RCC PROJECT 651475

DETERMINATION OF PHOTOTOXICITY WITH

CGF-C-1607

IN ALBINO GUINEA PIGS

REPORT

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Study Completion: 29-ARP-1997

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CONTENTS (CONT'D)

PREFACE

GENERAL

Title	Determination of Phototoxicity with CGF-C-1607 in Albino Guinea Pigs.
Sponsor	Ciba Chemikalien GmbH Werk Grenzach Geb. 9001.6.23 Postfach 1266 D-79630 Grenzach/Wyhlen
Monitoring Scientist	Dr. U. Mentzel
Testing Facility	RCC, Research & Consulting Company Ltd. Zelgliweg 1, 4452 Itingen / Switzerland
RCC Project Number	651475
Test Article	CGF-C-1607
Test System	Albino Guinea Pigs

PROJECT STAFF

Study Director	G. Arcelin
Technical Coordinator	R. Sacher

SCHEDULE

Acclimatization	05-MAR-1997 to 10-MAR-1997
Treatment / Observation	11-MAR-1997 to 14-MAR-1997
Termination	14-MAR-1997
Reported	29-APR-1997

ARCHIVING

RCC, Research & Consulting Company Ltd, CH-4452 Itingen will archive the following data for at least ten years: Protocol, report, raw data and test article reference sample. No data will be discarded without the sponsor's consent.

PROJECT STAFF SIGNATURES

Study Director

G. Arcelin

date: 29-APR-1997

Management

for T. R. Allen

date: 29-Apr.-97

QUALITY ASSURANCE STATEMENT

R C C, RESEARCH & CONSULTING COMPANY LTD,
CH-4452 ITINGEN / SWITZERLAND

PROJECT NUMBER : 651475

TEST ARTICLE : CGF-C-1607

STUDY DIRECTOR : G. Arcelin

TITLE : Determination of Phototoxicity with CGF-C-1607 in Albino Guinea Pigs.

Study procedures were periodically inspected and this report was audited by the RCC Quality Assurance Unit. The dates are given below.

Dates of QAU Inspections / Audits	Dates of Reports to the Study Director and to Management
05-MAR-1997	05-MAR-1997
21-MAR-1997	21-MAR-1997
16-APR-1997	16-APR-1997

Manager, Quality Assurance Unit — Dr. G. Menne

i.A. M. Dörre
date: 29-APR-1997

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE / GLP GUIDELINES

PROJECT NUMBER	:	651475
TEST ARTICLE	:	CGF-C-1607
STUDY DIRECTOR	:	G. Arcelin
TITLE	:	Determination of Phototoxicity with CGF-C-1607 in Albino Guinea Pigs.

The study described in this report was conducted in compliance with the following Good Laboratory Practice Standards:

Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles, March 1986

The OECD Principles of Good Laboratory Practice, Environmental Monograph Number 45. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring - Number 1.
Environment Directorate, Organisation for Economic Co-operation and Development. Paris, 1992

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

Study Director G. Arcelin

Date: 29-APR-1997

TEST GUIDELINES

The study procedures described in this report are based on the following methods:

CTFA Safety Testing Guidelines, The Cosmetic, Toiletry and Fragrance Association, Inc. Washington, D.C. 20036; "Guidelines for Evaluating Photodermatitis", 1991.

OECD Guidelines for Testing of Chemicals. Draft proposal for a New Guideline: "Acute Dermal Photoirritation Dose-Response Test", February 1995.

REFERENCES

Harber, L.C. and Shalita, A.R. (1975). "The Guinea Pig as an Effective Model for the Demonstration of Immunologically Mediated Contact Photosensitivity." In Animal Models in Dermatology (H. Maibach, Ed.) Ch. 10, Churchill Livingstone, New York.

SUMMARY OF PROTOCOL DEVIATION

The test article dilution is stable for at least 48 hours.

