

8472-8EHQ-0898-14232

CYTEC

Toxicology & Product Regulatory Compliance
5 Garret Mountain Plaza
West Paterson, NJ 07424

Contains No CBI

**CERTIFIED MAIL
RETURN RECEIPT REQUESTED**

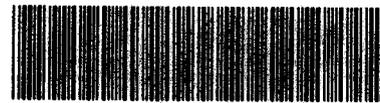
July 31, 1998

Document Control Office (7407)
Attn: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics (OPPT)
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

RECEIVED
OPPT/CBIC
98 AUG -3 PM 3:15

Attention: **TSCA SECTION 8(E) COORDINATOR**

REFERENCE: No 8(e) Number Has Been Assigned



8EHQ-98-14232

Dear Sir/Madam:

As a follow-up to our previous 8(e) submission dated July 22, 1998 for a chemical identified as Carbamothioic acid, 2-propenyl-, O-(2-methylpropyl) ester [CAS Number 86329-09-1], I am enclosing a copy of the final report entitled "AERO® 5100 Promoter - Skin Sensitization To The Guinea Pig".

This report was received by CYTEC on July 30, 1998. This report **does not** contain confidential business information.

If you have any questions please contact me at (201) 357-3375.

Sincerely,

Patricia Ann Vernon
Product Regulatory Compliance
Manager, Asia-Pacific

RECEIVED
OPPT/CBIC
98 AUG 10 PM 12:05

cc: K. E. Koster - CY3
S. J. Sherman - CY3



89980000272

**PUBLIC COPY
DOES NOT CONTAIN
CONFIDENTIAL BUSINESS INFORMATION**

Huntingdon
Life Sciences

RECEIVED

APR 2 1999

AERO[®]5100 PROMOTER

SKIN SENSITIZATION TO THE GUINEA-PIG

Report

CONFIDENTIAL

CTI 057/983631/SS

PUBLIC COPY
DOES NOT CONTAIN
CONFIDENTIAL BUSINESS INFORMATION

RECEIVED
OPPT CRIC
98 AUG -3 PM 3:15

AERO® 5100 PROMOTER
SKIN SENSITIZATION TO THE GUINEA-PIG

Sponsor

Cytec Industries Inc
5 Garret Mountain Plaza
West Paterson
NJ 07424
USA

Research Laboratory

Huntingdon Life Sciences Ltd
P O Box 2
Huntingdon
Cambridgeshire
PE18 6ES
ENGLAND

Report issued 28 July 1998

CONTENTS

	Page
COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS	3
QUALITY ASSURANCE STATEMENT	4
RESPONSIBLE PERSONNEL	5
SUMMARY	6
INTRODUCTION	7
TEST SUBSTANCE	8
EXPERIMENTAL PROCEDURE	9
RESULTS	15
CONCLUSION	16
FIGURE	
1. Position of intradermal injections and topical induction application	17
TABLES	
1. Dermal reactions observed after each induction	18
2. Dermal reactions observed after the challenge application	19
APPENDICES	
1. Individual bodyweights	22
2. Results of preliminary investigations	23
3. Summary of positive control data	25

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

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

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45 1992 and subsequently 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.


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

28 July 1998
Date

**PUBLIC COPY
DOES NOT CONTAIN
CONFIDENTIAL BUSINESS INFORMATION**

QUALITY ASSURANCE STATEMENT

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

Study Phases Inspected	Date of Inspection	Date of Reporting
Process Based Inspections		
Generic Standard Protocol Review	23 February 1998	24 February 1998
Animal Husbandry	01 May 1998	01 May 1998
Housing and Environment	01 May 1998	01 May 1998
Test Material Control	01 May 1998	01 May 1998
Treatment Procedures	01 May 1998	01 May 1998
Scoring Procedures	01 May 1998	01 May 1998
Report	9 July 1998	10 July 1998

Protocol: An audit of the standard protocol generated for this type of study design was conducted and reported to Company Management as indicated above.

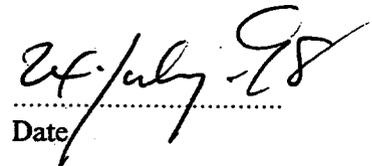
Process based inspections: At or about the time this study was in progress inspections and audits of routine and repetitive procedures employed on this type of study were carried out. These were conducted and reported to appropriate Company Management as indicated above

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.



