CONFIDENTIAL

DPT 432/985069/SS

[redacted]

SKIN SENSITIZATION TO THE GUINEA-PIG

(MAGNUSSON & KLIGMAN METHOD)

Sponsor

DuPont Specialty Chemicals
Jackson Laboratory.
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Deepwater
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COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The study described in this report was conducted in compliance with the following Good Laboratory Practice Standards and with the exception of that noted below I consider the data generated to be valid.

The United Kingdom Good Laboratory Practice Regulations 1997 (Statutory Instrument No 654).

EC Council Directive 87/18/EEC of 18 December 1986 (Official Journal No L 15/29) and, from 1 May 1999, EC Commission Directive 1999/11/EC of 8 March 1999 (Official Journal No L 77/8).

OECD Principles of Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17.

In line with normal practice in this type of short term study, the protocol did not require chemical analysis of formulated test and control articles for determination of stability, homogeneity and concentration.

D G Coleman

David G. Coleman, B.Sc. (Hons.),
Study Director,
Huntingdon Life Sciences Ltd.

27 July 1999
Date

QUALITY ASSURANCE STATEMENT

The following have been inspected or audited in relation to this study

Study Phases Inspected	Date of Inspection	Date of Reporting
Protocol Audit	20 October 1998	20 October 1998
Process Based Inspections		
Housing/Environment	13 August 1998	14 August 1998
Husbandry	13 August 1998	14 August 1998
Test Material:-		
Control/Administration	13 August 1998	14 August 1998
Treatment Procedure	13 August 1998	14 August 1998
Scoring	13 August 1998	14 August 1998
Records Audit	13 August 1998	14 August 1998
Training Records	13 August 1998	14 August 1998
Report Audit	8 January 1999	13 January1999

Protocol Audit: An audit of the protocol for this study was conducted and reported to the Study Director and Company Management as indicated above.

Process based inspections: At or about the time this study was in progress inspections and audits of routine and repetitive procedures employed on this type of study were carried out. These were conducted and reported to appropriate Company Management as indicated above

Report Audit: This report has been audited by the Quality Assurance Department. This audit was conducted and reported to the Study Director and Company Management as indicated above.

The methods, procedures and observations were found to be accurately described and the reported results to reflect the raw data.

Margaret Blows

Margaret Blows,
Quality Assurance Group Leader,
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.

26 July 1999
Date

RESPONSIBLE PERSONNEL

David G. Coleman B.Sc. (Hons.),
Study Director,
Department of Acute Toxicology.

SUMMARY

This study was performed to assess the skin sensitization potential of [redacted] using the guinea-pig. The method followed was that described in:

EEC Methods for the determination of toxicity, Annex to Directive 96/54/EC (Official Journal No. L248, 30.9.96), Part B, Method B.6. Skin sensitization;

OECD Guideline for Testing of Chemicals No. 406 "Skin Sensitization". Adopted: 17 July 1992.

MAGNUSSON, B. And KLIGMAN, A.M. (1970) *Allergic Contact Dermatitis in the Guinea-pig: Identification of contact allergens*, Thomas, C.C., Springfield, Illinois, U.S.A.

The guinea pigs were dosed by intradermal injection and topical application as these are the routes of exposure required by the test guideline and method.

Based on the results of a preliminary study and in compliance with the guideline, the following dose levels were selected:

Intradermal injection:	0.25% v/v in water for irrigation
Topical application:	as supplied
Challenge application:	50 and 25% v/v in distilled water

Ten test and five control guinea-pigs were used in this study.

In this study [redacted] did not produce evidence of skin sensitization (delayed contact hypersensitivity) in any of the ten test animals.

[redacted] does not require labelling with the risk phrase R43 "May cause sensitization by skin contact" in accordance with Commission Directive 93/21/EEC.

INTRODUCTION

This study was designed to assess the skin sensitization potential of [redacted] using the guinea-pig.

Following initial exposure to the test substance (the 'induction' period comprising intradermal injections and topical application) the animals were subjected, approximately two weeks after the topical induction exposure, to a 'challenge' exposure of the test substance in order to establish if a hypersensitive state had been induced. Sensitization is determined by examining the skin reaction of test animals to the challenge exposure in comparison to skin reactions demonstrated by control animals.

The test substance may come into contact with skin during handling or use.

The study was conducted in compliance with:

EEC Methods for the determination of toxicity, Annex to Directive 96/54/EC (Official Journal No. L248, 30.9.96), Part B, Method B.6. Skin sensitization.