SUMMARY

In order to assess the cutaneous phototoxic potential of CGF-C-1607 a phototoxicity test was carried out in 15 female (10 test and 5 control) Albino Dunkin Hartley guinea pigs.

CGF-C-1607 dissolved in polyethylene glycol (PEG 400) was applied epicutaneously at concentrations of 30%, 25%, 15% and 10%, each, to skin areas of 2 cm^2 on both flanks. Thirty minutes after application of the test article the left flank of the animals was exposed to 20 J/cm^2 UVA irradiation. The right flank remained unexposed to light after treatment and served as reference site. Control animals were exposed to UVA similarly, except they were treated with the solvent polyethylene glycol (PEG 400) only. Cutaneous reactions, i.e. erythema and oedema formation were evaluated at 24, 48 and 72 hours after application.

SUMMARY TABLE

The following findings were recorded:

	CONCENTRATION %	LEFT FLANK UV-A irradiated (20 J/cm^2) Hours after application			RIGHT FLANK non-irradiated Hours after application		
		24	48	72	24	48	72
CONTROL GROUP WITH VEHICLE	PEG 400	0/5*	0/5	0/5	0/5	0/5	0/5
TEST GROUP WITH CGF-C-1607	30	0/10	0/10	0/10	0/10	0/10	0/10
	25	0/10	0/10	0/10	0/10	0/10	0/10
	15	0/10	0/10	0/10	0/10	0/10	0/10
	10	0/10	0/10	0/10	0/10	0/10	0/10

* Number of animals showing an erythema/total number of treated animals

All animals were pretreated with 2 % DMSO in ethanol to enhance the skin penetration of the test article.

CONCLUSION

Due to the results determined it must be concluded, that under the test conditions used, the test article CGF-C-1607 showed no phototoxic potential for the guinea pigs of this strain and age.

OBJECTIVE

PURPOSE AND RATIONALE

The purpose of this study was to assess the phototoxic potential of CGF-C-1607 when applied to albino guinea pigs.

This study should provide a rational basis for phototoxic potential risk assessment in man.

MATERIALS AND METHODS

Experimental Design

TEST SYSTEM

Test system	Albino Dunkin Hartley Guinea Pigs, HsdPoc:DH SPF
Rationale	Recognized by the international guidelines as a recommended test system (e.g. CTFA).
Source	Harlan Winkelmann Gartenstrasse 27 D-33178 Borchen
Total Number of animals	15 females, nulliparous and non-pregnant
Age at start of acclimatization	5 - 7 weeks
Body weight at start of acclimatization	271 - 307 g
Identification	By unique cage number and corresponding ear tags.
Acclimatization	Six days under test conditions after health examination. Only animals without any visible signs of illness were used for the study.

The animals were distributed as follows:

Five animals for the control group (only treated with the vehicle) and 10 animals for the test group (treated with the test article).

GROUP	ANIMAL NUMBERS FEMALES
CONTROL GROUP	401 - 405
TEST GROUP	406 - 415

The sensitivity and reliability of the experimental technique used was assessed by use of 8-METHOXYPSORALENE. The most recent test was performed from October 21 to 31, 1996 under the RCC Project 901091, see Appendix D.

HUSBANDRY

Room Number: E 22/RCC

Conditions:
Standard Laboratory Conditions.
Air-conditioned with 10-15 air changes per hour and continuously monitored environment with a target range for temperature of 22 ± 3 degrees centigrade and for relative humidity of 40-70 % (values above 70 % during cleaning process possible). The animals were provided with a 12-hour light, 12-hour dark cycle. Fluorescent "Gold" lamps (Silvania Gold F40T12CO) were used. Music was played during the daytime light period.

Accommodation:
With the exception of the exposure period the animals were kept individually in type-3 wire mesh cages. During the exposure with UV-A irradiation: 10 test animals and 5 control animals were positioned on the right flank in a plastic box (32 x 52 cm) and irradiated together.

