

8EHQ-0601-14948

48563

RECEIVED
OPPT/NOIC

2001 JUN -7 11:11-04



Ciba

June 4, 2001

Via Federal Express

US Environmental Protection Agency
OPPT Document Control Office TS 7407
Attention: Section 8(e)
Ariel-Rios Building
1200 Pennsylvania Avenue NW
Washington, DC 20460

Contain NO CBI

Subject: TSCA 8(e) Notice - Chimassorb 81 (TK 10050)

Dear Section 8(e) Coordinator:

This letter and attached report do not contain Confidential Business Information.

In accordance with EPA's March 16, 1978 Policy Statement on Section 8(e) reporting under the Toxic Substances Control Act (TSCA), the EPA's June, 1991 TSCA Section 8(e) Reporting Guide, Ciba Specialty Chemicals Corporation wishes to bring to the attention of the Environmental Protection Agency the results observed in a contact hypersensitivity maximization test conducted with Chimassorb 81. Chimassorb 81 chemically is 2-Hydroxy-4-(octyloxy) benzophenone, CASRN 1843-05-6.

We are enclosing a copy of the study "**TK 10050 (Chimassorb 81): Contact Hypersensitivity in Albino Guinea Pigs, Maximization Test**" RCC,Ltd, CH-4452, Itingen, Switzerland; RCC Study Number 798704.

A contact hypersensitivity maximization-test was conducted in albino guinea pigs with Chimassorb 81. For the challenge procedure, the test article was prepared as a 40% solution in PEG300 (the highest technically applicable concentration). Seven of nine (7/9) animals showed a positive skin response (erythema) after the challenge. No toxic symptoms were evident. One animal was found dead on test day 14, but the death was not considered to be treatment-related.

Based upon current EPA guidelines, it is felt these results warrant reporting under TSCA 8(e). Please call the undersigned if you have any questions concerning this submittal.

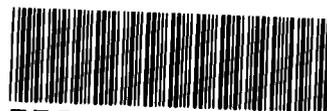
Respectfully,

Ciba Specialty Chemicals Corporation

Thomas Barber
Manager, Regulatory Compliance



8EHQ-01-14948



88010000159

2001 JUN 23 9:28
OPPT/NOIC

RCC Study Number 798704

TK 10050 (CHIMASSORB 81):

Contact Hypersensitivity in Albino Guinea
Pigs, Maximization-Test

Report

Author: G. Arcelin

Page 1 of 57



Table of contents

1	PREFACE	5
1.1	GENERAL	5
1.2	RESPONSIBILITIES	5
1.3	SCHEDULE.....	5
1.4	ARCHIVING	6
1.5	SIGNATURE PAGE	7
1.6	QUALITY ASSURANCE UNIT.....	8
1.7	STATEMENT OF COMPLIANCE / GLP GUIDELINES.....	9
1.8	CERTIFICATION OF GLP AND VERIFICATION OF THE REPORT	10
1.9	ACCREDITATION.....	11
1.10	TEST GUIDELINES	11
1.11	REFERENCES.....	11
1.12	CLASSIFICATION GUIDELINES	11
1.13	SUMMARY OF STUDY PLAN AMENDMENT	11
2	SUMMARY	12
3	PURPOSE.....	14
4	MATERIALS AND METHODS.....	14
4.1	TEST SYSTEM	14
4.2	ALLOCATION	15
4.3	HUSBANDRY	15
4.4	TEST ITEM	16
4.5	VEHICLE.....	16
4.6	AUXILIARY COMPOUNDS	16
4.7	TEST ITEM PREPARATION.....	17
4.8	RATIONALE.....	17
4.9	READINGS AND SCORING	18
4.10	SELECTION OF CONCENTRATION OF TEST ITEM FOR MAIN STUDY	18
5	STUDY CONDUCT - TREATMENT PROCEDURE.....	19
5.1	PRETEST PERFORMED WITH TK 10050 (CHIMASSORB 81) BEFORE AND DURING THE ACCLIMATIZATION PERIOD OF THE CONTROL AND TEST GROUP	19

Table of contents (cont'd)

5.2	MAIN STUDY	20
5.2.1	INDUCTION	20
5.2.1.1	INTRADERMAL INJECTIONS / PERFORMED ON TEST DAY 1	20
5.2.1.2	EPIDERMAL APPLICATIONS / PERFORMED ON TEST DAY 8	21
5.2.2	CHALLENGE / PERFORMED ON TEST DAY 22	22
5.3	INTERPRETATION	22
5.4	RATING OF ALLERGENICITY ACCORDING TO MAGNUSSON AND KLIGMAN	23
5.5	OBSERVATIONS	23
6	PATHOLOGY	23
6.1	NECROPSY	23
7	STATISTICAL ANALYSIS	24
8	DATA COMPILATION	24
9	RESULTS	25
	Main Study	25
9.1	SKIN EFFECTS AFTER INTRADERMAL INDUCTION - PERFORMED ON TEST DAY 1	25
9.2	SKIN EFFECTS AFTER EPIDERMAL INDUCTION - PERFORMED ON TEST DAY 8	25
9.3	SKIN EFFECTS AFTER THE CHALLENGE - PERFORMED ON TEST DAY 22	25
9.4	VIABILITY / MORTALITY / MACROSCOPIC FINDINGS	26
9.5	CLINICAL SIGNS, SYSTEMIC	26
9.6	BODY WEIGHTS	26

Table of contents (cont'd)

APPENDIX A	
PRETEST.....	28
MAIN STUDY	
- INDUCTION	30
- CHALLENGE.....	32
APPENDIX B	
NECROPSY	
- MACROSCOPIC FINDINGS	37
APPENDIX C	
BODY WEIGHTS	
- SUMMARY	39
- INDIVIDUAL	42
APPENDIX D	
RESULTS OF POSITIVE CONTROL	44
APPENDIX E	
SUMMARY TABLE OF STUDY INFORMATION AND RESULTS	54
APPENDIX F	
CERTIFICATION	
- ACCREDITATION / EUROPEAN STANDARD EN 45001	56
- GLP – CERTIFICATION.....	57
 LAST PAGE OF REPORT	 57

1 PREFACE

1.1 GENERAL

Title	TK 10050 (CHIMASSORB 81): Contact Hypersensitivity in Albino Guinea Pigs, Maximization-Test
Sponsor	Ciba Specialty Chemicals Inc. Additives Division P.O. Box CH-4002 Basel / Switzerland
Study Monitor	Dr. S. Müller
Test Facility	RCC Ltd Toxicology Division Wölferstrasse 4 CH-4414 Füllinsdorf / Switzerland

1.2 RESPONSIBILITIES

Study Director	G. Arcelin
Technical Coordinator	P. Reissbrodt
Head of RCC Quality Assurance	I. Wüthrich

1.3 SCHEDULE

Experimental Starting Date	24-JAN-2001
Experimental Completion Date	06-MAR-2001
Delivery of the Animals	24-JAN-2001 (pretest) 31-JAN-2001 (main study)
Pretest Start	24-JAN-2001
Acclimatization (main study)	31-JAN-2001 to 06-FEB-2001
Observation (main study)	31-JAN-2001 to 06-MAR-2001
Treatment (main study)	07-FEB-2001 to 01-MAR-2001
Termination	06-MAR-2001
Reported	09-APR-2001

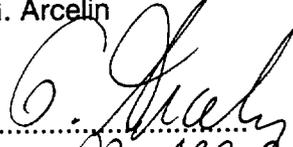
1.4 ARCHIVING

RCC Ltd (CH-4452 Itingen / Switzerland) will retain the study plan, raw data, a sample of test item(s), amendment and the final report of the present study for at least ten years. No data will be discarded without the Sponsor's consent.