OECD Guideline for Testing of Chemicals No. 406 "Skin Sensitization". Adopted: 17 July 1992.

The method used was the guinea-pig maximisation test described by MAGNUSSON, B. and KLIGMAN, A.M. (1970) *Allergic Contact Dermatitis in the Guinea-pig: Identification of contact allergens*, Thomas, C.C., Springfield, Illinois, U.S.A.

On this occasion ten test and five control animals were used.

The albino guinea-pig was chosen as the test species as it had been shown to be a suitable model for skin sensitization studies and is the species recommended by the test guidelines.

The dose levels for the study were chosen on the basis of a preliminary study in compliance with the guideline.

The protocol was approved by Huntingdon Life Sciences Management on 7 July 1998, by the Sponsor on 17 July 1998 and by the Study Director on 14 October 1998.

The experimental phase of the study was undertaken between 20 October and 28 November 1998.

TEST SUBSTANCE

Identity:	[redacted]
Chemical name:	[redacted]
Intended use:	[redacted]
Appearance:	[redacted]
Storage conditions:	[redacted]
Lot number:	[redacted]
Expiry:	Two years from date of receipt
Purity:	[redacted]
Date received:	23 June 1998

EXPERIMENTAL PROCEDURE

ANIMAL MANAGEMENT

Fifteen healthy female (which were nulliparous and non-pregnant) albino guinea-pigs of the Dunkin/Hartley strain were obtained from D. Hall, Newchurch, Staffs, UK.

The animals were approximately four to seven weeks of age on arrival and were acclimatised to the experimental environment for six days prior to the start of the main study. The guinea-pigs were within the weight range 343 - 429 g at the start of the study (Day 1).

An additional six animals from the same supplier were used for the preliminary investigations.

The animals on the main study were allocated without conscious bias to two groups as follows:

Group	Number of animals	Animal numbers
Control animals	5	4895 to 4899
Test animals	10	4900 to 4909

The guinea-pigs were housed in groups of five in suspended metal cages with wire mesh floors in Building R17 Room 13.

A vitamin C enriched guinea-pig diet (Harlan Teklad 9600 FD2 SQC) and drinking water were provided *ad libitum*. Hay was given thrice weekly.

The batch of diet used for the study was analysed for nutrients, possible contaminants or micro-organisms, likely to be present in the diet, and which, if in excess, may have had an undesirable effect on the test system. The certificates of analyses were lodged in Huntingdon Life Sciences Limited Archives. There were no known contaminants present in the diet which were expected to be capable of interfering with the study outcome.

Results of routine physical and chemical examination of drinking water, as conducted by the supplier are made available to Huntingdon Life Sciences Ltd as quarterly summaries.

Animal room temperature was controlled within the range 16 - 26.5°C and relative humidity within the range 38-68%. These environmental parameters were recorded daily. Lighting was controlled by means of a time switch to give 12 hours of artificial light (0700 - 1900 hours) in each 24 hour period.

Each animal was identified by ear tattoo number. This number was unique within the Huntingdon Life Sciences Acute Toxicology Department throughout the duration of the study. Each cage was identified by a coloured label displaying the study schedule number, animal numbers and the initials of the Study Director and Home Office licensee.

POSITIVE CONTROL

The sensitivity of the guinea-pig strain used is checked periodically at Huntingdon Life Sciences with known sensitisers hexyl cinnamic aldehyde (HCA), Benzocaine and 2-mercaptobenzothiazole (MBT). The results of recent tests are presented in Appendix 3.

TEST SUBSTANCE PREPARATION

The test substance was prepared prior to each application on the day of dosing in water for irrigation (intradermal injections) and distilled water (topical application). The concentrations used are described in the treatment procedure.

The absorption of the test substance was not determined.

The homogeneity, stability and purity of the test substance were the responsibility of the Sponsor.

TREATMENT PROCEDURE

Preliminary study

The intradermal and topical irritancy of a range of dilutions of the test substance was investigated to identify where possible (a) concentrations of the test substance that would produce irritation suitable for the induction phase of the main study and (b) a maximum non-irritant concentration by the topical route of administration for the challenge phase.

The animals for the topical irritancy investigations were pre-treated with an intradermal injection of Freund's complete adjuvant, 50 : 50 with water for irrigation[1] (Ph.Eur.), approximately one week prior to the start of the preliminary investigations.

