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INITIAL SUBMISSION: CGI 551 (TKA 40210) CONTACT HYPERSENSITIVITY IN ALBINO GUINEA PIGS, MAXIMIZATION-TEST, WITH COVER LETTER DATED 1/10/2001 (SANITIZED)		
Chemical Category		
CGI-0551 (CONFIDENTIAL)		

A 03

Ciba Specialty Chemicals
North America
Additives

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January 10, 2001

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Attention: Section 8(e)
Office of Pollution Prevention and Toxics
Environmental Protection Agency
401 M Street, SW
Washington, DC 20460-0001

8EHQ-01-14849
820100000615

Subject: TSCA 8(e) Notice - CGI-551 (TKA 40210)

SANITIZED
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2001 JAN 17 PM 8:30

Dear Section 8(e) Coordinator:

This letter contains Confidential Business Information. Confidential Information bracketed { }.

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 test conducted on albino Guinea pigs with CGI-551. CGI-551 chemically is {
}). A CASRN has not been assigned to this material.

We are enclosing a copy of the study entitled "**CGI 551 (TKA 40210) Contact Hypersensitivity in Albino Guinea Pigs, Maximization-Test (RCC Report No. 783134)**". Following the epidermal challenge treatment with the test substance, all treated animals (10/10) showed a moderate redness at the site of application. Based on the Magnusson/Kligman rating scale, the test substance is an extreme sensitizer. There were no deaths and no other toxic symptoms observed. The control animals did not show a response.

In addition, two workers in a Ciba chemistry laboratory in Schwarzwaldallee, Switzerland, experienced a reddening and swelling of facial skin after handling the compound. The first individual affected volunteered for a patch test that confirmed a sensitization-type response to the compound.

As a result of this information, Ciba has informed all employees and has suspended normal development research on the compound while an investigation is conducted with the aim to produce a formulation that can be handled without risk of sensitization. Ciba has notified customers that have received samples of this new compound of these findings and requested that they also suspend work with the material until further notice from Ciba.

540 White Plains Road
Tarrytown, New York 10591

Tel. 914-785-4311
Fax 914-785-4147

COMPANY SANITIZED

A 04

Based upon current EPA guidelines, it is felt these results warrant reporting under TSCA 8(e). A sanitized copy of this letter is also enclosed; the report does not contain Confidential Business Information (CBI) . A CBI Substantiation for this product was submitted to the Agency on February 7, 2000 as part of an earlier 8(e) submission (8EHQ-00-14651). Please call the undersigned if you have any questions concerning this submittal.

Respectfully,

Ciba Specialty Chemicals Corporation



Thomas Barber
Manager, Regulatory Compliance

RCC Study Number 783134

**CGI 551 (TKA 40210):
Contact Hypersensitivity in Albino Guinea
Pigs, Maximization-Test**

Report

Author: G. Arcelin

Contain NO CBI



RCC STUDY NUMBER 783134
CGI 551 (TKA 40210)

Report

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CGI 551 (TKA 40210)

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RCC STUDY NUMBER 780134
CGI 551 (TKA 40210)

Report

1. PREFACE

1.1 GENERAL

Title	CGI 551 (TKA 40210): 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. H.-J. Weideli
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	18-SEP-2000
Experimental Completion Date	01-NOV-2000
Delivery of the Animals	18-SEP-2000 (pretest) 25-SEP-2000 (main study)
Pretest Start	18-SEP-2000
Acclimatization (main study)	25-SEP-2000 to 01-OCT-2000
Observation (main study)	25-SEP-2000 to 01-NOV-2000
Treatment (main study)	02-OCT-2000 to 24-OCT-2000
Termination	01-NOV-2000
Reported	28-NOV-2000

RCC STUDY NUMBER 783134
CGI 551 (TKA 4021C)

Report

1.4 ARCHIVING

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

RCC STUDY NUMBER 783134
CGI 591 (TKA 40210)

Report

1.5 SIGNATURE PAGE

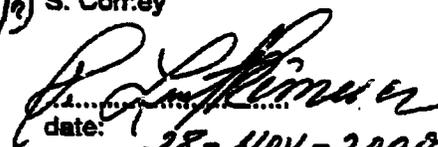
Study Director:

G. Arcelin


.....
date: 28-NOV-2000

Management:

(J2) S. Correy


.....
date: 28-NOV-2000

RCC STUDY NUMBER 783134
CGI 551 (TKA 40210)

Report

1.6 QUALITY ASSURANCE UNIT

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

STATEMENT

RCC STUDY NUMBER : 783134
TEST ITEM : CGI 551 (TKA 40210)
STUDY DIRECTOR : G. Arcelin
TITLE : CGI 551 (TKA 40210);
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.

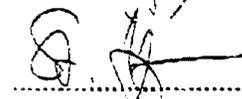
Study procedures were periodically inspected. The study plan and this report were audited by the Quality Assurance Unit. The dates are given below.

