

BFGoodrich

BFGoodrich Performance Materials
9911 Brecksville Road
Cleveland, Ohio 44141-3247
Tel: (216) 447-5636
Fax: (216) 447-5760

RECEIVED
OPPT CBIC

Kenneth J. Willings
Vice President
Health, Safety & Environmental

1999 MAY 10 AM 11: 56

8EHQ - 0599 - 14437

May 5, 1999

Document Processing Center (TS-790)
(Attn: TSCA 8(e) Coordinator)
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

MR 22321
PDCN
PDCN - 84-990000 156

Re: Supplement to the Notice of Substantial Risk Under TSCA Section 8(e)
Regarding Evidence of Skin Sensitization in a Guinea Pig MK
Maximization Study with Hycar® ATBN

Dear Sir/Madam:

The B.F. Goodrich Company (BFG) is submitting the final report on the guinea pig sensitization studies with Hycar® ATBN (see enclosure) as a supplement to our submission under Section 8(e) of the Toxic Substances Control Act (TSCA) dated April 28, 1999. This submission does not contain confidential business information.

If you have any questions regarding this submission, please contact Dr. Robert K. Hinderer at (216) 447-5181.

Sincerely,

Kenneth J. Willings

Kenneth J. Willings
Vice President
Health, Safety, & Environmental



8EHQ-99-14437

KJW/lr
Enclosure

Contains No CB

RECEIVED
OPPT CBIC
99 MAY 20 AM 12: 11

VIA CERTIFIED MAIL



89990000202

RECEIVED
OPPT CBIC
1999 MAY 10 AM 11: 56

CONFIDENTIAL

HYCAR ATBN:
MAGNUSSON & KLIGMAN MAXIMISATION
STUDY IN THE GUINEA PIG
SPL PROJECT NUMBER: 826/088

AUTHOR: A Sanders

STUDY SPONSOR:

BF Goodrich Company
Specialty Polymers & Chemicals Division
9911 Brecksville Road
Cleveland
OHIO 44141-3247
UNITED STATES OF AMERICA

ISSUED BY:

Safeparm Laboratories Limited
P.O. Box No. 45
DERBY
DE1 2BT
UK

GEASR00964

Telephone: DERBY (01332) 792896

Facsimile: (01332) 799018

Contains No CBI

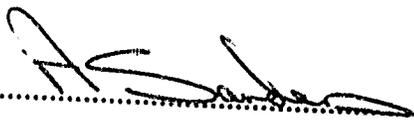
RECEIVED
MAY 05 1999
R. HINDERER

GLP COMPLIANCE STATEMENT

I, the undersigned, hereby declare that the objectives laid down in the protocol were achieved and as nothing occurred to adversely affect the quality or integrity of the study, I consider the data generated to be valid. This report fully and accurately reflects the procedures used and data generated.

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1997 (SI 1997/654)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 87/18/EEC and 88/320/EEC.

These international standards are acceptable to the United States Environmental Protection Agency and Food and Drug Administration, and fulfil the requirements of 40 CFR Part 160, 40 CFR Part 792 and 21 CFR Part 58 (as amended).



DATE: 28 APR 1999

A Sanders
Study Director
for Safepfarm Laboratories

QUALITY ASSURANCE REPORT

The routine inspection of short term studies at Safepharm is carried out as a continuous process designed to encompass all major phases of each study type once per month. Inspection findings are reported to Management/Study Directors on the day of inspection in each case. Dates of relevant monthly inspections are as follows:

04, 08, 16, 26 March 1999

This report has been audited by Safepharm Quality Assurance Unit. It is considered to be an accurate account of the data generated and of the procedures followed.

Date of Report Audit:

20 April 1999

..... *G. Wren* DATE: 28 APR 1999

For Safepharm Quality Assurance Unit

Authorised QA Signatures:

Head of Department: JR Pateman CBIol MIBiol DipRQA
Deputy Head of Department: JM Crowther MScT
Senior Audit Staff: JV Johnson BSc; G Wren ONC; AJ Cooper HNC; RJ Gilbert BSc

CONTENTS

	PAGE
SUMMARY	
1. INTRODUCTION	6
2. TEST MATERIAL AND EXPERIMENTAL PREPARATION	7
2.1 Description, Identification and Storage Conditions	7
2.2 Experimental Preparation	7
3. METHODS	8
3.1 Animals and Animal Husbandry	8
3.2 Procedure	8
3.2.1 Selection of Concentrations for Main Study (Sighting Tests)	9
3.2.2 Main Study	9
3.3 Interpretation of Results	10
4. ARCHIVES	13
5. RESULTS	13
5.1 Intradermal and Topical Sighting Tests	14
5.2 Main Study	14
5.3 Bodyweight	14
6. CONCLUSION	16
TABLES	
TABLE 1 Individual Skin Reactions in Test Animals at Challenge	17
TABLE 2 Individual Skin Reactions in Control Animals at Challenge	18