The numerical values given to the dermal reactions observed in the preliminary tests are shown in Appendix 2.

[1] Sterile water for injection

Selection of concentrations of test substance for the main study

Based on the results of the preliminary investigations, the following concentrations of [redacted] were selected:

Induction intradermal injection - 0.25% v/v in water for irrigation

This was the highest concentration that caused irritation but did not adversely affect the animals.

Induction topical application - as supplied

The test material applied topically as supplied produced some irritation but did not adversely affect the animals.

Topical challenge - 50 and 25% v/v in distilled water

From preliminary investigations 50% v/v in distilled water was the highest concentration not giving rise to irritating effects.

Main study

The procedure may be considered in two parts, Induction and Challenge.

Induction

Induction intradermal injections - test animals

A 40 × 60 mm area of dorsal skin on the scapular region of the guinea-pig was clipped free of hair with electric clippers. Three pairs of intradermal injections were made into a 20 × 40 mm area within the clipped area as shown in Figure 1.

Injectables for the test animals were prepared as follows:

1. Freund's complete adjuvant** was diluted with an equal volume of water for irrigation (Ph.Eur.).
2. [redacted] 0.25% v/v in water for irrigation.
3. [redacted] 0.25% v/v in a 50 : 50 mixture of Freund's complete adjuvant and water for irrigation.

** Difco Laboratories, Detroit, Michigan, U.S.A.

Induction topical application - test animals

One week after the injections, the same 40 x 60 mm interscapular area was clipped and shaved free of hair.

A 20 x 40 mm patch of Whatman No. 3 paper was saturated with approximately 0.4 ml of [redacted]s supplied. The patch was placed on the skin of the test animals and covered by a length of impermeable plastic adhesive tape (50 mm width "Blenderm"). This in turn was firmly secured by elastic adhesive bandage (50 mm width "Elastoplast") wound round the torso of the animal and fixed with "Sleek" impervious plastic adhesive tape. The dressing was left in place for 48 hours.

Induction - control animals

During the induction phase, the control animals were treated similarly to the test animals with the exception that the test substance was omitted from the intradermal injections and topical application.

The dermal reactions observed after each induction phase in both control and test animals are shown in Table 1.

Challenge

Challenge - control and test animals

The control and test animals were challenged topically two weeks after the topical induction application using [redacted]50 and 25% v/v in distilled water.

Hair was removed by clipping and then shaving from an area on the left flank of each guinea-pig. A 20 x 20 mm patch of Whatman No. 3 paper was saturated with approximately 0.2 ml of [redacted]50% v/v in distilled water and applied to an anterior site on the flank. [redacted] 25% v/v in distilled water was applied in a similar manner to the posterior site. The patches were sealed to the flank for 24 hours under strips of "Blenderm" covered by "Elastoplast" wound round the trunk and secured with "Sleek".

OBSERVATIONS

Clinical signs

All animals were observed daily for signs of ill health or toxicity.

Bodyweight

The bodyweight of each guinea-pig on the main study was recorded on Day 1 (day of intradermal injections) and on the last day observations were made of dermal responses to the challenge application.

Dermal responses

The dermal reactions resulting from intradermal injection and topical application on the preliminary study, and topical application at the challenge were assessed using the following numerical system:

Erythema and eschar formation:

No erythema	0
Slight erythema	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Oedema formation:

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

The approximate diameter (mm) of the dermal response at the intradermal injection sites was recorded in the preliminary study only to assist in the choice of concentrations for the main study.

Any lesion not covered by this scoring system was described.

The challenge sites were evaluated 24 and 48 hours after removal of the patches.

On completion of the study all animals were killed by cervical dislocation.

INTERPRETATION OF THE RESULTS

Dermal reactions in the test animals elicited by the challenge application were compared with the findings simultaneously obtained in the control animals.

A test animal was considered to show positive evidence of delayed contact hypersensitivity if the observed dermal reaction at challenge was definitely more marked and/or persistent than the maximum reaction seen in animals of the control group.

If the dermal reaction seen in a test animal at challenge was slightly more marked and/or persistent than (but not clearly distinguishable from) the maximum reaction seen in control animals, the result for that test animal was classified as inconclusive.

A test animal was considered to show no evidence of delayed contact hypersensitivity if the dermal reaction resulting from the challenge application was the same as, or less marked and/or persistent than the maximum reaction seen in animals of the control group.

ARCHIVES

All raw data and study related documents generated during the course of the study at Huntingdon Life Sciences, together with a copy of the final report will be lodged in the Huntingdon Life Sciences Ltd Archive, Huntingdon.

