OECD GUIDELINE FOR TESTING OF CHEMICALS

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"Genetic Toxicology: *In vitro* Sister Chromatid Exchange Assay in Mammalian Cells"

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

The sister chromatid exchange (SCE) assay is a short-term test for the detection of reciprocal exchanges of DNA between two sister chromatids of a duplicating chromosome. SCEs represent the interchange of DNA replication products at apparently homologous loci. The exchange process presumably involves DNA breakage and reunion, although little is known about its molecular basis. Detection of SCEs requires some means of differentially labelling sister chromatids, which can be achieved e.g. by incorporation of bromodeoxyuridine (BrdU) into chromosomal DNA for two cell cycles. SCEs can also be measured in mammals and in non-mammalian systems.

• Principle of the method

Mammalian cells *in vitro* are exposed to the test chemical with and without an exogenous mammalian metabolic activation system and cultured for two rounds of replication in BrdU-containing medium. After treatment with a spindle inhibitor (e.g. colchicine) to accumulate cells in a metaphase-like stage of mitosis (c-metaphase), cells are harvested and chromosome preparations are made.

B. DESCRIPTION OF THE METHOD

- Preparations

Test substance

The test substance may be prepared in the culture medium or dissolved in an appropriate vehicle just prior to treatment of the cells. The final concentration of a vehicle in the culture system should not significantly affect cell viability, growth rate or SCE frequency.

Cells and culture technique

Primary cultures (e.g. human lymphocytes) or established cell lines (e.g. Chinese hamster ovary or lung cells) may be used in the assay. Cell lines should be checked for mycoplasma contamination, and karyotype stability should be monitored.

Appropriate culture media and incubation conditions (e.g. temperature, culture vessels, CO_2 concentration and humidity) should be used.

Metabolic activation

Cells should be exposed to the test substance both in the presence and absence of an appropriate mammalian metabolic activation system. Examples of such activation systems include cofactor-supplemented post-mitochondrial fractions prepared from the livers of mammals treated with enzyme inducers, and primary cultures of mammalian hepatocytes.

- Test Conditions

Exposure concentrations

At least three adequately spaced concentrations of the test substance should be used. The highest concentration should give rise to a significant toxic effect but must still allow adequate cell replication to occur. Relatively water-insoluble substances should be tested up to their limit of solubility using appropriate procedures. For freely water-soluble, non-toxic substances, the highest concentration used should be determined on a case-by-case basis.

Number of replicates

At least duplicate cultures should be used for each experimental point.

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Controls

Positive controls, using both a direct acting compound and a compound requiring metabolic activation, should be included in each study. A vehicle control should be used. The following are examples of substances which might be used as positive controls:

– ethylmethanesulphonate, mitomycin C (direct acting compounds);
– cyclophosphamide (indirect acting compound).

• Performance of the test

Preparation of cultures

Established cell lines are generated from stock cultures (e.g. by trypsinization or by shaking off), seeded in culture vessels at appropriate density and incubated at 37° C. For monolayer cultures, the number of cells per culture vessel should be adjusted so that the cultures are less than confluent at the time of harvest. Cells may be used in suspension culture. Human lymphocyte cultures are set up from blood, using appropriate techniques and incubated at 37° C.

Treatment

Established cell lines in an exponential stage of growth are exposed to the test substance for a suitable period of time. In most cases one to two hours may be effective, but the treatment time may be extended up to two complete cell cycles. In certain cases it may be more effective to treat the cells in serum-free medium. Cells should be exposed to the test chemical in the presence and absence of an appropriate metabolic activation system. At the end of the exposure period, cells are washed free of test substance and cultured for two rounds of replication in the presence of BrdU. As an alternative procedure, cells may be exposed simultaneously to the test substance and BrdU for the complete culture time of two cell cycles.

Human lymphocyte cultures are treated while they are in a semisynchronous condition.

Cells are analysed in their second mitotic division after initiation of treatment ensuring that the most sensitive cell cycle stages have been exposed to the chemical.

All cultures to which BrdU is added should be handled in a way to minimize photolysis of BrdU-containing DNA up to harvesting of cells.

Harvesting of cells

Cell cultures are treated with a spindle inhibitor (e.g. colchicine) 1-4 hours prior to harvesting. Each culture is harvested and processed separately for the preparation of chromosomes.

Chromosome preparation and staining

Chromosome preparations are made by standard cytogenetic techniques. Staining of slides to show SCEs can be performed by several techniques (e.g. the fluorescence plus Giemsa method).

Analysis

Usually, at least 25 well-spread metaphases per culture are analysed for SCEs, but the number may be influenced by the spontaneous control frequency. Slides are coded before analysis. In human lymphocytes, only metaphases containing 46 centromeres are analysed. In established cell lines only metaphases containing +/- 2 centromeres of the modal number are analysed. It should be stated whether or not centromeric switch of label is scored as an SCE. The result should be confirmed in a independent experiment.

3. DATA AND REPORTING

- Treatment of results

Data should be presented in tabular form. The number of SCEs, the number of chromosomes and the calculated mean number of SCEs per chromosome should be listed separately for each metaphase for all treated and control cultures.

The data should be evaluated 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 mean number of SCEs per cell. Another criterion may be the 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 mean number of SCEs per cell nor a statistically significant and reproducible positive response at any one of the test points is considered not active in this system.

- Test report

The test report should include the following information:

- cells used, methods of maintenance of cell culture;
- test conditions: composition of media, CO_2 concentration, concentrations of test substance, vehicle used, incubation temperature, treatment time, spindle inhibitor used, itsconcentration and duration of treatment, type of mammalian metabolic activation system used, positive and negative controls, BrdU concentration used;
- number of cell cultures per experimental point;
- details of the technique used for slide preparation;
- number of metaphases analysed (data given separately for each culture);
- mean number of SCEs per cell and per chromosome (data given separately for each culture);
- criteria for scoring SCEs;
- rationale for dose selection;
- dose-response relationship, if applicable;
- statistical evaluation;
- discussion of results;
- interpretation of results.

4. LITERATURE

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