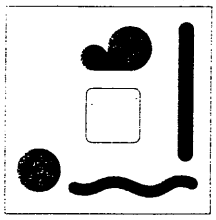

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Project ID of the contracting Institute
RCC 701853

RCC-CCR PROJECT 615904

CHROMOSOME ABERRATION ASSAY *IN VITRO:* PHOTOMUTAGENICITY IN CHINESE HAMSTER V79 CELLS WITH CGF-C-1607

REPORT

Study Completion Date:
October 05, 1998

RCC

COPY OF GLP CERTIFICATE

HESSISCHES MINISTERIUM
FÜR UMWELT, ENERGIE,
JUGEND, FAMILIE UND
GESUNDHEIT

GLP-Bescheinigung

Bescheinigung

Hiermit wird bestätigt, daß die Prüfeinrichtung
RCC Cytotest Cell Research GmbH
in 64380 Roßdorf
In den Leppsteinswiesen 19
(Ort, Anschrift)
der RCC/CCR Holding Verwaltungs GmbH
(Firma)
am 25./26. Februar 1998
(Datum)

von der für die Überwachung zuständigen Behörden über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist.

Es wird hiermit bestätigt, daß folgende Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden:

Prüfungen zur Bestimmung der toxikologischen Eigenschaften
Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)

Certificate

It is hereby certified that the test facility
RCC Cytotest Cell Research GmbH
in 64380 Roßdorf
In den Leppsteinswiesen 19
(location, address)
of RCC/CCR Holding Verwaltungs GmbH
(company name)
on 25./26. Februar 1998
(date)

was (inspected by the competent authority regarding compliance with the Principles of Good Laboratory Practice.

It is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice:

Toxicity studies

Mutagenicity studies

Im Auftrag

Dr. Hecker

HESSISCHES MINISTERIUM FÜR UMWELT, ENERGIE, JUGEND, FAMILIE UND GESUNDHEIT
2

(Dr. Hecker) Wiesbaden, den 30 März 1998

CONTENTS

PREFACE

General

Sponsor:	CIBA Spezialitätenchemie Grenzach GmbH Postfach 1266 D-79630 Grenzach-Wyhlen
Study Monitor:	Dr. U. Mentzel
Testing Facility:	RCC CYTOTEST CELL RESEARCH GMBH In den Leppsteinswiesen 19 D-64380 Roßdorf
RCC-CCR Project No.:	615904
Contracting Institute:	R C C REGISTRATION AND CONSULTING COMPANY LTD. CH-4452 Itingen; Switzerland
RCC Project No.:	701853
Test Article:	CGF-C-1607
RCC-CCR Test Article No.:	S 121322
Title:	Chromosome Aberration Assay *in vitro*: Photomutagenicity in Chinese Hamster V79 Cells with CGF-C-1607

Project Staff

Study Director:	Dr. Andreas Czich
Management:	Markus Arenz
Quality Assurance Unit:	Frauke Hermann

Schedule

Date of Protocol:	July 20, 1998
Start of Experiments:	July 22, 1998
End of Experiments:	September 30, 1998
Date of Final Report:	October 05, 1998

Project Staff Signatures

Study Director — Dr. Andreas Czich

Date: October 05, 1998

Management — Markus Arenz

Date: October 05, 1998

Quality Assurance

The study was performed in compliance with:

„Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, „Anhang 1" (Annexe 1), dated July 25, 1994 („BGBl I 1994", pp. 1703), last revision May 14, 1997.

„The OECD Principles of Good Laboratory Practice", Paris 1981.

Guidelines

This study was conducted according to the procedures indicated by the following internationally accepted guidelines and recommendations:

SCC Guideline CSC/803-5/90 (1990). Commission of the European Communities Scientific Committee for Cosmetology. Guidelines for assessing the potential for toxicity of compounds used as sunscreen agents in cosmetics, Annex 1, Notes of guidance for the toxicity testing of cosmetic ingredients.

Ninth Addendum to the OECD Guidelines for Testing of Chemicals, February 1998, adopted July 21, 1997, Guideline Nr.473 „*In vitro* Mammalian Cytogenetic Test".

EEC Directive 92/69, L 383 A, Annexe V, B 10, dated December 29, 1992.

Archiving

RCC Cytotest Cell Research, D-64380 Roßdorf will archive the following data for 15 years:

Raw data, protocol, and a copy of the report.

A sample of the test article will be archived for at least 2 years following the date on which the report is audited by the Quality Assurance Unit and also at least until the next inspection of RCC Cytotest Cell Research by the GLP-authority.

Microscopic slides will be archived for at least 12 years.

If there are no other instructions by the sponsor the raw data and the above mentioned material will be discarded at the end of the archiving period.

Deviation to the protocol

a) Positive Control Substances

Without Irradiation

Endkonzentration: **800 µg/ml = 6,4 mM**

b) Experimental Performance

Schedule

Exposure time: **0.5 h**

c) Treatment

Exposure time 18 and 28 hours:

After two days of incubation ....

30 min after start of irradiation the test-article in the PBS solution was removed and culture medium was added. This medium ...

d) Preparation of the Cultures

16 h (18 h) and 26 h (28 h) after the start of the **irradiation** colcemid was added (0.2 µg/ml culture medium) to the cultures. 2.0 h later, the cells were treated on the slides in the Quadriperm chambers with hypotonic solution (0.4 % KCl) for 20 min at 37° C.

Reason for the deviations (printed in bold letters): updating

These deviations have no detrimental impact on the outcome of the study.

STATEMENT OF COMPLIANCE

Project Number:	615904
Test Material :	CGF-C-1607
Study Director:	Dr. Andreas Czich
Title:	Chromosome Aberration Assay *in vitro*: Photomutagenicity in Chinese Hamster V79 Cells with CGF-C-1607

This study performed in the testing facility of RCC-CCR was conducted in compliance with Good Laboratory Practice Regulations.

„Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, „Anhang 1" (Annexe 1), dated July 25, 1994 („BGBl I 1994", pp. 1703), last revision May 14, 1997.

„The OECD Principles of Good Laboratory Practice", Paris 1981.

There were no circumstances that may have affected the quality or integrity of the study.

Study Director

RCC Cytotest Cell Research GmbH
Dr. Andreas Czich

A. Czich

.....

