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Protocol for chromosome
aberration studies on
various coded samples
using cultured Chinese
hamster ovary cells

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1. STUDY ORGANIZATION**1.1. BIBRA Project No.**

1363/2/0
June 1993

1.2. Title

Protocol for chromosome aberration studies on various coded samples using cultured Chinese hamster ovary cells

1.3. Testing facility

BIBRA Toxicology International
Woodmansterne Road
Carshalton
Surrey SM5 4DS
UK

1.4. Sponsor

B.A.T. (UK & Export) Ltd
Research & Development Centre
Regent's Park Road
Southampton SO9 1PE
UK

1.5. Critical dates

A detailed timetable will be issued as a protocol addendum following receipt of instructions from the sponsor to proceed.

1.6. Responsible staff

Study Director : B.J. Phillips, BSc, PhD, MIBiol.

1.7. Good Laboratory Practice

The study described in this protocol will be carried out to the highest scientific standards and in accordance with written Standard Operating Procedures.

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1.8. Sponsor representative

The sponsors will appoint a representative to assure them of the adequate conduct of the study and to act as the contact between the sponsor and BIBRA.

Instructions to modify the study will be accepted only from this representative or an individual nominated in writing by the representative.

1.8.1. Definition of representative

The sponsor representative will be:

Dr E.D. Massey
B.A.T. (UK & Export) Ltd
Research & Development Centre
Regent's Park Road
Southampton SO9 1PE
UK

1.8.2. Access of representative to the study

The sponsor representative or any nominated individual will have access at all times within normal working hours (Monday to Friday 09.00 to 17.00 hr) to all stages of the study, all data generated as part of the study and to the scientific staff responsible for its conduct.

It is expected that such access would normally be by agreement with the Study Director.

Access to various parts of the BIBRA facility will depend upon compliance with codes of practice relating to use and safety.

1.8.3. Contact

The sponsor representative will be informed of the progress of the study by:
- receiving copies of any documents passed from BIBRA to the sponsor relating to the conduct of the study

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- any visits made (1.8.2). If such visits are made by a nominated individual, it will be the responsibility of that individual to report to the sponsor representative
- contact by the most rapid means available should unexpected events occur. All contacts will be confirmed in writing

1.8.4. Integrity of the study

In the event of unexpected occurrences requiring action, BIBRA will contact the sponsor representative (1.8.3.). Should the representative not be available or not respond in reasonable time, BIBRA will take the action considered to be in the best interests of the study. Details of the reason and the action will be documented to the representative and will form part of the study record.

2. SCIENTIFIC OBJECTIVE

To assess the potential of various coded samples to cause chromosome aberrations in cultured Chinese hamster ovary (CHO) cells.

3. TEST AND CONTROL ARTICLES

3.1. Test articles

The test articles will be various coded samples provided by the sponsor with any relevant data relating to stability and storage conditions. Unless otherwise indicated, samples will be stored at room temperature in the containers provided.

3.2. Positive control article

Ethyl methanesulphonate will be provided by BIBRA.

3.3. Negative control article

This will be the solvent used for the test article and will be provided by BIBRA.

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4. TEST SYSTEM

4.1. Cell line

The Chinese hamster ovary cell line, isolated by Kao and Puck (Proc. Natl. Acad. Sci. USA, 1967, 60, 1275) and cloned by O'Neill *et al.* (Mutation Res. 1977, 145, 91) is designated CHO-KI-BH₁.

4.2. Cell culture

The stock of cells is stored in liquid nitrogen. A sample will be recovered approximately 10 days before the start of the study and grown in Ham's F12 medium, supplemented with 5% foetal bovine serum and antibiotics.

4.3. Justification

The study of chromosome aberrations in cultured cells has been widely used for detecting the potential of radiation and chemicals to cause genetic damage (e.g. Ishidate, M. Jr., Harnois, M.C. and Sofuni, T., Mutation Res. 1988, 195, 151). The method described in this protocol is based on the recommendations of the United Kingdom Environmental Mutagen Society (Report of the UKEMS Sub-Committee on Guidelines for Mutagenicity Testing Part I, 1989).

5. EXPERIMENTAL DESIGN

The following studies will be conducted on each of the test articles.

5.1. Preliminary toxicity test

A range of test article concentrations will be suggested by the sponsor. The effects of these concentrations on cell viability will be checked as follows. A series of cultures will be established and incubated for 24 hr. One culture will be treated with each of the suggested concentrations for 2 hr. The cultures will be examined visually after 24 hr and 3 doses selected for chromosome aberration tests on the basis of sufficient mitotic cells.

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5.2. Chromosome aberration test

Sixteen cultures will be established and incubated for 24 hr. They will then be treated as follows:

Number of cultures	Treatment
3	Test article - Conc. 1
3	Test article - Conc. 2
3	Test article - Conc. 3
2	Ethylmethanesulphonate (positive control)
5	Solvent used for test article

After 2 hr, the test solutions will be replaced with fresh medium.

Slides will be prepared (6.1.) 24 hr after the start of treatment and evaluated under code for mitotic index (6.2.) and chromosome aberrations (6.3.).

6. PROCEDURES AND OBSERVATIONS

6.1. Preparation of chromosome slides

Starting 2 hr before the designated harvest time, cultures will be incubated with colcemid (0.1 $\mu\text{g}/\text{ml}$) to arrest mitosis. The cells will then be suspended, treated for 10 min with 0.075 KCl and fixed in several changes of methanol:acetic acid (3:1 v/v). Slides will be prepared by air drying and stained with Giemsa solution.

6.2. Evaluation of mitotic index

The slides from each culture will be scanned and the number of mitotic figures and interphase nuclei recorded up to a total of 500. The mitotic index will be expressed as the percentage of mitotic cells in the sample.

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6.3. Evaluation of chromosome aberrations

A total of 100 well-spread metaphases, comprising 18-21 chromosomes, will be evaluated from the set of slides from each culture. Chromatid and chromosome gaps, breaks and exchanges will be noted according to a classification system based on that recommended by the UKEMS guidelines for mutagenicity testing.

The proportion of metaphases showing one or more aberrations (both including and excluding gaps) will be used as the basic statistic for evaluation using the Fisher's Exact test and analysis for linear trend.

7. REPORTING

A report will be prepared which:-

- defines the responsible staff
- defines the test article
- defines the test system
- defines the procedures used
- summarizes the findings
- evaluates the findings
- lists the data generated

8. PROTOCOL ACCEPTANCE

I hereby declare that the study described in this protocol will be conducted under my supervision to the highest scientific standards and in accordance with written Standard Operating Procedures.

Study Director:
B.J. Phillips, BSc, PhD, MIBiol.

Signature *B. J. Phillips* Date *8th June 1993*

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