8EHQ-0602-15155S
MR 59549

8EHQ-02-15155
88020000 14/51

June 11, 2002

TSCA Document Processing Center
U.S. EPA 7407 M
Ariel Rios Building
EPA East Bldg, Room 6428
1200 Pennsylvania Avenue NW
Washington, DC 20460-0001
Attn: Section 8(e) Submission

Re: TSCA § 8(e) Submission

Dear Sir or Madame:

On behalf of [ ] is submitting a TSCA § 8(e) submission describing the results of an assessment of a skin sensitization study performed on the guinea pig on one of its products, [ ].

This submission asserts confidential business information claims. Accordingly, the substantiation of confidentiality claims is also enclosed.

Any questions regarding this submission should be directed to [ ].

Sincerely

[ ]
[ ]

Enclosures

June 10, 2002

TSCA Document Processing Center
U.S. EPA 7407 M
Ariel Rios Building
EPA East Bldg, Room 6428
1200 Pennsylvania Avenue NW
Washington, DC 20460-0001
Attn: Section 8(e) Submission

Re: TSCA § 8(e) Submission

Dear Sir or Madame:

Pursuant to EPA's TSCA § 8(e) policy for the reporting of substantial risk information, [ ] is submitting the results of an assessment of a skin sensitization study performed on the guinea pig on [ ]. The study, which was received by [ ] on May 23, 2002, is enclosed.

The study concluded that the chemical substance caused skin sensitization in all ten test animals.

The [ ] is a textile chemical formulation used as a soil repellent whose components are:

A) [ ]
B) [ ]
C) [ ]
D) [ ]
E) [ ]
F) [ ]
G) [ ]
H) [ ]
I) [ ]

[ ]

This submission contains confidential business information.

Any questions regarding this submission should be directed to [ ].

Sincerely

[ ]

[ ]
[ ]

Enclosure

CBI CLAIMS MADE UNDER TSCA 8(E)

Substantiating Claims of Confidentiality

1. Is your company asserting this confidential business information (CBI) claim on its own behalf? If the answer is no, please provide company name, address and telephone number of entity asserting claim.

   >[ ]is asserting CBI claims on its own behalf.

2. For what period do you assert four claim(s) of confidentiality? If the claim is to extend until a certain event or point in time, please indicate that event or time period. Explain why the information should remain confidential until such point.

   >[ ]asserts the CBI claims indefinitely. The product formulation information is an extremely valuable competitive asset that would be lost upon releasing CBI claims at any time. Divulgence of this information would cause[ ]to lose competitive advantages by allowing competitors to copy the successful formulations.

3. Has the information that you are claiming as confidential been disclosed to any other governmental agency, or to this agency at any other time? Identify the agency to which the information was disclosed and provide the date and circumstance of the same. Was the disclosure accompanied by a claim of confidentiality? If yes, attach a copy of said document reflecting the confidentiality agreement.

   > No.

4. Briefly describe any physical or procedural restrictions within you company relating to the use and storage of the information you are claiming CBI.

   > R&D and regulatory compliance personnel tightly manage the product formulation information. The information is stamped confidential and kept in locked cabinets and access to this information is severely limited.

5. If anyone outside your company has access to any of the information claimed as CBI, are they restricted by confidentiality agreement(s)? If so, explain the content of the agreement(s).

   >[ ]consultants and lawyers have access to this information after signing confidentiality agreements with[ ]The agreements state that this information cannot be disseminated to outside parties and must be maintained confidential.

6. Does the information claimed as confidential appear or is it referred to in any of the following:

   (a) Advertising or promotional materials for the chemicals substance or the resulting end product;

   >The formulation is not divulged in these types of documents. Only the product name appears.

   (b) Material safety data sheets or other similar materials (such as technical data sheets) for the substance or resulting end product (include copies of this information as it appears when accompanying the substance and/or product at the time of transfer or sale);

   > The formulation is not divulged in the MSDS. Only the product name appears.

   (c) Professional or trade publications; or

   > The formulation is not divulged in these types of documents. Only the product name appears.

   (d) Any other media or publications available to the public or to your competitors.

   >The formulation is not divulged in these types of documents. Only the product name appears.

