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July 24, 1997

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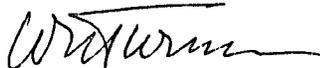
In accordance with Section 8(e) of the Toxic Substance Control Act (TSCA), Clariant Corporation is submitting the following final report of a sensitization study in guinea pigs. The test material is 2,2-Dimethoxy ethanal (CAS:51673-84-8).

In this study, 2,2-Dimethoxy ethanal elicited a positive response, indicative of skin sensitization (delayed contact hypersensitivity) in thirteen of the test animals following the challenge application. An inconclusive result was seen in a further six animals and a negative result in the remaining test animal.

This submission does not contain confidential business information.

If the agency would like additional information or assistance, please contact me at the address on this letterhead. Note that effective July 1, 1997, the Hoechst Celanese Corporation Specialty Chemical Group was merged into Clariant Corporation.

Sincerely,

William N. Turner  
Manager, Product Safety**88970000240**cc: D. Lisman  
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# CORNING Hazleton

## FINAL REPORT

2,2-Dimethoxy ethanal 60% (water solution)

Skin Sensitisation Study in the Guinea Pig

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Report number: 1433/4-1032

Report issue: November 1995

Page number: 1 of 28

REPRODUCTION INTERDITE - EIGENTUM DER SOCIÉTÉ FRANÇAISE HOECHST - ALLE RECHTE VORBEHALTEN SOCIÉTÉ FRANÇAISE HOECHST - OWNERSHIP - ALL RIGHTS RESERVED

TABLE OF CONTENTS

TITLE PAGE	1
PREFACE PAGES	
TABLE OF CONTENTS	2
STUDY DIRECTOR AUTHENTICATION AND GLP COMPLIANCE STATEMENT	4
RESPONSIBLE PERSONNEL	5
SCIENTIFIC REVIEW	5
ARCHIVE STATEMENT	5
QUALITY ASSURANCE RECORD AND AUTHENTICATION STATEMENT	6
1 SUMMARY	7
2 INTRODUCTION	8
3 MATERIALS AND METHODS	9
3.1 Protocol adherence	9
3.2 Test article and vehicle	9
3.3 Experimental design	9
3.4 Main study	11
3.5 Test article formulation	13
3.6 Test system	13
3.7 Animal health and welfare	14
3.8 Identification of the test system	14
3.9 Experimental observations	14
3.10 Terminal procedures	15
3.11 Data interpretation	15

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## TABLE OF CONTENTS (continued)

	Page
<b>4 RESULTS</b>	17
4.1 Screening studies	17
4.2 Clinical observations	17
4.3 Body weights	17
4.4 Induction reactions	17
4.5 Challenge response	18
<b>5 CONCLUSION</b>	18
<b>6 REFERENCES</b>	19
<b>TABLES</b>	20
1 First screening study	20
2 Second screening study	21
3 Third screening study	21
4 Summary of induction phase dermal reactions	22
5 Dermal reactions observed after challenge - controls	23
6 Dermal reactions observed after challenge - test	24
7 Body weights	26
<b>APPENDICES</b>	27
1 Positive control data	27
2 Deviations from protocol	28

**STUDY DIRECTOR AUTHENTICATION  
AND GLP COMPLIANCE STATEMENT**

**2,2-Dimethoxy ethanal 60% (water solution)  
Skin Sensitisation Study in the Guinea Pig**

I, the undersigned, hereby declare that the work described in this report was performed under my supervision, as Study Director, and that the report provides a true and accurate record of the results obtained.

The study was performed in accordance with the agreed protocol, unless otherwise stated, and the study objectives were achieved. The study was also performed in accordance with Corning Hazleton (Europe) Standard Operating Procedures and the principles of the following codes of Good Laboratory Practice:

UK Principles of Good Laboratory Practice  
The UK Compliance Programme  
Department of Health  
London 1989

OECD  
Good Laboratory Practice in the Testing of Chemicals  
Final Report ISBN 92-64-12367-9  
Paris 1982

United States Environmental Protection Agency  
Title 40 Code of Federal Regulations part 792  
Toxic Substances Control Act  
Good Laboratory Practice Standards  
Issued 29 November 1983 Federal Register, plus subsequent amendments



S M Denton BSc CBiol MIBiol  
Study Director

Date: 16 November 1985

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**RESPONSIBLE PERSONNEL**

**2,2-Dimethoxy ethanal 60% (water solution)  
Skin Sensitisation Study in the Guinea Pig**

The following staff were responsible for key elements of the study:

- Animal house supervisor : Dave Moss
- Animal health and welfare : Anthony Basford
- Formulation : Chris Wood

**SCIENTIFIC REVIEW**

I, the undersigned, hereby declare that I have reviewed this report in conjunction with the Study Director and that the interpretation and presentation of the data in the report are consistent with the results obtained.



