

Kopie entspricht Original  
27.05.98 148

**RCC - CCR PROJECT 609003**

Project ID of the Contracting Institute: RCC 689354

**PHOTOMUTAGENICITY IN A  
SALMONELLA TYPHIMURIUM  
AND  
ESCHERICHIA COLI  
REVERSE MUTATION ASSAY**

**WITH**

**CGF-C-1607**

**REPORT**

**Study Completion Date:**

**May 22, 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)

Im Auftrag

*Dr. Hecker*

(Dr. Hecker) Wiesbaden, den 30. März 1998



### 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

## CONTENTS

COPY OF GLP CERTIFICATE	2
PREFACE	4
General	4
Project Staff	4
Schedule	4
Project Staff Signatures	5
Quality Assurance	5
Guidelines	5
Archiving	6
Deviations to Protocol	6
STATEMENT OF COMPLIANCE	7
QUALITY ASSURANCE UNIT	8
SUMMARY OF RESULTS	9
Conclusion	9
OBJECTIVE	10
Aims of the Study	10
Reasons for the Study	10
MATERIALS AND METHODS	11
Test Article	11
Controls	12
Test System	12
Pre-Experiment for Toxicity	13
Dose Selection	14
Pre-Experiment on Photomutagenicity and Phototoxicity	14
Experimental Performance	14
Data Recording	15
Acceptability of the Assay	15
Evaluation of Results	15
Biometry	15
DISCUSSION OF RESULTS	16
REFERENCES	17
Distribution of the Report	17
ANNEXE: TABLES OF RESULTS	18
Pre-Experiment for Toxicity	18
Pre-Experiment on Phototoxicity and Photomutagenicity	19
Table 2 and Figure 1 Strain TA 102	19
Table 2 and Figure 2 Strain WP2	20
Experiment I: Plate Incorporation Test	21
Experiment II: Pre-Incubation Test	23
Summary of Results	25
ANNEXE: DATA OF THE LIGHT SOURCE	26

## PREFACE

### General

Sponsor: Ciba Spezialitätenchemie Grenzach GmbH  
P.O. Box 1266  
D-79630 Grenzach-Wyhlen

Study Monitor: Dr. U. Mentzel

Testing Facility: **R C C**  
CYTOTEST CELL RESEARCH GMBH  
In den Leppsteinswiesen 19  
D-64380 Roßdorf

RCC-CCR Project No.: 609003

Contracting Institute: **R C C**  
REGISTRATION AND CONSULTING  
COMPANY LTD.  
CH-4452 Itingen

RCC Project No.: 689354

Test Article: CGF-C-1607

RCC-CCR Test Article No.: S1213 32

Title: Photomutagenicity in a Salmonella Typhimurium and  
Escherichia Coli Reverse Mutation Assay  
with CGF-C-1607

### Project Staff

Study Director: Dr. Hans-Eric Wollny

Management: Markus Arenz

Quality Assurance Unit: Frauke Hermann

### Schedule

Date of Protocol: March 23, 1998

Start of Experiments: March 31, 1998

End of Experiments: April 27, 1998

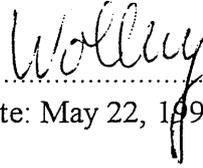
Date of Draft: May 05, 1998

Date of Final Report: May 22, 1998

## Project Staff Signatures

Study Director

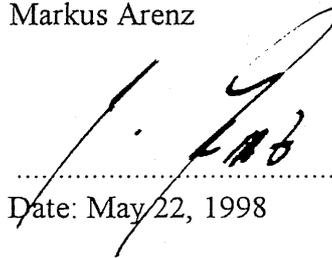
Dr. Hans-Eric Wollny



.....  
Date: May 22, 1998

Management

Markus Arenz



.....  
Date: May 22, 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 („BGBI. I 1994“, pp. 1703), last revision: May 14, 1997

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

## Guidelines

This study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

Ninth Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 471, "Bacterial Reverse Mutation Test", adopted June 21, 1997.