1.5 SIGNATURE PAGE

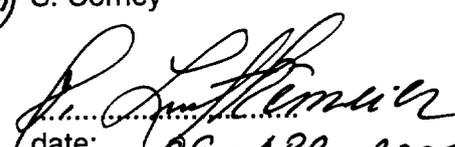
Study Director:

G. Arcelin


.....
date: 09-APR-2001

Management:

(b) S. Corney


.....
date: 09-APR-2001

1.6 QUALITY ASSURANCE UNIT

RCC Ltd, Toxicology Division, CH-4452 Itingen / Switzerland

STATEMENT

RCC STUDY NUMBER : 798704
TEST ITEM : TK 10050 (CHIMASSORB 81)
STUDY DIRECTOR : G. Arcelin
TITLE : TK 10050 (CHIMASSORB 81):
Contact Hypersensitivity in Albino Guinea Pigs,
Maximization-Test

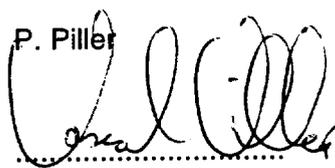
The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

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

Dates and Types of QAU Inspections	Dates of Reports to the Study Director and to Management
15-JAN-2001 Study Plan Audit	15-JAN-2001
23-JAN-2001 Process Based Inspection	23-JAN-2001
29-MAR to 05-APR-2001 Draft Report Audit	05-APR-2001

This statement also confirms that this final report reflects the raw data.

Quality Assurance:

P. Piller

date: 09-Apr-2001

GOOD LABORATORY PRACTICE

1.7 STATEMENT OF COMPLIANCE / GLP GUIDELINES

RCC STUDY NUMBER : 798704
TEST ITEM : TK 10050 (CHIMASSORB 81)
STUDY DIRECTOR : G. Arcelin
TITLE : TK 10050 (CHIMASSORB 81):
Contact Hypersensitivity in Albino Guinea Pigs,
Maximization-Test

The stability of the test item in a 1:1 (v/v) mixture of FCA/physiological saline is unknown and therefore is excluded from this statement.

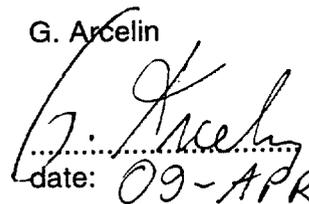
The study described in this report was conducted in compliance with the following Good Laboratory Practice Standards:

Swiss Ordinance relating to Good Laboratory Practice, adopted February 2nd, 2000 [RS 813.016.5]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97)186/Final].

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

Study Director:

G. Arcelin


date: 09-APR-2001

1.8 CERTIFICATION OF GLP AND VERIFICATION OF THE REPORT

The statement of Compliance with Good Laboratory Practice found in this report, and signed by the Study Director is truthful and accurate, and this report as provided by the test facility is complete and unaltered.

Signature of the Sponsor:

.....

.....

Date:

1.9 ACCREDITATION

The test facility "RCC Ltd, Toxicology Division" is accredited according to EN 45001 under accreditation number STS 085 by the Swiss Accreditation Service.

1.10 TEST GUIDELINES

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

Directive 96/54/EEC, B.6. "Acute Toxicity - Skin Sensitization", July 30, 1996.

OECD Guidelines for Testing of Chemicals, Number 406 "Skin Sensitization", adopted by the Council on July 17, 1992 (reported Paris, April 29, 1993).

1.11 REFERENCES

Magnusson B.; Kligman A.M., 1969.

The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximization Test. J. Invest. Dermatol. 52: 268-276.

1.12 CLASSIFICATION GUIDELINES

The evaluation of the results is based on the criteria of the EEC Commission Directive 96/54/EEC, July 30, 1996 adapting to technical progress for the 22nd time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. A potential contact sensitizer is classified as any article that produces in an adjuvant assay at least 30 % of test animals with allergic contact dermatitis. The test item is then classified as „may cause sensitization by skin contact" and labelled with the risk phrase R43.

1.13 SUMMARY OF STUDY PLAN AMENDMENT

Study Plan Amendment No. 1:

The purity and formulation of the test item was changed.

2 SUMMARY

In order to assess the cutaneous allergenic potential of TK 10050 (CHIMASSORB 81), the Maximization-Test was performed in 15 (10 test and 5 control) female albino guinea pigs, in accordance with OECD Guideline No. 406 and the Directive 96/54/EEC, B.6.

The intradermal induction of sensitization in the test group was performed in the nuchal region with a 15 % dilution of the test item in PEG 300 and in an emulsion of Freund's Complete Adjuvant (FCA) / physiological saline. The epidermal induction of sensitization was conducted for 48 hours under occlusion with the test item at 40 % in PEG 300 one week after the intradermal induction and following pretreatment of the test areas with 10 % Sodium-Lauryl-Sulfate (SLS) 23 hours prior to application of the test item. The animals of the control group were intradermally induced with PEG 300 and FCA/physiological saline and epidermally induced with PEG 300 under occlusion following pretreatment with 10 % SLS.

Two weeks after epidermal induction the control and test animals were challenged by epidermal application of the test item at 40 % in PEG 300 and PEG 300 alone under occlusive dressing.

Cutaneous reactions were evaluated at 24 and 48 hours after removal of the dressing.

Results

Skin Reactions after the Challenge Procedure

	after 24 hours	after 48 hours
	positive / total % positive of total	positive / total % positive of total
CONTROL GROUP		
TK 10050 (CHIMASSORB 81) 40 % in PEG 300 (left flank)	$\frac{0}{5}$ 0	$\frac{0}{5}$ 0
PEG 300 only (right flank)	$\frac{0}{5}$ 0	$\frac{0}{5}$ 0
TEST GROUP		
TK 10050 (CHIMASSORB 81) 40 % in PEG 300 (left flank)	$\frac{7}{9^*}$ 78	$\frac{7}{9}$ 78
PEG 300 only (right flank)	$\frac{0}{9}$ 0	$\frac{0}{9}$ 0

* One animal of the test group was found dead on test day 14 (i.e. 2 days after the 48-hour reading of the epidermal induction). At necropsy, no macroscopic findings were noted. The cause of death could not be established. The death was considered treatment-unrelated.