CONTENTS (continued)

	PAGE
APPENDICES	
APPENDIX I	Intradermal Sighting Test - Summary of Results 19
APPENDIX II	Topical Sighting Test for Induction Application (48-hour Exposure) - Individual Skin Reactions 20 21
APPENDIX III	Topical Sighting Test for Challenge Application (24-hour Exposure) - Individual Skin Reactions 22
APPENDIX IV	Intradermal Induction - Individual Skin Reactions in Test Animals 23
APPENDIX V	Intradermal Induction - Individual Skin Reactions in Control Animals 24
APPENDIX VI	Topical Induction - Individual Skin Reactions in Test Animals 25
APPENDIX VII	Topical Induction - Individual Skin Reactions in Control Animals 26
APPENDIX VIII	Individual Bodyweights and Bodyweight Gains of Test Animals 27
APPENDIX IX	Individual Bodyweights and Bodyweight Gains of Control Animals 28
APPENDIX X	Scales for Evaluation of Skin Reactions 29
APPENDIX XI	Summary of Positive Control Data 30
APPENDIX XII	Statement of GLP Compliance in Accordance with Directive 88/320/EEC 31

SUMMARY

STUDY SPONSOR : BF GOODRICH COMPANY

STUDY TITLE : MAGNUSSON & KLIGMAN
MAXIMISATION STUDY IN THE
GUINEA PIG

TEST MATERIAL : HYCAR ATBN

1. A study was performed to assess the contact sensitisation potential of the test material in the albino guinea pig. The study was performed in compliance with the OECD Guidelines for Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992) and Method B6 of Commission Directive 96/54/EC (which constitutes Annex V of Council Directive 67/548/EEC).

The results may be used as a basis for classification and labelling under Annex VI of Council Directive 67/548/EEC (as adapted to technical progress by Commission Directive 93/21/EEC).

2. Twenty test and ten control animals were used for the main study.

Based on the results of sighting tests, the concentrations of test material for the induction and challenge phases were selected as follows:

Intradermal Induction : 1% w/v in arachis oil BP

Topical Induction : undiluted as supplied

Topical Challenge : undiluted as supplied and 75% v/v in arachis oil BP

3. The test material produced a 75% (15/20) sensitisation rate and was classified as a strong sensitiser to guinea pig skin. The test material was also classified as a sensitiser according to EU labelling regulations. The symbol "Xi", the indication of danger 'irritant' and the risk phrase R 43 "MAY CAUSE SENSITISATION BY SKIN CONTACT" are required.

**HYCAR ATBN:
MAGNUSSON & KLIGMAN MAXIMISATION
STUDY IN THE GUINEA PIG**

1. INTRODUCTION

The study was performed to assess the contact sensitisation potential of the test material. The study was performed in compliance with the recommendations of the OECD Guidelines for the Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992) and Method B6 of Commission Directive 96/54/EC (which constitutes Annex V of Council Directive 67/548/EEC).

The results may be used as a basis for classification and labelling under Annex VI of Council Directive 67/548/EEC (as adapted to technical progress by Commission Directive 93/21/EEC).

The test system was chosen because the guinea pig has been shown to be a suitable species for this type of study and is recommended in the test method. The strain used in these laboratories has been shown to produce satisfactory sensitisation responses using known positive sensitisers (see Appendix XI). The results of the study are believed to be of value in predicting the likely contact sensitisation potential of the test material to man.

The study was performed between 8 February 1999 and 20 March 1999.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	HYCAR ATBN
Date received	:	25 January 1999
Description	:	extremely viscous turbid light amber liquid
Storage conditions	:	room temperature in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the sponsor.

2.2 Experimental Preparation

For the purpose of this study the test material was freshly prepared as follows:

Intradermal Induction	:	1% w/v in arachis oil BP
		1% w/v in a mixture of Freund's Complete Adjuvant plus distilled water (1:1)
Topical Induction	:	undiluted as supplied
Topical Challenge	:	undiluted as supplied and 75% v/v in arachis oil BP

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Thirty-six female albino Dunkin Hartley guinea pigs supplied by David Hall Limited, Burton-on-Trent, Staffordshire, UK were used. At the start of the main study the animals weighed 318 to 405g, and were approximately eight to twelve weeks old. After an acclimatisation period of at least five days, each animal was selected at random and given a number unique within the study which was written on a small area of clipped rump using a black indelible marker-pen.

