"Genetic Toxicology: Micronucleus Test"

1. INTRODUCTORY INFORMATION

• Prerequisites

- Solid, liquid, gaseous or vapour test substance
- Chemical identification of test substance
- Purity (impurities) of test substance
- Solubility characteristics
- Melting point/boiling point
- pH (where appropriate)

• Standard documents

There are no relevant international standards.

2. METHOD

A. INTRODUCTION, PURPOSE, SCOPE, RELEVANCE, APPLICATION AND LIMITS OF TEST

The micronucleus test is a mammalian *in vivo* test which detects damage to the chromosomes or the mitotic apparatus induced by chemicals (1) (4) (6).

Polychromatic erythrocytes in the bone marrow of rodents are used in this assay. When an erythroblast develops into an erythrocyte, the main nucleus is extruded and may leave micronuclei in the cytoplasm. Visualisation of micronuclei is facilitated in these cells because they lack a nucleus. Micronuclei form under normal conditions. The assay is based on an increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow of treated animals.

• Definitions

Micronuclei are small particles consisting of acentric fragments of chromosomes or entire chromosomes which lag behind at anaphase stage of cell division. After telophase, these fragments may not be included in the nuclei of daughter cells and form single or multiple micronuclei in the cytoplasm.

- Principle of the test method

Animals are exposed to the test substance by an appropriate route. They are sacrificed, the bone marrow extracted and smear preparations made and stained. Polychromatic erythrocytes are scored for micronuclei under the microscope.

B. DESCRIPTION OF THE TEST PROCEDURE

- Preparations

Test substances

Solid and liquid test substances should be dissolved in isotonic saline. If insoluble, they should be dissolved or suspended in appropriate vehicles. Freshly prepared solutions of the test substance should be employed.

- Experimental animals

Selection of species

Mice are recommended. However, any appropriate mammalian species may be used. 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 if several sampling times after treatment are included in the experimental schedule. The use of a single sex or a different number of animals should be justified.

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

Treatment schedule

Test substances should generally be administered only once. However, based on pharmacokinetic and metabolic information, a repeated treatment schedule may be employed. A repeated treatment schedule can only be applied if the test substance at the dose(s) used does not exhibit cytotoxic effects in the bone marrow.

Dose levels

For the initial assessment of genotoxicity, 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. a change in the ratio of polychromatic to normochromatic erythrocytes. Additional dose levels may be used when these are indicated by scientific reasons. If the test is being used for verification, at least two additional dose levels should be used.

Controls

A substance known to produce micronuclei *in vivo* is employed as a positive control, and a negative (solvent) control group is also included in the design of each experiment.

- Performance of the test

The test may be performed in two ways:

(a) Animals are treated with the test substance once at the highest dose. Sampling times should coincide with the maximum response of the assay, which varies with the test substance. Therefore, using the highest dose, samples of bone marrow are taken at least three times, starting not earlier than 12 hours after treatment, with appropriate intervals following the first sample, but not extending beyond 72 hours. When other doses are used, sampling should be at the maximum sensitive period, or, if that is not known, approximately 24 hours after treatment. Other appropriate sampling times may be used in addition.

(b) If pharmacokinetic and metabolic information indicates a repeated treatment schedule, repeated dosage can be employed, and samples should be taken at least three times, starting

not earlier than 12 hours after the last treatment and then at appropriate intervals following the first sample, but not extending beyond 72 hours.

Bone marrow is obtained from both femurs of freshly killed animals. Cells are separated, put on slides, spread as a smear and stained.

Analysis

Slides are coded before microscopic analysis. At least 1000 polychromatic erythrocytes per animal are scored for the incidence of micronuclei. The ratio of polychromatic to normochromatic erythrocytes is determined for each animal by counting a total of 1000 erythrocytes. Additional information may be obtained by scoring normochromatic erythrocytes for micronuclei.

3. DATA AND REPORTING

- Treatment of results

Data should be presented in tabular form, including positive and negative control and experimental groups. The number of polychromatic erythrocytes scored, the number of micronucleated polychromatic erythrocytes and the percentage of micronucleated cells are listed separately for each experimental animal. The ratio of polychromatic to normochromatic erythrocytes and, if considered applicable, the percentage of micronucleated normochromatic erythrocytes is given for each animal. Data should be evaluated by using 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 micronucleated polychromatic erythrocytes. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

A test substance producing neither a statistically significant dose-related increase in the number of micronucleated polychromatic erythrocytes nor a statistically significant and reproducible positive response at any one of the test points is considered non-mutagenic in this system.

- Test report

The test report should also include the following information:

- animals: species and strain of animals used, age and weight of animals, number of animals for each sex in experimental and control groups
- test conditions: detailed description of and rationale for treatment and sampling schedule, dose levels, toxicity data, negative (solvent) and positive controls
- criteria for identification of micronucleated polychromatic erythrocytes
- dose/response relationship, where possible

- Interpretation of results

Positive results in the micronucleus test indicate that a substance induces micronuclei in the polychromatic erythrocytes in the test species, which may have been the result of chromosomal damage or damage to the mitotic apparatus. Negative results indicate that, under the test conditions, the test substance does not produce micronuclei in the polychromatic erythrocytes in the test species.

4. LITERATURE

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