Such records will be retained for a minimum period of five years from the date of issue of the final report. At the end of the five year retention period the Client will be contacted and advice sought on the future requirements. Under no circumstances will any item be discarded without the Client's knowledge.

DEVIATIONS FROM PROTOCOL

There were no deviations from the protocol that were considered to have affected the integrity or validity of the study, however the following is of note:

On occasion the temperature of the animal room was outside the range given in the protocol, however this was not considered to have had an adverse effect on the animals.

RESULTS

CLINICAL SIGNS

No signs of ill health or toxicity were observed.

BODYWEIGHT

Individual bodyweights are shown in Appendix 1.

Bodyweight increases were recorded for all guinea-pigs over the period of the study.

INDUCTION

Dermal reactions seen following the induction applications are summarised in Table 1.

Intradermal injections

Necrosis was recorded at sites receiving Freund's Complete Adjuvant in test and control animals.

Slight irritation was seen in four test animals at sites receiving [redacted] 0.25% v/v in water for irrigation and slight irritation was observed in one control animal receiving water for irrigation. No reactions were noted for the remaining test or control animals.

Topical application

Slight erythema was observed in most test animals following topical application with [redacted] as supplied.

Slight erythema was seen in one control guinea-pig.

CHALLENGE

The numerical values given to the dermal reactions elicited by the challenge applications are shown in Table 2.

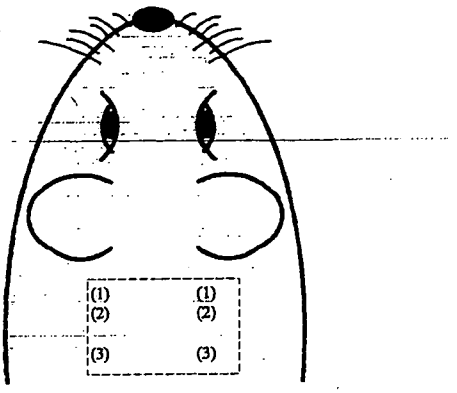
There were no dermal reactions seen in any of the test or control animals, therefore all ten test animals gave negative responses.

CONCLUSION

In this study, [redacted] did not produce evidence of skin sensitization (delayed contact hypersensitivity) in any of the ten test animals.

FIGURE 1

Position of intradermal injections and topical induction application



The rectangle outlines the 20 × 40 mm clipped scapular area in which injections were made and to which the topical induction application was made one week later.

Control animals:

(1) 0.1 ml of Freund's complete adjuvant 50 : 50 with water for irrigation (Ph.Eur.).

(2) 0.1 ml of water for irrigation.

(3) 0.1 ml of Freund's complete adjuvant 50 : 50 with water for irrigation.

Test animals:

(1) 0.1 ml of Freund's complete adjuvant 50 : 50 with water for irrigation (Ph.Eur.).

(2) 0.1 ml of [redacted] 0.25% v/v in water for irrigation.

(3) 0.1 ml of [redacted] 0.25% v/v in a 50 : 50 mixture of water for irrigation and Freund's complete adjuvant.

A volume of 0.1 ml was injected into both the left and right injection sites.

TABLE 1

Dermal reactions observed after each induction

Group	Animal number	Intradermal injections Site number			Topical application
		1	2	3	
Control	4895	N	0	N	0
	4896	N	0	N	0
	4897	N	1	N	1
	4898	N	0	N	0
	4899	N	0	N	0
Test	4900	N	0	N	0
	4901	N	0	N	1
	4902	N	0	N	1
	4903	N	1	N	1
	4904	N	1	N	1
	4905	N	0	N	1
	4906	N	1	N	0
	4907	N	0	N	1
	4908	N	1	N	1
	4909	N	0	N	1

Intradermal injections

Control animals: See figure 1 (previous page)
Test animals: See figure 1 (previous page)

N Necrosis
0 No irritation
1 Slight irritation
2 Well-defined irritation
3 Moderate irritation
4 Severe irritation

Topical application

Control animals: distilled water
Test animals: [redacted] as supplied

0 No erythema
1 Slight erythema
2 Well-defined erythema
3 Moderate erythema
4 Severe erythema

TABLE 2

Dermal reactions observed after the challenge application with [redacted]