.....
 Kevin P. de-Salis, B.A. (Hons.), C.Biol., M.I.Biol., Dip.R.Q.A.,
 Quality Assurance Unit Head,
 Department of Quality Assurance,
 Huntingdon Life Sciences Ltd.



.....
 Date

**PUBLIC COPY
 DOES NOT CONTAIN
 CONFIDENTIAL BUSINESS INFORMATION**

RESPONSIBLE PERSONNEL

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

**PUBLIC COPY
DOES NOT CONTAIN
CONFIDENTIAL BUSINESS INFORMATION**

SUMMARY

This study was performed to assess the skin sensitization potential of AERO[®] 5100 Promoter using the guinea-pig. The method followed was that described in:

EEC Methods for the determination of toxicity, Annex to Directive 96/54/EEC (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:	2.5% v/v in Alembicol D
Topical application:	as supplied
Challenge application:	75 and 37.5% v/v in Alembicol D

Twenty test and ten control guinea-pigs were used in this study.

In this study AERO[®] 5100 Promoter produced evidence of skin sensitization (delayed contact hypersensitivity) in thirteen of the twenty test animals. The remaining seven test animals gave negative responses. Overall, AERO[®] 5100 Promoter is considered to have the potential to cause skin sensitization.

AERO[®] 5100 Promoter requires labelling with the risk phrase R43 "May cause sensitization by skin contact" in accordance with Commission Directive 93/21/EEC.

**PUBLIC COPY
DOES NOT CONTAIN
CONFIDENTIAL BUSINESS INFORMATION**

INTRODUCTION

This study was designed to assess the skin sensitization potential of AERO[®] 5100 Promoter 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 has 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/EEC (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 twenty test and ten 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 3 April 1998, by the Sponsor on 14 April 1998 and by the Study Director on 30 April 1998.

The experimental phase of the study was undertaken between 5 May and 6 June 1998.

**PUBLIC COPY
DOES NOT CONTAIN
CONFIDENTIAL BUSINESS INFORMATION**

TEST SUBSTANCE

Identity:	AERO [®] 5100 Promoter
Alternative name:	CT-637-97
Chemical name:	Carbamothioic acid, 2-propenyl-, O-(2-methylpropyl) ester
Intended use:	Flotation mineral collector
Appearance:	Pale brown liquid
Storage conditions:	Room temperature in the dark
Lot number:	95
Expiry:	March 1999
Purity:	~ 98%
Date received:	4 March 1998

**PUBLIC COPY
DOES NOT CONTAIN
CONFIDENTIAL BUSINESS INFORMATION**

EXPERIMENTAL PROCEDURE

ANIMAL MANAGEMENT

Thirty healthy male 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 352 - 433 g at the start of the study (Day 1).

Additional 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	10	1780 to 1789
Test animals	20	1790 to 1809

The guinea-pigs were housed in groups of five in suspended metal cages with wire mesh floors in Building R17 Room 14.

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 19 to 26 °C and relative humidity within the range 37 to 68%. These environmental parameters were recorded daily. Air exchange was maintained at approximately 15 air changes per hour and lighting was controlled by means of a time switch to give 12 hours of artificial light (0700 - 1900 hours) in each 24 hours 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 Alembicol D[#]. 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.

[#] *A product of coconut oil, supplied by Alembic Products, Saltney, Chester, England*

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.

Animals for these investigations were pre-treated with an intradermal injection of Freund's complete adjuvant, 50 : 50 with water for irrigation (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.

**PUBLIC COPY
DOES NOT CONTAIN**

Selection of concentrations of test substance for the main study

Based on the results of the preliminary investigations, the following concentrations of AERO[®] 5100 Promoter were selected:

Induction intradermal injection - 2.5% v/v in Alembicol D

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 - 75 and 37.5% v/v in Alembicol D

From preliminary investigations 75% v/v in Alembicol D 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. AERO[®] 5100 Promoter, 2.5% v/v in Alembicol D.
3. AERO[®] 5100 Promoter, 2.5% v/v in a 50 : 50 mixture of Freund's complete adjuvant and Alembicol D.