Diet:
Pelleted standard Nafag Ecosan 845 25W4, Batch no. 01/97 guinea pig breeding/ maintenance diet ("Nafag", Nähr- und Futtermittel AG, CH-9202 Gossau), ad libitum. Results of analysis for contaminants are included in this report.
See Appendix C.

Water:
Community tap water from Itingen, ad libitum. Once weekly additional supply of ascorbic acid (approx. 1 g/l) via the drinking water. Results of bacteriological, chemical and contaminant analyses are included in this report.
See Appendix C.

TEST ARTICLE

Identification	CGF-C-1607
Description	Yellow solid
Batch Number	002
Purity	> 98 %
Stability of Test Article	Stable under storage condition; expiration date: 31-AUG-1998
Stability of Test Article Dilution	Stable in polyethylene glycol (PEG 400) for at least 48 hours
Storage Conditions	In the original container at room temperature away from direct sunlight.
Safety precautions	Gloves, goggles and face mask were obligatory to ensure personnel health and safety.

TEST ARTICLE PREPARATION

The test article and vehicle PEG 400 were placed into a glass beaker on a tared Mettler PM 460 balance. Weight/weight dilutions were prepared using a magnetic stirrer as homogenizer. Homogeneity of the test article in vehicle was maintained during treatment. The preparations were made immediately prior to each dosing.

LIGHT SOURCE

Philips Actinic "TLD" lamps (36W/08)

Energy:	1 x 10 exp.4 Ergs/cm²/sec.
Spectrum:	320 - 400 nm
Irradiation dose:	20 J/cm²-UV-A

The duration of the exposure was regulated by a time control device.

SPECIFIC CONSIDERATIONS

The test article was applied at concentrations of 30%, 25%, 15% and 10%.

The test sites on all animals were pretreated approximately 30 to 50 minutes prior to the test article (respectively solvent) application with 2 % DMSO diluted in ethanol (0.025 ml/2cm²) to enhance the skin penetration of the test article.

Rationale

To determine the influence of UV-A irradiation to the skin after test article treatment.

TEST PROCEDURE

INDUCTION PHASE

One day before application the animals were fasted and both flanks of the animals were shaved by electric clipper. One day later the animals were sedated with COMBELEN (0.2 ml/kg) and narcotized with VETANARCOL (0.2 ml/kg) by intra-peritoneal injection. Thereafter, the 4 test sites of 2 cm² were marked on both flanks by circular stamp.

To the test areas of 2cm² of the different test article concentrations were applied with a spatula to the left flank in order to saturate the test sites. Thirty minutes after application of the test article he left flank of the animals was exposed to non-erythematogenic UV-A irradiation (20 J/cm²). After irradiation the same treatment was performed on the right flanks but the test sites remained unexposed to light after treatment and served as control sites.

After treatment the animals were returned to their individual cages and provided with the same diet.

The animals of the control group were treated with the vehicle alone.

The right flank was used to evaluate the highest non-irritating concentration, therefore no pretest was performed.

The allocation of the different test dilutions to the sites on the animals was alternated in order to minimize the site to site variations in responsiveness. Which test site used for the different concentrations is described in the tables attached (Appendix A).

OBSERVATION AND SCORING

Animals were examined 24, 48 and 72 hours after application of the test article for signs of erythema and edema.

The skin responses are scored according to the scale below:

0 = no reaction

1 = defined erythema

2 = erythema and defined edema

3 = marked erythema and edema

4 = severe erythema and edema with deep injuries

All data are summarized on the tables under Appendix A.

OBSERVATIONS

Mortality / Viability	Daily from delivery of the animals to the termination of test
Body weights	At acclimatization start, at test day one and at termination of test.
Clinical signs (local/systemic)	Daily from delivery of the animals to the termination of test
Skin reactions	At the time of reaction readings

Records were maintained on all additional and standard observations.

PATHOLOGY

Necropsy

No necropsy was performed in the animals euthanized at termination of study.