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, Annexe 1, Notes of guidance for the toxicity testing of cosmetic ingredients.

## 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.

The following sample 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:

A sample of the test article

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.

## Deviations to Protocol

### Guidelines

Present:

First Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 471, "Salmonella typhimurium, Reverse Mutation Assay", adopted May 26, 1983 and Section 4, No. 472, "Escherichia coli, Reverse Mutation Assay", adopted May 26, 1983

There were no deviations to protocol.

New:

Ninth Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 471, "Bacterial Reverse Mutation Test", adopted June 21, 1997.

Reason for the Alteration: updating

### RCC-CCR Test Article No.:

Present:

S1490 32

New:

S1213 32

Reason for the Alteration: typing error

### Positive control substances

Present:

Name: 8-Methoxypsoralen (8-MOP)

Concentration: 5 µl/plate

New:

Name: 8-Methoxypsoralen (8-MOP)

Concentration: 125 µg/plate in strain TA 102 and 12.5 µg/plate in strain WP2

Reason for the Alteration: typing error

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

## STATEMENT OF COMPLIANCE

Project Number: 609003  
Test Article: CGF-C-1607  
Study Director: Dr. Hans-Eric Wollny  
Title: Photomutagenicity in a Salmonella Typhimurium and Escherichia Coli Reverse Mutation Assay with CGF-C-1607

This study performed in the testing facility of RCC Cytotest Cell Research 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 („BGBI. 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 - CCR**  
Dr. Hans-Eric Wollny

*Wollny*  
.....  
Date: *May 25, 1998*

## QUALITY ASSURANCE UNIT

R C C Cytotest Cell Research GmbH  
In den Leppsteinswiesen 19,  
D-64380 Roßdorf

### Statement

Project Number: 609003  
Test Article: CGF-C-1607  
Study Director: Dr. Hans-Eric Wollny  
Title: Photomutagenicity in a Salmonella Typhimurium and  
Escherichia Coli Reverse Mutation Assay  
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 and Dates of QAU Inspections/ Audits	Dates of Reports to the Study Director and to Management
Protocol Audit: March 24, 1998	March 24, 1998
Study Inspection: April 22, 1998	April 22, 1998
Draft Audit: May 11, 1998	May 11, 1998

Head of Quality Assurance Unit

Frauke Hermann

F. Hermann  
Date: May 25, 1998

## SUMMARY OF RESULTS

This study was performed to investigate the potential of CGF-C-1607 to induce gene mutations under irradiation with artificial sunlight according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the *Salmonella typhimurium* strain TA 102 and the *Escherichia coli* strain WP2. These strains were chosen since they tolerate relatively high doses of UV irradiation used to assess the possible photomutagenic potential of sunblockers.

The assay was performed in two independent experiments. Each concentration, including the controls, was tested in triplicate. The test article was tested at the following concentrations:

33; 100; 333; 1000; 2500; and 5000 µg/plate

Slight toxic effects, evident as a reduction in the number of revertants, occurred in the strains TA 102 and WP2 in experiment II.

The plates incubated with the test article showed normal background growth up to 5000 µg/plate in all strains used.

No substantial increase in revertant colony numbers of any of the two tester strains was observed following treatment with CGF-C-1607 at any dose level. There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

## Conclusion

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test article did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Therefore, CGF-C-1607 is considered to be non-mutagenic in this *Salmonella typhimurium* and *Escherichia coli* photomutagenicity assay.

## OBJECTIVE

### Aims of the Study

The experiments were performed to assess the potential of the test article to induce gene mutations under irradiation with artificial sunlight by means of two independent *Salmonella typhimurium* and *Escherichia coli* reverse mutation assays. Experiment I was performed as a plate incorporation assay. Since a negative result was obtained in this experiment, experiment II was performed as a pre-incubation assay.