   If you answered yes to any of the above, indicate where the information appears, include copies, and explain why it should nonetheless be treated as confidential.

7. Has EPA, another Federal Agency, or court made any confidentiality determinations regarding information associated with this chemical substance? If so, provide copies of such determinations.

   >No.

8. Describe the substantial harmful effects that would result to your competitive position if the CBI information is made available to the public. In your answer, explain the causal relationship between disclosure and any resulting harmful effects. Consider in your answer such constraints as capital and marketing cost, specialized technical expertise, or unusual processes and your competitors' access to customers. Address each piece of information claimed CBI separately.

>[ ]would suffer losses in market share after competitors learned of the product name and the product formulation. Competitors would be able to replicate the formulations and offer them commercially. The substantial investment in developing the formulation technology would also be lost. The competitors in the textile soil repellent business are limited, so with[ ]technology in hand, could easily entice customers away from[ ]

9. Has the substance been patented in the U.S. or elsewhere? If a patent for the substance currently pending?

   > No. No patent is pending.

10. Is this substance/product commercially available and if so, for how long has it been available on the commercial market?

    (a) If on the commercial market, are your competitors aware that the substance is commercially available in the U.S.?

    >Yes, for 3 years.

    (b) If not already commercially available, describe what stage of research and development (R&D) the substance is in, and estimate how soon a market will be established.

    >Not applicable.

    (c) What is the substance used for and what type of products(s) does it appear in?

    >The formulated product is offered as a water/oil repellent agent.

11. Describe whether a competitor could employ reverse engineering to identically recreate the substance.

    > A sophisticated competitor with modern analytical tools could reverse engineer the formulation.

12. Do you assert that disclosure of this information you are claiming CBI would reveal:

    (a) Confidential processes used in the manufacturing the substance;

    >No.

    (b) If a mixture, the actual portions of the substance in the mixture; or

>Yes.

(c) Information unrelated to the effects of the substance on human health or the environment?

>Yes.

If your answer to any of the above questions is yes, explain how such information would be revealed.

> The product name and detailed product formulation are revealed in the cover letter so that EPA can evaluate and assess all of the formulation ingredients with respect to the reported health effect.

13. Provide the Chemical Abstract Registry Number for the product, if known. Is you company applying for a CAS number now or in the near future? If you have applied for a CAS number, include a copy of the contract with CAS.

>This information is disclosed in the cover letter.

14. Is the substance or any information claimed CBI the subject of FIFRA regulation or reporting? If so, explain.

>No.

[ ]

[ ]

SKIN SENSITIZATION TO THE GUINEA-PIG

(MAGNUSSON & KLIGMAN METHOD)

Sponsor

[ ]

Research Laboratory

Huntingdon Life Sciences Limited
Woolley Road
Alconbury
Huntingdon
Cambridgeshire
PE28 4HS
ENGLAND

Report issued 3 July 2001

[ ]

CONTENTS

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 Regulations 1999 (Statutory Instrument No 3106).

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.

Information regarding test substance characterisation, namely purity/composition, was not made available to Huntingdon Life Sciences as required for compliance with Good Laboratory Practice Standards given above.

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.

3 July 2001
Date

QUALITY ASSURANCE STATEMENT

The following inspections and audits have been carried out in relation to this study

Study Phase	Date of Inspection	Date of Reporting
Protocol Audit	13 March 2001	13 March 2001
Process Based Inspections		
Housing/Environment	2 April 2001	6 April 2001
Husbandry	2 April 2001	6 April 2001
Intradermal injections	3 April 2001	6 April 2001
Topical induction	3 April 2001	6 April 2001
Challenge application	3 April 2001	6 April 2001
Scoring (ID topical)	4 April 2001	6 April 2001
Study Documentation	5 April 2001	6 April 2001
Records Maintenance	6 April 2001	6 April 2001
Report Audit	28 June 2001	28 June 2001

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.

.....

Andrew Gilbert,
Group Manager,
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.

.....

Date

CONTRIBUTING SCIENTIST

David G. Coleman B.Sc. (Hons.),
Study Director,
Short Term Studies Group,
Division of Toxicology.