The animals were euthanized at the end of the test period with an intraperitoneal injection of NARCOREN (Rhone Merieux GmbH, D-88471 Laupheim) at a dose of at least 5.1 ml/kg body weight (equivalent to 810 mg sodium pentobarbitone/kg body weight) and discarded.

STATISTICAL ANALYSIS

Mean values with standard deviations are described under the body weight tables.

DATA COMPILATION

The following data were recorded on data sheets and transcribed for compilation and analysis:

mortality / viability,

clinical signs (local/systemic),

skin reactions.

The following data were recorded on-line:

body weights.

RESULTS

MORTALITY / VIABILITY / MACROSCOPIC FINDINGS

As there were no deaths during the course of the treatment period no necropsies were performed.

CLINICAL SIGNS (LOCAL/SYSTEMIC)

Neither local nor systemic signs were observed during the study.

PHOTOTOXIC REACTIONS

No phototoxic reactions were observed after test article administration at 30%, 25%, 15% and 10% in PEG 400.

See pp. 19-21

BODY WEIGHTS

The body weight of the animals was within the range of physiological variability known for this strain and age.

See pp. 23-25

APPENDIX A

RESULT TABLES OF PHOTOTOXIC REACTIONS

Test Article: CGF-C-1607

Vehicle: PEG 400

CONTROL GROUP

Hrs. after application	LEFT FLANK UV-A irradiated (20 J/cm^2)			RIGHT FLANK non-irradiated		
	24	48	72	24	48	72
Animal no. / Sex						
401 / Female						
vehicle	0	0	0	0	0	0
402 / Female						
vehicle	0	0	0	0	0	0
403 / Female						
vehicle	0	0	0	0	0	0
404 / Female						
vehicle	0	0	0	0	0	0
405 / Female						
vehicle	0	0	0	0	0	0

Test Article: CGF-C-1607

Vehicle: PEG 400

TEST GROUP

Hrs. after application	LEFT FLANK UV-A irradiated (20 J/cm^2) 24	48	72	RIGHT FLANK non-irradiated 24	48	72
Animal no. / Sex						
406 / Female						
A = 30 % B = 25 % C = 15 % D = 10 %	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
407 / Female						
D = 10 % A = 30 % B = 25 % C = 15 %	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
408 / Female						
C = 15 % D = 10 % A = 30 % B = 25 %	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
409 / Female						
B = 25 % C = 15 % D = 10 % A = 30 %	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
410 / Female						
A = 30 % B = 25 % C = 15 % D = 10 %	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0

PHOTOTOXIC REACTIONS

Test Article: CGF-C-1607

Vehicle: PEG 400

TEST GROUP

Hrs. after application	LEFT FLANK UV-A irradiated (20 J/cm^2) 24	48	72	RIGHT FLANK non-irradiated 24	48	72
Animal no. / Sex						
411 / Female						
D = 10 % A = 30 % B = 25 % C = 15 %	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
412 / Female						
C = 15 % D = 10 % A = 30 % B = 25 %	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
413 / Female						
B = 25 % C = 15 % D = 10 % A = 30 %	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
414 / Female						
A = 30 % B = 25 % C = 15 % D = 10 %	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
415 / Female						
D = 10 % A = 30 % B = 25 % C = 15 %	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0

APPENDIX B

BODY WEIGHTS - Summary
Individual

BODY WEIGHTS (GRAM) SUMMARY
FEMALES

ACCLIMATIZATION			GROUP 1 CONTROL GROUP	GROUP 2 TEST GROUP
DAY	1	MEAN	283	276
WEEK	1	ST.DEV.	15.1	5.2
		MINIMUM	271	271
		MAXIMUM	307	283
		N	5	10

BODY WEIGHTS (GRAM) SUMMARY
FEMALES

TREATMENT		GROUP 1 CONTROL GROUP	GROUP 2 TEST GROUP
DAY 1	MEAN	312	301
WEEK 1	ST.DEV.	19.7	7.7
	MINIMUM	300	289
	MAXIMUM	347	314
	N	5	10
DAY 4	MEAN	345	327
WEEK 1	ST.DEV.	18.6	13.6
	MINIMUM	330	298
	MAXIMUM	375	348
	N	5	10