J R Gardner BSc CBiol MIBiol

Date:

13 November 1995

**ARCHIVE STATEMENT**

All primary data, or authenticated copies thereof, specimens and the final report will be retained in the Corning Hazleton (Europe) archives for three years after the submission of the final report. At that time the Sponsor will be contacted to determine whether data should be returned, retained or destroyed on their behalf.

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*CHE Study Number 1433/4*

**QUALITY ASSURANCE RECORD  
AND AUTHENTICATION STATEMENT**

**2,2-Dimethoxy ethanal 60% (water solution)  
Skin Sensitisation Study in the Guinea Pig**

The study described in this report was subject to audit by the independent Quality Assurance Unit as indicated below. The findings of each audit were reported to the Study Director and management as prescribed by Standard Operating Procedures.

The report audit was designed to confirm that as far as can be reasonably established the methods described and results incorporated in the report accurately reflect the raw data produced during the study.

<b>Inspection programme</b>	<b>Inspection date</b>	<b>Report date</b>
Protocol review	8 August 1995	8 August 1995
Procedure inspection	5 September 1995	5 September 1995
Data review	8 November 1995	8 November 1995
Draft study report	8 November 1995	8 November 1995

*Gaul wood*

**G Wood  
Section Head Quality Assurance**

**Date: 16 November 1995**

## 1 SUMMARY

This study was conducted to assess the potential of 2,2-dimethoxy ethanal to elicit skin sensitisation (delayed contact hypersensitivity) in the guinea pig. The method followed was based on the procedures developed by Magnusson and Kligman (1,2,3) and was in compliance with that described in US EPA TSCA Health Effects Testing Guidelines, Section 798.4100; Annex to Commission Directive 92/69/EC, Method B6 and OECD Guidelines for Testing of Chemicals, Method 406 (4,5).

Based on the results of the preliminary screening studies, the following dose levels were chosen:

Intradermal injection:	20% m/v in distilled water and/or adjuvant
Topical induction:	undiluted liquid test article
Challenge application:	undiluted test article and 50% m/v in distilled water

Twenty test and ten control animals were used in this study.

2,2-Dimethoxy ethanal elicited a positive response, indicative of skin sensitisation (delayed contact hypersensitivity) in thirteen of the test animals following the challenge application. An inconclusive response was seen in a further six animals and a negative result in the remaining test animal.

Based on the results of this study, the General Classification and Labelling Requirements for Dangerous Substances and Preparations, as stated in Annex IV to Commission Directive 93/21/EC (6) indicate that the risk phrase R43 "May cause sensitisation by skin contact" is required for 2,2-dimethoxy ethanal in respect of its skin sensitisation potential.

## 2 INTRODUCTION

The study was designed to assess the skin sensitisation potential of 2,2-dimethoxy ethanal. The study objective was to assess the potential of the test article to elicit delayed contact hypersensitivity in guinea pigs using methods based on those first developed by Magnusson and Kligman. Determination of the sensitisation potential of a particular chemical is among the first investigations carried out to assess the hazard it presents to manufacturer and consumer populations, providing information on likely consequences to man of exposure to the chemical during handling or use and is a common basis for classification and labelling.

The study was conducted in compliance with US EPA TSCA Health Effects Testing Guidelines, Section 798.4100; Annex to Commission Directive 92/69/EC, Method B6 and OECD Guidelines for Testing of Chemicals, Section 4, Method No 406.

EC and OECD guidelines for the Magnusson & Kligman test method specify use of the albino guinea pig as the only appropriate species. The strain of guinea pig selected for this study has been subject to regular (six monthly) testing to confirm its susceptibility to a moderately strong skin sensitiser 2-mercaptobenzothiazole.

The dose levels for the main study were selected on the basis of screening studies conducted in compliance with the guidelines. The maximum practical concentrations that could be achieved in the optimum vehicle for intradermal injection or topical administration were determined prior to conducting the screening studies.