Freund's treated controls

Guinea-pig number	E = Erythema O = Oedema	Score			
		24 Hours		48 Hours	
		A	P	A	P
4895	E	0	0	0	0
	O	0	0	0	0
4896	E	0	0	0	0
	O	0	0	0	0
4897	E	0	0	0	0
	O	0	0	0	0
4898	E	0	0	0	0
	O	0	0	0	0
4899	E	0	0	0	0
	O	0	0	0	0

A Anterior site, exposed to [redacted] 50% v/v in distilled water
P Posterior site, exposed to [redacted] 25% v/v in distilled water

TABLE 2

Dermal reactions observed after the challenge application with [redacted] (continued)

Test animals

Guinea-pig number	E = Erythema O = Oedema	Score				Results Positive (+) Negative (-) Inconclusive (±)
		24 Hours		48 Hours		
		A	P	A	P	
4900	E	0	0	0	0	-
	O	0	0	0	0	
4901	E	0	0	0	0	-
	O	0	0	0	0	
4902	E	0	0	0	0	-
	O	0	0	0	0	
4903	E	0	0	0	0	-
	O	0	0	0	0	
4904	E	0	0	0	0	-
	O	0	0	0	0	
4905	E	0	0	0	0	-
	O	0	0	0	0	
4906	E	0	0	0	0	-
	O	0	0	0	0	
4907	E	0	0	0	0	-
	O	0	0	0	0	
4908	E	0	0	0	0	-
	O	0	0	0	0	
4909	E	0	0	0	0	-
	O	0	0	0	0	

A Anterior site, exposed to [redacted] 50% v/v in distilled water
P Posterior site, exposed to [redacted] 25% v/v in distilled water

APPENDIX 1

Individual bodyweights (g)

Group	Guinea-pig number	Day 1 4 November 1998	Last observation day 28 November 1998
Control	4895	343	514
	4896	406	627
	4897	387	544
	4898	362	540
	4899	379	579
Test	4900	370	588
	4901	359	537
	4902	375	555
	4903	365	555
	4904	368	547
	4905	361	527
	4906	348	546
	4907	404	628
	4908	379	533
	4909	429	672

APPENDIX 2

Results of preliminary investigations with [redacted]

Intradermal injections

Vehicle: Water for irrigation

Guinea-pig number	Concentration % v/v	Score		
		Hours	24	72
4449	10.0	D	10	8
		E	N	N
		O	2	2
	7.5	D	10	10
		E	N	N
		O	2	2
	5.0	D	10	8
		E	N	N
		O	2	2
	2.5	D	8	8
		E	N	N
		O	2	2
	1.0	D	8	8
		E	SN	N
		O	2	2
	0.5	D	8	8
		E	SN	SN
		O	2	2
	0.25	D	8	8
		E	2	2
		O	2	2
	0.1	D	6	8
		E	2	2
		O	2	2
	Vehicle control	D	6	8
		E	2	2
		O	2	2

Guinea-pig number	Concentration % v/v	Score		
		Hours	24	72
4450	10.0	D	10	10
		E	N	N
		O	2	2
	7.5	D	10	10
		E	N	N
		O	2	2
	5.0	D	8	8
		E	N	N
		O	2	2
	2.5	D	8	8
		E	SN	N
		O	2	2
	1.0	D	8	8
		E	SN	N
		O	2	2
	0.5	D	8	8
		E	SN	SN
		O	2	2
	0.25	D	8	8
		E	2	2
		O	2	2
	0.1	D	6	8
		E	2	2
		O	2	2
	Vehicle control	D	6	6
		E	2	2
		O	2	2

Key:

D Diameter (mm)
E Erythema (0 - 4 numerical scores)
O Oedema (0 - 4 numerical scores)
SN Slight necrosis
N Necrosis

APPENDIX 2

Results of preliminary investigations with [redacted] (continued)

Topical application

Vehicle: distilled water

Guinea-pig number	Concentration % v/v	Score					
		0 Hours		24 Hours		48 Hours	
		E	O	E	O	E	O
4466	75	1	1	1	0	L1*	0
	50	0	0	0	0	0	0
	25	0	0	0	0	0	0
	10	0	0	0	0	0	0
4467	75	1	0	1	0	1*	0
	50	0	0	0	0	0	0
	25	0	0	0	0	0	0
	10	0	0	0	0	0	0
4470	75	1	0	1	0	1*	0
	50	0	0	0	0	0	0
	25	0	0	0	0	0	0
	10	0	0	0	0	0	0
4471	75	1	0	1	0	0*	0
	50	0	0	0	0	0	0
	25	0	0	0	0	0	0
	10	0	0	0	0	0	0
4761	100	1	0	1	0	0	0
	90	0	0	0	0	0	0
	100	0	0	0	0	0	0
	90	0	0	0	0	0	0
4762	100	1	0	L1	0	0	0
	90	1	0	0	0	0	0
	100	0	0	1*	0	0	0
	90	0	0	1*	0	0	0