No toxic symptoms were evident in the guinea pigs of the control or test group.

Seven out of 9 surviving test animals showed discrete/patchy to moderate/confluent erythema at the 24- and 48-hour reading after the challenge treatment with TK 10050 (CHIMASSORB 81) at 40 % (w/w) in PEG 300. No skin effect was observed in the control group.

Conclusion

Based on the above mentioned findings in an adjuvant sensitization test (M&K-test) in guinea pigs and in accordance to Commission Directive 96/54/EEC, TK 10050 (CHIMASSORB 81) has to be classified and labelled as a skin sensitizer.

3 PURPOSE

The purpose of this skin sensitization study was to assess the allergenic potential of TK 10050 (CHIMASSORB 81) when administered to the skin of albino guinea pigs.

This study should provide a rational basis for risk assessment of the sensitizing potential of the test item in man.

The sensitivity and reliability of the experimental technique employed was assessed by use of 2-MERCAPTOBENZOTHIAZOLE which is recommended by the OECD 406 Guidelines and is known to have moderate skin sensitization properties in the guinea pig strain. The results from the most recent test run (RCC study number 905095, performed from 03-OCT-2000 to 10-NOV-2000) are included in this report under the APPENDIX D.

4 MATERIALS AND METHODS

4.1 TEST SYSTEM

Test system	Ibm: GOHI; SPF-quality guinea pigs (synonym: Himalayan spotted)
Rationale	Recognized by the international guidelines as a recommended test system (e.g. OECD, EEC).
Source	RCC Ltd, Biotechnology & Animal Breeding Division, Wölferstrasse 4, CH-4414 Füllinsdorf / Switzerland
Number of animals for main study / pretest	15 female / 3 female animals (nulliparous and non-pregnant)
Age at delivery	4 - 6 weeks
Age at pretest start/beginning of acclimatization period	4 - 6 weeks
Body weight at pretest start	Pretest groups: 367 - 399 g
Body weight at beginning of acclimatization period	Control and test group 319 - 394 g
Identification	By unique cage number and corresponding ear tags.
Randomization	Selected by hand at time of delivery. No computer randomization.
Acclimatization	One week for the control and test group under test conditions after health examination. No acclimatization for the animals of the pretest. Only animals without any visible signs of illness were used for the study.

4.2 ALLOCATION

The animals were distributed as follows:

	NUMBER OF ANIMALS PER GROUP	ANIMAL NUMBERS PER GROUP
1 Intradermal Pretest	1	83
2 Epidermal Pretest	2	84 - 85
3 Control Group	5	86 - 90
4 Test Group	10	91 - 100

4.3 HUSBANDRY

Room no. 109 / RCC Ltd, Füllinsdorf

Conditions

Standard Laboratory Conditions

Air-conditioned with target ranges for room temperature 20 ± 3 °C, relative humidity 30-70 % and approximately 10-14 air changes per hour. Room temperature and humidity were monitored continuously and values outside of these ranges occasionally occurred, usually following room cleaning. These transient variations are considered not to have any influence on the study and, therefore, these data are not reported but are retained at RCC. The animals were provided with an automatically controlled light cycle of 12 hours light and 12 hours dark. Music was played during the light period.

Accommodation

Individually in Makrolon type-4 cages with standard softwood bedding ("Lignocel", Schill AG, CH-4132 Muttenz).

Diet

Pelleted standard Nafag Ecosan 845 25W4, batch no. 114/00 ("Nafag", Nähr- und Futtermittel AG, CH-9202 Gossau) and Provimi Kliba 3418, batch no. 33/00 (Provimi Kliba AG, CH-4303 Kaiseraugst), guinea pig breeding / maintenance diet, containing Vitamin C, *ad libitum*. Results of analyses for contaminants are archived at RCC Ltd, Itingen.

Water

Community tap water from Füllinsdorf, *ad libitum*. Results of bacteriological, chemical and contaminant analyses are archived at RCC Ltd, Itingen.

4.4 TEST ITEM

The following information was provided by the sponsor:

Identification	TK 10050 (CHIMASSORB 81)
Description	light yellowish solid
Batch number	8101880P
Purity / Formulation	Confidential information; available in sponsor's file.
Stability of test item	Stable under storage conditions; expiration date: 01-DEC-2002
Stability of test item dilution	Stable in polyethylene glycol for at least a few hours at room temperature. Unknown in a 1:1 (v/v) mixture of FCA/physiological saline; is excluded from the statement of compliance.
Storage conditions	In the original container, at room temperature (range of 20 ± 3 °C), away from direct sunlight.

4.5 VEHICLE

The following information was provided by RCC Ltd:

Identification	Polyethylene glycol 300 (PEG 300)
Description	colorless viscous liquid
Lot number	405374/1 30600
Source	FLUKA Chemie AG, CH-9471 Buchs
Stability of vehicle	Stable under storage conditions; expiration date: 18-APR-2001
Storage conditions	In the original container, at room temperature (range of 20 ± 3 °C), away from direct sunlight.

4.6 AUXILIARY COMPOUNDS

The following information was provided by RCC Ltd:

FCA

Identification	Freund's Adjuvant - Complete
Description	clear, amber liquid containing light colored particles
Batch No.	20K8933
Source	Sigma, 3050 Spruce Street, Saint Louis, Missouri 63103 USA

Purity	each ml contains 1 mg Mycobacterium Tuberculosis (H 37Ra, ATCC 25177), heat killed and dried, 0.85 ml mineral oil and 0.15 ml mannide monooleate
Expiry date	06-DEC-2001
Storage conditions	In the original container, in the refrigerator (range of 4 ± 3 °C), away from direct sunlight.

Physiological saline

Identification	Natrium chloratum 0.9 %
Description	colorless liquid
Batch No.	720504/2
Source	G. Streuli & Co. AG, CH-8730 Uznach/Switzerland
Expiry date	July 2004
Storage conditions	In the original container, in the refrigerator (range of 4 ± 3 °C), away from direct sunlight.

4.7 TEST ITEM PREPARATION

The test item and vehicle* or auxillary compound were placed into a glass beaker on a tared Mettler PM 460 balance and a weight by weight dilution was prepared. Homogeneity of the test item preparation was ensured and maintained during treatment using a magnetic stirrer. The preparations were made immediately prior to each dosing.

Dose levels were in terms of material as supplied by the sponsor.

4.8 RATIONALE

The application procedure was used to detect a possible allergenic potential of the test item applied.

* PEG 300 was used for the intradermal and epidermal pretests. It was also used for the intradermal and epidermal induction and the challenge in the main study. The 1:1 mixture (v/v) of Freund's Complete Adjuvant:physiological saline was used for the pretest and the intradermal induction in the main study.

4.9 READINGS AND SCORING

The scoring system was performed by visual scoring of erythema, oedema and other clinical changes of skin conditions. They were assessed using the following Magnusson and Kligman grading scale:

- 0 = no visible change
- 1 = discrete or patchy erythema
- 2 = moderate and confluent erythema
- 3 = intense erythema and swelling

Grading of all animals was done by positioning the animal under true-light (Philips TLD 36W/84 or Osram 36W/31 830).