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

PUBLIC COPY
DOES NOT CONTAIN
CONFIDENTIAL BUSINESS INFORMATION

Induction topical application - test animals

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

A 20 × 40 mm patch of Whatman No. 3 paper was saturated with approximately 0.4 ml of AERO[®] 5100 Promoter, as 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 AERO[®] 5100 Promoter, 75 and 37.5% v/v in Alembicol D.

Hair was removed by clipping and then shaving from an area on the left flank of each guinea-pig. A 20 × 20 mm patch of Whatman No. 3 paper was saturated with approximately 0.2 ml of AERO[®] 5100 Promoter, 75% v/v in Alembicol D and applied to an anterior site on the flank. AERO[®] 5100 Promoter, 37.5% v/v in Alembicol D was applied in a similar manner to a 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.

**PUBLIC COPY
DOES NOT CONTAIN
CONFIDENTIAL BUSINESS INFORMATION**

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 other 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 above the range given in the protocol, however this was not considered to have had an adverse effect on the animals.

The dermal reactions of the control and test animals to the induction intradermal injections and induction topical application were scored and reported individually for each animal (rather than by group as given in the protocol).

**PUBLIC COPY
DOES NOT CONTAIN
CONFIDENTIAL BUSINESS INFORMATION**

RESULTS

CLINICAL SIGNS

No signs of ill health or toxicity were recorded.

**PUBLIC COPY
DOES NOT CONTAIN
CONFIDENTIAL BUSINESS INFORMATION**

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 to well-defined irritation was seen in test animals at sites receiving AERO[®] 5100 Promoter, 2.5% v/v in Alembicol D and slight to well-defined irritation was observed in control animals receiving Alembicol D.

Topical application

Slight to well-defined erythema was observed in test animals following topical application with AERO[®] 5100 Promoter, as supplied.

Slight erythema was seen in the control guinea-pigs.

CHALLENGE

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

Dermal reactions were observed in thirteen of the twenty test animals compared to none in the controls, therefore these thirteen animals were considered to have given positive responses. The remaining seven animals gave negative responses. Overall, AERO[®] 5100 Promoter is considered to have the potential to cause skin sensitization.

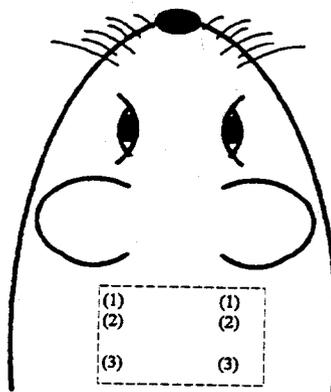
CONCLUSION

In this study AERO[®] 5100 Promoter produced evidence of skin sensitization (delayed contact hypersensitivity) in thirteen of the twenty test animals. The remaining seven test animals gave negative responses. Overall, AERO[®] 5100 Promoter is considered to have the potential to cause skin sensitization.

**PUBLIC COPY
DOES NOT CONTAIN
CONFIDENTIAL BUSINESS INFORMATION**

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 sterile water for injection (Ph.Eur.).
- (2) 0.1 ml of Alembicol D.
- (3) 0.1 ml of Freund's complete adjuvant 50 : 50 with Alembicol D.

Test animals:

- (1) 0.1 ml of Freund's complete adjuvant 50 : 50 with sterile water for injection (Ph.Eur.).
- (2) 0.1 ml of AERO[®] 5100 Promoter, 2.5% v/v in Alembicol D.
- (3) 0.1 ml of AERO[®] 5100 Promoter, 2.5% v/v in a 50 : 50 mixture of Alembicol D and Freund's complete adjuvant.