The animals were housed singly or in pairs in solid-floor polypropylene cages furnished with woodflakes. Free access to mains tap water and food (Guinea Pig FD1 Diet, Special Diets Services Limited, Witham, Essex, UK) was allowed throughout the study.

The temperature and relative humidity were controlled to remain within target ranges of 17 to 23 °C and 30 to 70% respectively. The rate of air exchange was approximately fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light and twelve hours darkness.

3.2 Procedure

The method used for assessing the sensitising properties of the test material was based on the Guinea Pig Maximisation test of Magnusson B & Kligman A M, J. Invest. Dermatol. (1969) 52: 268 - 276.

3.2.1 Selection of Concentrations for Main Study (Sighting Tests)

The concentrations of test material to be used at each stage of the main study were determined by 'sighting tests' in which groups of guinea pigs were treated with various concentrations of test material. The procedures were as follows:

3.2.1.1 Selection of Concentration for Intradermal Induction

Two concentrations of test material were investigated (1% and 5% w/v in arachis oil BP). A total of two guinea pigs were used, each guinea pig receiving four 0.1 ml injections of only one concentration of test material. The degree of erythema at the injection sites was assessed approximately 24, 48 and 72 hours and 7 days after injection according to the scale shown in Appendix X. The degree of oedema was not evaluated. Any evidence of systemic toxicity was also recorded. The highest concentration that caused only mild to moderate skin irritation, and which was well tolerated systemically, was selected for the intradermal induction stage of the main study.

3.2.1.2 Selection of Concentration for Topical Induction

Two guinea pigs (intradermally injected with Freund's Complete Adjuvant fifteen days earlier) were treated with the undiluted test material and three preparations of the test material (75%, 50% and 25% v/v in arachis oil BP). Applications were made to the clipped flanks under occlusive dressings for an exposure period of 48 hours. The degree of erythema and oedema was evaluated approximately 1, 24 and 48 hours after dressing removal. The highest concentration producing only mild to moderate dermal irritation was selected for the topical induction stage of the main study.

3.2.1.3 Selection of Concentration for Topical Challenge

The undiluted test material and three preparations of the test material (75%, 50% and 25% v/v in arachis oil BP) were applied to the clipped flanks of two guinea pigs under occlusive dressings for an exposure period of 24 hours. These guinea pigs did not form part of the main study but had been treated identically to the control animals of the main study, up to Day 14. The degree of erythema and oedema was evaluated approximately 1, 24 and 48 hours after dressing removal. The highest non-irritant concentration of the test material and one lower concentration were selected for the topical challenge stage of the main study.

3.2.2 Main Study

A group of thirty guinea pigs was used for the main study, twenty test and ten control. The bodyweight of each animal was recorded at the start and end of the study.

Two main phases were involved in the main study; (a) an induction of a response and (b) a challenge of that response.

3.2.2.1 Induction

Induction of the Test Animals: Shortly before treatment on Day 0 the hair was removed from an area approximately 40 mm x 60 mm on the shoulder region of each animal with veterinary clippers. A row of three injections (0.1 ml each) was made on each side of the mid-line. The injections were:

- a) Freund's Complete Adjuvant plus distilled water in the ratio 1:1
- b) a 1% w/v formulation of the test material in arachis oil BP
- c) a 1% w/v formulation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus distilled water.

Approximately 24 and 48 hours after intradermal injection the degree of erythema at the test material injection sites (ie. injection site b) was evaluated according to the scale shown in Appendix X.

One week later (Day 7), the same area on the shoulder region used previously for intradermal injections was clipped again and treated with a topical application of the undiluted test material. A filter paper patch (WHATMAN No.4: approximate size 40 mm x 20 mm), saturated with the undiluted test material was applied to the prepared skin and held in place with a strip of surgical adhesive tape (BLENDERM: approximate size 50 mm x 30 mm) covered with an overlapping length of aluminium foil. The patch and foil were further secured with a strip of elastic adhesive bandage (ELASTOPLAST: approximate size 250 mm x 35 mm) wound in a double layer around the torso of each animal. This occlusive dressing was kept in place for 48 hours.

The degree of erythema and oedema was quantified one and twenty-four hours following removal of the patches using the scale shown in Appendix X.

Any other reactions were also recorded.

Induction of the Control Animals: Intradermal injections were administered using an identical procedure to that used for the test animals, except that the injections were:

- a) Freund's Complete Adjuvant plus distilled water in the ratio 1:1
- b) arachis oil BP
- c) a 50% w/v formulation of arachis oil BP in a 1:1 mixture of Freund's Complete Adjuvant/distilled water

Approximately 24 and 48 hours after intradermal injection the degree of erythema at the vehicle injection sites (ie injection site b) was evaluated according to the scale shown in Appendix X.