4.10 SELECTION OF CONCENTRATION OF TEST ITEM FOR MAIN STUDY

Intradermal Induction:

The 15 % concentration of test item used for the intradermal induction exposure was well-tolerated systemically and was the highest technically applicable concentration causing mild skin irritation.

Epidermal Induction:

The 40 % concentration of test item used for the epidermal induction exposure was the highest attainable concentration which could be applied and was well-tolerated systemically. This concentration did not cause any skin irritation but was nevertheless selected because it was the highest technically applicable concentration.

Epidermal Challenge:

The 40 % concentration was the maximum tested non-irritant concentration.

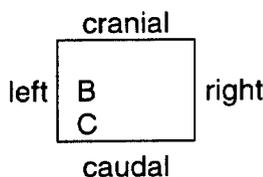
To determine the different concentrations an intradermal and epidermal pretest was performed as described below.

5 STUDY CONDUCT - TREATMENT PROCEDURE

5.1 PRETEST PERFORMED WITH TK 10050 (CHIMASSORB 81) BEFORE AND DURING THE ACCLIMATIZATION PERIOD OF THE CONTROL AND TEST GROUP

INTRADERMAL INJECTIONS:

Four intradermal injections (0.1 ml/site) of a 1:1 (v/v) mixture of Freund's Complete Adjuvant/physiological saline were made into the shaved neck of one guinea pig (no. 83). One week later intradermal injections (0.1 ml/site) were made into the clipped flank of the same guinea pig at concentrations of B = 15 % and C = 10 % of the test item in PEG 300. Although the third concentration of A = 20 % was technically not applicable, the three concentrations were determined during non-GLP formulation trials performed prior to the pretest. The concentration of 15 % was considered to be the highest technically applicable concentration which could be injected into the intra-cellular space in spite of the high viscosity of the application dilution and the obstacle caused by the tissues.



Dermal reactions were assessed 24 hours later.

Based on the results, the test item concentration of 15 % was selected for intradermal induction in the main study.

The skin reactions are listed on page 28 in APPENDIX A.

EPIDERMAL APPLICATIONS:

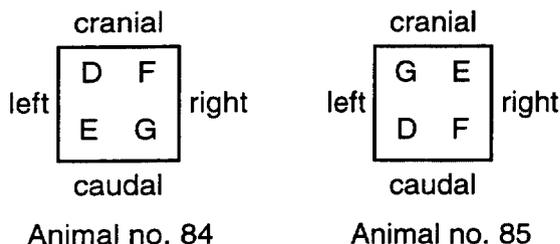
Four intradermal injections (0.1 ml/site) of a 1:1 (v/v) mixture of Freund's Complete Adjuvant/physiological saline were made into the shaved neck of two guinea pigs. One week later both flanks of each of the guinea pigs were clipped and shaved just prior to the application. Thereafter 4 patches of filter paper (3 x 3 cm) were saturated with the test item at D = 40 % (technically the highest possible concentration to be applied sufficiently), E = 25 %, F = 15 % and G = 10 % in PEG 300 and applied to the clipped and shaved flanks. The amount of test item preparation applied was approximately 0.2 g for the test item at 40 % and 25 % and a volume of approximately 0.2 ml was applied for the remaining test item concentrations. The patches were covered by a strip of aluminum foil and firmly secured by elastic plaster wrapped around the trunk and covered with impervious adhesive tape. This procedure ensured the intensive contact of the test item. The dressings were removed after an exposure period of 24 hours.

Twenty-one hours after removal of the dressing the application site was depilated with an approved depilatory cream (VEET Cream, Reckitt & Colman AG, CH-4123 Allschwil) in order to visualize any resulting erythema.

The depilatory cream was placed on the patch sites and surrounding areas, and left on for 3-5 minutes. It was then thoroughly washed off with a stream of warm, running water. Thereafter, the animals were dried with a disposable towel, and returned to their cages.

The reaction sites were assessed 24 and 48 hours after removal of the bandage for erythema and oedema according to the method of Magnusson and Kligman (see 4.9).

The position of the epidermal applications is shown below:



The allocation of the different test item dilutions to the sites (D, E, F, G) on the two animals was alternated in order to minimize site-to-site variation in responsiveness.

Results are listed on page 29 in APPENDIX A. Based on the results obtained the concentration selected for induction and challenge in the main study was 40 %.

5.2 MAIN STUDY

5.2.1 INDUCTION

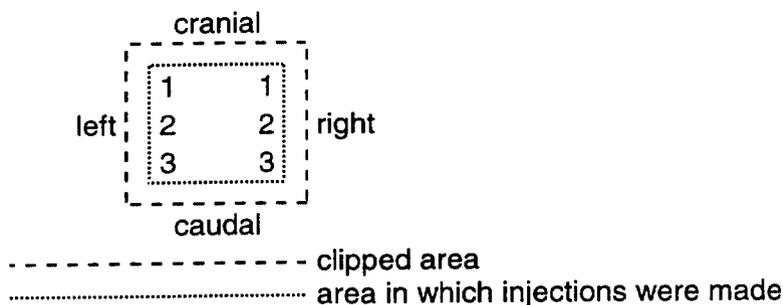
5.2.1.1 INTRADERMAL INJECTIONS / PERFORMED ON TEST DAY 1

An area of dorsal skin from the scapular region (approximately 6 x 8 cm) was clipped free of hair. Three pairs of intradermal injections (0.1 ml/site) were made at the border of a 4 x 6 cm area in the clipped region as follows:

- Test Group:
- 1) 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline.
 - 2) The test item, at 15 % in PEG 300.
 - 3) The test item at 15 % in a 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline.

- Control Group:
- 1) 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline.
 - 2) PEG 300
 - 3) 1:1 (w/w) mixture of PEG 300 in a 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline.

The positions of the intradermal injections are shown below:



5.2.1.2 EPIDERMAL APPLICATIONS / PERFORMED ON TEST DAY 8

On test day 7 and 23 hours prior to the epidermal application the scapular area (approximately 6 x 8 cm) of the animals of the control and test group was clipped, shaved free of hair and the test area was pretreated with 0.5 ml of 10 % Sodium-Lauryl-Sulfate (SLS) in *paraffinum perliquidum* as no primary irritation had been observed in the pretest. The SLS was massaged into the skin with a glass rod without bandaging. This 10 % concentration of SLS enhances sensitization by provoking a mild inflammatory reaction (Magnusson and Kligman 1970).

On test day 8, a 2 x 4 cm patch of filter paper was saturated with the test item (40 % in PEG 300) and placed over the injection sites of the test animals. The amount of test item preparation applied was approximately 0.3 g. The patch was covered with aluminum foil and firmly secured by an elastic plaster wrapped around the trunk of the animal and secured with impervious adhesive tape. The occlusive dressings were left in place for 48 hours. The epidermal application procedure described ensured intensive contact of the test item.