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

**PUBLIC COPY
DOES NOT CONTAIN
CONFIDENTIAL BUSINESS INFORMATION**

TABLE 1

Dermal reactions observed after each induction

Group	Animal number	Intradermal injections			Topical application
		Site number			
		1	2	3	
Control	1780	N	1	N	1
	1781	N	1	N	0
	1782	N	0	N	0
	1783	N	2	N	0
	1784	N	1	N	0
	1785	N	2	N	0
	1786	N	1	N	1
	1787	N	1	N	0
	1788	N	1	N	0
	1789	N	1	N	0
Test	1790	N	2	N	2
	1791	N	2	N	2
	1792	N	1	N	2
	1793	N	1	N	1
	1794	N	1	N	2
	1795	N	1	N	1
	1796	N	2	N	1
	1797	N	1	N	1
	1798	N	2	N	1
	1799	N	1	N	2
	1800	N	2	N	1
	1801	N	N	N	N
	1802	N	2	N	1
	1803	N	2	N	1
	1804	N	2	N	2
	1805	N	2	N	1
	1806	N	2	N	1
	1807	N	N	N	1
	1808	N	2	N	1
	1809	N	2	N	2

Intradermal injections

Topical application

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

Control animals: Alembicol D
 Test animals: AERO® 5100
 Promoter, as supplied

N Necrosis
 0 No irritation
 1 Slight irritation
 2 Well-defined irritation

0 No erythema
 1 Slight erythema
 2 Well-defined erythema

PUBLIC COPY

DOES NOT CONTAIN

CONFIDENTIAL BUSINESS INFORMATION

TABLE 2

Dermal reactions observed after the challenge application with AERO[®] 5100 Promoter

Freund's treated controls

Guinea-pig number	E = Erythema O = Oedema	Score			
		24 Hours		48 Hours	
		A	P	A	P
1780	E	0	0	0	0
	O	0	0	0	0
1781	E	0	0	0	0
	O	0	0	0	0
1782	E	0	0	0	0
	O	0	0	0	0
1783	E	0	0	0	0
	O	0	0	0	0
1784	E	0	0	0	0
	O	0	0	0	0
1785	E	0	0	0	0
	O	0	0	0	0
1786	E	0	0	0	0
	O	0	0	0	0
1787	E	0	0	0	0
	O	0	0	0	0
1788	E	0	0	0	0
	O	0	0	0	0
1789	E	0	0	0	0
	O	0	0	0	0

A Anterior site, exposed to AERO[®] 5100 Promoter, 75% v/v in Alembicol D
 P Posterior site, exposed to AERO[®] 5100 Promoter, 37.5% v/v in Alembicol D

**PUBLIC COPY
 DOES NOT CONTAIN
 CONFIDENTIAL BUSINESS INFORMATION**

TABLE 2

Dermal reactions observed after the challenge application with AERO[®] 5100 Promoter
(continued)

Test animals

Guinea-pig number	E = Erythema O = Oedema	Score				Results Positive (+) Negative (-) Inconclusive (±)
		24 Hours		48 Hours		
		A	P	A	P	
1790	E	0	0	0	0	-
	O	0	0	0	0	
1791	E	*1	1	*1	0	+
	O	1	0	1	0	
1792	E	1	0	1	0	+
	O	0	0	0	0	
1793	E	1	1	1	1	+
	O	1	1	1	0	
1794	E	0	0	0	0	-
	O	0	0	0	0	
1795	E	0	0	0	0	-
	O	0	0	0	0	
1796	E	1	1	1	1	+
	O	1	0	1	0	
1797	E	0	0	0	0	-
	O	0	0	0	0	
1798	E	1	1	1	1	+
	O	0	0	0	0	
1799	E	1	1	1	1	+
	O	1	0	1	0	

* Dryness and sloughing of the epidermis

A Anterior site, exposed to AERO[®] 5100 Promoter, 75% v/v in Alembicol D

P Posterior site, exposed to AERO[®] 5100 Promoter, 37.5% v/v in Alembicol D

**PUBLIC COPY
DOES NOT CONTAIN
CONFIDENTIAL BUSINESS INFORMATION**

TABLE 2

Dermal reactions observed after the challenge application with AERO[®] 5100 Promoter
(continued)

Test animals

Guinea-pig number	E = Erythema O = Oedema	Score				Results Positive (+) Negative (-) Inconclusive (±)
		24 Hours		48 Hours		
		A	P	A	P	
1800	E	2	2	2	1	+
	O	1	1	1	1	
1801	E	2	*2	2	*1	+
	O	1	1	1	1	
1802	E	0	0	0	0	-
	O	0	0	0	0	
1803	E	1	1	1	1	+
	O	1	0	1	0	
1804	E	1	1	1	0	+
	O	0	0	0	0	
1805	E	1	1	1	1	+
	O	1	1	1	0	
1806	E	1	0	1	0	+
	O	0	0	0	0	
1807	E	0	0	0	0	-
	O	0	0	0	0	
1808	E	1	0	0	0	-
	O	0	0	0	0	
1809	E	1	1	1	0	+
	O	0	0	0	0	