### Reasons for the Study

The most widely used assays for detecting gene mutations are those using bacteria. They are relatively simple and rapid to perform, and give reliable data on the ability of an agent to interact with DNA and produce mutations.

Reverse mutation assays determine the frequency with which an agent reverses or suppresses the effect of the forward mutation. The genetic target presented to an agent is therefore small, specific and selective. Several bacterial strains, or a single strain with multiple markers are necessary to overcome the effects of mutagen specificity. The reversion of bacteria from growth-dependence on a particular amino acid to growth in the absence of that amino acid (reversion from auxotrophy to prototrophy) is the most widely used marker.

The *Salmonella typhimurium* histidine (*his*) and the *E. coli* tryptophan (*trp*) reversion system measures  $his^- \rightarrow his^+$  and  $trp^- \rightarrow trp^+$  reversions, respectively.

According to the direct plate incorporation or the pre-incubation method the bacteria are exposed to the test article under UV-irradiation and plated on selective medium. After a suitable period of incubation, revertant colonies are counted.

To establish a dose response effect 6 dose levels with adequately spaced intervals were tested. The maximum dose level was 5000 µg/plate.

A pre-experiment was performed with eight equally spaced concentrations of the test material to measure toxicity of the test substance.

The optimal duration of the UV irradiation was established in a separate pre-experiment. The bacteria were exposed to different doses of UV light using different exposure times. Following irradiation the bacteria were plated on selective medium. After a suitable period of incubation, revertant colonies were counted to measure both photomutagenicity and phototoxicity of the irradiation. According to literature (3) a UV dose was chosen that did not strongly increase the mutant frequency. With the bacteria used in this experiment the optimal UV-dose was in the range of 20 - 80 mJ/cm<sup>2</sup> of UVA and approx. 1 - 4 mJ/cm<sup>2</sup> of UVB.

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

## 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
Colour:	yellow
Purity:	> 98 %
Stability in solvent:	unknown
Storage:	room temperature, light protected
Expiration Date:	July 30, 1999

On the day of the experiment, the test article CGF-C-1607 was dissolved in acetone. The solvent was chosen because of its solubility properties and its relative non-toxicity to the bacteria.

The test article was tested as a suspension. The undissolved particles had no influence on the data recording.

## Controls

### Negative Controls

Concurrent untreated and solvent controls were performed.

### Positive Control Substances

Name: 8-Methoxypsoralen (8-MOP)  
Supplier: SIGMA, D-82041 Deisenhofen  
Catalogue No.: 820775  
Purity: > 99.0 %  
Dissolved in: DMSO  
Concentration: 125 µg/plate in strain TA 102 and 12.5 µg/plate in strain WP2

The stability of the positive control substance in solution was unknown but a mutagenic response in the expected range means a biological stability demonstration. The dilutions of the stock solutions were prepared on the day of the experiment and used immediately.

## Test System

### Characterisation of the *Salmonella typhimurium* and *E. coli* strain

The bacterial strains used in this assay are excision repair proficient since excision repair deficient strains are extremely sensitive towards UV-irradiation. Furthermore, DNA crosslinking which is known to be a characteristic damage introduced by UV-light is not recognised by excision repair deficient strains.

The histidine dependent strain TA 102 is derived from *S. typhimurium* strain LT2 through mutations in the histidine locus. Additionally due to the "deep rough" (*rfa*<sup>-</sup>) mutation the bacteria possess a faulty lipopolysaccharide envelope which enables substances to penetrate the cell wall more easily. In the strain TA 102 the R-factor plasmid pKM 101 carries the ampicillin resistance marker and the multicopy plasmid pAQ1, which carries the hisG428 mutation and a tetracycline resistance gene. TA 102 contains the ochre mutation in the hisG gene.