E Erythema (0 - 4 numerical scores)
O Oedema (0 - 4 numerical scores)
L Localised dermal reaction
* Dryness and sloughing ofd the epidermis

APPENDIX 3

Skin sensitization positive control study with 2-Mercaptobenzothiazole (MBT) to the Magnusson & Kligman method (Sch. No. HLS/010)

This study was performed to confirm the sensitivity and reliability of the experimental technique used at Huntingdon Life Sciences to detect skin sensitization potential. The study was performed using the guinea-pig and a known weak/moderate sensitizer - 2-Mercaptobenzothiazole (MBT). The method followed was that described in:

MAGNUSSON, B. and KLIGMAN, A.M. (1970) *Allergic Contact Dermatitis in the Guinea-pig: Identification of contact allergens*, Thomas, C.C., Springfield, Illinois, U.S.A.

This positive control study was conducted between 23 June and 17 July 1998 using fifteen female (nulliparous and non pregnant) albino guinea-pigs of the Dunkin Hartley strain supplied by D Hall, Staffs, UK. The MBT used for this study was a pale yellow powder, batch number 16H3435, expiry date 3 April 2000, supplied by Sigma Chemicals St Louis, USA.

Based on preliminary investigations previously conducted at this laboratory, the following concentrations of MBT were administered:

Intradermal injection:	10 % w/v in Alembicol D
Topical application:	83.3% w/v in Alembicol D
Challenge application:	83.3 and 40% w/v in Alembicol D

RESULTS

INDUCTION

Intradermal injections - Slight to well-defined irritation was seen in test animals at sites receiving MBT, 10% w/v in Alembicol D and slight irritation was observed in control animals receiving Alembicol D.

Topical application - Well-defined erythema was observed in test animals following topical application with MBT, 83.3% w/v in Alembicol D. Well-defined erythema was seen in the control animals receiving Alembicol D.

CHALLENGE

Dermal reactions were noted in all of the ten test animals compared to none in the controls therefore all ten test animals were considered to have given positive sensitization responses.

CONCLUSION

In this study MBT produced evidence of skin sensitisation (delayed contact hypersensitivity) in all of the ten animals, thus confirming the sensitivity and reliability of the experimental technique.

APPENDIX 3

(continued)

Individual dermal reactions after challenge application of MBT

CONTROL ANIMALS					
Guinea-pig number	E = Erythema O = Oedema	Score			
		24 Hours		48 Hours	
		A	P	A	P
2460	E	0	0	0	0
	O	0	0	0	0
2461	E	0	0	0	0
	O	0	0	0	0
2462	E	0	0	0	0
	O	0	0	0	0
2463	E	0	0	0	0
	O	0	0	0	0
2464	E	0	0	0	0
	O	0	0	0	0

TEST ANIMALS						
Guinea-pig number	E = Erythema O = Oedema	Score				Results Positive (+) Negative (-) Inconclusive (±)
		24 Hours		48 Hours		
		A	P	A	P	
2465	E	2	1	Ø2	Ø1	+
	O	1	0	1	0	
2466	E	1	1	1	1	+
	O	1	0	1*	0*	
2467	E	0	1	0	1	+
	O	0	1	0	1*	
2468	E	1	1	Ø1	Ø1	+
	O	1	0	1	1	
2469	E	1	1	Ø1	Ø1	+
	O	1	0	1	0	
2470	E	2	2	Ø2	NE2	+
	O	2	2	2	Ø2	
2471	E	2	2	2	1	+
	O	2	1	1*	0	
2472	E	2	1	Ø2	Ø1	+
	O	1	0	1	0	
2473	E	2	2	Ø2	2	+
	O	2	1	2	1	
2474	E	2	2	2	2	+
	O	2	2	2*	2*	

* Dryness and sloughing of the epidermis
Ø Thickening, dryness and sloughing of the epidermis
NE Necrotic edge
A Anterior site, exposed to MBT, 83.3% w/v in Alembicol D
P Posterior site, exposed to MBT, 40% w/v in Alembicol D