The protocol was approved by the Study Director and CHE Management on 31 July 1995 and by the Sponsor on 1 August 1995. A protocol amendment was issued on 9 October 1995 and countersigned by the client on 19 October 1995. The study was undertaken between 23 August and 29 September 1995. The study completion date is considered to be the date on which the Study Director signs the final report. All phases of the study were completed at Corning Hazleton (Europe), Otley Road, Harrogate, North Yorkshire, England.

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### 3 MATERIALS AND METHODS

#### 3.1 Protocol adherence

The study was conducted in accordance with the agreed protocol and one amendment. Deviations from the protocol are detailed in Appendix 2.

#### 3.2 Test article and vehicle

##### 3.2.1 Description, identification and storage

The test article was identified as 60% m/m 2,2-dimethoxy ethanal in water. A consignment of 3 kg of the test article, batch number D43221, was received at Corning Hazleton (Europe), Harrogate [CHE] on 28 July 1995 and was given the CHE Dispensary number 0628/95-1433.

The test article was a yellow liquid. The sponsor advised that it had an active component purity of 60% w/w in aqueous solution and an expiry date of 1 December 1995. The identity, stability, purity and composition or other defining characteristics of the test article remained the responsibility of the sponsor. When not in use the test article was stored in a refrigerator.

The vehicle used in the induction phase for intradermal injection was distilled water. Freund's Complete Adjuvant (FCA) emulsion was prepared for injection by mixing approximately equal volumes of water and FCA.

The vehicle used in the challenge phase was also distilled water. The undiluted test article was administered in the topical induction phase.

Test concentrations are expressed in this report in terms of the test article as supplied, no correction for the active component has been included.

#### 3.3 Experimental design

##### 3.3.1 Screening test for intradermal injection phase of induction

The vehicle and six formulations were selected. The dorsum of two guinea pigs were clipped on the day prior to dosing. On Day 1, intradermal injections (0.1 mL per site), incorporating a range of concentrations from 0.1% m/v to the maximum practical concentration, 20 % m/v test article in distilled water, were made into the scapular zone of the denuded

dorsum. Dermal reactions were assessed and recorded individually approximately 24 and 72 hours later.

### 3.3.2 Screening test for topical application phase of induction

Three formulations incorporating the test article at concentrations from 40% up to 80% m/v test article in distilled water were selected together with the undiluted test article as supplied. Two guinea pigs were prepared by receiving two 0.1 mL intradermal injections of FCA emulsion into the suprascapular dorsum at least five days prior to application of the test formulation. The dorsum and flanks were clipped and shaved on the day before application of the test formulations. The animals were subject to occluded, topical application of four 20 x 20 mm patches of Whatman No 4 filter paper each saturated with approximately 0.2 mL of one of the test formulations. Occlusion was effected by covering the Whatman patches with successive layers of "Blenderm" adhesive dressing from 3M Co, Loughborough, aluminium foil and "Steroban" open-weave, elasticated bandage from Steroplast Ltd, Bredbury. The last layer completely enveloped the torso to ensure the patches remained secure. The dressings and bandages were removed approximately 48 hours after application and the location of each patch was marked with indelible ink. Dermal reactions were assessed approximately 24 and 96 hours after removal of the patches.

### 3.3.3 Screening test for topical application at challenge

Four formulations were chosen to identify the maximum non-irritant concentration of test article after occluded, topical application to skin. Two guinea pigs were prepared by receiving two 0.1 mL intradermal injections of FCA emulsion into the suprascapular dorsum at least fourteen days prior to application of the test formulations. The flanks were clipped on the day before application. The same areas were shaved approximately two hours before treatment. Each animal was subject to occluded topical application of four 12 mm Finn chambers from Biodiagnostics Ltd, Upton-upon-Severn, each loaded with approximately 0.1 mL of one of the four selected test formulations. The Finn chambers were secured by successive applications of Blenderm and Steroban. The dressings and chambers were removed after 24 hours and the treated areas of skin were washed with water. The location of each challenge site was marked on the skin using indelible ink. The treated areas of skin were reshaved 21 hours after removal of the chambers. Dermal reactions were assessed 24 and 48 hours after removal of the chambers.

### 3.3.4 Criteria for selection of main study treatment regime

Treatment should not cause systemic toxic effects.