BODY WEIGHTS (GRAM)
FEMALES

	ACCLIMATIZATION	TREATMENT	
DAYS	1	1	4
WEEKS	1	1	1
ANIMAL			
GROUP 1 (CONTROL GROUP)			
401	278	302	332
402	286	308	351
403	307	347	375
404	271	300	330
405	271	303	337
GROUP 2 (TEST GROUP)			
406	278	301	331
407	280	310	318
408	271	305	348
409	272	303	330
410	282	296	326
411	283	303	339
412	280	314	336
413	271	297	322
414	271	292	298
415	271	289	321

APPENDIX C

WATER ANALYSES

- BACTERIOLOGICAL AND CHEMICAL ASSAYS
- CONTAMINANT ANALYSIS OF DRINKING WATER

CONTAMINANT ANALYSIS OF FOOD

RCC PROJECT 651475
CGF-C-1607

BACTERIOLOGICAL ASSAY OF DRINKING WATER, ITINGEN

Official Laboratory	Liestal, 23.10.96
Basel-Landschaft	Ref. No. 96100286
Sampling point:	59.99.N Net water RCC, Room No. 10
Sampled on:	23.10.96
Sample:	
Time of sampling	08.45 a.m.
Water temperature (°C)	15.0

BACTERIOLOGICAL TEST:

Aerobic mesophilic bacteria (per ml)	9
E.coli (per 100 ml)	0
Enterococci (per 100 ml)	0

ASSESSMENT:

At the time of sampling, the tested bacteriological parameters met the requirements for drinking water according to article 260 of the "Eidg. Lebensmittelverordnung".

Official Laboratory
The Official Chemist

(signed Dr. W. Stutz)

RCC PROJECT 651475
CGF-C-1607

CHEMICAL WATER ANALYSIS, ITINGEN

Official Laboratory
Basel-Landschaft

Liestal, 23.10.96
Ref. No. 96100286

Sampling point: 59.99.N Net water RCC, Room No. 10

Sampled on: 23.10.96

Time of sampling 08.45 a.m.
Water temperature (°C) 15.0

Chemical Test

Appearance		clear, colourless
Odor		not remarkable
Taste		not remarkable

Total hardness		fr.H°	38.7
Alkaline hardness		fr.H°	26.3
Non carbonate hardness		fr.H°	12.4
Conductivity		µS/cm	694.0
Oxygen demand	($KMnO_4$ cons.)	mg/l	2.4
Free ammonia	NH_4+	mg/l	<0.01
Nitrite	NO_2-	mg/l	<0.002
Nitrate	NO_3-	mg/l	22.8
Chloride	Cl-	mg/l	21.4
Calcium	Ca++	mg/l	134.5
Magnesium	Mg++	mg/l	12.5
Phosphate		mg/l	<0.005

At the time of sampling, the tested chemical parameters met the requirements for drinking water according to article 260 of the "Eidg. Lebensmittelverordnung".

Official Laboratory
The Official Chemist

(signed Dr. W. Stutz)

RCC PROJECT 651475
CGF-C-1607

CONTAMINANT ASSAY OF DRINKING WATER, ITINGEN

RCC Project: 900720

Date of sampling: 23.10.1996

Sample: H_2O I of room 10, 1st ground-floor, 8.45 h, 15 °C

Parameter	Assay level µg/l	Limit * µg/l
Lindane	<0.05	0.1
Heptachlor	<0.05	0.1
Malathion	<0.05	0.1
DDT, total	<0.05	0.1
Dieldrin	<0.05	0.1
Cadmium	<0.2	5
Arsenic	<0.15	50
Lead	<0.25	50
Mercury	<0.05	1
Selenium	<0.15	10
Copper	<0.15	1500
PCBs (28, 52, 101, 138, 153, 180)	<0.05	0.1
Nitrosamines total (DMN, DEN, NPIP, NMORPH)	<0.05	---