The topical applications followed the same procedure as for the test animals except that nothing was applied to the filter paper. Skin reactions were quantified as for the test animals.

3.2.2.2 Challenge

Shortly before treatment on Day 21, an area of approximately 50 mm x 70 mm on both flanks of each animal, was clipped free of hair with veterinary clippers.

A square filter paper patch (WHATMAN No.4: approximate size 20 mm x 20 mm), saturated with the undiluted test material was applied to the shorn right flank of each animal and was held in place with a strip of surgical adhesive tape (BLENDERM: approximate size 40 mm x 50 mm). To ensure that the maximum non-irritant concentration was used at challenge, the test material at a concentration of 75% v/v in arachis oil BP was similarly applied to a skin site on the left shorn flank. The patches were occluded with an overlapping length of aluminium foil and secured with a strip of elastic adhesive bandage (ELASTOPLAST: approximate size 250 mm x 75 mm) wound in a double layer around the torso of each animal.

After 24 hours, the dressing was carefully cut using blunt-tipped scissors, removed and discarded. The challenge sites were swabbed with cotton wool soaked in diethyl ether to remove residual material. The position of the treatment sites was identified by using a black indelible marker-pen.

Prior to the 24-hour observation the flanks were clipped using veterinary clippers to remove regrown hair.

3.2.2.3 Evaluation of Skin Reactions

Approximately 24 and 48 hours after challenge dressing removal, the degree of erythema and oedema was quantified using the scale shown in Appendix X.

Any other reactions were also recorded.

3.3 Interpretation of Results

The percentage of test animals that showed a more severe reaction at the test material challenge site than the most severe reaction seen in the control animals, was compared with the following scale:

Percentage of animals sensitised	Classification of sensitisation potential
0	non-sensitiser
> 0 - 8	weak sensitiser
> 8 - 28	mild sensitiser
> 28 - 64	moderate sensitiser
> 64 - 80	strong sensitiser
> 80 - 100	extreme sensitiser

The data obtained may be used to classify the test material according to Commission Directive 93/21/EEC adapting Council Directive 67/548/EEC on the classification, packaging and labelling of dangerous substances.

The test material will be classified as sensitising and assigned the symbol "Xi", the indication of danger 'irritant' and the risk phrase R 43 "MAY CAUSE SENSITISATION BY SKIN CONTACT" if 30% or more of the test animals show a sensitisation response.

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for a period of five years. After this period, the Sponsor's instructions will be sought.

5. RESULTS

5.1 Intradermal and Topical Sighting Tests

A summary of the results of the intradermal sighting test and the individual skin reactions observed in the topical sighting tests are given in Appendices I to III.

Based on these results, the following concentrations were selected for the main study:

Intradermal Induction	:	1% w/v in arachis oil BP
Topical Induction	:	undiluted as supplied
Topical Challenge	:	undiluted as supplied and 75% v/v in arachis oil BP

5.2 Main Study

5.2.1 Skin Reactions Observed After Intradermal Induction

Individual reactions observed at the test material intradermal injection sites of the test group animals and vehicle intradermal injection sites of the control group animals are given in Appendices IV and V respectively.

Moderate and confluent to intense erythema was noted at the intradermal induction sites of all test group animals at the 24 and 48-hour observations.

Discrete or patchy erythema was noted at the intradermal induction sites of all control group animals at the 24 and 48-hour observations.

5.2.2 Skin Reactions Observed After Topical Induction

Individual skin reactions observed at the topical induction sites of the test and control group animals are given in Appendices VI and VII.

Discrete or patchy to moderate and confluent erythema with very slight or slight oedema was noted at the topical induction sites of all test group animals at the 1-hour observation. Discrete or patchy to moderate and confluent erythema with or without very slight oedema was noted at the topical induction sites of eighteen test group animals at the 24-hour observation. Bleeding from the intradermal injection sites was noted in eleven test group animals at the 1-hour observation. Adhered test material

was noted at the topical induction sites of five test group animals at the 1-hour observation. Residual test material was noted at the topical induction sites of seventeen test group animals at the 1-hour observation and all test group animals at the 24-hour observation.

No signs of erythema or oedema were noted at the topical induction sites of control group animals at the 1 or 24-hour observations.

Bleeding from the intradermal injection sites was noted in two control group animals at the 1-hour observation.

5.2.3 Skin Reactions Observed After Topical Challenge

Individual skin reactions at the challenge sites of the test and control group animals are given in Tables 1 and 2.

Undiluted as Supplied

Positive skin responses (discrete or patchy to moderate and confluent erythema - grade 1 to 2 with or without very slight to slight oedema) were noted at the challenge sites of fifteen test group animals at the 24, 48 and 72-hour observations. Desquamation was noted at the challenge sites of five test group animals at the 48 and/or 72-hour observations. Fur loss was noted at the challenge site of one test group animal at the 24-hour observation. Physical damage caused by the removal of adhered test material was noted in two test group animals.