Dates and Types of QAU Inspections	Dates of Reports to the Study Director and to Management
14-SEP-2000 Study Plan Audit	14-SEP-2000
07-NOV-2000 Process Based Inspection	07-NOV-2000
23-NOV-2000 Draft Report Audit	23-NOV-2000

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

Quality Assurance:

P. Piller



date: 28-NOV-2000

RCC STUDY NUMBER 783134
CGI 551 (TKA 40210)

Report

GOOD LABORATORY PRACTICE

1.7 STATEMENT OF COMPLIANCE / GLP GUIDELINES

RCC STUDY NUMBER : 783134
TEST ITEM : CGI 551 (TKA 40210)
STUDY DIRECTOR : G. Arcelin
TITLE : CGI 551 (TKA 40210):
Contact Hypersensitivity in Albino Guinea Pigs
Maximization-Test

The stability of the test item in polyethylene glycol and 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: 28 - NOV - 2000

RCC STUDY NUMBER 783134
CGI 551 (TKA 40210)

Report

**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:

RCC STUDY NUMBER 783134
CGI 551 (TKA 40210)

Report

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 AMENDMENTS

Study Plan Amendment No. 1:

The concentrations and animals used for the intradermal-, epidermal induction and challenge procedure as well as the date of the applications were defined.

Study Plan Amendment No. 2:

The concentration for the intradermal induction procedure was re-defined.

RCC STUDY NUMBER 783134
CGI 551 (TKA 40210)

Report

2. SUMMARY

In order to assess the cutaneous allergenic potential of CGI 551 (TKA 40210), the Maximization-Test was performed in 15 (10 test and 5 control) male 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 3 % 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 50 % in PEG 300 one week after the intradermal induction and following pretreatment of the test areas with 10 % Sodium-Lauryl-Sulfate (SLS) approximately 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 50 % 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		
CGI 551 (TKA 40210), 50 % 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		
CGI 551 (TKA 40210), 50 % in PEG 300 (left flank)	$\frac{8}{10}$ 80	$\frac{10}{10}$ 100
PEG 300 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.

**RCC STUDY NUMBER 783134
CGI 551 (TKA 40210)**

Report

Eight (at the 24-hour reading) and 10 (at the 48-hour reading) out of 10 test animals showed discrete/patchy to moderate/confluent erythema after the challenge treatment with CGI 551 (TKA 40210) at 50 % (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, CGI 551 (TKA 40210) has to be classified and labelled as an extreme skin sensitizer.

RCC STUDY NUMBER 783134
CGI 551 (TKA 40210)

Report

3. PURPOSE -

The purpose of this skin sensitization study was to assess the allergenic potential of CGI 551 (TKA 40210) 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 alpha-hexylcinnamaldehyde 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 904522, performed from 22-MAR-2000 to 03-MAY-2000) are included in this report under the APPENDIX C.

4. MATERIALS AND METHODS

4.1 TEST SYSTEM

Test system	Ibm: GOH1; 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 males / 3 males
Age at delivery	4 - 6 weeks
Age at pretest start/beginning of acclimatization period	4 - 6 weeks
Body weight at pretest start	Pretest groups: 373 - 390 g
Body weight at beginning of acclimatization period	Control and test group 329 - 390 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.

RCC STUDY NUMBER 783134
CGI 551 (TKA 40210)

Report

4.2 ALLOCATION

The animals were distributed as follows:

	NUMBER OF ANIMALS PER GROUP	ANIMAL NUMBERS PER GROUP
1 Intradermal Pretest	1	851
2 Epidermal Pretest	2	852 - 853
3 Control Group	5	854 - 858
4 Test Group	10	859 - 868

4.3 HUSBANDRY

Rooming.

110 / RCC Ltd, Füllinsdorf

Conditions

Standard Laboratory Conditions

The animal room was air-conditioned with 10-15 air changes per hour and the air was continuously monitored with a range for room temperature of 19-21.5 °C and for relative humidity between 48-75 % (values above 70 % during cleaning process possible). 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. 45/00 and 73/00, guinea pig breeding / maintenance diet, containing Vitamin C ("Nafag", Nähr- und Futtermittel AG, CH-9202 Gossau), *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

CGI 551 (TKA 40210)

Description

off-white solid

Batch number

4339-39-V46-Op1

Purity / Formulation

analytical certificate in progress / Confidential information, available in Sponsor's file.

RCC STUDY NUMBER 783134
CGI 551 (TKA 40210)

Report

Stability of test item - Stable under storage conditions;
expiration date: 17-JUL-2002

Stability of test item dilution Unknown in polyethylene glycol and 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 (light-
sensitive test item).

4.5 VEHICLE

The following information was provided by RCC Ltd:

Identification Polyethylene glycol 300 (PEG 300)

Description colorless viscous liquid

Batch 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. 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
mineral 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.