< 0.05 = less than 0.05 microgram per liter

* Schweizer Lebensmittelbuch

December 10, 1996

K.Biedermann

RCC PROJECT 651475
CGF-C-1607

Biochemistry & Environmental Sciences Division RCC

RCC Umweltchemie AG
Postfach
CH-4452 Itingen
Switzerland
Phone: 41/61 975 11 11
Fax: 41/61 971 52 66

ANALYTICAL TEST REPORT FOR FEED

RCC Project 647335
09.01.97

Prepared for	EBERLE NAFAG AG NAFAG ECOSAN Postfach 9201 Gossau
Attention of	Mr. F. Wetter
Authorized by	Letter of 07.01.1997
Materials tested	GLP No. 1/97 Nr. 845
Test performed	AAS, GC, GC-MS, HPLC
Test results	See attached Table 1
Submitted	J. Walker
Issued by	K. Biedermann

January 27, 1997/waj

Biochemistry & Environmental Sciences Division RCC

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Postfach
CH-4452 Itingen
Switzerland
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ATTACHMENT

RCC Project 647335
09.01.97

Table 1 - Test Results GLP No. 1/97 Nr. 845

PARAMETER	ASSAY LEVEL mg/kg	LIMIT* mg/kg
Aflatoxins (B1, B2, G1, G2), total	< 0.001	0.005
Estrogens (DES, Hexestrol, Dienestrol), total	< 0.001	0.001
Lindane	< 0.005	0.02
Heptachlor	< 0.005	0.02
Malathion	< 0.5	2.5
DDT, total	< 0.025	0.100
Dieldrin	< 0.005	0.02
Cadmium	0.04	0.160
Arsenic	< 0.15	1.0
Lead	0.50	1.5
Mercury	< 0.05	0.1
Selenium	< 0.15	0.6
Copper	15	---
PCBs	< 0.025	0.05
Nitrosamines (DMN, DEN, NPIP, NMORPH), total	< 0.01	0.010

< 0.001 = less than 0.001 milligram per kilogram
* = USP EPA, Federal Register, Vol. 44, No. 91, May 9, 1979

APPENDIX D

RESULTS OF POSITIVE CONTROL

RCC PROJECT 901091

TEST OF PHOTOTOXICITY WITH

8-METHOXYPSORALENE

IN ALBINO GUINEA PIGS

Positive Control

SUMMARY AND CONCLUSION

Harber, L.C. and Shalita, A.R. (1975). "The Guinea Pig as an Effective Model for the Demonstration of Immunological Mediated Contact Photosensitivity". In Animal Models in Dermatology (H. Maibach, Ed.) Ch. 10, Churchill Livingstone, New York.

For validation of the sensitivity of test method and test system used, a known photosensitizer 8-METHOXYPSORALENE described below was selected as a positive control. This was performed in accordance with CTFA Safety Testing Guidelines, The Cosmetic, Toiletry and Fragrance Association, Inc., Washington, D.C. 20036; "Guidelines for Evaluating Photodermatitis" 1991.

The raw data from this project are kept in a separate file at RCC. The test described above was performed under GLP-conditions with a final QA-check.

TEST ARTICLE

Identification	8-METHOXYPSORALENE
Description	light yellow crystalline
Date of test article receipt	06-APR-1994
Lot Number	62H0454
Purity	unknown
Stability of test article	unknown
Stability of test article dilution	unknown
Storage Conditions	Stored in BRL at room temperature, protected from light

According to the procedures used in this experiment (performed from October 21 to 31, 1996) clear positive results were observed in the treated animals after the epidermal challenge application.