The guinea pigs of the control group were treated as described above with PEG 300 only, applied at a volume of approximately 0.3 ml.

The reaction sites were assessed 24 and 48 hours after removal of the bandage for erythema and oedema according to the method of Magnusson and Kligman (see 4.9).

The skin reactions are listed in tables 1-2 in APPENDIX A.

5.2.2 CHALLENGE / PERFORMED ON TEST DAY 22

The test and control guinea pigs were challenged two weeks after the epidermal induction application and were treated in the same way.

Hair was clipped and shaved from a 5 x 5 cm area on the left and right flank of each guinea pig just prior to the application. Two patches (3 x 3 cm) of filter paper were saturated with the test item at the highest tested non-irritating concentration of 40 % (applied to the left flank) and the vehicle only (PEG 300 applied to the right flank) using the same method as for the epidermal application. The amount of test item preparation applied was approximately 0.2 g and a volume of approximately 0.2 ml was used for the vehicle. The dressings were left in place for 24 hours.

Twenty-one hours after removal of the dressing the test sites treated with the test item were depilated as described in the epidermal pretest.

The reaction sites were assessed 24 and 48 hours after removal of the bandage for erythema and oedema according to the method of Magnusson and Kligman (see 4.9).

The skin reactions are listed in tables 3-6 in APPENDIX A.

5.3 INTERPRETATION

The results obtained from test animals following the challenge application were compared with the results seen in control animals.

An allergic reaction was defined by visible reddening of the challenge site.

If the dermal reactions of test animals following the challenge were more marked and/or persistent than those of the control animals, the animals were considered to show evidence of contact hypersensitivity.

If the dermal reactions of test animals following the challenge were not clearly different from the reactions seen in the control group animals, the results for the test animals were considered "inconclusive".

The test animals were considered to show no evidence of contact hypersensitivity if the dermal reactions to the challenge application were identical or less marked and/or persistent than the reactions observed in the control animals.

By "maximizing" the exposure and enhancing allergenicity, some problems could arise, particularly in relation to specificity, especially the potential for false-positive reactions. An inflammatory response at challenge may not necessarily be due to allergenicity, but instead may be a false-positive irritant response caused by an inducing hyperirritability.

5.4 RATING OF ALLERGENICITY ACCORDING TO MAGNUSSON AND KLIGMAN

Based upon the percentage of animals sensitized (24- and 48-hour reading), the test item was assigned to one of the following five grades of allergenic potency according to Magnusson and Kligman, ranging from weak to extreme:

Sensitization Rate (%)	Grade	Classification
0 - 8	1	weak
9 - 28	2	mild
29 - 64	3	moderate
65 - 80	4	strong
81 - 100	5	extreme

5.5 OBSERVATIONS

The following observations and data were recorded during the study:

Viability / Mortality	Daily from delivery of the animals to the termination of the test.
Clinical signs (systemic)	Daily from delivery of the animals to the termination of the test.
Skin reactions	At the times specified during the pretest, induction and challenge periods.
Body weights	At pretest and acclimatization start, day 1 and termination of the test.

Records were maintained on all additional and standard observations.

6 PATHOLOGY

6.1 NECROPSY

Necropsy was performed in one animal (no. 94) of the test group which was found dead on test day 14 (i.e. 2 days after the 48-hour reading of the epidermal induction).

No necropsies were performed in the animals of the control and test group sacrificed at termination of the observation period nor in the animals of the intradermal and epidermal pretest sacrificed on test day 1 of the main study.

The surviving animals were sacrificed by intraperitoneal injection of NARCOREN (Rhône Mérieux GmbH, D-88471 Laupheim) at a dose of at least 2.0 ml/kg body weight (equivalent to 320 mg sodium pentobarbitone/kg body weight) and discarded.

7 STATISTICAL ANALYSIS

Descriptive statistics (means and standard deviations) were calculated for body weights. No inferential statistics were used.

8 DATA COMPILATION

The following data were recorded on data sheets and transcribed in the report:
skin reactions, viability/mortality and clinical signs.

The following data were compiled into the RCC computer system during recording:
macroscopic findings.

The following data were recorded on-line:
body weights.

9 RESULTS

Main Study

9.1 SKIN EFFECTS AFTER INTRADERMAL INDUCTION - PERFORMED ON TEST DAY 1

The expected and common findings were observed in the control and test group after the different applications using FCA intradermally. These findings consisted of erythema, oedema, necrotizing dermatitis, encrustation and exfoliation of encrustation.

No detailed description of the effects is given in the report as these FCA effects are well-known.

9.2 SKIN EFFECTS AFTER EPIDERMAL INDUCTION - PERFORMED ON TEST DAY 8

CONTROL GROUP

No erythematous or oedematous reaction was observed in the animals treated with PEG 300 only.

TEST GROUP

Discrete/patchy erythema was observed in all animals at the 24- and 48-hour reading after treatment with the test item at 40 % in PEG 300.

The animals of **both groups** were pretreated with 10 % SLS in *paraffinum perliquidum*.

See TABLE 1 and TABLE 2, pp. 30 - 31

9.3 SKIN EFFECTS AFTER THE CHALLENGE - PERFORMED ON TEST DAY 22

CONTROL GROUP

No skin reactions were observed in the animals when treated with either PEG 300 only or when treated with the test item at 40 % in PEG 300.

See TABLE 3 and TABLE 4, pp. 32 - 33

TEST GROUP

Discrete/patchy to moderate/confluent erythema were observed at the 24- and 48-hour reading in seven out of 9 surviving animals after treatment with the test item at 40 % in PEG 300. No skin reactions were observed in the animals treated with PEG 300 only.

See TABLE 5 and TABLE 6, pp. 34 - 35

9.4 VIABILITY / MORTALITY / MACROSCOPIC FINDINGS

One animal (no. 94) of the test group was found dead on test day 14 (i.e. 2 days after the 48-hour reading of the epidermal induction). At necropsy, no macroscopic findings were noted. The cause of death could not be established. The death was considered treatment-unrelated.

See p. 37

9.5 CLINICAL SIGNS, SYSTEMIC

No signs of systemic toxicity were observed in the animals.

9.6 BODY WEIGHTS

The body weight of the animals was within the range commonly recorded for animals of this strain and age.

See pp. 39 - 42

APPENDIX A

PRETEST

MAIN STUDY

- INDUCTION** **- Epidermal Reactions**
- CHALLENGE** **- Epidermal Reactions**

PRETEST

The following reactions were observed in the pretest:

INTRADERMAL INJECTION /
performed before and during the acclimatization period of the control and test group

Vehicle: PEG 300

Animal No.	Sex	Concentration (%)	REACTION READING AFTER 24 HOURS
83	female	20	technically not applicable
		15	1
		10	1

The concentration selected for the main study was 15 %

PRETEST (CONTINUED)

EPIDERMAL PRETEST /
 performed before and during the acclimatization period of the control and test group

Vehicle: PEG 300

Animal No.	Sex	Concentration (%)	REACTION READINGS AFTER REMOVAL OF BANDAGE	
			24 hours	48 hours
84	female	D = 40	0	0
		E = 25	0	0
		F = 15	0	0
		G = 10	0	0
85	female	G = 10	0	0
		D = 40	0	0
		E = 25	0	0
		F = 15	0	0

Three hours prior to the 24-hour reading the test sites were depilated.