RCC STUDY NUMBER 783134
CGI 55II (TKA 40210)

Report

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 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 in a suitable vehicle* was ensured during preparation 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.

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).

* PEQ 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 as vehicle for the pretest and the intradermal induction in the main study.

RCC STUDY NUMBER 783134
CGI 551 (TKA 40210)

Report

4.10 SELECTION OF CONCENTRATION OF TEST ITEM FOR MAIN STUDY

Intradermal Induction:

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

Intradermal and Epidermal Induction:

The concentration of test item used for the epidermal induction exposure was well-tolerated and should have been the highest to cause mild-to-moderate skin irritation. In this study, no skin reactions were observed from the lowest to the highest tested concentration during the pretest.

RCC STUDY NUMBER 783134
CGI 551 (TKA 40210)

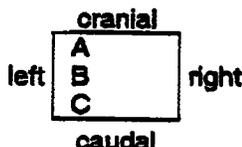
Report

5. STUDY CONDUCT - TREATMENT PROCEDURE

5.1 PRETEST PERFORMED WITH CGI 551 (TKA 40210) 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. 851). One week later intradermal injections (0.1 ml/site) were made into the clipped flank of the same guinea pig at concentrations of A = 10 %, B = 5 % and C = 3 % of the test item in PEG 300. The three concentrations were determined during formulation trials performed prior to the pretest. The concentration of 10 % 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 3 % was selected for intradermal induction in the main study.

The skin reactions are listed on page 27 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 = 50 % (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 volume of test item preparation applied was approximately 0.2 ml. 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.

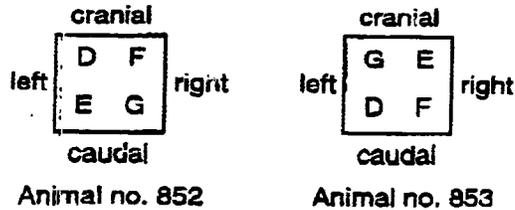
RCC STUDY NUMBER 783134
CGI 551 (TKA 40210)

Report

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 28 in APPENDIX A. Based on the results obtained the concentration selected for induction and challenge in the main study was 50 %.

5.2 MAIN STUDY

5.2.1 INDUCTION

5.2.1.1 INTRADERMAL INJECTIONS / PERFORMED ON TEST DAY 1

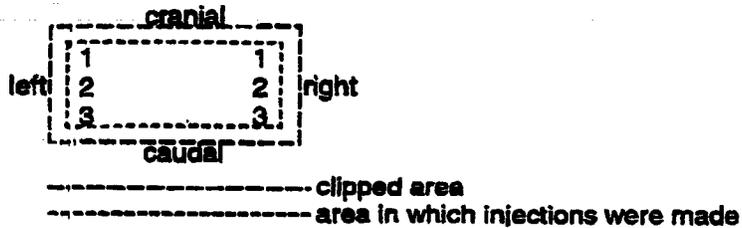
An area of dorsal skin from the scapular region (approximately 6 x 8 cm) was clipped free of hair. Three pairs of intradermal injections (0.1 ml/site) were made at the border of a 4 x 6 cm area in the clipped region as follows:

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

RCC STUDY NUMBER 783134
CGI 551 (TKA 40210)

Report

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 approximately 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 Kilgman 1970).

On test day 8, a 2 x 4 cm patch of filter paper was saturated with the test item (50 % in PEG 300) and placed over the injection sites of the test animals. The volume of test item preparation applied was approximately 0.3 ml. The patch was covered with aluminum foil and firmly secured by an elastic plaster wrapped around the trunk of the animal and secured with irripervious 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, also 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 Kilgman (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 50 % (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 volume of test item preparation and vehicle applied was approximately 0.2 ml. 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.

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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

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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 (local / 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

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 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

No statistical analysis was performed. Only mean values with standard deviations were calculated in the body weight tables.

8. DATA COMPILATION

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

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

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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

Discrete/patchy erythema was observed in all animals at the 24- and 48-hour reading after treatment 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 50 % in PEG 300.

The reactions observed in **both groups** occurred following pretreatment with 10 % SLS in *paraffinum perliquidum*.

See TABLE 1 and TABLE 2, pp. 29 - 30

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 50 % in PEG 300.

See TABLE 3 and TABLE 4, pp. 31 - 32

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TEST GROUP

Discrete/patchy to moderate/confluent erythema were observed in eight (at the 24-hour reading) and in 10 (at the 48-hour reading) out of 10 animals after treatment with the test item at 50 % in PEG 300. No skin reactions were observed in the animals treated with PEG 300 only.

See TABLE 5 and TABLE 6, pp. 33 - 34

9.4 VIABILITY / MORTALITY / MACROSCOPIC FINDINGS

There were no deaths during the course of the study, hence no necropsies were performed.

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. 36 - 39