The highest concentration of test article that caused no more than moderate irritation (Grade 3 erythema) and no more than minute central foci of necrosis or other marked tissue damage at sites of injection in the first screen was selected for the intradermal injection phase of induction.

The highest concentration of test article that caused no more than moderate irritation (Grade 3 erythema) and no indication of necrosis or other marked tissue change at the site of application in the second screen was selected for the topical application phase of induction.

The highest concentration of test article that caused no skin irritation in the third screen was selected for challenge.

## 3.4 Main study

The main study consisted of a test group of twenty female guinea pigs and a control group of ten females. Each animal was weighed on Day 1, before induction commenced; with one exception (No 395 = 502g) initial body weights were less than 500g.

### 3.4.1 Induction phase - intradermal injection

The dorsum of each test and control guinea pig was clipped on the day before treatment commenced. The denuded area was confirmed to be free from injury or irritation. On Day 1 single, straight rows of three intradermal injections (0.1 mL per site) were placed parallel to and on either side of the dorsal mid-line of each guinea pig. The anterior and posterior injection sites marked the corners of a 20 x 40 mm area of the clipped dorsum overlying the scapulae. The middle injection sites were positioned close to the anterior sites.

The concentration of test article selected after the first screen was administered as follows:

<u>Site</u>	<u>Test group</u>	<u>Control group</u>
Anterior	FCA emulsion	FCA emulsion
Middle	Test article 20% m/v in distilled water	distilled water
Posterior	Test article 20% m/v in FCA emulsion	50% v/v distilled water in FCA emulsion

Irritation or other dermal changes at the injection site were recorded by group on Day 2.

### 3.4.2 Induction phase - topical application

On Day 7 the areas of dorsum denuded for the first phase of induction were clipped. On Day 8 the dorsum of all animals were shaved. At least two hours later the area of skin including the intradermal injection sites was subject to application of a 25 x 45 mm patch of Whatman No 4 filter paper loaded with approximately 0.5 mL of undiluted test article as supplied (test animals) or distilled water alone (control group). Occlusion of the treated skin was effected by successive layers of Blenderm, aluminium foil and Steroban. The patches and dressings remained in place for 48 hours. Irritation or other dermal changes at the sites of occluded topical application were recorded by group on Day 11.

### 3.4.3 Challenge phase

On Day 21 both flanks of all guinea pigs were clipped. On Day 22 the denuded flanks were shaved. At least two hours later the left flank of each animal was subject to application of a 12 mm Finn chamber loaded with approximately 0.1 mL vehicle. Finn chambers containing the formulation selected for challenge, undiluted test article and a second, lower concentration, 50% m/v test article in distilled water, were applied to the right flank. The chambers were kept in place by successive layers of Blenderm and Steroban. The chambers and dressings were removed 24 hours after application and the treated areas of skin were washed with water. The location of the challenge sites were marked with indelible ink immediately after the washing procedure. The challenge sites were reshaved

approximately 23 hours after removal of the chambers. Dermal responses to challenge were assessed 24 and 48 hours after removal of the chambers. Responses to challenge were recorded individually.

### 3.5 Test article formulation

Concentrations were expressed gravimetrically and in terms of test article received (without regard to purity or active content). All formulations were used on the day of preparation.

The test article was prepared at various concentrations in CA emulsion or distilled water as indicated under 3.4 Main Study.

### 3.6 Test system

#### 3.6.1 Species, strain and supplier

Male and/or nulliparous, non-pregnant female Dunkin-Hartley guinea pigs were obtained from D Hall Ltd, Burton on Trent on 10 and 31 July and 14 August 1995 for the preliminary study and on 14 August 1995 for the main study.

The condition of the animals was assessed daily throughout an acclimatisation period of at least nine days. A clinical inspection was performed prior to study commencement to ensure the animals were suitable for the study. Overtly healthy animals were arbitrarily allocated to the study groups on the day prior to commencement of treatment.

Animals in the main study were in a body weight range of 307 to 502 g prior to dosing on Day 1. Based on information from the supplier the guinea pigs were six to eight weeks old on Day 1.

#### 3.6.2 Caging

Up to five guinea pigs were accommodated in suspended polypropylene cages (six per battery) with open tops and stainless steel mesh front panels providing minimum internal dimensions of 61 x 81 x 25 cm. The cage had a solid floor which was relined with a whitewood bedding, Grade 10 Woodflakes from Datesand Ltd, Brooklands, three times each week. Each batch of bedding had been analysed for specific constituents.