Strain WP2 and its derivatives all carry the same defect in one of the genes for tryptophan biosynthesis. Tryptophan-independent (*Trp*<sup>+</sup>) mutants (revertants) can arise either by a base change at the site of the original alteration or by a base change elsewhere in the chromosome so that the original defect is suppressed. This second possibility can occur in several different ways so that the system seems capable of detecting all types of mutagen which substitute one base for another.

When summarised, the mutations of the TA strain and the *E. coli* strain used in this study can be described as follows:

*Salmonella typhimurium*

TA 102: his G 428; *rfa*<sup>-</sup>; *uvrB*<sup>-</sup>; R-factor: base-pair substitutions

*Escherichia coli*

WP2: *trp*<sup>-</sup>: base-pair substitutions and others

Regular checking of the properties of the strains regarding the membrane permeability and ampicillin resistance as well as spontaneous mutation rates is performed in RCC Cytotest

Cell Research according to Ames et al. (1). In this way it was ensured that the experimental conditions set down by Ames were fulfilled.

The bacterial strain TA 102 was obtained from Dr. B.N. Ames (University of California, 94720 Berkeley, U.S.A.). The bacterial strain WP2 was obtained from Dr. Heinz Träger, Knoll AG, D-67008 Ludwigshafen.

### Storage

The strain cultures were stored as stock cultures in ampoules with nutrient broth + 5 % DMSO (MERCK, D-64293 Darmstadt) in liquid nitrogen.

### Precultures

From the thawed ampoules of the strains 0.5 ml suspension was transferred into 250 ml Erlenmeyer flasks containing 20 ml nutrient medium. A solution of 20 µl ampicillin (25 µg/ml) and 20 µl tetracycline (2 µg/ml) was added to strain TA 102. This nutrient medium contains per litre:

8 g Merck Nutrient Broth (MERCK, D-64293 Darmstadt)  
5 g NaCl (MERCK, D-64293 Darmstadt)

The bacterial culture was incubated in a shaking water bath for 8 hours at 37° C.

### Selective Agar

The plates with the minimal agar were obtained from E. Merck, D-64293 Darmstadt.

### Overlay Agar

The overlay agar contains per litre:

for Salmonella strain:

6.0 g MERCK Agar Agar\*  
6.0 g NaCl\*  
10.5 mg L-Histidine×HCl×H<sub>2</sub>O\*  
12.2 mg Biotin\*

\* (MERCK, D-64293 Darmstadt)

for Escherichia coli:

6.0 g MERCK Agar Agar\*  
6.0 g NaCl\*  
2.5 mg Tryptophan\*

Sterilisations were performed at 121° C in an autoclave.

### Pre-Experiment for Toxicity

To evaluate the toxicity of the test article a pre-experiment was performed with strains TA 102 and WP2. Eight concentrations were tested for toxicity and mutation induction with three plates each. The experimental conditions in this pre-experiment were the same as described below for the experiment I without irradiation (plate incorporation test).

Toxicity of the test article results in a reduction in the number of spontaneous revertants or a clearing of the bacterial background lawn.

## Dose Selection

Based upon the results of this pre-experiment the concentrations applied in the main experiments were chosen. The concentration range covered two logarithmic decades. Two independent experiments were performed.

According to the dose selection criteria the test article was tested at the following concentrations:

33; 100; 333; 1000; 2500; and 5000 µg/plate

## Pre-Experiment on Photomutagenicity and Phototoxicity

The optimal UV dose was established in a separate pre-experiment. The bacteria were exposed to different doses of UV light using different exposure times. Following irradiation with a discharge lamp emitting a spectrum similar to sunlight, the bacteria are plated on selective medium. After a suitable period of incubation, revertant colonies are counted to measure both, photomutagenicity and phototoxicity of the irradiation. According to the literature (3) an UV dose was chosen that increased the number of revertant colonies to approximately twice the number of spontaneous revertants without irradiation. (10 seconds of irradiation, approx. 20 mJ/cm<sup>2</sup> UVA and approx. 1 mJ/cm<sup>2</sup> UVB in strain WP2 and 40 seconds totalling in 80 mJ/cm<sup>2</sup> UVA and about 4 mJ/cm<sup>2</sup> UVB in strain TA 102).

