chemical class-related positive control chemicals may be considered, when available. Examples of positive control substances include:

| **Substance** | **CAS No.** | **EINECS No.** |
|---|---|---|
| Cyclophosphamide<br><br>Cyclophosphamide monohydrate | 50-18-0<br><br>6055-19-2 | 200-015-4 |
| Cyclohexylamine | 108-91-8 | 203-629-0 |
| Mitomycin C | 50-07-7 | 200-008-6 |
| Monomeric acrylamide | 79-06-1 | 201-173-7 |
| Triethylenemelamine | 51-18-3 | 200-083-5 |

Negative controls, treated with solvent or vehicle alone, and otherwise treated in the same way as the treatment groups, should be included for every sampling time, unless acceptable inter-animal variability and frequency of cells with chromosome aberrations are demonstrated by historical control data. In addition, untreated controls should also be used unless there are historical or published control data demonstrating that no deleterious or mutagenic effects are induced by the chosen solvent/vehicle.

1.5 PROCEDURE

1.5.1 **Number of animals**

Each treated and control group must include at least 5 analysable males.

1.5.2 **Treatment schedule**

Test substances are preferably administered once or twice (i.e. as a single treatment or as two treatments). Test substances may also be administered as a split dose, i.e. two treatments on the same day separated by no more than a few hours, to facilitate administering a large volume of material. Other dose regimens should be scientifically justified.

In the highest dose group two sampling times after treatment are used. Since cell cycle kinetics can be influenced by the test substance, one early and one late sampling time are used around 24 and 48 hours after treatment. For doses other than the highest dose, a sampling time of 24 hours or 1.5 cell cycle length after treatment should be taken, unless another sampling time is known to be more appropriate for detection of effects (6).

In addition, other sampling times may be used. For example in the case of chemicals which may induce chromosome lagging, or may exert S-independent effects, earlier sampling times may be appropriate (1).

The appropriateness of a repeated treatment schedule needs to be identified on a case-by-case basis. Following a repeated treatment schedule the animals should then be sacrificed 24 hours (1.5 cell cycle length) after the last treatment. Additional sampling times may be used where appropriate.

Prior to sacrifice, animals are injected intraperitoneally with an appropriate dose of a metaphase arresting substance (e.g. Colcemid® or colchicine). Animals are sampled at an appropriate interval thereafter. For mice this interval is approximately 3 - 5 hours, for Chinese hamsters this interval is approximately 4 - 5 hours.

1.5.3 **Dose levels**

If a range finding study is performed because there are no suitable data available, it should be performed in the same laboratory, using the same species, strain and treatment regimen to be used in the main study (7). If there is toxicity, three dose levels are used for the first sampling time. These dose levels should cover a range from the maximum to little or no toxicity. At the later sampling time only the highest dose needs to be used. The highest dose is defined as the dose producing signs of toxicity such that higher dose levels, based on the same dosing regimen, would be expected to produce lethality.

Substances with specific biological activities at low non-toxic doses (such as hormones and mitogens) may be exceptions to the dose-setting criteria and should be evaluated on a case-by-case basis. The highest dose may also be defined as a dose that produces some indication of toxicity in the spermatogonial cells (e.g. a reduction in the ratio of spermatogonial mitoses to first and second meiotic metaphases; this reduction should not exceed 50%).

1.5.4 **Limit test**

If a test at one dose level of at least 2000 mg/kg body weight/day using a single treatment, or as two treatments on the same day, produces no observable toxic effects, and if genotoxicity would not be expected based upon data from structurally related substances, then a full study using three dose levels may not be considered necessary. Expected human exposure may indicate the need for a higher dose level to be used in the limit test.

1.5.5 **Administration of doses**

The test substance is usually administered by gavage using a stomach tube or a suitable intubation cannula, or by intraperitoneal injection. Other routes of exposure may be acceptable where they can be justified. The maximum volume of liquid that can be administered by gavage or injection at one time depends on the size of the test animal. The volume should not exceed 2 ml/100g body weight. The use of volumes higher than these must be justified. Except for irritating or corrosive substances, which will normally reveal exacerbated effects with higher concentrations, variability in test volume should be minimised by adjusting the concentration to ensure a constant volume at all dose levels.