Date: October 09, 1998

QUALITY ASSURANCE UNIT

RCC Cytotest Cell Research GmbH
In den Leppsteinswiesen 19
D-64380 Roßdorf

Statement

Project Number: 615904

Test Material : CGF-C-1607

Study Director: Dr. Andreas Czich

Title: Chromosome Aberration Assay *in vitro*: Photomutagenicity in Chinese Hamster V79 Cells with CGF-C-1607

This report was audited by the Quality Assurance Unit and the conduct of this study was inspected on the following dates:

Phases of QAU Inspections/Audits	Dates of QAU Inspections/Audits	Dates of Reports to the Study Director and to Management
Protocol Audit	July 22, 1998	July 22, 1998
Study Inspection	August 28, 1998	August 28, 1998
Final Report Audit	Ocotber 07, 1998	October 07, 1998

Head of Quality Assurance Unit Frauke Hermann

F. Hermann

Date: October 09, 1998

SUMMARY OF RESULTS

The test article CGF-C-1607, dissolved in acetone, was assessed for its potential to induce structural chromosome aberrations in V79 cells (Chinese hamster cell line) in the absence and presence of a UVA/UVB light source (Suntest CPS, ATLAS, D-63558 Gelnhausen). In experiment I cultures with the test article-PBS solution (containing 1 % (v/v) acetone with the test article) were irradiated with 200 mJ/cm^2 UVA and 22 mJ/cm^2 UVB. In experiment II the cultures were irradiated with the dose mentioned above and additionally with 300 mJ/cm^2 UVA and 33 mJ/cm^2 UVB. The chromosomes were prepared 18 h (exp. I) and 28 h (exp. II) after start of irradiation with the test article. In each experimental group two parallel cultures were set up. In the test article groups and in the negative control groups per culture 100 metaphases were scored for structural chromosomal aberrations.

The test article concentrations were chosen with regard to the solubility of the test article in PBS and the results of the Chromosome Aberration Assay with CGF-C-1607 (RCC-CCR Project 597700). The irradiation dose was chosen according to its none toxicity to the cells.

In this study, precipitation of the test article in culture medium was observed after treatment with 25.0 µg/ml and above in the **absence** and **presence** of irradiation. No influence of the test article on the pH value or osmolarity was observed.

In both independent experiments, in the **absence** and the **presence** of UV-light no reduction of the mitotic indices or the cell numbers could be observed up to the highest applied test article concentration of 100.0 µg/ml.

In both independent experiments (exp. I 18 h and exp. II 28 h preparation interval) in the **absence** and **presence** of UV irradiation the test article did not increase the frequency of cells carrying structural chromosomal aberrations. In the **absence** and **presence** of UV irradiation the aberration rates of the cells after treatment with the test article were near to the range of the solvent control values and within the range of our historical control data.

In both experiments, no biologically relevant increase in the frequency of polyploid metaphases was found after treatment with the test article as compared to the frequencies of the controls.

EMS and 8-Methoxypsoralen was used as positive control and induced distinct increases in cells with structural chromosomal aberrations.

Conclusion

In conclusion, it can be stated that in the study described and under the experimental conditions reported, the test article did not induce structural chromosome aberrations under and after irradiation with UV light as determined by the chromosomal aberration test in the V79 Chinese hamster cell line.

Therefore, CGF-C-1607 is considered to be none mutagenic in this chromosomal aberration test.

Table 1: Summary of results of the chromosomal aberration study with CGF-C-1607

Exp	Prep. interval	Irradiation UVA/UVB in mJ/cm²	Concentration of CGF-C-1607 in µg/ml	Polyploid cells in %	Mitotic index in % of control	Aberrant cells in % incl gaps	excl. gaps*	exchanges
I	18 h	0/0	solvent control	3.8	100.0	2.5	0.5	0.0
		0/0	negative control	4.1	100.0	2.0	1.0	0.5
		0/0	positive control	3.0	109.1	13.5	11.0**	4.5
		0/0	6.25	2.8	94.8	2.0	1.5	0.0
G		0/0	12.5	2.9	86.5	4.0	1.5	0.5
		0/0	25.0^{P}	4.7	103.1	2.5	1.0	0.0
		0/0	100.0^{P}	3.8	87.8	2.5	2.0	0.0
II	28 h	0/0	solvent control	3.4	100.0	2.0	1.0	0.0
		0/0	negative control	3.5	100.0	1.0	0.5	0.0
		0/0	positive control	4.0	100.8	14.0	11.0**	4.0
		0/0	12.5	3.4	99.3	0.5	0.5	0.0
		0/0	25.0^{P}	2.8	95.0	2.0	1.0	0.0
		0/0	50.0^{P}	3.3	103.9	3.0	2.0	0.0
		0/0	100.0^{P}	3.4	103.2	0.5	0.5	0.0
I	18 h	200/22	solvent control	5.7	100.0	1.5	1.5	0.5
		200/22	negative control	3.4	100.0	3.5	1.0	0.0
		200/22	positive control	2.1	68.0	13.0	13.0**	7.5
		200/22	6.25	2.6	88.9	1.5	1.0	1.0
		200/22	12.5	5.7	95.4	6.0	3.5	1.0
		200/22	25.0^{P}	5.4	102.7	3.0	2.0	0.5
		200/22	100.0^{P}	4.4	94.7	5.0	4.0	0.5
II	28 h	200/22	solvent control	3.8	100.0	3.5	2.5	0.5
		200/22	negative control	3.8	100.0	4.0	2.5	0.5
		200/22	positive control	2.6	86.0	7.5	7.0**	2.5
		200/22	12.5	3.3	106.7	2.0	1.5	0.5
		200/22	25.0^{P}	2.3	113.3	1.5	0.5	0.0
		200/22	50.0^{P}	2.5	123.1	1.0	0.5	0.0
		200/22	100.0^{P}	2.7	130.8	0.5	0.5	0.0
II	28 h	300/33	solvent control	4.4	100.0	2.5	1.5	0.0
		300/33	negative control	3.3	100.0	3.0	2.5	0.5
		300/33	positive control	2.8	78.5	26.5	24.0**	9.5
		300/33	12.5	4.2	82.1	3.5	3.0	0.0
		300/33	25.0^{P}	3.4	99.6	1.5	1.0	0.5
		300/33	50.0^{P}	3.0	72.3	2.0	1.5	0.0
		300/33	100.0^{P}	2.4	81.7	3.5	2.5	1.0

* inclusive cells carrying exchanges
** Aberration frequency statistically significant higher than corresponding negative control values
P precipitation occurred

INTRODUCTION

According to legal requirements cosmetics should be tested for possible hazards to humans and environment before use. Genotoxicity studies provide important information for the assessment of the mutagenic potential of these articles (1, 2). The *in vitro* chromosome aberration assay performed in this study is an essential part for genotoxicity studies of substances and test article formulations.