According to Magnusson - Kligman and to the findings observed, the test item at 40 % was considered to be the maximally tolerated concentration to stimulate a state of immune hypersensitivity and the highest tested non-irritating concentration to be used for the challenge.

Technically, 40 % test item concentration was the highest which could be applied to the skin to ensure optimal skin contact during the treatment period and to avoid mechanical irritation caused by the test item.

MAIN STUDY - INDUCTION

TABLE 1 : CONTROL GROUP

SKIN RESPONSE AFTER THE EPIDERMAL APPLICATION OF THE VEHICLE
(PEG 300) DURING INDUCTION PERIOD (SCAPULAR AREA)

Animal No.	Sex	REACTION READINGS AFTER REMOVAL OF BANDAGE	
		24 hours	48 hours
86	female	0	0
87	female	0	0
88	female	0	0
89	female	0	0
90	female	0	0

The animals of the control group were pretreated with a 10 % Sodium-Lauryl-Sulfate solution 23 hours prior to the epidermal induction application.

MAIN STUDY - INDUCTION (CONTINUED)

TABLE 2 : TEST GROUP

SKIN RESPONSE AFTER THE EPIDERMAL APPLICATION OF
TK 10050 (CHIMASSORB 81) (40 % IN PEG 300)
DURING INDUCTION PERIOD (SCAPULAR AREA)

Animal No.	Sex	REACTION READINGS AFTER REMOVAL OF BANDAGE	
		24 hours	48 hours
91	female	1	1
92	female	1	1
93	female	1	1
94	female	1	1
95	female	1	1
96	female	1	1
97	female	1	1
98	female	1	1
99	female	1	1
100	female	1	1

The animals of the test group were pretreated with a 10 % Sodium-Lauryl-Sulfate solution 23 hours prior to the epidermal induction application.

MAIN STUDY - CHALLENGE

TABLE 3 : CONTROL GROUP

SKIN RESPONSE AFTER THE CHALLENGE APPLICATION OF PEG 300
(RIGHT FLANK)

Animal No.	Sex	REACTION READINGS AFTER REMOVAL OF BANDAGE	
		24 hours	48 hours
86	female	0	0
87	female	0	0
88	female	0	0
89	female	0	0
90	female	0	0

MAIN STUDY - CHALLENGE (CONTINUED)

TABLE 4 : CONTROL GROUP

SKIN RESPONSE AFTER THE CHALLENGE APPLICATION OF
TK 10050 (CHIMASSORB 81), 40 % IN PEG 300 (LEFT FLANK)

Animal No.	Sex	REACTION READINGS AFTER REMOVAL OF BANDAGE	
		24 hours	48 hours
86	female	0	0
87	female	0	0
88	female	0	0
89	female	0	0
90	female	0	0

Three hours prior to the 24-hour reading of the challenge the test sites were depilated.

MAIN STUDY - CHALLENGE (CONTINUED)

TABLE 5 : TEST GROUP

SKIN RESPONSE AFTER THE CHALLENGE APPLICATION OF PEG 300
(RIGHT FLANK)

Animal No.	Sex	REACTION READINGS AFTER REMOVAL OF BANDAGE	
		24 hours	48 hours
91	female	0	0
92	female	0	0
93	female	0	0
94	female	EXITUS	
95	female	0	0
96	female	0	0
97	female	0	0
98	female	0	0
99	female	0	0
100	female	0	0

MAIN STUDY - CHALLENGE (CONTINUED)

TABLE 6 : TEST GROUP

SKIN RESPONSE AFTER THE CHALLENGE APPLICATION OF
TK 10050 (CHIMASSORB 81), 40 % IN PEG 300 (LEFT FLANK)

Animal No.	Sex	REACTION READINGS AFTER REMOVAL OF BANDAGE	
		24 hours	48 hours
91	female	1	1
92	female	1	1
93	female	0	0
94	female	EXITUS	
95	female	1	1
96	female	1	1
97	female	0	0
98	female	1	1
99	female	2	2
100	female	1	1

Three hours prior to the 24-hour reading of the challenge the test sites were depilated.

RCC STUDY NUMBER 798704
TK 10050 (CHIMASSORB 81)

Report

APPENDIX B

NECROPSY

- MACROSCOPIC FINDINGS

RCC STUDY NUMBER 798704
TK 10050 (CHIMASSORB 81)

MACROSCOPIC FINDINGS
FEMALES
GROUP 4 (TEST GROUP)

ANIMAL 94

(SPONTANEOUS DEATH, 20-FEB-01)

NO FINDINGS NOTED

RCC STUDY NUMBER 798704
TK 10050 (CHIMASSORB 81)

Report

APPENDIX C

BODY WEIGHTS

- SUMMARY

- INDIVIDUAL

RCC STUDY NUMBER 798704
 TK 10050 (CHIMASSORB 81)

**BODY WEIGHTS (GRAM) SUMMARY
 FEMALES**

PRETEST		GROUP 1 INTRADERMAL PRETEST	GROUP 2 EPIDERMAL PRETEST	GROUP 3 CONTROL GROUP
DAY 1	MEAN	399	379	---
WEEK 1	ST.DEV.	---	16.9	---
	MINIMUM	399	367	---
	MAXIMUM	399	391	---
	N	1	2	0
GROUP 4 TEST GROUP				
	MEAN	---		
	ST.DEV.	---		
	MINIMUM	---		
	MAXIMUM	---		
	N	0		

RCC STUDY NUMBER 798704
 TK 10050 (CHIMASSORB 81)

BODY WEIGHTS (GRAM) SUMMARY FEMALES

TREATMENT		GROUP 1 INTRADERMAL PRETEST	GROUP 2 EPIDERMAL PRETEST	GROUP 3 CONTROL GROUP
DAY 1	MEAN	498	432	416
WEEK 1	ST.DEV.	---	31.3	33.8
	MINIMUM	498	410	374
	MAXIMUM	498	454	453
	N	1	2	5

GROUP 4
 TEST GROUP

MEAN	399
ST.DEV.	27.7
MINIMUM	365
MAXIMUM	453
N	10

TREATMENT		GROUP 1 INTRADERMAL PRETEST	GROUP 2 EPIDERMAL PRETEST	GROUP 3 CONTROL GROUP
DAY 28	MEAN	---	---	506
WEEK 4	ST.DEV.	---	---	20.9
	MINIMUM	---	---	485
	MAXIMUM	---	---	541
	N	0	0	5

GROUP 4
 TEST GROUP

MEAN	503
ST.DEV.	44.8
MINIMUM	431
MAXIMUM	562
N	9

RCC STUDY NUMBER 798704
 TK 10050 (CHIMASSORB 81)