1.5.6 **Chromosome preparation**

Immediately after sacrifice, cell suspensions are obtained from one or both testes, exposed to hypotonic solution and fixed. The cells are then spread on slides and stained.

1.5.7 **Analysis**

For each animal at least 100 well-spread metaphase should be analysed (i.e. a minimum of 500 metaphases per group). This number could be reduced when high numbers of aberrations are observed. All slides, including those of positive and negative controls, should be independently coded before microscopic analysis. Since fixation procedures often result in the breakage of a proportion of metaphases with loss of chromosomes, the cells scored should contain a number of centromeres equal to the number $2n \pm 2$.

2. **DATA**

2.1 TREATMENT OF RESULTS

Individual animal data should be presented in a tabular form. The experimental unit is the animal. For each individual animal the number of cells with structural chromosome aberrations and the number of chromosome aberrations per cell should be evaluated. Different types of structural chromosome aberrations should be listed with their numbers and frequencies for treated and control groups. Gaps are recorded separately and reported but generally not included in the total aberration frequency.

If mitosis as well as meiosis is observed, the ratio of spermatogonial mitoses to first and second meiotic metaphases should be determined as a measure of cytotoxicity for all treated and negative control animals in a total sample of 100 dividing cells per animal to establish a possible cytotoxic effect. If only mitosis is observed, the mitosis index should be determined in at least 1000 cells for each animal.

2.2 EVALUATION AND INTERPRETATION OF RESULTS

There are several criteria for determining a positive result, such as a dose-related increase in the relative number of cells with chromosome aberrations or a clear increase in the number of cells with aberrations in a single dose at a single sampling time. Biological relevance of the results should be considered first. Statistical methods may be used as an aid in evaluating the test results (8). Statistical significance should not be the only determining factor for a positive response. Equivocal results should be clarified by further testing preferably using a modification of experimental conditions.

A test substance for which the results do not meet the above criteria is considered non-mutagenic in this test.

Although most experiments will give clearly positive or negative results, in rare cases the data set will preclude making a definite judgement about the activity of the test substance. Results may remain equivocal or questionable regardless of the number of times the experiment is repeated.

Positive results from the *in vivo* spermatogonial chromosome aberration test indicate that the test substance induces structural chromosome aberrations in the germ cells of the species tested. Negative results indicate that, under the test conditions, the test substance does not induce chromosome aberrations in the germ cells of the species tested.

The likelihood that the test substance or its metabolites reach the target tissue should be discussed.

3. **REPORTING**

TEST REPORT

The test report must include the following information:

Solvent/Vehicle

— justification for choice of vehicle;

— solubility and stability of the test substance in solvent/vehicle, if known;

Test animals:

— species/strain used;

— number and age of animals;

— source, housing conditions, diet, etc.;

— individual weight of the animals at the start of the test, including body weight range, mean and standard deviation for each group;

Test conditions :

— data from range finding study, if conducted;

— rationale for dose level selection;

— rationale for route of administration;

— details of test substance preparation;

— details of the administration of the test substance;

— rationale for sacrifice times;

— conversion from diet/drinking water test substance concentration (ppm) to the actual dose (mg/kg body weight/day), if applicable;

— details of food and water quality;

— detailed description of treatment and sampling schedules;

— methods for measurement of toxicity;

— identity of metaphase arresting substance, its concentration and duration of treatment;

— methods of slide preparation;

— criteria for scoring aberrations;

— number of cells analysed per animal;

— criteria for considering studies as positive, negative or equivocal;

Results:

— signs of toxicity;

— mitotic index;

— ratio of spermatogonial mitoses cells to first and second meiotic metaphases;

— type and number of aberrations, given separately for each animal;

— total number of aberrations per group;

— number of cells with aberrations per group;

— dose-response relationship, if possible;

— statistical analyses, if any;

— concurrent negative control data;

— historical negative control data with ranges, means and standard deviations;

— concurrent positive control data;

— changes in ploidy, if seen.

Discussion of results.

Conclusions.

## 4. REFERENCES

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