POSITIVE ERYTHEMA REACTIONS AFTER CHALLENGE PROCEDURE

	CONCENTRATION %	LEFT FLANK UV-A irradiated (20 J/cm²) Hours after application			RIGHT FLANK non-irradiated Hours after application		
		24	48	72	24	48	72
CONTROL GROUP	Ethanol	0/5*	0/5	0/5	0/5	0/5	0/5
TEST GROUP (8-METHOXY-PSORALENE)	0.3%	9/10	10/10	10/10	1/10	0/10	0/10
	0.1%	9/10	9/10	8/10	0/10	0/10	0/10
	0.03%	5/10	5/10	5/10	0/10	0/10	0/10
	0.01%	4/10	3/10	2/10	0/10	0/10	0/10

*) number of animals showing an erythema/total

All animals were pretreated with 2% DMSO in ethanol to enhance the skin penetration of the test article.

CONCLUSION

From the results previously described, overt difference was observed at the test article concentration of 0.3%, 0.1%, 0.03% and 0.01% comparing the observation made on the non-irradiated and the irradiated skin sites.

8-METHOXYPSORALENE showed phototoxic potential for the guinea pigs of this strain and age.

PHOTOTOXIC REACTIONS/ INDIVIDUAL FINDINGS

Test Article: 8-METHOXYPSORALENE

Vehicle: ethanol

CONTROL GROUP

Hrs. after application	LEFT FLANK UV-A irradiated (20 J/cm^2)			RIGHT FLANK non-irradiated		
	24	48	72	24	48	72
Animal no. / Sex						
84 / female						
vehicle	0	0	0	0	0	0
85 / female						
vehicle	0	0	0	0	0	0
86 / female						
vehicle	0	0	0	0	0	0
87 / female						
vehicle	0	0	0	0	0	0
88 / female						
vehicle	0	0	0	0	0	0

PHOTOTOXIC REACTIONS/ INDIVIDUAL FINDINGS

Test Article: 8-METHOXYPSORALENE

Vehicle: ethanol

TEST GROUP

Hrs. after application	LEFT FLANK UV-A irradiated (20 J/cm^2) 24	48	72	RIGHT FLANK non-irradiated 24	48	72
Animal no. / Sex						
89 / female						
A = 0.3%	2	2	2	0	0	0
B = 0.1%	2	2	1	0	0	0
C = 0.03%	1	1	1	0	0	0
D = 0.01%	1	1	0	0	0	0
90 / female						
D = 0.01%	1	1	1	0	0	0
A = 0.3%	2	2	2	0	0	0
B = 0.1%	2	2	2	0	0	0
C = 0.03%	1	1	1	0	0	0
91 / female						
C = 0.03%	0	0	0	0	0	0
D = 0.01%	0	0	0	0	0	0
A = 0.3%	1	1	1	0	0	0
B = 0.1%	1	1	0	0	0	0
92 / female						
B = 0.1%	1	1	1	0	0	0
C = 0.03%	1	1	1	0	0	0
D = 0.01%	0	0	0	0	0	0
A = 0.3%	2	2	2	0	0	0
93 / female						
A = 0.3%	1	1	1	0	0	0
B = 0.1%	1	1	1	0	0	0
C = 0.03%	0	1	1	0	0	0
D = 0.01%	0	0	0	0	0	0

PHOTOTOXIC REACTIONS

Test Article: 8-METHOXYPSORALENE

Vehicle: ethanol

TEST GROUP

Hrs. after application	LEFT FLANK UV-A irradiated (20 J/cm^2) 24	48	72	RIGHT FLANK non-irradiated 24	48	72
Animal no. / Sex						
94 / female						
D = 0.01%	1	0	0	0	0	0
A = 0.3%	2	2	2	1	0	0
B = 0.1%	1	1	1	0	0	0
C = 0.03%	1	1	1	0	0	0
95 / female						
C = 0.03%	0	0	0	0	0	0
D = 0.01%	0	0	0	0	0	0
A = 0.3%	1	1	1	0	0	0
B = 0.1%	1	1	1	0	0	0
96 / female						
B = 0.1%	1	1	1	0	0	0
C = 0.03%	1	0	0	0	0	0
D = 0.01%	1	1	1	0	0	0
A = 0.3%	1	1	1	0	0	0
97 / female						
A = 0.3%	1	1	1	0	0	0
B = 0.1%	1	1	1	0	0	0
C = 0.03%	0	0	0	0	0	0
D = 0.01%	0	0	0	0	0	0
98 / female						
D = 0.01%	0	0	0	0	0	0
A = 0.3%	0	1	1	0	0	0
B = 0.1%	0	0	0	0	0	0
C = 0.03%	0	0	0	0	0	0