* Dryness and sloughing of the epidermis

A Anterior site, exposed to AERO[®] 5100 Promoter, 75% v/v in Alembicol D

P Posterior site, exposed to AERO[®] 5100 Promoter, 37.5% v/v in Alembicol D

**PUBLIC COPY
DOES NOT CONTAIN
CONFIDENTIAL BUSINESS INFORMATION**

APPENDIX 1

Individual bodyweights (g)

Group	Guinea-pig number	Day 1 13 May 1998	Last observation day 6 June 1998
Control	1780	373	611
	1781	433	686
	1782	386	623
	1783	382	600
	1784	383	583
	1785	385	604
	1786	426	680
	1787	396	511
	1788	352	556
	1789	388	607
Test	1790	369	523
	1791	384	596
	1792	373	590
	1793	360	552
	1794	373	550
	1795	355	591
	1796	398	579
	1797	380	656
	1798	418	683
	1799	395	656
	1800	410	552
	1801	387	535
	1802	384	625
	1803	358	549
	1804	399	655
	1805	394	551
	1806	400	615
	1807	396	603
	1808	405	699
	1809	375	620

**PUBLIC COPY
DOES NOT CONTAIN
CONFIDENTIAL BUSINESS INFORMATION**

APPENDIX 2

Results of preliminary investigations with AERO® 5100 Promoter

Intradermal injections

Vehicle: Alembicol D

Guinea-pig number	Concentration % v/v	Score		
		Hours	24	72
1641	10.0	D	10	10
		E	SN	N
		O	2	2
	7.5	D	10	10
		E	2	2
		O	2	2
	5.0	D	10	10
		E	2	2
		O	2	2
	2.5	D	10	8
		E	2	2
		O	2	2
	1.0	D	10	8
		E	2	2
		O	2	2
	0.5	D	8	8
		E	2	2
		O	2	2
	0.25	D	8	8
		E	2	2
		O	2	2
	0.1	D	8	8
		E	2	2
		O	2	2
	Vehicle control	D	8	8
		E	2	2
		O	2	2

Guinea-pig number	Concentration % v/v	Score		
		Hours	24	72
1642	10.0	D	10	10
		E	SN	N
		O	2	2
	7.5	D	8	10
		E	N	N
		O	2	2
	5.0	D	8	8
		E	2	SN
		O	2	2
	2.5	D	8	8
		E	2	2
		O	2	2
	1.0	D	8	8
		E	2	2
		O	2	2
	0.5	D	8	8
		E	2	2
		O	2	2
	0.25	D	8	8
		E	2	2
		O	2	2
	0.1	D	8	8
		E	2	2
		O	2	2
	Vehicle control	D	8	8
		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

PUBLIC COPY
DOES NOT CONTAIN
CONFIDENTIAL BUSINESS INFORMATION

APPENDIX 2

Results of preliminary investigations with AERO[®] 5100 Promoter
(continued)

Topical application

Vehicle: Alembicol D

Guinea-pig number	Concentration % v/v	Score					
		0 Hours		24 Hours		48 Hours	
		E	O	E	O	E	O
1643	As supplied	0	0	0	0	0	0
	75	0	0	0	0	0	0
	50	0	0	0	0	0	0
	25	0	0	0	0	0	0
1644	As supplied	0	0	1	0	1	*0
	75	0	0	0	0	0	0
	50	0	0	0	0	0	0
	25	0	0	0	0	0	0
1645	As supplied	0	0	L1	0	*1	0
	75	0	0	0	0	0	0
	50	0	0	0	0	0	0
	25	0	0	0	0	0	0
1646	As supplied	0	0	0	0	0	0
	75	0	0	0	0	0	0
	50	0	0	0	0	0	0
	25	0	0	0	0	0	0

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

PUBLIC COPY
DOES NOT CONTAIN
CONFIDENTIAL BUSINESS INFORMATION

APPENDIX 3

Skin sensitization positive control study with hexyl cinnamic aldehyde (HCA) to the Magnusson & Kligman method (Sch. No. HLS/009)

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 - hexyl cinnamic aldehyde (HCA). 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 15 December 1997 and 8 January 1998 using fifteen guinea-pigs of the Dunkin Hartley strain supplied by D Hall, Staffs, UK.