## Experimental Performance

For each strain and dose level including the controls, three plates were used.

The following materials were mixed in a test tube and poured onto the minimal agar plates:

- 100 µl Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
- 500 µl phosphate buffered saline, pH 7.4
- 100 µl Bacteria suspension (cf. test system, pre-culture of the strains),
- 2000µl Overlay agar

The plates were irradiated with UV light for duration specified above.

In the pre-incubation method 100 µl test solution, 500 µl phosphate buffered saline and 100 µl bacterial suspension were mixed in a six-well plate without lid. After irradiation of the plates with an intended dose of UV-light, the solution was transferred to test tubes and incubated at 37°C for 60 minutes. After pre-incubation 2.0 ml overlay agar (45° C) were added to each tube. The mixture was poured onto minimal agar plates.

After solidification the plates were incubated upside down for at least 48 hours at 37° C in the dark.

## Data Recording

The colonies were counted using the AUTOCOUNT (Artek Systems Corporation, BIOSYS GmbH, D-61184 Karben). The counter was connected to an IBM AT compatible PC with printer to print out the individual values and the means from the plates for each concentration together with standard deviations and enhancement factors as compared to the spontaneous reversion rates (see tables of results) In the pre-experiment some of the colonies were counted manually from 100 up to 5000 µg/plate due to precipitation of the test article.

## Acceptability of the Assay

The Salmonella typhimurium and Escherichia coli reverse mutation assay is considered acceptable if it meets the following criteria:

- corresponding background growth on both negative control and test plates
- normal range of spontaneous reversion rates without irradiation.

Range of spontaneous reversion frequencies of strain WP2: 26 - 64\*  
Range of spontaneous reversion frequencies of strain TA 102: 121-293\*

## Evaluation of Results

A test article is considered positive if either a dose related and reproducible increase in the number of revertants or a reproducible increase for at least one test concentration is induced.

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

A mutagenic response is described as follows:

A test article is considered mutagenic if the number of revertants is at least twice as high as compared to the irradiated control (4).

Also, a dose-dependent and reproducible increase in the number of revertants is regarded as an indication of possibly existing mutagenic potential of the test article regardless whether the highest dose induced the above described enhancement factors or not.

## Biometry

No statistical evaluation of the data is required.

---

\*These values are referring to the negative control group without metabolic activation and represent our historical control range since 1993

## DISCUSSION OF RESULTS

This study was performed to investigate the potential of CGF-C-1607 to induce gene mutations under irradiation with artificial sunlight according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the Salmonella typhimurium strain TA 102 and the Escherichia coli strain WP2. These strains were chosen since they tolerate relatively high doses of UV irradiation used to assess the possible photomutagenic potential of sunblockers.

The assay was performed in two independent experiments. Each concentration and the controls were tested in triplicate. The test article was tested at the following concentrations:

33; 100; 333; 1000; 2500; and 5000 $\mu$ g/plate

Slight toxic effects, evident as a reduction in the number of revertants, occurred in the strains TA 102 and WP2 in experiment II.

The plates incubated with the test article showed normal background growth up to 5000  $\mu$ g/plate in all strains used.

No substantial increase in revertant colony numbers of any of the two tester strains was observed following treatment with CGF-C-1607 at any dose level. There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies.

The number of spontaneous revertants without irradiation was within the historical range of negative control values (strain TA 102 = 223 colonies in exp. I and 152 colonies in exp. II (mean value) strain WP2 = 47 colonies in exp. I, and 37 colonies in exp. II (mean value)).