**BODY WEIGHTS (GRAM)
 FEMALES**

	PRETEST	ACCLIMATIZATION	TREATMENT	
DAYS	1	1	1	28
WEEKS	1	1	1	4
ANIMAL				
GROUP 1 (INTRADERMAL PRETEST)				
83	399	460	498	---
GROUP 2 (EPIDERMAL PRETEST)				
84	367	405	410	---
85	391	442	454	---
GROUP 3 (CONTROL GROUP)				
86	---	328	374	485
87	---	370	392	507
88	---	394	453	500
89	---	357	417	541
90	---	394	445	497
GROUP 4 (TEST GROUP)				
91	---	371	422	558
92	---	319	371	480
93	---	339	387	486
94	---	338	397	---
95	---	367	395	466
96	---	380	453	562
97	---	345	404	531
98	---	363	425	534
99	---	328	365	431
100	---	343	375	482

RCC STUDY NUMBER 798704
TK 10050 (CHIMASSORB 81)

Report

APPENDIX D

RESULTS OF POSITIVE CONTROL

RCC Study Number 905095

2-MERCAPTOBENZOTHAZOLE:

Contact Hypersensitivity in Albino Guinea
Pigs, Maximization-Test

POSITIVE CONTROL

performed from 03-OCT-2000 to 10-NOV-2000

1. SUMMARY

For validation of sensitivity of the Maximization-Test of B. Magnusson and A.M. Kligman (1969) as well as the sensitivity of the test system used, a known sensitizer 2-MERCAPTOBENZOTHIAZOLE was selected as a positive control. This was performed in accordance with the recommendation of the OECD for testing of chemicals number 406 "Skin Sensitization Test", adopted by the Council on July 17, 1992 (reported Paris, April 29, 1993).

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

The study was performed with 15 (10 test and 5 control) male albino guinea pigs (GOH1), delivered by RCC Ltd, Biotechnology & Animal Breeding Division (CH-4414 Füllinsdorf / Switzerland).

The intradermal induction of sensitization in the test group was performed in the nuchal region with a 5 % dilution of the test item in mineral oil and in an emulsion of Freund's Complete Adjuvant (FCA) / physiological saline. The epidermal induction of sensitization was conducted for 48 hours under occlusion with the test item at 15 % in mineral oil one week after the intradermal induction. The animals of the control group were intradermally induced with mineral oil and FCA/physiological saline and epidermally induced with mineral oil under occlusion. Two weeks after epidermal induction the control and test animals were challenged by epidermal application of the test item at 1 % in mineral oil and mineral oil alone under occlusive dressing.

Cutaneous reactions were evaluated at 24 and 48 hours after removal of the dressing.

Results

Skin Reactions after the Challenge Procedure

	after 24 hours	after 48 hours
	positive / total % positive of total	positive / total % positive of total
CONTROL GROUP		
2-MERCAPTOBENZOTHIAZOLE, 1 % in mineral oil (left flank)	$\frac{0}{5}$ 0	$\frac{0}{5}$ 0
Mineral oil only (right flank)	$\frac{0}{5}$ 0	$\frac{0}{5}$ 0
TEST GROUP		
2-MERCAPTOBENZOTHIAZOLE, 1 % in mineral oil (left flank)	$\frac{10}{10}$ 100	$\frac{10}{10}$ 100
Mineral oil only (right flank)	$\frac{0}{10}$ 0	$\frac{0}{10}$ 0

No toxic symptoms were evident in the guinea pigs of the control or test group.

No deaths occurred.

All test animals showed discrete/patchy to moderate/confluent erythema after the challenge treatment with 2-MERCAPTOBENZOTHIAZOLE at 1 % (w/w) in mineral oil. No skin effect was observed in the control group.

2. CONCLUSION

Based on the above mentioned findings in an adjuvant sensitization test (M&K-test) in guinea pigs and in accordance to Commission Directive 96/54/EEC, 2-MERCAPTOBENZOTHIAZOLE has to be classified and labelled as a skin sensitizer.

3. TEST ITEM

Identification	2-MERCAPTOBENZOTHAZOLE
Description	Solid
Date of test item receipt	12-March-1999
Batch number	08924-118
Purity	98 % (T)
Stability of test item	Stable under storage conditions; expiration date: 12-MAR-2004
Stability of test item dilution	Stable in mineral oil for at least 2 hours (determined at RCC Ltd, Environmental Chemistry & Pharamalytics Division, RCC project 903611, under non GLP conditions; is excluded from the statement of compliance). Unknown in a 1:1 (v/v) mixture of FCA/physiological saline; is excluded from the statement of compliance.
Storage conditions	In the original container, at room temperature (range of 20 ± 3 °C), away from direct sunlight.
Safety precautions	Routine hygienic procedures were used to ensure the health and safety of the personnel.

4. VEHICLE

Identification	Mineral oil
Description	colorless viscous liquid
Lot number	28504-108
Source	Sigma-Aldrich, D-89555 Steinheim, Germany
Stability of vehicle	Stable under storage conditions; expiration date: 31-DEC-2001
Storage conditions	In the original container, at room temperature (range of 20 ± 3 °C), away from direct sunlight.
Safety precautions	Routine hygienic procedures were used to ensure the health and safety of the personnel.

5. AUXILIARY COMPOUNDS

FCA

Identification	Freund's Adjuvant - Complete
Description	clear, amber liquid containing light colored particles
Batch No.	79H8938
Source	Sigma, 3050 Spruce Street, Saint Louis, Missouri 63103 USA
Purity	each ml contains 1 mg Mycobacterium Tuberculosis (H 37Ra, ATCC 25177), heat killed and dried, 0.85 ml min- eral oil and 0.15 ml mannide monooleate
Expiry date	04-AUG-2001
Storage conditions	In the original container, in the refrigerator (range of 4 ± 3 °C), away from direct sunlight.

Physiological saline

Identification	Natrium chloratum 0.9 %
Description	colorless liquid
Batch No.	720504/2
Source	G. Streuli & Co. AG, CH-8730 Uznach/Switzerland
Expiry date	July 2004
Storage conditions	In the original container, in the refrigerator (range of 4 ± 3 °C), away from direct sunlight.

6. RESULTS OF THE MAIN STUDY – CHALLENGE

TABLE 1: CONTROL GROUP

SKIN RESPONSE AFTER THE CHALLENGE APPLICATION OF MINERAL OIL
(RIGHT FLANK)

Animal No.	Sex	REACTION READINGS AFTER REMOVAL OF BANDAGE	
		24 hours	48 hours
822	male	0	0
823	male	0	0
824	male	0	0
825	male	0	0
826	male	0	0

TABLE 2: CONTROL GROUP

SKIN RESPONSE AFTER THE CHALLENGE APPLICATION OF
2-MERCAPTOBENZOTHAZOLE, 1 % IN MINERAL OIL (LEFT FLANK)

Animal No.	Sex	REACTION READINGS AFTER REMOVAL OF BANDAGE	
		24 hours	48 hours
822	male	0	0
823	male	0	0
824	male	0	0
825	male	0	0
826	male	0	0

Three hours prior to the 24-hour reading of the challenge the test sites were depilated.