This *in vitro* study with V79 cells is an assay for the detection of the potential to induce structural chromosomal aberrations after treatment with the UV-irradiated test article. It was shown that the evaluation of the photomutagenic potential is indicated for compounds wich are used by man concomitantly with light exposure (3, 4). An obvious example are cosmetic ingredients used as UV absorbers.

Chromosome aberrations are frequently lethal to the damaged cells. However, cytogenetic damage in somatic cells is an indicator of a potential to induce more subtle chromosomal damage that may be compatible with cell division. Similar damage induced in germ cells may lead to heritable cytogenetic abnormalities. Heritable cytogenetic abnormalities are known to have deleterious effects in man, e.g. induction of neoplastic events or birth defects. Also, chromosome abnormalities in somatic cells may become one of the reasons why a transformed cell population possibly is leading to neoplastic processes.

Chromosome aberrations should be evaluated in first post-treatment mitoses. With the majority of chemical mutagens induced aberrations are therefore of the chromatid type. However, chromosome type aberrations mainly occur if the scored cell has passed the second post treatment mitosis..

For treatment, cells should be in exponential growth to guarantee that there are cells in all stages of the cell cycle (i.e. an asynchronous population). Since the normal cell cycle time of V79 cells is 12 h and the guidelines require preparation times of about 1.5-fold of the normal cell cycle, a preparation time of around 18 hours is appropriate. Because there may be chemicals which induce very extensive mitotic delay at clastogenic concentrations or may be clastogenic only when cells have gone through more than one cell cycle since the beginning of treatment an additional later sampling time (28 h) will be included (5).

At preparation time 18 h, cultures after treatment with three concentrations of the test article were evaluated for cytogenetic damage. At the later preparation time (28 h) one culture treated with the highest concentration was evaluated for cytogenetic damage.

The frequency of polyploid cells was checked so that the level in control cultures can be monitored and their induction by the test agent can be recorded especially when a later sampling time is used.

To validate the test, reference mutagens are tested in parallel to the test article.

Aims of the Study

This *in vitro* assay was performed to assess the potential of the test article to induce structural chromosome aberrations in the presence of UV irradiation by means of two independent chromosome aberration experiments using V79 cells (Chinese hamster cell line).

MATERIALS AND METHODS

Test Article

The test article and the information concerning the test article were provided by the sponsor.

Name:	CGF-C-1607
Batch No.:	6
Aggregate State at Room Temperature:	solid
Molecular Weight:	627.80
Colour:	yellow
Purity:	> 98 %
Analysis:	HPLC
Stability in solvent:	not indicated
Storage:	room temperature
Expiration Date:	July 30, 1999

On the day of the experiment (immediately before treatment), the test article was dissolved in acetone (E. MERCK, D-64293 Darmstadt; purity 99.5 %). The final concentration of acetone in the culture medium was 1 % (v/v).

Controls

Negative Controls

Concurrent negative (culture medium) and solvent controls were performed to exclude an influence of the solvent on the results.

Positive Control Substances

With Irradiation

Name:	8-methoxypsoralene (8-MOP)
Supplier:	SIGMA, D-82041 Deisenhofen, Germany
Catalogue No.:	820775
Purity:	> 99.0 %
Dissolved in:	DMSO
Concentration:	0.5 µg/ml

Without Irradiation

Name:	EMS; Ethylmethansulfonat
Hersteller:	Merck-Schuchardt, D-85662 Hohenbrunn
Katalog Nr.:	820774; (Reinheit: > 98%)
Gelöst in:	Nährmedium
Endkonzentration:	800 µg/ml = 6,4 mM

Solution prepared on day of experiment.

The stability of the positive control substance in solution was proven by the mutagenic response in the expected range.

Test System

Reasons for the Choice of the Cell Line V79

The V79 cell line has been used successfully in *in vitro* experiments for many years. Especially the high proliferation rate (doubling time of the V79/T5 cell clone in stock cultures: 12 h; determined on March 17, 1994) and a high plating efficiency of untreated cells (as a rule more than 70 %) both necessary for the appropriate performance of the study, recommend the use of this cell line. The cells have a stable karyotype with a modal chromosome number of 22.

Cell Cultures

Large stocks of the V79 cell line (supplied by Laboratory for Mutagenicity Testing, LMP, Technical University Darmstadt, D-64287 Darmstadt) were stored in liquid nitrogen in the cell bank of RCC-CCR allowing the repeated use of the same cell culture batch in experiments. Before freezing each batch was screened for mycoplasm contamination and checked for karyotype stability. Consequently, the parameters of the experiments remain similar because of standardized characteristics of the cells.

Thawed stock cultures were propagated at 37° C in 80 cm^2 plastic flasks (GREINER, D-72632 Frickenhausen). About 5 x 10^5 cells per flask were seeded into 15 ml of MEM (Minimal Essential Medium; SEROMED; D-12247 Berlin) supplemented with 10 % fetal calf serum (FCS; Boehringer Mannheim, D-68261 Mannheim). The cells were subcultured twice weekly. The cell cultures were incubated at 37° C in a humidified atmosphere with 4.5 % carbon dioxide (95.5 % air).

Source of Light

Irradiation was performed with a Xenon light source which emits a continuous spectrum simulating sunlight (obtained from Suntest CPS, ATLAS, D-63558 Gelnhausen). The exposure rates were determined with a Dr. Hönle UV-meter (obtained from Dr. Hönle GmbH, D-82152 Planegg/München, Germany) using two individual detectors, one for UV-A and one for UV-B light. Due to low UV-B dose levels the UV-B dose was calculated from the UV-A value.

Dose Selection

The test article concentrations were chosen with regard to the OECD Guideline 473, the solubility of the test article in PBS and the results of the Chromosome Aberration Assay with CGF-C-1607 (CCR Project 597700).