In conclusion, it can be stated that during the described photomutagenicity test and under the experimental conditions reported, the test article did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

## REFERENCES

1. Green, M.H.L. and W.J. Muriel (1976)  
Mutagen testing using Trp<sup>+</sup> Reversion in Escherichia coli  
Mutation Res. 38, 3-32
2. McCann, J. and B.N. Ames (1976)  
Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 300  
chemicals: discussion.  
Proc. Natl. Acad. Sci. (USA) 73, 950-954
3. Chételat, A.; Albertini, S.; Dresp, J.H.; Strobel, R. and Gocke, E. (1993)  
Photomutagenesis test development: I. 8-Methoxypsoralen, chlorpromazine and  
sunscreen compounds in bacterial and yeast assays  
Mutation Res. 292, 241-250
4. Hollstein, M., J. McCann, F.A. Angelosanto and W.W. Nichols (1979)  
Short-term tests for carcinogens and mutagens  
Mutation Res. 65, 133-226

## Distribution of the Report

Sponsor	2 × (1x original, 1x copy)
Study Director	1 × (copy)

## ANNEXE: TABLES OF RESULTS

### Pre-Experiment for Toxicity

8 concentrations were tested for toxicity and mutation induction with each 3 plates.

The results are given in the following table:

Table 1:

Substance	Concentration per plate [µg]	Revertants per plate	
		TA 102	WP2
		-	-
Negative control	-	200	37
Solvent control	-	234	33
methyl methane sulfonate	5µl/plate	1528	669
Test article	3	239	36
	10	232	41
	33	238	38
	100	205	27
	333	227	40
	1000	236	36
	2500	203	12
	5000	251	23

The plates with the test article showed normal background growth up to 5000.0 µg/plate in strain TA 98 and TA 100.

According to the dose selection criteria, the test article was tested at the following concentrations:

33; 100; 333; 1000; 2500; and 5000 µg/plate

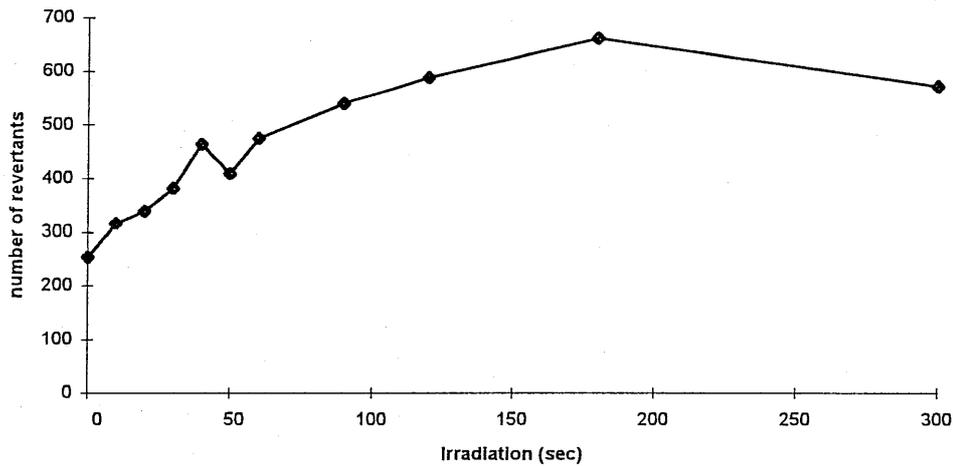
## Pre-Experiment on Phototoxicity and Photomutagenicity

8 concentrations were tested for toxicity and mutation induction with each 2 plates.

