



"Genetic Toxicology: *In vivo* Mammalian Bone Marrow Cytogenetic Test - Chromosomal Analysis"

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

The *in vivo* bone marrow cytogenetic test is a mutagenicity test for the detection of structural chromosomal aberrations. Chromosomal aberrations are generally evaluated in first post-treatment mitoses. With the majority of chemical mutagens, induced aberrations are of the chromatid type, but chromosome-type aberrations also occur.

• Definitions

Chromosome-type aberration: a change which results from damage expressed in both sister chromatids at the same site.

Chromatid-type aberration: change expressed as breakage of single chromatids or breakage and reunion between chromatids.

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- Principle of the test method

Animals are exposed to test substances by appropriate routes and are sacrificed at sequential intervals. Chromosome preparations are made from bone marrow cells. The stained preparations are examined and metaphase cells are scored for chromosomal aberrations.

B. DESCRIPTION OF THE TEST PROCEDURE

The method employs bone marrow of laboratory rodents which have been exposed to test substances. Prior to sacrifice, animals are further treated with a spindle inhibitor (e.g. colchicine or Colcemid[®]) to arrest the cells in c-metaphase. Chromosome preparations from the cells are stained and scored for aberrations.

- Preparations

Test substances

Where appropriate, test substances should be dissolved in water or isotonic saline; if insoluble, they should be dissolved or suspended in appropriate vehicles. Freshly prepared solutions or suspensions of the test substance should be employed.

- Experimental animals

Selection of species

Any appropriate mammalian species may be used. Examples of commonly used rodent species are rats, mice and hamsters. Healthy young adult animals are randomised and assigned to treatment and control groups.

Number and sex

At least five female and five male animals per experimental and control group are employed. Thus, ten animals would be sacrificed per time per group treated with the test substance if several test times after treatment are included in the experimental schedule. The use of a single sex or a different number of animals should be justified.

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Housing and feeding conditions

Animals may be caged in groups by sex or individually; the number of animals per cage should not interfere with clear observation of each animal. Appropriate diet and drinking water should be supplied *ad libitum*. Temperature, humidity and light cycles should be controlled as dictated by good animal husbandry procedures.

• Test conditions

Route of administration

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

Treatment schedule

In general, test substances should be administered once only. However, based on toxicological information a repeated treatment schedule may be employed.

Dose levels

For an initial assessment, one dose of the test substance may be used, the dose being the maximum-tolerated dose or that producing some indication of cytotoxicity (e.g. partial inhibition of mitosis). Additional dose levels may be used. For determination of dose-response, at least three dose levels should be used.

Controls

A positive and a negative (vehicle) control are included. The positive control substance should be known to produce chromosome aberrations *in vivo*.

• Performance of the test

Generally the test may be performed in two ways:

- (a) Animals are treated with the test substance once at the selected dose(s). Samples are taken at three times after treatment. For rodents the central sampling interval is 24 hours. Since

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cell cycle kinetics can be influenced by the test substance, one earlier and one later sampling interval adequately spaced within the range of 6 to 48 hours are applied. Where the additional dose levels are tested in a subsequent experiment, samples should be taken at the predetermined most sensitive interval or, if this is not established, at the central sampling time.

- (b) If a repeated treatment schedule is used at the selected dose(s), samples should be taken 6 and 24 hours after the last treatment; other sampling times may be used if justified. Where the additional dose levels are tested in a subsequent experiment samples should be taken at the predetermined most sensitive interval or, if this is not established, at six hours after the last treatment.

Prior to sacrifice, animals are injected intraperitoneally with an appropriate dose of a spindle inhibitor (e.g. colchicine or Colcemid ®) to arrest cells in c-metaphase.

Immediately after sacrifice, bone marrow is obtained, exposed to hypotonic solution and fixed. The cells are then spread on slides and stained.

Analysis

The number of cells to be analysed per animal should be based upon the number of animals used, the negative control frequency, the predetermined sensitivity and the power chosen for the test. Slides should be coded before microscopic analysis.

3. DATA AND REPORTING

• Treatment of results

Data should be presented in tabular form for both cells and animals. Different types of structural chromosomal aberrations should be listed with their numbers and frequencies for treated and control groups. Gaps (achromatic lesions) are recorded separately and not included

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in the total aberration frequency. Differences among animals within each group should be considered before making comparisons between treated and control groups. Data should be evaluated by appropriate statistical methods.

• 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 structural chromosomal aberrations.

A test substance which does not produce a statistically significant dose-related increase in the number of structural chromosomal aberrations 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:

- animals: species and strain of animals used, age and weights of animals, number of animals of each sex in treated and control groups
- rationale for selection of dose(s)
- test conditions: detailed description of treatment and sampling schedules, dose levels, identity of spindle-inhibitor, its concentration and duration of treatment
- mitotic index, where applicable
- criteria for scoring aberrations
- number of cells analysed per animal
- type and number of aberrations, given separately for each animal
- total number of aberrations per group
- number of cells with aberrations per group

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– dose-response relationship, where possible

• Interpretation of results

Positive results in the *in vivo* bone marrow cytogenetic assay demonstrate the ability of the test substance to induce chromosomal aberrations in the bone marrow of the test species.

Negative results indicate that under the test conditions the test substance does not induce chromosomal aberrations in the bone marrow of the test species.

4. LITERATURE

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