In this study, precipitation of the test article in culture medium was observed after treatment with 25.0 µg/ml and above in the absence and presence of irradiation. No influence of the test article on the pH value or osmolarity was observed.

Table 2: Doses applied in this study with CGF-C-1607

Preparation interval	Experiment	Concentrations in µg/ml					
		without irradiation					
18 h	I	**6.25**	**12.5**	**25.0**	50.0	75.0	**100.0**
28 h	II		**12.5**	**25.0**	**50.0**	75.0	**100.0**
		200 mJ/cm² UVA und 22 mJ/cm² UVB					
18 h	I	**6.25**	**12.5**	**25.0**	50.0	75.0	**100.0**
28 h	II		**12.5**	**25.0**	**50.0**	75.0	**100.0**
		300 mJ/cm² UVA und 33 mJ/cm² UVB					
28 h	II		**12.5**	**25.0**	**50.0**	75.0	**100.0**

evaluated experimental points were printed in bold letter

Experimental Performance

Schedule

	with irradiation			without irradiation	
	Exp. I	Exp. II		Exp. I	Exp. II
Pre incubation of the Test article in PBS	30 min	30 min	30 min	30 min	30 min
Power of light	0,4 mW/cm^2	0,4 mW/cm^2	0,4 mW/cm^2	0 mW/cm^2	0 mW/cm^2
Irradiation time	8 min 20 sec	8 min 20 sec	12 min 30 sec	0 min	0 min
UVA dose	200 mJ/cm^2	200 mJ/cm^2	300 mJ/cm^2	0 mJ/cm^2	0 mJ/cm^2
UVB dose	22 mJ/cm^2	22 mJ/cm^2	33 mJ/cm^2	0 mJ/cm^2	0 mJ/cm^2
Exposure time	0.5 h	0.5 h	0.5 h	0.5 h	0.5 h
Recovery	17.5 h	27.5h	27.5 h	17.5 h	27.5 h
Preparation interval	18 h	28 h	28 h	18 h	28 h

Seeding of the Cultures

Three days old exponentially growing stock cultures more than 50 % confluent were trypsinized at 37 °C for approximately 5 minutes. Then the enzymatic digestion was stopped by adding complete culture medium and a single cell suspension was prepared. The trypsin concentration was 0.2 % in Ca-Mg-free salt solution (Trypsin: Difco Laboratories, Detroit, USA).

The Ca-Mg-free salt solution was composed as follows (per litre):

NaCl	8000 mg
KCl	400 mg
Glucose	1000 mg
$NaHC0_3$	350 mg

Prior to the trypsin treatment the cells were rinsed with Ca-Mg-free salt solution containing 200 mg/l EDTA (Ethylene diamine tetraacetic acid).

The cells were seeded into Quadriperm dishes (Heraeus, D-63450 Hanau) which contain microscopic slides (at least 2 chambers per dish and test group). Into each chamber about 1 x 10^4 - 6 x 10^4 cells were seeded with regard to preparation time. The medium was MEM + 10 % FCS.

Treatment

Exposure time 18 and 28 hours:

After two days of incubation cell cultures have reached a confluency of about 50 %. Before irradiation the culture medium was replaced by phosphate buffered saline (PBS) containing the solvent or several dilutions of the test article. PBS was chosen since it only minimally absorbs the UV wavelengths applied (3). After 30 min pre-incubation with the test article in the dark, the culture dishes were placed under the UV source. The lids had been removed and the cultures were treated with UV light. Four Quadriperm dishes were irradiated in a field emitting 0.4 mW/cm^2 UVA and 0.044 mW/cm^2 UVB. The culteres were irradiated as described in the schedule on page 15. Concurrent solvent and positive controls were run in parallel. 30 min after start of irradiation the test-article in the PBS solution was removed and culture medium was added. This medium was not changed until preparation of the cells.

The cells were incubated until preparation at 37 °C in a humidified atmosphere with 4.5 % CO_2.

Preparation of the Cultures

16 h (18 h) and 26 h (28 h) after the start of the irradiation colcemid was added (0.2 µg/ml culture medium) to the cultures. 2.0 h later, the cells were treated on the slides in the Quadriperm chambers with hypotonic solution (0.4 % KCl) for 20 min at 37° C. After incubation in the hypotonic solution the cells were fixed with 3 + 1 methanol + glacial acetic acid. Per experiment both slides per group were prepared. After preparation the cells were stained with Giemsa (E. Merck, D-64293 Darmstadt).

Analysis of Metaphase Cells

Evaluation of the cultures was performed (according to standard protocol of the "Arbeitsgruppe der Industrie, Cytogenetik" (4)) using NIKON microscopes with 100x oil immersion objectives. Breaks, fragments, deletions, exchanges and chromosomal desintegrations were recorded as structural chromosomal aberrations. Gaps were recorded as well but not included in the calculation of the aberration rates. A gap was defined as an achromatic region in one or both chromatids independent of its width. The chromosome structure must not be altered in the way that lateral or longitudinal dislocation of chromatid parts occurs. In the test article treatment groups 100 well spread metaphases per culture were scored for cytogenetic damage on coded slides. In the positive control groups 100 well spreaded metaphases per culture were scored. Only metaphases with the characteristic chromosome number of 22 ± 1 were included in the analysis. To describe a cytotoxic effect the mitotic index (% cells in mitosis) was determined. In addition, the number of polyploid cells (% polyploid metaphases; in the case of this aneuploid cell line polyploid means a near tetraploid karyotype) was scored.

Acceptability of the Assay

The chromosomal aberration assay is considered acceptable if it meets the following criteria:

a) the number of aberrations found in the negative and/or solvent controls without irradiation fall within the laboratory historical control data range: 0.0 % - 6.0 %.

b) the positive control substance(s) should produce significant increases in the number of cells with structural chromosome aberrations.

Data Recording

The data generated were recorded in the laboratory protocol. The results were presented in tabular form, including experimental groups with the test article, negative and positive controls.

Evaluation of Results

A test article is classified as mutagenic if it induces reproducibly either a concentration-related increase in the number of structural chromosomal aberrations or a significant and reproducibly positive response for at least one of the test points.

A test article producing reproducibly neither a significant concentration-related increase in the number of structural chromosomal aberrations nor a significant and reproducible positive response at any one of the test points is considered non-mutagenic in this system.