The results are given in the following table and corresponding figure:

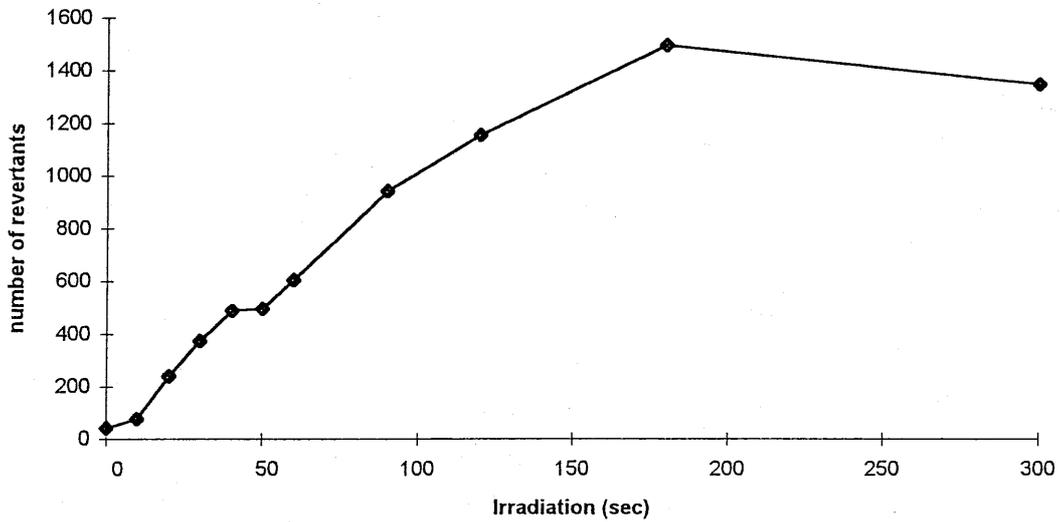
**Table 2 and Figure 1 Strain TA 102**

Irradiation (sec)	number of revertants			mean	factor
	plate 1	plate 2	plate 3		
0	228	259	273	253	
10	326	302	320	316	1.2
20	324	373	319	339	1.3
30	356	362	424	381	1.5
40	494	493	403	463	1.8
50	399	409	417	408	1.6
60	438	506	479	474	1.9
90	566	532	517	538	2.1
120	590	591	576	586	2.3
180	674	614	691	660	2.6
300	569	582	556	569	2.2



**Table 2 and Figure 2 Strain WP2**

Irradiation (sec)	number of revertants			mean	factor
	plate 1	plate 2	plate 3		
0	37	35	51	41	
10	77	73	77	76	1.8
20	255	218	239	237	5.8
30	361	387	365	371	9.0
40	501	513	444	486	11.9
50	430	518	534	494	12.0
60	601	608	599	603	14.7
90	874	950	993	939	22.9
120	1178	1232	1042	1151	28.1
180	1500	1575	1397	1491	36.4
300	1233	1337	1449	1340	32.7



## Experiment I: Plate Incorporation Test

Test article: CGF-C-1607

Test strain: TA 102

### without S9 mix

Concentration µg/plate	Plate			Revertants / plate		
	1	2	3	mean	s.d.	factor*
Negative Control	318	350	438	369	62.1	
Solvent Control	291	323	364	326	36.6	1.0
Positive Control <sup>#</sup>	792	804	785	794	9.6	2.4
33	233	299	225	252	40.6	0.8
100	210	282	290	261	44.1	0.8
333	316	289	236	280	40.7	0.9
1000	290	312	328	310	19.1	1.0
2500	269	308	337	305	34.1	0.9
5000	295	316	269	293	23.5	0.9

$$\text{* enhancement factor} = \frac{\sum \text{revertants} / \text{concentr. test article}}{\sum \text{revertants} / \text{solvent control}}$$

<sup>#</sup> 8-methoxypsoralen 125 µg/plate

**Experiment I: Plate Incorporation Test**

Test article: CGF-C-1607

Test strain: WP2

**without S9 mix**

Concentration µg/plate	Plate			Revertants / plate		
	1	2	3	mean	s.d.	factor*
Negative Control	78	109	105	97	16.9	
Solvent Control	87	113	84	95	15.9	1.0
Positive Control <sup>#</sup>	197	204	188	196	8.0	2.1
33	62	72	76	70	7.2	0.7
100	79	97	92	89	9.3	0.9
333	54	59	72	62	9.3	0.7
1000	104	72	74	83	17.9	0.9
2500	59	71	73	68	7.6	0.7
5000	75	71	97	81	14.0	0.9

$$* \text{ enhancement factor} = \frac{\sum \text{ revertants / concentr. test article}}{\sum \text{ revertants / solvent control}}$$