No signs of erythema or oedema were noted at the challenge sites of control group animals at the 24, 48 or 72-hour observations.

Physical damage caused by the removal of adhered test material was noted in one control group animal at the 24-hour observation.

75% v/v in Arachis Oil BP

Positive skin responses (discrete or patchy to moderate and confluent erythema - grade 1 to 2 with or without very slight to slight oedema) were noted at the challenge sites of twelve test group animals at the 24-hour observation. The severity of the reaction persisted at the challenge sites of

eight test group animals and increased in three test group animals at the 48-hour observation. Positive skin responses (discrete or patchy to moderate and confluent erythema - grade 1 to 2 and an isolated incident of very slight oedema) developed at the challenge sites of three test group animals at the 48-hour observation. Positive skin responses (discrete or patchy to moderate and confluent erythema - grade 1 to 2 with or without very slight to slight oedema) were noted at the challenge sites of fifteen test group animals at the 72-hour observation. Desquamation was noted at the challenge sites of four test group animals at the 48 and/or 72-hour observations. Small superficial scattered scabs were noted at the challenge site of one test group animal at the 72-hour observation. Physical damage caused by the removal of adhered test material was noted in one test group animal at the 48 and 72-hour observations.

No signs of erythema or oedema were noted at the challenge sites of control group animals at the 24, 48 or 72-hour observations.

Physical damage caused by the removal of adhered test material was noted in one control group animal at the 48-hour observation.

5.3 Bodyweight

Individual bodyweights and bodyweight gains of the test and control group animals are given in Appendices VIII and IX.

Bodyweight gains of guinea pigs in the test group, between Day 0 and Day 24, were comparable to those observed in the control group animals over the same period.

6. CONCLUSION

The test material, HYCAR ATBN, produced a 75% (15/20) sensitisation rate and was classified as a STRONG SENSITISER to guinea pig skin. The test material was also classified as a sensitiser according to EU labelling regulations. The symbol "Xi", the indication of danger 'irritant' and the risk phrase R 43 "MAY CAUSE SENSITISATION BY SKIN CONTACT" are required.

HYCAR ATBN : MAGNUSSON & KLIGMAN MAXIMISATION STUDY IN THE GUINEA PIG
 TABLE 1
 INDIVIDUAL SKIN REACTIONS IN TEST ANIMALS AT CHALLENGE

CHALLENGE CONCENTRATIONS: UNDILUTED AS SUPPLIED AND 75% v/v

VEHICLE: ARACHIS OIL 8P

Animal Number	Skin Reactions (Hours After Removal of Dressing)														
	24 Hours				48 Hours				72 Hours						
	100%	75%	100%	75%	100%	75%	100%	75%	100%	75%	100%	75%			
Er	Oe	Other	Er	Oe	Other	Er	Oe	Other	Er	Oe	Other	Er	Oe	Other	
1	2	1	-	1	0	-	2	1	-	2	1	-	2	1	-
2	1	0	-	1	0	-	1	0	-	1	0	-	1	0	-
3	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-
4	1	0	-	1	0	-	1	0	-	1	0	-	1	0	-
5	1	0	-	2	1	-	2	2	-	2	1	-	2	1	-
6	1	0	-	0	0	-	1	0	-	0	0	-	2	1	-
7	1	0	-	1	0	-	1	0	-	1	0	-	1	0	-
8	2	0	-	1	0	-	1	0	-	2	1	-	0	0	-
9	2	1	-	0	0	-	2	1	-	PdD	1	0	1	1	-
10	1	0	-	0	0	-	2	1	-	-	0	0	2	1	-
11	2	0	-	1	0	-	1	0	-	-	2	1	2	1	-
12	0	0	-	2	1	-	2	0	-	-	2	1	1	0	-
13	2	1	-	0	0	-	0	0	-	-	2	1	2	1	-
14	1	0	-	1	0	-	2	1	-	-	2	1	2	1	-
15	2	0	-	2	1	-	1	1	-	-	1	1	2	1	-
16	0	0	-	0	0	-	0	0	-	-	1	1	1	1	-
17	2	1	-	2	2	-	2	2	-	-	0	0	0	0	-
18	0	0	-	0	0	-	0	0	-	D	2	2	2	2	-
19	0	0	-	0	0	-	0	0	-	-	0	0	0	0	-
20	1	0	-	0	0	-	0	0	-	Pd	0	0	0	0	-