### 3.6.3 Diet and water

SQC FD1 (pelleted) diet from Special Diets Services Ltd., Witham was freely available to the animals at all times. Each batch of diet has been analysed for specific constituents.

Mains water was provided, *ad libitum*, via cage mounted water bottles. The water is periodically analysed for specific contaminants. No contaminants were expected to be present in diet or water at levels which might interfere with achieving the objective of the study.

### 3.6.4 Environment

The animal room used during the acclimatisation and observation periods was designed to permit at least 10 air changes per hour and to maintain environmental conditions of 16 to 22°C. Humidity was not actively controlled but was expected to remain within the range 40-80% RH. Recordings of maximum and minimum temperature and humidity were made twice daily. The rooms were illuminated by fluorescent strip-lights for fourteen hours daily.

## 3.7 Animal health and welfare

All procedures carried out on five animals as part of this study were subject to the provisions of United Kingdom National Law, in particular the Animals (Scientific Procedures) Act, 1986.

## 3.8 Identification of the test system

The guinea pigs were individually identified by a number tattooed on the ear. A colour coded card on each cage gave information including study number, cage number, animal numbers and sex.

## 3.9 Experimental observations

### 3.9.1 Clinical signs

Test and control guinea pigs were observed daily for clinical signs of reaction to treatment.

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### 3.9.2 Body weights

The guinea pigs were weighed on Day 1 (day of dosing) and Day 25 following completion of the challenge phase.

### 3.9.3 Dermal reactions

Changes at sites of intradermal injection or occluded topical application were made in a well-illuminated area. Dermal changes were recorded using the following scheme:

Dermal change	Record
No erythema	0
Slight erythema	1
Well defined erythema	2
Moderate erythema	3
Severe erythema	4
In-depth damage/eschar	E
Desquamation	Q
Discoloration	D

Any other lesions not covered by this scheme have been described in detail.

## 3.10 Terminal procedures

### 3.10.1 Necropsy

Guinea pigs were killed by intraperitoneal injection of sodium pentobarbitone following completion of the challenge procedure. No tissue preservation or histopathological assessment of tissues was undertaken.

## 3.11 Data interpretation

Dermal reactions to challenge among test animals were compared with those of the controls. The response of each test animal was classified as positive, negative or equivocal according to the following system:

A test animal developing a dermal response to challenge that was more marked or more persistent than the maximum reaction apparent among controls was considered sensitised to the test article (individual result - positive).

If the challenge response of a test animal was only marginally more marked or marginally more persistent than the maximum reaction among controls, this was considered to be equivocal evidence of sensitisation (individual result - equivocal).

A test animal developing no reaction to challenge, reactions that were the same as the maximum control response or reactions that were less marked or less sustained than the maximum reaction apparent among controls was considered not to have become sensitised to the test article (individual result - negative).

Where there was evidence of a reaction to the vehicle in either test or control animals, assessment of the challenge responses to the test article among test animals took account of the vehicle effect.

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## 4 RESULTS

### 4.1 Screening studies

Results for the three screening studies are presented in Tables 1, 2 and 3.

### 4.2 Clinical observations

No clinical observations of ill health or toxicity were noted during the study.

### 4.3 Body weights (Table 7)

Body weight increases were recorded for all animals on Day 25 in comparison with Day -1.

### 4.4 Induction reactions

A summary of reactions seen in the induction phase is presented in Table 4.

#### 4.4.1 Intradermal injection

Slight erythema was noted at both anterior and posterior injection sites (treated with Freund's Complete Adjuvant) for both test and control animals.

Dark foci with associated necrotic tissue was observed at the middle injection sites in test animals receiving 20% m/v test article in water and also at the posterior site injected with 20% m/v test article in FCA emulsion.

No erythema was observed at the middle injection site in control animals receiving water alone.

#### 4.4.2 Topical application

Slight erythema, desquamation and yellow discoloration of the dose site was apparent for the test group animals following application of the undiluted test article, as supplied.

Slight erythema was apparent at the topical application sites in the control guinea pigs.