<sup>#</sup> 8-methoxypsoralen 12.5 µg/plate

## Experiment II: Pre-Incubation Test

Test article: CGF-C-1607

Test strain: TA 102

### without S9 mix

Concentration µg/plate	Plate			Revertants / plate		
	1	2	3	mean	s.d.	factor*
Negative Control	114	138	146	133	16.7	
Solvent Control	160	164	141	155	12.3	1.0
Positive Control#	1100	1061	944	1035	81.2	6.7
33	120	127	120	122	4.0	0.8
100	114	127	168	136	28.2	0.9
333	153	155	167	158	7.6	1.0
1000	156	160	141	152	10.0	1.0
2500	157	150	142	150	7.5	1.0
5000	75	62	83	73	10.6	0.5

$$* \text{ enhancement factor} = \frac{\sum \text{revertants / concentr. test article}}{\sum \text{revertants / solvent control}}$$

# 8-methoxypsoralen 125 µg/plate

**Experiment II: Pre-Incubation Test**

Test article: CGF-C-1607

Test strain: WP2

**without S9 mix**

Concentration µg/plate	Plate			Revertants / plate		
	1	2	3	mean	s.d.	factor*
Negative Control	62	69	64	65	3.6	
Solvent Control	53	44	53	50	5.2	1.0
Positive Control <sup>#</sup>	135	133	129	132	3.1	2.6
33	33	46	47	42	7.8	0.8
100	42	26	30	33	8.3	0.7
333	51	50	45	49	3.2	1.0
1000	45	35	38	39	5.1	0.8
2500	40	34	28	34	6.0	0.7
5000	32	27	21	27	5.5	0.5

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

<sup>#</sup> 8-methoxypsoralen 12.5 µg/plate

## Summary of Results

Test article: CGF-C-1607

Concentration µg/plate	Revertants/plate mean from three plates			
	TA 102		WP2	
	I	II	I	II
Negative control	369	133	97	65
Solvent control	326	155	95	50
Positive control <sup>#</sup>	794	1035	196	132
33	252	122	70	42
100	261	136	89	33
333	280	158	62	49
1000	310	152	83	39
2500	305	150	68	34
5000	293	73	81	27

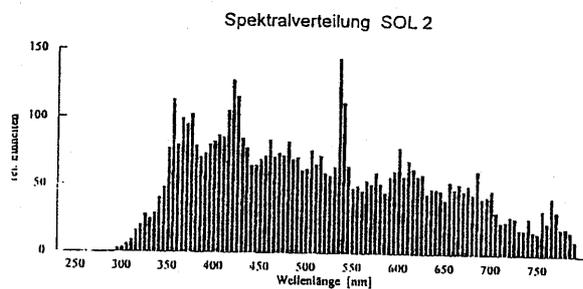
<sup>#</sup> 8-methoxypsoralen 125 µg/plate in strain TA 102 and 12.5 µg/plate in strain WP2

## ANNEXE: DATA OF THE LIGHT SOURCE

Type of equipment: SOL 2 made by Dr. K. Hönle GmbH UV-Technologie,  
Fraunhoferstrasse 5, D-82152 Planegg/München)

Type of light source: SOL 500 combined with filter H2 (Dr. K. Hönle GmbH)

Spectrum:



Dr. Hönle

Light intensity: 120000 lux in a distance of 25 cm

Total dose: 900 W/m<sup>2</sup> in a distance of 25 cm

Power consumption: 400 W

Max. current: 5 A