APPENDIX E

SUMMARY TABLE OF STUDY INFORMATION AND RESULTS

Test article identification: Name: CGF-C-1607					SUMMARY TABLE
Batch No: 002					
SKIN TOLERANCE STUDIES / IMMUNOSTIMULATION (Phototoxicity potential by epidermal administration) **Phototoxicity Test**					Study No: 651475 Report date: 29-APR-1997
Species/Strain: Albino Dunkin Hartley GP					Number of exp. animals: 15
Procedure	Administration route/site		Irradiation	Day	Vehicle
Induction	epicutaneous/left flank epicutaneous/right flank		20J/cm² UVA none	1	PEG 400

Study group		Control Group		Test Group	
		Conc. of test article	No. of appl. and dose	Conc. of test article in %	No. of appl. and dose
Induction	A	PEG 400	1x25µl/2cm²	30	1x25µl/2cm²
	B			25	"
	C			15	"
	D			10	"
Sex		female		female	
Number of animals		5		10	
Test flank/irradiated		left/yes	right/no	left/yes	right/no
Animals with positive reactions on irra-diated/ non-irra-diated sites	Observed (24h) A	0(5)	0(5)	0(10)	0(10)
	B			0(10)	0(10)
	C			0(10)	0(10)
	D			0(10)	0(10)
	Observed (48h) A	0(5)	0(5)	0(10)	0(10)
	B			0(10)	0(10)
	C			0(10)	0(10)
	D			0(10)	0(10)
	Observed (72h) A	0(5)	0(5)	0(10)	0(10)
	B			0(10)	0(10)
	C			0(10)	0(10)
	D			0(10)	0(10)
Summary of salient findings: The test article CGF-C-1607 showed no phototoxic potential.					
Study conducted by the applicant: yes < > no <X> Study in compliance with GLP: yes <X> no < > QAU inspected: yes <X> no < >					

( ) = number of animals tested

APPENDIX F

GLP-CERTIFICATE

RCC PROJECT 651475
CGF-C-1607

EIDGENÖSSISCHES DEPARTEMENT DES INNERN
DÉPARTEMENT FÉDÉRAL DE L'INTÉRIEUR
DIPARTIMENTO FEDERALE DELL'INTERNO

GLP Compliance Statement

It is hereby certified that

on February 12-16, 1996
February 19-23, 1996
June 14, 1996

the testing facilities of RCC Holding Company Ltd
4414 Füllinsdorf
Switzerland

were inspected by the Federal Office of Public Health, the Federal Office of Environment, Forests and Landscape and the Intercantonal Office for the Control of Medicaments with respect to the compliance with the Swiss GLP Principles. The inspection was performed in agreement with the OECD Guidelines for National GLP Inspections and Audits and comprised the following testing facilities:

- RCC Research and Consulting Company Ltd, Itingen
- RCC Umweltchemie AG, Itingen
- RCC Pharmanalytics Ltd, Itingen
- BRL Biological Research Laboratories Ltd/Microbiology, Füllinsdorf

It was found that the aforementioned testing facilities were operating in compliance with the Swiss Principles of Good Laboratory Practice (Good Laboratory Practice [GLP] in Switzerland, Procedures and Principles, March 1986) at the time they were inspected.

FEDERAL DEPARTMENT OF THE INTERIOR

Bern, July 9, 1996

Ruth Dreifuss
Federal Councillor