#### 4.5 Challenge response

Individual test and control reactions are presented in Tables 5 and 6. The dermal reactions in thirteen test animals were greater than in the controls. Generally for these animals well defined erythema was apparent within 24 hours of bandage removal and commonly accompanied by eschar and in one animal fissuring of the dermis and necrosis at the site of application. In the six animals whose response was considered to be inconclusive, slight erythema was present at the application site of the undiluted test article at the 24 hour assessment but had resolved by the 48 hour assessment.

### 5 CONCLUSION

The results from the challenge procedure indicated that thirteen animals gave a positive response indicative of delayed contact hypersensitivity, the response for six animals was inconclusive and the result for the remaining guinea pig was negative.

Based on the results of this study, the General Classification and Labelling Requirements for Dangerous Substances and Preparations, as stated in Annex IV to Commission Directive 93/21/EC, indicate that the risk phrase R43 "May cause sensitisation by skin contact" is required for 2,2-dimethoxy ethanal in respect of its skin sensitisation potential.

**6 REFERENCES**

- [1] Magnusson, B and Kligman, A M (1969). The identification of contact allergens by animal assay. The Guinea-Pig Maximisation Test. *J Invest Dermatol*, 52, 268-276.
- [2] Magnusson, B, Fregert, S and Wahlberg, J (1979). Determination of skin sensitization potential of chemicals. Predictive testing in guinea pigs. *Arbete och Halsa*, 26(E), 1-31.
- [3] Wahlberg, J and Boman, A (1985). Guinea-Pig Maximisation Test. in *Curr Prob Derm*, Vol 14, 59-106.
- [4] Annex to Commission Directive 92/69/EC of 31 July 1992 adapting to technical progress for the seventeenth time Council Directive 67/548/EC. Method B6. *Official Journal of the European Communities* L383A, Vol 35, 29 December 1992.
- [5] OECD Guidelines for Testing of Chemicals, Section 4, Method No 406, adopted 17 July 1992. OECD Publications Office, Paris, France.
- [6] US Environmental Protection Agency, Toxic Substances Control Act, Health Effects Testing Guidelines, CFR 40 Section 798.4100 Dermal sensitisation. Revised July 1992.
- [7] Annex IV of Commission Directive 93/21/EC of 27 April 1993 adapting to technical progress for the eighteenth time Council Directive 67/548/EC. *Official Journal of the European Communities* L110A, Vol 36, 4 May 1993.

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TABLE 1

## Results of first screening study

Concentration % m/v	Animal number			
	315F		316F	
	24 hours	72 hours	24 hours	72 hours
20	2	1	1	1
10	2	1	0	0
6	1	0	0	0
3	0	0	0	0
1	0	0	0	0
0.1	0	0	0	0
Vehicle	0	0	0	0

## Key:

- 0 No erythema
- 1 Slight erythema
- 2 Well defined erythema

TABLE 2

Results of second screening study

Concentration % m/v	Animal number			
	273M		274M	
	24 hours	96 hours	24 hours	96 hours
100	1	0	1	0
80	0	0	0	0
60	0	0	0	0
40	0	0	0	0

Key:

- 0 No erythema
- 1 Slight erythema

TABLE

Results of third screening study

Concentration % m/v	Animal number			
	260F		261F	
	24 hours	48 hours	24 hours	48 hours
80	0	0	0	0
70	0	0	0	0
60	0	0	0	0
50	0	0	0	0

Key:

- 0 No erythema

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TABLE 4

Summary of induction phase dermal reactions

Site	Intradermal injection		Topical application	
	Test animals	Control animals	Test animals	Control animals
Anterior	Slight erythema	Slight erythema	Slight erythema with desquamation and yellow discoloration	Slight erythema
Middle	N+	No erythema		
Posterior	N+ Slight erythema	Slight erythema		

N+ Dark foci present at injection site, surrounding tissue appeared necrotic

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TABLE 5

## Dermal reactions observed after challenge

## Controls

Guinea pig number and sex	Score					
	24 hours			48 hours		
	A	P	C	A	P	C
370F	0	0	0	0	0	0
371F	0	0	0	0	0	0
372F	0	0	0	0	0	0
373F	0	0	0	0	0	0
374F	0	0	0	0	0	0
375F	0	0	0	0	0	0
376F	0	0	0	0	0	0
377F	0	0	0	Q	0	0
378F	0	0	0	0	0	0
379F	0	0	0	0	0	0