Er = erythema
 Oe = oedema
 - = no other reactions noted
 D = desquamation
 Fl = fur loss
 Pd = physical damage to skin caused by attempted removal of adhered test material
 Ss = small superficial scattered scabs

HYCAR ATBN : MAGNUSSON & KLIGMAN MAXIMISATION STUDY IN THE GUINEA PIG
 TABLE 2
 INDIVIDUAL SKIN REACTIONS IN CONTROL ANIMALS AT CHALLENGE

CHALLENGE CONCENTRATIONS: UNDILUTED AS SUPPLIED AND 75% v/v

VEHICLE: ARACHIS OIL BP

Animal Number	Skin Reactions (Hours After Removal of Dressing)											
	24 Hours			48 Hours			72 Hours			75%		
	Er	Oe	Other	Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
21	0	0	-	0	0	-	0	0	-	0	0	-
22	0	0	Pd	0	0	-	0	0	-	0	0	-
23	0	0	-	0	0	-	0	0	-	0	0	-
24	0	0	-	0	0	-	0	0	-	0	0	-
25	0	0	-	0	0	-	0	0	-	0	0	-
26	0	0	-	0	0	-	0	0	-	0	0	-
27	0	0	-	0	0	-	0	0	-	0	0	-
28	0	0	-	0	0	-	0	0	-	0	0	-
29	0	0	-	0	0	-	0	0	-	0	0	-
30	0	0	-	0	0	-	0	0	-	0	0	-

Er = erythema
 Oe = oedema

- = no other reactions noted
 Pd = physical damage to skin caused by attempted removal of adhered test material

A P P E N D I C E S

HYCAR ATBN : MAGNUSSON & KLIGMAN MAXIMISATION STUDY IN THE GUINEA PIG
 A P P E N D I X I
 INTRADERMAL SIGHTING TEST - SUMMARY OF RESULTS

VEHICLE: ARACHIS OIL BP

Animal identification	Time of Observation	Concentration of Test Material (% w/v)	Grade of Erythema at Injection Sites	Evidence of Systemic Toxicity
A	24 Hours		2-3	None
	48 Hours	1	3	None
	72 Hours		2-3	None
	7 Days		2	None
B	24 Hours		3	None
	48 Hours	5	N	None
	72 Hours		N	None
	7 Days		E	None

E = eschar
 N = pale green necrosis

The concentration of the test material selected for the intradermal induction stage of the main study was 1% w/v in arachis oil BP

HYCAR ATBN : MAGNUSSON & KLICMAN MAXIMISATION STUDY IN THE GUINEA PIG
 A P P E N D I X I I
 TOPICAL SIGHTING TEST FOR INDUCTION APPLICATION
 (48-HOUR EXPOSURE) - INDIVIDUAL SKIN REACTIONS

VEHICLE: ARACHIS OIL BP

Animal Identification	Concentration of Test Material (% v/v)	Skin Reactions (Hours After Removal of Patches)														
		1				24				48						
		Er	Oe	Other	Other	Er	Oe	Other	Other	Er	Oe	Other				
C	100	1	0	-	-	1	1	-	-	-	-	-	-	-	-	-
	75	2	0	Rt	-	1	1	-	-	-	-	-	-	-	-	-
	50	0	0	-	-	1	0	-	-	-	-	-	-	-	-	-
	25	0	0	-	-	0	0	-	-	-	-	-	-	-	-	-
D	100	2	0	Rt	-	2	1	-	-	-	-	-	-	-	-	-
	75	2	0	-	-	1	1	-	-	-	-	-	-	-	-	-
	50	2	0	-	-	1	1	-	-	-	-	-	-	-	-	-
	25	1	0	-	-	1	1	-	-	-	-	-	-	-	-	-

Er - erythema
 Oe - oedema

- - no other reactions noted
 Rt - residual test material

The undiluted test material was selected for the main study topical induction

HYCAR ATBN : MAGNUSON & KLIGMAN MAXIMISATION STUDY IN THE GUINEA PIG
 A P P E N D I X I I I
 TOPICAL SIGHTING TEST FOR CHALLENGE APPLICATION
 (24-HOUR EXPOSURE) - INDIVIDUAL SKIN REACTIONS

VEHICLE: ARACHIS OIL BP

Animal Identification	Concentration of Test Material (% v/v)	Skin Reactions (Hours After Removal of Patches)											
		24				48							
		Er	Oe	Other	Other	Er	Oe	Other	Other	Er	Oe	Other	
E	100	2	1	.	.	0	0	0	0	0	0	0	0
	75	2	0	.	.	0	0	0	0	0	0	0	0
	50	1	0	.	.	0	0	0	0	0	0	0	0
	25	1	0	.	.	0	0	0	0	0	0	0	0
F	100	2	1	.	.	0	0	0	0	0	0	0	0
	75	2	1	.	.	0	0	0	0	0	0	0	0
	50	2	0	.	.	0	0	0	0	0	0	0	0
	25	1	0	.	.	0	0	0	0	0	0	0	0