The Fisher´s Exact Test (9) was applied as statistical method.

However, both biological and statistical significance of the results should be considered together.

RESULTS AND DISCUSSION

The test article CGF-C-1607, dissolved in acetone, was assessed for its potential to induce structural chromosome aberrations in V79 cells (Chinese hamster cell line) in the absence and the presence of a UVA/UVB light source. In experiment I cultures with the test article-PBS solution (containing 1 % (v/v) acetone with the test article) were irradiated with 200 mJ/cm^2 UVA and 22 mJ/cm^2 UVB. In experiment II the cultures were irradiated with the dose mentioned above and additionally with 300 mJ/cm^2 UVA and 33 mJ/cm^2 UVB. The chromosomes were prepared 18 h (exp. I) and 28 h (exp. II) after start of irradiation with the test article. In each experimental group two parallel cultures were set up. In the test article groups and in the negative control groups per culture 100 metaphases were scored for structural chromosomal aberrations.

The test article concentrations were chosen with regard to the solubility of the test article in PBS and the results of the Chromosome Aberration Assay with CGF-C-1607 (CCR Project 597700). The irradiation dose was chosen according to its none toxicity to the cells. 200 mJ/cm^2 was described as top dose without an effect on the cells previously (3). At 300 mJ/cm^2 increased aberration frequencies were expected (data from validation experiment without GLP).

In the cytogenetic experiments precipitation of the test article in culture medium was observed after treatment with 25 µg/ml and above in the **absence** and **presence** of irradiation. No influence of the test article on the pH value or osmolarity was observed at both experiments (solvent control: 353-392 mOsm, pH 6.4 - 6.6 versus 377 - 394 mOsm and pH 6.4 -6.6 at 100 µg/ml).

Table 3: Evaluated experimental points after treatment with CGF-C-1607

Preparation interval	Experiment	concentrations in µg/ml			
without irradiation					
18 h	I	6.25	12.5	25.0 ^{P}	100.0 ^{P}
28 h	II	12.5	25.0 ^{P}	50.0 ^{P}	100.0 ^{P}
with irradiation 200/22 mJ/cm2 UVA/UVB					
18 h	I	6.25	12.5	25.0 ^{P}	100.0 ^{P}
28 h	II	12.5	25.0 ^{P}	50.0 ^{P}	100.0 ^{P}
with irradiation 300/33 mJ/cm^2 UVA/UVB					
28 h	II	12.5	25.0 ^{P}	50.0 ^{P}	100.0 ^{P}

^{P} precipitation occurred

In both independent experiments, in the **absence** and the **presence** of UV-light neither reduced mitotic indices nor reduced cell numbers could be observed up to the highest applied test article concentration of 100.0 µg/ml.

In both independent experiments in the **absence** and **presence** of UV radiation (200 mJ/cm^2 UVA and 22 mJ/cm^2 UVB and 300 mJ/cm^2 UVA and 33 mJ/cm^2 UVB) the test article did not increase the frequency of cells carrying structural chromosomal aberrations. The aberration rates of the cells after treatment with the test article in the presence of irradiation (0.5 % - 4.0 % aberrant cell exclusive gaps) were near to the range of the frequencies in the absence of irradiation (0.5 % - 2.0 % aberrant cells exclusive gaps). In addition no relevant increase was observed when compared to the control values (0.5 % - 1.0 % without irradiation; 1.5 % - 2.5 % with irradiation) and our historical control data: range 0.0 % - 6.0 %.

In experiment II increased aberration frequencies were expected after irradiation with 300 mJ/cm^2 UVA and 33 mJ/cm^2 UVB according to the data of the validation experiment. However, no increased aberration frequencies were observed after irradiation with 300 mJ/cm^2 UVA and 33 mJ/cm^2 UVB. A clear increase of the aberration frequencies of the positive controls between 200 and 300 mJ/cm^2 was observed (see table 9 and 10) considered as clear indication that a higher irradiation dose was used. For the outcome of the study, the missing increase has no influence.

Tables 4 and 7 show the status of polyploid metaphases. In the **absence** and the **presence** of UV irradiation, no biologically relevant increase in the frequency of polyploid metaphases was found after treatment with the test article (with irradiation 2.3 % - 5.7 %; without irradiation: 2.8 % - 4.7 %) as compared to the frequencies of the controls (with irradiation 3.3 % - 5.7 %; without irradiation: 3.4 % - 4.1 %).

In both experiments, EMS and 8-Methoxypsoralen were used as positive control substance. Distinct statistically significant ($p < 0.05$) increases in cells carrying structural chromosome aberrations were induced.

In conclusion, it can be stated that in the study described and under the experimental conditions reported the test article CGF-C-1607 was not photoactivated to a clastogenic metabolite.

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Experiment I and II: Determination of Toxicity

The toxicity of the test article was examined using the determination of the cell number.
Cell numbers of two cultures (10 coordinate defined fields per culture) were determined for each experimental group.

Table 4: Number of cells in % of solvent control

Experiment I

without irradiation		with irradiation 200 mJ/cm² UVA and 22 mJ/cm² UVB	
Concentration in µg/ml	Cells in % of solvent control	Concentration in µg/ml	Cells in % of solvent control
6.25	80,0	6.25	91,5
12.5	83.1	12.5	106.5
25.0	74.0	25.0	105.8
50.0	70.4	50.0	86.5
75.0	80.2	75.0	93.9
100.0	91.0	100.0	76.1

Experiment II

without irradiation		with irradiation 200 mJ/cm² UVA and 22 mJ/cm² UVB		with irradiation 300 mJ/cm² UVA and 33 mJ/cm² UVB	
Concentration in µg/ml	Cells in % of solvent control	Concentration in µg/ml	Cells in % of solvent control	Concentration in µg/ml	Cells in % of solvent control
12.5	114.3	12.5	78.4	12.5	83.1
25.0	119.5	25.0	119.7	25.0	108.3
50.0	62.2	50.0	78.4	50.0	84.5
75.0	73.2	75.0	79.0	75.0	98.6
100.0	112.1	100.0	75.1	100.0	78.4