## Key:

- A Anterior site exposed to undiluted test article  
 P Posterior site exposed to 50% m/v test article in distilled water  
 C Control site exposed to distilled water
- 0 No erythema  
 Q Desquamation

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TABLE 6

## Dermal reactions observed after challenge

Test

Guinea pig number	Score						Result Positive[+] Inconclusive [±] Negative [-]
	24 hours			48 hours			
	A	P	C	A	P	C	
380F	0	0	0	Q	0	0	-
381F	1	1	0	Q	Q	0	±
382F	1	0	0	Q	Q	0	±
383F	1	0	0	Q	0	0	±
384F	1	0	0	Q	0	0	±
385F	Q2	1	0	Q	Q	0	+
386F	1	0	0	Q	Q	0	±
387F	Q2	2	0	Q2	Q1	0	+
388F	1	0	0	Q	0	0	±
389F	2	2	0	Q1	Q1	0	+

Key:

- A Anterior site exposed to undiluted test article
- P Posterior site exposed to 50% m/v test article in distilled water
- C Control site exposed to distilled water
  
- 0 No erythema
- 1 Slight erythema
- 2 Well defined erythema
- Q Desquamation

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TABLE 6

## Dermal reactions observed after challenge

Test

Guinea pig number	Score						Result Positive [ + ] Inconclusive [ ± ] Negative [ - ]
	24 hours			48 hours			
	A	P	C	A	P	C	
390F	Q2	Q2	0	Q1	Q	0	+
391F	2FN	0	0	QE	Q		+
392F	Q1	Q1	0	Q2	Q1	0	+
393F	Q2	1	0	QE	Q	0	+
394F	Q2	Q2	0	Q2	Q2	0	+
395F	2	0	0	Q	0	0	+
396F	Q2	Q1	0	Q	Q	0	+
397F	2	2E	0		QE	0	+
398F	Q2	Q1	0	QE	Q	0	+
399F	2E	2E	0	QE	QE	0	+

Key:

- A Anterior site exposed to undiluted test article  
 P Posterior site exposed to 50% m/v test article in distilled water  
 C Control site exposed to distilled water

- 0 No erythema  
 1 Slight erythema  
 2 Well defined erythema  
 E In depth eschar  
 Q Desquamation  
 N Necrosis  
 F Fissuring

TABLE 7

## Body weights

## Controls

Guinea pig number	Day 1 5 September 1995	Day 25 29 September 1995
370	474	646
371	487	670
372	450	590
373	468	634
374	446	614
375	415	557
376	442	627
377	418	576
378	430	533
379	395	525

## Test

Guinea pig number	Day 1 5 September 1995	Day 25 29 September 1995
380	534	604
381	472	574
382	446	640
383	459	638
384	408	563
385	307	446
386	333	527
387	330	511
388	345	558
389	385	589
390	434	626
391	413	625
392	435	615
393	387	580
394	407	580
395	502	643
396	462	606
397	475	599
398	471	620
399	450	617

APPENDIX 1

Summary of positive control Magnusson and Kligman studies using 2-mercaptobenzothiazole (MBT)

M&K positive control study number	No. of animals used (♀)		Study dates		Date of receipt of MBT	Concentrations (% m/m) in Alembicol D		Results			
	Test	Control	Start	Finish		Induction phase	Challenge phase	Positive	Inconclusive	Negative	
0000/137	10	5	25.07.94	02.09.94	16 June 1994	Intradermal 2.5	Topical 60	50 and 25	8/8*	0/8	0/8
0000/153	10	5	31.01.95	24.02.95	16 June 1994	2.5	60	30 and 15	10/10	0/10	0/10
0000/175	10	5	18.07.95	11.08.95	17 October 1994	2.5	60	30 and 15	8/10	1/10	1/10

Animals supplied by D. Hall, Newchurch, Staffordshire, England  
 MBT supplied by Sigma Chemical Co

\* One animal killed on humane grounds, in one animal the test sites could not be determined on bandage removal

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APPENDIX 2

Deviations from protocol

The animal holding room was expected to maintain temperature and humidity in the ranges 16° to 22°C and 40% to 80% RH. On two occasions during the study the maximum temperature exceeded the expected range by 1°C. These environmental conditions had no overt effect upon the study animals and were not considered to have compromised the study integrity.

Only two suitably prepared animals were available for the third screening study.

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