Er - erythema Oe - oedema . - no other reactions noted

The concentrations of the test material selected for the main study topical challenge were undiluted as supplied and 75% v/v in arachis oil BP

HYCAR ATBN : MAGNUSSON & KLIGMAN MAXIMISATION STUDY IN THE GUINEA PIG
 APPENDIX I V
 INTRADERMAL INDUCTION - INDIVIDUAL SKIN REACTIONS IN TEST ANIMALS
 INDUCTION CONCENTRATION: 1% w/v

VEHICLE: ARACHIS OIL BP

Animal Number	Grade of Erythema at Observation Time					
	24 Hours		48 Hours			
	Left Side	Right Side	Left Side	Right Side	Left Side	Right Side
1	2	2				
2	2	2	2			2
3	3	3	2			2
4	2	2	3			2
5	2	2	2			2
6	2	2	2			2
7	2	3	2			2
8	2	2	2			2
9	2	2	2			2
10	2	2	2			2
11	3	3	2			2
12	2	2	2			2
13	2	3	2			2
14	3	3	2			2
15	3	3	2			2
16	3	3	2			2
17	2	2	2			2
18	3	2	2			2
19	2	2	3			2
20	2	2	2			2

HYCAR ATBN : MAGNUSSON & KLIGMAN MAXIMISATION STUDY IN THE GUINEA PIG
APPENDIX V
INTRADERMAL INDUCTION - INDIVIDUAL SKIN REACTIONS IN CONTROL ANIMALS
VEHICLE: ARACHIS OIL BP

Animal Number	Grade of Erythema at Observation Time			
	24 Hours		45 Hours	
	Left Side	Right Side	Left Side	Right Side
21	1	1		1
22	1	1	1	1
23	1	1	1	1
24	1	1	1	1
25	1	1	1	1
26	1	1	1	1
27	1	1	1	0
28	1	1	1	1
29	1	1	1	1
30	1	1	0	1

HYCAR ATBN : MAGNUSSON & KLIGMAN MAXIMISATION STUDY IN THE GUINEA PIG
 A P P E N D I X V I
 TOPICAL INDUCTION - INDIVIDUAL SKIN REACTIONS IN TEST ANIMALS
 INDUCTION CONCENTRATION: UNDILUTED AS SUPPLIED

Animal Number	Skin Reactions (Hours After Removal of Dressing)					
	1 Hour			24 Hours		
	Er	Oe	Other	Er	Oe	Other
1	2					
2	2	2	RIBs	1	1	Rt
3	2	1	Rt	1	0	Rt
4	1	1	Rt	1	0	Rt
5	2	1	RIBs	0	0	Rt
6	2	1	TaRt	1	0	Rt
7	2	1	TaRt	1	0	Rt
8	2	1	RIBs	1	0	Rt
9	1	1	RIBs	1	0	Rt
10	2	1	Rt	1	0	Rt
11	2	2	RIBs	1	0	Rt
12	2	2	TaBs	1	0	Rt
13	1	1	Ta	2	1	Rt
14	1	1	Rt	1	0	Rt
15	2	1	TaBs	0	0	Rt
16	1	1	Rt	1	0	Rt
17	2	1	RIBs	1	0	Rt
18	1	1	RIBs	1	0	Rt
19	1	1	RIBs	1	0	Rt
20	2	1	RIBs	1	0	Rt
	2	1	Rt	1	0	Rt

Er = erythema
 Oe = oedema
 Bs = bleeding from intradermal injection sites

Rt = residual test material
 Ta = test material adhered to treatment site

HYCAR ATBN : MAGNUSSON & KLIGMAN MAXIMISATION STUDY IN THE GUINEA PIG
 A P P E N D I X V I I
 TOPICAL INDUCTION - INDIVIDUAL SKIN REACTIONS IN CONTROL ANIMALS
 BLANK PATCH APPLIED

Animal Number	Skin Reactions (Hours After Removal of Dressing)					
	1 Hour			24 Hours		
	Er	Oe	Other	Er	Oe	Other
21	0	0	-	0	0	-
22	0	0	-	0	0	-
23	0	0	-	0	0	-
24	0	0	Bs	0	0	-
25	0	0	-	0	0	-
26	0	0	-	0	0	-
27	0	0	-	0	0	-
28	0	0	-	0	0	-
29	0	0	-	0	0	-
30	0	0	Bs	0	0	-