Experiment I

Table 5: Number of polyploid cells and mitotic index

Treatment group	conc. per ml	Irradiation UVA/UVB mJ/cm²	preparation interval	polyploid cells* culture 1	culture 2	total	%	mitotic index** absolute 1	absolute 2	mean	%***
without irradiation											
Neg. control		0/0	18 h	20	21	41	4.1	13.9	16.9	15.4	100.0
Solv. control#	1.0 %	0/0	18 h	20	18	38	3.8	15.9	12.9	14.4	100.0
Pos. control##	800.0 µg	0/0	18 h	17	13	30	3.0	15.6	18.0	16.8	109.1
Test article	6.25 µg	0/0	18 h	14	14	28	2.8	13.8	13.5	13.7	94.8
"	12.5 µg	0/0	18 h	16	13	29	2.9	11.3	13.6	12.5	86.5
"	25.0 µg	0/0	18 h	17	30	47	4.7	14.9	14.8	14.9	103.1
"	100.0 µg	0/0	18 h	17	21	38	3.8	11.7	13.6	12.7	87.8
with irradiation 200 mJ/cm2 UVA and 22 mJ/cm2 UVB											
Neg. control		200/22	18 h	16	18	34	3.4	13.9	15.5	14.7	100.0
Solv. control#	1.0 %	200/22	18 h	21	36	57	5.7	12.6	13.6	13.1	100.0
Pos. control###	0.5 µg	200/22	18 h	8	13	21	2.1	10.1	9.9	10.0	68.0
Test article	6.25 µg	200/22	18 h	13	13	26	2.6	13.1	10.2	11.7	88.9
"	12.5 µg	200/22	18 h	28	29	57	5.7	12.9	12.1	12.5	95.4
"	25.0 µg	200/22	18 h	25	29	54	5.4	14.6	12.3	13.5	102.7
"	100.0 µg	200/22	18 h	17	27	44	4.4	12.6	12.2	12.4	94.7

* The number of polyploid cells was determined of each test group in a sample of 500 cells per culture

** The mitotic index was determined in a sample of 1000 cells per culture of each test group

*** For the positive control groups, the relative values of the mitotic index are related to the negative control; for the test article treatment groups (CGF-C-1607 dissolved in Acetone) the values are related to the solvent controls (Acetone).

\# acetone

\## EMS

\### 8-methoxypsoralene

Table 6: Structural chromosome aberrations Experiment I
preparation interval 18 h; without irradation

slide no.	cells scored	% aberrant cells incl. gaps	excl. gaps*	with ex-changes	aberrations: gaps g	gaps ig	chromatid type b	f	d	ex	chromosome type ib	if	id	cx	other ma	cd
without irradiation																
Negative control																
1	100				2	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	3	0	0	1	0	0	0	0	0	0
1 + 2	200	2.0	1.0	0.5	2	0	3	0	0	1	0	0	0	0	0	0
Solvent control: acetone 1.0 %																
1	100				1	0	1	0	0	0	0	0	0	0	0	0
2	100				3	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	2.5	0.5	0.0	4	0	1	0	0	0	0	0	0	0	0	0
Positive control: EMS 800.0 µg / ml																
1	100				3	0	3	1	0	9	2	0	0	0	0	0
2	100				5	0	1	4	1	3	1	0	0	0	1	0
1 + 2	200	13.5	11.0	4.5	8	0	4	5	1	12	3	0	0	0	1	0
Test article: 6.25 µg / ml																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				1	0	2	0	0	0	1	0	0	0	0	0
1 + 2	200	2.0	1.5	0.0	1	0	2	0	0	0	1	0	0	0	0	0
Test article: 12.5 µg /ml																
1	100				1	0	0	0	0	2	1	0	0	0	0	0
2	100				5	0	1	0	0	0	1	0	0	0	0	0
1 + 2	200	4.0	1.5	0.5	6	0	1	0	0	2	2	0	0	0	0	0
Test article: 25.0 µg / ml																
1	100				3	0	0	0	0	0	0	0	0	0	1	0
2	100				0	0	1	0	0	0	0	0	0	0	0	0
1 + 2	200	2.5	1.0	0.0	3	0	1	0	0	0	0	0	0	0	1	0
Test article: 100.0 µg / ml																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				1	0	1	1	0	0	0	0	1	0	1	0
1 + 2	200	2.5	2.0	0.0	1	0	1	1	0	0	0	0	1	0	1	0

* inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap, gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Table 7: Structural chromosome aberrations Experiment I
preparation interval 18 h; with irradation 200 mJ/cm^2 UVA and 22 mJ/cm^2 UVB

slide no.	cells scored	% aberrant cells incl. gaps	excl. gaps*	with ex-changes	aberrations gaps g	ig	chromatid type b	f	d	ex	chromosome type ib	if	id	cx	other ma	cd
with irradiation 200 mJ/cm2 UVA and 22 mJ/cm2 UVB																
Negative control																
1	100				3	0	2	0	0	0	0	1	0	0	0	0
2	100				2	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	3.5	1.0	0.0	5	0	2	0	0	0	0	1	0	0	0	0
Solvent control: acetone 1.0 %																
1	100				2	0	1	0	0	2	1	0	0	0	0	0
2	100				0	0	2	0	0	0	0	0	0	0	0	0
1 + 2	200	1.5	1.5	0.5	2	0	3	0	0	2	1	0	0	0	0	0
Positive control: 8-MOP 0.5 µg /ml																
1	100				0	0	1	0	0	7	1	1	0	0	1	0
2	100				1	0	4	2	0	9	6	0	0	0	2	0
1 + 2	200	13.0	13.0	7.5	1	0	5	2	0	16	7	1	0	0	3	0
Test article: 6.25 µg / ml																
1	100				1	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	2	0	0	0	0	0	0
1 + 2	200	1.5	1.0	1.0	1	0	0	0	0	2	0	0	0	0	0	0
Test article: 12.5 µg /ml																
1	100				3	0	2	1	0	0	0	0	0	0	0	0
2	100				2	0	3	0	0	5	0	0	0	0	0	0
1 + 2	200	6.0	3.5	1.0	5	0	5	1	0	5	0	0	0	0	0	0
Test article: 25.0 µg / ml																
1	100				1	0	0	0	0	0	0	0	0	0	0	0
2	100				1	0	2	1	0	1	1	1	0	0	0	0
1 + 2	200	3.0	2.0	0.5	2	0	2	1	0	1	1	1	0	0	0	0
Test article: 100.0 µg / ml																
1	100				0	0	3	0	0	1	0	0	0	0	0	0
2	100				2	0	4	0	0	0	0	1	0	0	0	0
1 + 2	200	5.0	4.0	0.5	2	0	7	0	0	1	0	1	0	0	0	0

* inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap, gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Experiment II

Table 8: Number of polyploid cells and mitotic index

Treatment group	conc. per ml	Irradiation UVA/UVB mJ/cm²	preparation interval	polyploid cells* culture 1	culture 2	total	%	mitotic index** absolute 1	absolute 2	mean	%***
without irradiation											
Neg. control		0/0	28 h	19	16	35	3.5	12.6	12.8	12.7	100.0
Solv. control#	1.0 %	0/0	28 h	18	16	34	3.4	15.4	12.6	14.0	100.0
Pos. control##	800.0 µg	0/0	28 h	18	22	40	4.0	13.9	11.7	12.8	100.8
Test article	12.5 µg	0/0	28 h	21	13	34	3.4	15.0	12.8	13.9	99.3
"	25.0 µg	0/0	28 h	15	13	28	2.8	12.5	14.1	13.3	95.0
"	50.0 µg	0/0	28 h	15	18	33	3.3	14.2	14.9	14.6	103.9
"	100.0 µg	0/0	28 h	15	19	34	3.4	14.2	14.7	14.5	103.2
with irradiation 200 mJ/cm2 UVA and 22 mJ/cm2 UVB											
Neg. control		200/22	28 h	16	22	38	3.8	11.9	10.9	11.4	100.0
Solv. control#	1.0 %	200/22	28 h	16	22	38	3.8	7.4	12.1	9.8	100.0
Pos. control###	0.5 µg	200/22	28 h	9	17	26	2.6	9.6	10.0	9.8	86.0
Test article	12.5 µg	200/22	28 h	15	18	33	3.3	11.0	9.8	10.4	106.7
"	25.0 µg	200/22	28 h	10	13	23	2.3	12.7	9.4	11.1	113.3
"	50.0 µg	200/22	28 h	14	11	25	2.5	10.9	13.1	12.0	123.1
"	100.0 µg	200/22	28 h	14	13	27	2.7	12.2	13.3	12.8	130.8
with irradiation 300 mJ/cm2 UVA and 33 mJ/cm2 UVB											
Neg. control		300/33	28 h	20	13	33	3.3	10.1	12.2	11.2	100.0
Solv. control#	1.0 %	300/33	28 h	21	23	44	4.4	12.6	9.8	11.2	100.0
Pos. control###	0.5 µg	300/33	28 h	12	16	28	2.8	8.8	8.7	8.8	78.5
Test article	12.5 µg	300/33	28 h	22	20	42	4.2	11.2	7.2	9.2	82.1
"	25.0 µg	300/33	28 h	14	20	34	3.4	10.1	12.2	11.2	99.6
"	50.0 µg	300/33	28 h	12	18	30	3.0	3.9	12.3	8.1	72.3
"	100.0 µg	300/33	28 h	16	8	24	2.4	12.3	6.0	9.2	81.7

* The number of polyploid cells was determined of each test group in a sample of 500 cells per culture

** The mitotic index was determined in a sample of 1000 cells per culture of each test group

*** For the positive control groups, the relative values of the mitotic index are related to the negative control; for the test article treatment groups (CGF-C-1607 dissolved in Acetone) the values are related to the solvent controls (Acetone).

\# acetone

\## EMS

\### 8-metoxypsoralene

Table 9: Structural chromosome aberrations Experiment II
preparation interval 28 h; without irradiation

slide no.	cells scored	% aberrant cells incl. gaps	excl. gaps*	with ex-changes	aberrations gaps g	ig	chromatid type b	f	d	ex	chromosome type ib	if	id	cx	other ma	cd
without irradiation																
Negative control																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				1	0	1	0	0	0	0	0	0	0	0	0
1 + 2	200	1.0	0.5	0.0	1	0	1	0	0	0	0	0	0	0	0	0
Solvent control: acetone 1.0 %																
1	100				1	0	1	0	0	0	0	2	0	0	0	0
2	100				1	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	2.0	1.0	0.0	2	0	1	0	0	0	0	2	0	0	0	0
Positive control: EMS 800.0 µg / ml																
1	100				6	0	4	6	0	3	0	1	0	2	0	0
2	100				0	0	5	0	0	4	0	1	0	0	0	0
1 + 2	200	14.0	11.0	4.0	6	0	9	6	0	7	0	2	0	2	0	0
Test article: 12.5 µg / ml																
1	100				0	0	1	0	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	0.5	0.5	0.0	0	0	1	0	0	0	0	0	0	0	0	0
Test article: 25.0 µg /ml																
1	100				2	0	1	0	0	0	0	0	0	0	0	0
2	100				0	0	0	1	0	0	0	0	0	0	0	0
1 + 2	200	2.0	1.0	0.0	2	0	1	1	0	0	0	0	0	0	0	0
Test article: 50.0 µg / ml																
1	100				3	0	2	0	0	0	0	0	0	0	0	0
2	100				0	0	1	1	0	0	0	0	0	0	0	0
1 + 2	200	3.0	2.0	0.0	3	0	3	1	0	0	0	0	0	0	0	0
Test article: 100.0 µg / ml																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	1	0	0	0	0	0	0	0	0	0
1 + 2	200	0.5	0.5	0.0	0	0	1	0	0	0	0	0	0	0	0	0

* inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap, gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Table 10: Structural chromosome aberrations Experiment II
preparation interval 28 h; with irradiation 200 mJ/cm^2 UVA and 22 mJ/cm^2 UVB

slide no.	cells scored	% aberrant cells incl. gaps	excl. gaps*	with ex-changes	aberrations: gaps g	gaps ig	chromatid type b	f	d	ex	chromosome type ib	if	id	cx	other ma	cd
with irradiation 200 mJ/cm2 UVA and 22 mJ/cm2 UVB																
Negative control																
1	100				3	0	0	1	0	0	0	0	0	0	0	0
2	100				0	0	3	1	0	1	0	0	0	0	0	0
1 + 2	200	4.0	2.5	0.5	3	0	3	2	0	1	0	0	0	0	0	0
Solvent control: acetone 1.0 %																
1	100				2	0	1	2	0	1	0	0	0	0	0	0
2	100				0	0	1	0	0	0	1	1	0	0	0	0
1 + 2	200	3.5	2.5	0.5	2	0	2	2	0	1	1	1	0	0	0	0
Positive control: 8-MOP 0.5 µg /ml																
1	100				0	0	0	1	0	2	0	2	0	0	2	0
2	100				1	0	2	1	0	1	1	3	1	2	0	0
1 + 2	200	7.5	7.0	2.5	1	0	2	2	0	3	1	5	1	2	2	0
Test article: 12.5 µg / ml																
1	100				0	1	0	0	0	0	0	1	0	0	1	0
2	100				0	0	0	0	0	3	0	1	0	0	0	0
1 + 2	200	2.0	1.5	0.5	0	1	0	0	0	3	0	2	0	0	1	0
Test article: 25.0 µg /ml																
1	100				1	0	0	0	0	0	0	0	0	0	0	0
2	100				1	0	0	1	0	0	0	0	0	0	0	0
1 + 2	200	1.5	0.5	0.0	2	0	0	1	0	0	0	0	0	0	0	0
Test article: 50.0 µg / ml																
1	100				1	0	1	0	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	1.0	0.5	0.0	1	0	1	0	0	0	0	0	0	0	0	0
Test article: 100.0 µg / ml																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	0	0	1	0	0	0	0
1 + 2	200	0.5	0.5	0.0	0	0	0	0	0	0	0	1	0	0	0	0

* inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap, gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Table 11: Structural chromosome aberrations Experiment II
preparation interval 28 h; with irradiation 300 mJ/cm^2 UVA and 33 mJ/cm^2 UVB

slide no.	cells scored	% aberrant cells incl. gaps	excl. gaps*	with ex-changes	aberrations: gaps g	gaps ig	chromatid type b	f	d	ex	chromosome type ib	if	id	cx	other ma	cd
with irradiation 300 mJ/cm2 UVA and 33 mJ/cm2 UVB																
Negative control																
1	100				0	0	1	0	0	0	0	0	0	1	0	0
2	100				1	0	0	1	0	0	0	1	0	1	0	0
1 + 2	200	3.0	2.5	0.5	1	0	1	1	0	0	0	1	0	2	0	0
Solvent control: acetone 1.0 %																
1	100				1	0	1	0	0	0	0	0	0	0	0	0
2	100				1	0	0	0	0	0	1	1	0	0	0	0
1 + 2	200	2.5	1.5	0.0	2	0	1	0	0	0	1	1	0	0	0	0
Positive control: 8-MOP 0.5 µg /ml																
1	100				12	0	5	4	1	8	1	2	0	1	3	0
2	100				2	0	13	1	0	10	8	8	1	2	16	0
1 + 2	200	26.5	24.0	9.5	14	0	18	5	1	18	9	10	1	3	19	0
Test article: 12.5 µg / ml																
1	100				0	0	1	0	0	0	0	0	1	0	1	0
2	100				1	0	1	1	0	0	1	0	0	0	0	0
1 + 2	200	3.5	3.0	0.0	1	0	2	1	0	0	1	0	1	0	1	0
Test article: 25.0 µg /ml																
1	100				1	0	0	0	0	0	0	0	0	0	1	0
2	100				0	0	0	1	0	0	0	0	0	0	0	0
1 + 2	200	1.5	1.0	0.5	1	0	0	1	0	0	0	0	0	0	1	0
Test article: 50.0 µg / ml																
1	100				0	0	1	0	0	0	0	0	0	0	0	0
2	100				1	0	1	0	0	0	1	0	0	0	0	0
1 + 2	200	2.0	1.5	0.0	1	0	2	0	0	0	1	0	0	0	0	0
Test article: 100.0 µg / ml																
1	100				1	0	1	0	0	0	0	1	0	0	0	0
2	100				0	1	1	1	0	0	0	1	0	0	0	0
1 + 2	200	3.5	2.5	1.0	1	1	2	1	0	0	0	1	0	0	0	0

* inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap, gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

BIOMETRY

Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of the Fisher´s-exact-test. Evaluation was performed only for cells carrying aberrations exclusive gaps.

Experiment I

Solvent control versus		Preparation interval	UVA/UVB in mJ/cm^2	p-value
Test group	6.25 µg/ml	18 h	0/0	0.187
"	12.5 µg/ml	18 h	0/0	0.187
"	25.0 µg/ml	18 h	0/0	0.312
"	100.0 µg/ml	18 h	0/0	0.108
"	6.25 µg/ml	18 h	200/22	n.c.
"	12.5 µg/ml	18 h	200/22	0.111
"	25.0 µg/ml	18 h	200/22	0.362
"	100.0 µg/ml	18 h	200/22	0.070
Negative control versusPositive control				
EMS	800 µg/ml	18 h	0/0	0.001 > p > 0*
8-MOP	0.5 µg/ml	18 h	200/22-	0.001 > p > 0*

n.c. = not tested as the aberration frequency is equal or lower than the control frequency
* aberration frequency is statistically significant higher than the control frequency

Experiment II

Solvent control versus		Preparation interval	UVA/UVB in mJ/cm^2	p-value
Test group	12.5 µg/ml	28 h	0/0	n.c.
"	25.0 µg/ml	28 h	0/0	n.c.
"	50.0 µg/ml	28 h	0/0	0.225
"	100.0 µg/ml	28 h	0/0	n.c.
"	12.5 µg/ml	28 h	200/22	n.c.
"	25.0 µg/ml	28 h	200/22	n.c.
"	50.0 µg/ml	28 h	200/22	n.c.
"	100.0 µg/ml	28 h	200/22	n.c.
"	12.5 µg/ml	28 h	300/33	0.169
"	25.0 µg/ml	28 h	300/33	n.c.
"	50.0 µg/ml	28 h	300/33	n.c.
"	100.0 µg/ml	28 h	300/33	0.252
Negative control versusPositive control				
8-MOP	0.5 µg/ml	28 h	200/22	0.018*
8-MOP	0.5 µg/ml	28 h	300/33	0.001 > p > 0*

n.c. = not tested as the aberration frequency is equal or lower than the control frequency
* aberration frequency is statistically significant higher than the control frequency