TABLE 3: TEST GROUP

SKIN RESPONSE AFTER THE CHALLENGE APPLICATION OF MINERAL OIL
(RIGHT FLANK)

Animal No.	Sex	REACTION READINGS AFTER REMOVAL OF BANDAGE	
		24 hours	48 hours
827	male	0	0
828	male	0	0
829	male	0	0
830	male	0	0
831	male	0	0
832	male	0	0
833	male	0	0
834	male	0	0
835	male	0	0
836	male	0	0

TABLE 4: TEST GROUP

SKIN RESPONSE AFTER THE CHALLENGE APPLICATION OF
2-MERCAPTOBENZOTHAZOLE, 1 % IN MINERAL OIL (LEFT FLANK)

Animal No.	Sex	REACTION READINGS AFTER REMOVAL OF BANDAGE	
		24 hours	48 hours
827	male	2	2
828	male	1	1
829	male	1	1
830	male	1	1
831	male	2	2
832	male	2	1
833	male	2	2
834	male	1	1
835	male	1	1
836	male	2	2

Three hours prior to the 24-hour reading of the challenge the test sites were depilated.

RCC STUDY NUMBER 798704
TK 10050 (CHIMASSORB 81)

Report

APPENDIX E

SUMMARY TABLE OF STUDY INFORMATION AND RESULTS

Test item identification: Name: TK 10050 (CHIMASSORB 81)			SUMMARY TABLE	
Batch No.: 8101880P			RCC Study No.: 798704	
SKIN TOLERANCE STUDIES / IMMUNOSTIMULATION (sensitization potential by intradermal and epidermal administration)			Report Date: 09-APR-2001	
Maximization Test (M&K Test)			Number of exp. animals: 15	
Species/Strain: Ibm: GOHI; SPF-quality guinea pigs (synonym: Himalayan spotted)				
Procedure	Administration route/site		Day	Vehicle
First induction	Intradermal/scapular		1	Test: 1. FCA:phys. saline 1:1 2. PEG 300 3. FCA:phys. saline 1:1 Control: 1. FCA:phys. saline 1:1 2. PEG 300 3. FCA:phys. saline 1:1 / PEG 300 50/50
Second induction	Epidermal occl./scapular		8	} PEG 300
Challenge	Epidermal occl./left flank		22	
Study group	Control group		Test group	
	Application	No. of appl. and dose/animal	Concentration of test item in %	No. of appl. and dose/animal
Intradermal induction	1. FCA:phys. saline 1:1	2x100 µl/i.d.	0 %	2x100 µl/i.d.
	2. PEG 300	2x100 µl/i.d.	15 %	2x100 µl/i.d.
	3. FCA:phys. saline 1:1/PEG 300 50/50	2x100 µl/i.d.	15 %	2x100 µl/i.d.
Epidermal occl. patch induction	PEG 300	Saturated patch/8 cm ²	40 %	Saturated patch/8 cm ²
Challenge	A 40 %	} Saturated patch/9 cm ²	40 %	} Saturated patch/9 cm ²
	B PEG 300		PEG 300	
No. of animals & Sex	5 females		10 females	
Animals with skin reactions / out of total				
First Challenge (24-hour reading)	A	0 / 5	7 / 9*	
	B	0 / 5	0 / 9	
First Challenge (48-hour reading)	A	0 / 5	7 / 9	
	B	0 / 5	0 / 9	
Summary of salient findings: The test item tested under the described conditions is considered to be a skin sensitizer.				
Study in compliance with GLP: yes <input checked="" type="checkbox"/> no <input type="checkbox"/>			QA inspected/audited: yes <input checked="" type="checkbox"/> no <input type="checkbox"/>	

A = left flank

B = right flank

* One animal of the test group was found dead on test day 14.

RCC STUDY NUMBER 798704
TK 10050 (CHIMASSORB 81)

Report

APPENDIX F

CERTIFICATION

- ACCREDITATION / EUROPEAN STANDARD EN 45001

- GLP – CERTIFICATION



Eidgenössisches Amt für Messwesen
Office fédéral de métrologie
Ufficio federale di metrologia
Swiss Federal Office of Metrology



S Schweizerische Akkreditierungsstelle
A Service d'accréditation suisse
S Servizio d'accreditamento svizzero
S Swiss Accreditation Service

ACCREDITATION

Based on the Accreditation and Designation Ordinance
dated 17th June 1996
the Swiss Federal Office of Metrology grants the

RCC Ltd
Toxicology Division
Zelgliweg 1
4452 Itingen

accreditation as a

**Testing Laboratory for toxicological investigation of
pharmaceuticals and medical devices, agrochemicals, industrial
chemicals, food- and feed-additives**

in accordance with SN EN 45001.

The accredited scope of testing is defined in the Official
Directory of the Accredited Testing Laboratories in Switzerland.
The relevant requirements of the ISO 9002 standard
are also covered by this accreditation.

Accreditation number: STS 085

Date of the accreditation: 16th September 1994

Date of the last renewal of the accreditation: 20th September 1999

The accreditation is valid until: 19th September 2004

Wabern, 20th September 1999

Swiss Federal Office of Metrology
The Director

Dr Wolfgang Schwitz

The head of SAS

Hanspeter Ischi

The Swiss GLP Monitoring Authorities



Swiss Federal
Office of
Public Health



Swiss Agency for the
Environment, Forests
and Landscape



Intercantonal Office
for the Control of
Medicines

Statement of GLP Compliance

It is hereby confirmed that
during the period of
the following Test Facilities of

August 15 – 17, 2000

RCC Ltd
4452 Itingen
Switzerland

were inspected by the Federal Office of Public Health, the Swiss Agency for the Environment, Forests and Landscape and the Intercantonal Office for the Control of Medicines with respect to the compliance with the Swiss legislation on Good Laboratory Practice.

Test Facilities

- Toxicology Division
- Environmental Chemistry and
Pharmanalytics Division
- Microbiological Diagnostics by
Biotechnology & Animal Breeding Division

areas of expertise*

TOX, ACC

ACC, ECT, ENF, PCT, RES,
OTH (Animal metabolism)

OTH (Microbiology)

The inspection was performed in agreement with the OECD Guidelines for National GLP Inspections and Audits. It was found that the aforementioned test facilities were operating in compliance with the Swiss Ordinance relating to Good Laboratory Practice [RS 813.016.5] at the time they were inspected.

Federal Office of Public Health
The Director

Prof. Th. Zeltner

Bern, November 2000

* TOX = Toxicology ; ACC = Analytical and Clinical Chemistry ; ECT = Environmental toxicity on aquatic and terrestrial organisms ; ENF = Behaviour in water, soil and air. Bioaccumulation ; PCT = Physical-chemical testing ; RES = Residue studies ; OTH = Other, to be specified.