Er = erythema
 Oe = oedema

- = no other reactions noted
 Bs = bleeding from intradermal injection sites

HYCAR ATBN : MAGNUSSON & KLIGMAN MAXIMISATION STUDY IN THE GUINEA PIG
A P P E N D I X V I I I
INDIVIDUAL BODYWEIGHTS AND BODYWEIGHT GAINS OF TEST ANIMALS

Animal Number	Bodyweight (g)		Bodyweight (g) Increase
	Day 0	Day 24	
1	405	591	186
2	357	558	201
3	365	547	182
4	385	622	237
5	386	550	164
6	359	531	172
7	339	529	190
8	355	585	230
9	358	534	176
10	363	606	243
11	377	578	201
12	333	517	184
13	397	648	251
14	346	527	181
15	357	576	219
16	318	482	164
17	324	512	188
18	390	649	259
19	364	562	198
20	357	593	236

HYCAR ATBN : MAGNUSSON & KLIGMAN MAXIMISATION STUDY IN THE GUINEA PIG
APPENDIX IX
INDIVIDUAL BODYWEIGHTS AND BODYWEIGHT GAINS OF CONTROL ANIMALS

Animal Number	Bodyweight (g)		Bodyweight (g) Increase
	Day 0	Day 24	
21	341	514	173
22	343	508	165
23	386	595	209
24	382	620	238
25	370	537	167
26	335	454	119
27	346	547	201
28	390	603	213
29	323	514	191
30	334	496	162

HYCAR ATBN : MAGNUSSON & KLIGMAN MAXIMISATION STUDY IN THE GUINEA PIG

A P P E N D I X X

SCALES FOR EVALUATION OF SKIN REACTIONS

EVALUATION OF ERYTHEMA

	VALUE
No erythema	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

From: OECD Test Guideline 406, 1992 and Method B6 Skin Sensitisation of Commission Directive 96/54/EC.

EVALUATION OF OEDEMA

	VALUE
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre extending beyond the area of exposure)	4

From: Draize, J H (1977) "Dermal and Eye Toxicity Tests" In: Principles and Procedures for Evaluating the Toxicity of Household Substances, National Academy of Sciences, Washington DC, p31.

HYCAR ATBN : MAGNUSON & KLIGMAN MAXIMISATION STUDY IN THE GUINEA PIG
 A P P E N D I X X I
 SUMMARY OF POSITIVE CONTROL DATA FOR THE MAGNUSON AND KLIGMAN MAXIMISATION STUDY

Project Number	Date Start	Date End	Number of Animals and Sex*		Positive Control Material	Concentration		Incidence of Sensitisation	
			Test	Control		Induction	Challenge		
413/26	19/08/96	21/09/96	10 Female	10 Female	2,4-Dinitrochlorobenzene	Intradermal 0.1% in arachis oil BP	Topical 0.75% in 80% aqueous ethanol	0.25% and 0.1% in 80% aqueous ethanol	100% (10/10)
039/239	11/11/96	06/12/96	10 Female	5 Female	2-Mercaptobenzothiazole	10% in arachis oil BP	50% in acetone: PEG 400 (70:30)	50% and 25% in acetone: PEG 400 (70:30)	90% (9/10)
039/249	22/05/97	15/06/97	10 Female	5 Female	2-Mercaptobenzothiazole	10% in arachis oil BP	50% in acetone: PEG 400 (70:30)	50% and 25% in acetone: PEG 400 (70:30)	70% (7/10)
039/258	17/10/97	10/11/97	10 Female	5 Female	2-Mercaptobenzothiazole	10% in arachis oil BP	50% in acetone: PEG 400 (70:30)	50% and 25% in acetone: PEG 400 (70:30)	90% (9/10)
039/284	11/05/98	04/06/98	10 Male	5 Male	2-Mercaptobenzothiazole	10% in arachis oil BP	50% in acetone: PEG 400 (70:30)	50% and 25% in acetone: PEG 400 (70:30)	100% (10/10)
039/333	22/12/98	05/02/99	10 Female	5 Female	2-Mercaptobenzothiazole	10% in arachis oil BP	50% in acetone: PEG 400 (70:30)	50% and 25% in acetone: PEG 400 (70:30)	90% (9/10)

* All animals supplied by David Hall Ltd., Burton-on-Trent, Staffordshire, UK

APPENDIX XII



THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 86/320 EEC

LABORATORY

TEST TYPE

SafePharm Laboratories Ltd.
Shardlow Business Park
London Road
Shardlow
Derbyshire DE72 2GD

Analytical Chemistry
Environmental Fate
Environmental Toxicity
Mutagenicity
Phys/Chem Tests
Toxicology

DATE OF INSPECTION

23rd March 1998

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

21st July 1998

UK GLP Monitoring Authority