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

Intradermal injection:	10 % v/v in Alembicol D
Topical application:	As supplied (neat)
Challenge application:	As supplied (neat) and 50% v/v in Alembicol D

RESULTS

INDUCTION

Intradermal injections

Slight irritation was seen in test animals at sites receiving HCA, 10% v/v in Alembicol D and slight irritation was observed in control animals receiving Alembicol D.

Topical application

Slight erythema was observed in test animals following topical application with HCA, as supplied. Slight erythema was seen in the control animals receiving Alembicol D.

CHALLENGE

Dermal reactions seen in all ten test animals were more marked than those seen for controls and therefore all ten test animals were considered to give positive responses.

CONCLUSION

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

**PUBLIC COPY
DOES NOT CONTAIN
CONFIDENTIAL BUSINESS INFORMATION**

APPENDIX 3

(continued)

Individual dermal reactions after challenge application of HCA

CONTROL ANIMALS					
Guinea-pig number	E = Erythema O = Oedema	Score			
		24 Hours		48 Hours	
		A	P	A	P
5410	E	1	L1	L1	0
	O	0	0	0*	0*
5411	E	L1	0	0	0
	O	0	0	0*	0*
5412	E	0	0	0	0
	O	0	0	0*	0*
5413	E	L1	0	L1	0
	O	0	0	0*	0*
5414	E	L1	0	L1	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	
5420	E	1	1	Ø1	1	+
	O	1	1	1	1*	
5421	E	2	1	2	1	+
	O	1	1	1*	1*	
5422	E	1	1	1	1	+
	O	1	1	1*	1*	
5423	E	1	1	1	1	+
	O	1	0	1*	1*	
5424	E	2	1	2	1	+
	O	1	1	1*	1*	
5425	E	2	1	2	1	+
	O	1	1	1*	0*	
5426	E	2	1	Ø2	1	+
	O	2*	1*	2	1*	
5427	E	2	1	2	1	+
	O	1*	0*	1*	0*	
5428	E	1	1	1	L1	+
	O	1	0	1*	0*	
5429	E	2	1	2	1	+
	O	2*	0	1*	0*	

- L Localised dermal reaction (restricted to a small area of the challenge site)
 * Dryness and sloughing of the epidermis
 Ø Thickening, dryness and sloughing of the epidermis
 A Anterior site, exposed to HCA, as supplied
 P Posterior site, exposed to HCA, 50% v/v in Alembicol D

PUBLIC COPY
 DOES NOT CONTAIN

Huntingdon Life Sciences Ltd
PO Box 2
Huntingdon
Cambridgeshire PE18 6ES
England
Tel: +44 (0) 1480 892300
Fax: +44 (0) 1480 892350

Huntingdon Life Sciences Ltd
Eye
Suffolk IP23 7PX
England
Tel: +44 (0) 1480 892300
Fax: +44 (0) 1480 892350

Huntingdon Life Sciences Ltd
Altrincham Road
Wilmslow
Cheshire SK9 4LY
England
Tel: +44 (0) 1480 892300
Fax: +44 (0) 1480 892350

Huntingdon Life Sciences Inc
P.O. Box 2360
Mettlers Road
East Millstone, NJ 08875-2360
USA
Tel: +1 (732) 873-2550
Fax: +1 (732) 873-3992

Huntingdon Life Sciences Co Ltd
Bancho Kaikan
12-1 Gobancho
Chiyoda-Ku
Tokyo 102
Japan
Tel: +81 (0) 3 3238 6381-7
Fax: +81 (0) 3 3238 6388-9

Shin Won Scientific Company
#407, New Family 2nd Officitel
99-2 Karak-Dong
Songpa-Ku
Seoul
Korea 138-160
Tel: +82 (0) 2 409 8240
Fax: +82 (0) 2 409 8241