[ ]

SUMMARY

This study was performed to assess the skin sensitization potential of [ ] 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.

EPA Health Effects Test Guidelines OPPTS 870.2600 "Skin Sensitization" EPA 712-C-98-197. August 1998.

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 guidelines and method.

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

Intradermal injection:	5% v/v in water for irrigation
Topical application:	As supplied
Challenge application:	As supplied and 50% v/v in water for irrigation

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

In this study [ ] produced evidence of skin sensitization (delayed contact hypersensitivity) in all of the ten test animals. [ ] is considered to have the potential to cause skin sensitization.

As all of the animals gave positive responses, [ ] requires 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 [ ] using the guinea-pig and was performed because the test substance may come into contact with skin during handling or use.

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 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.
>
> EPA Health Effects Test Guidelines OPPTS 870.2600 "Skin Sensitization" EPA 712-C-98-197. August 1998.

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 for the main study.

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 guidelines.

The protocol was approved by Huntingdon Life Sciences Management on 7 February 2001, by the Sponsor on 15 February 2001 and by the Study Director on 12 March 2001.

The experimental phase of the study was undertaken between 19 March and 20 April 2001.

[ ]

TEST SUBSTANCE

Identity:	[ ]
Chemical name:	[ ]
Intended use:	Water and oil repellent agent
Appearance:	Pale yellow emulsion
Storage conditions:	Room temperature
Lot number:	T472L04006
Expiry:	September 2001
Purity/composition:	Not advised
Date received:	5 February 2001

EXPERIMENTAL PROCEDURE

ANIMAL MANAGEMENT

Fifteen 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 main study guinea-pigs were within the weight range 371 - 449 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	180 to 184
Test animals	10	185 to 194

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*.

For environmental enrichment, autoclaved hay was given to the guinea-pigs three times weekly at irregular intervals and plastic tubular pipes were included in the cage. These procedures, which alleviate boredom and stereotype behaviours are standard practice at this laboratory and are not considered to have any influence on test results interpretation.

The batch(es) of diet used for the study was analysed by the supplier 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 are lodged in Huntingdon Life Sciences Ltd. 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 environmental controls were set to maintain temperature within the range 21 ± 3°C and relative humidity within 30 to 70%. Any minor deviations from these ranges would not have had an adverse effect on the animals and would not affect the integrity or validity of the study. These environmental parameters were continuously recorded and the permanent record archived with other departmental raw data. Lighting was controlled by means of a time switch to give 12 hours of artificial light (0600 - 1800 hours GMT) in each 24 hour period.

Each animal was identified by ear tattoo number. This number was unique within the Huntingdon Life Sciences Short Term Studies Group 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 hexyl cinnamic aldehyde (HCA) – a known moderate sensitizer. The results of the most recent test are presented in Appendix 3.

TEST SUBSTANCE PREPARATION

A vehicle trial conducted with [ ] showed that it formed a clear white opaque liquid in water for irrigation. The maximum practical concentration for intradermal and topical dosing were as supplied.

The test substance was prepared prior to each application on the day of dosing in water for irrigation. 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) the minimum irritant test substance concentrations suitable for the induction phase of the main study and (b) a maximum non-irritant concentration by the topical route of administration and a dilution of this 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 procedure employed for these investigations was as follows:

---

[1] *Also known as sterile water*

Intradermal injections - Intradermal injections (0.1 ml/site) were made into the clipped and shaved flank of two guinea-pigs, using a range of concentrations (0.1 to 10% v/v) of [ ] in water for irrigation. The resulting dermal responses were assessed approximately 24 and 72 hours later.

Topical application - Patches of Whatman No. 3 paper (2 cm x 2 cm) were saturated (volume approximately 0.2 ml per patch) with a range of concentrations (25% v/v to as supplied) of [ ] in water for irrigation and applied to the clipped and shaved flanks of each of four guinea-pigs. The patches were covered by a strip of "Blenderm" and firmly secured by "Elastoplast" wound round the trunk and fixed with an impervious plastic adhesive tape. The dressings were removed after an exposure period of approximately 24 hours and the reaction sites were assessed for erythema and oedema. Further examination of the sites was carried out approximately 24 and 48 hours after removal of the dressings.

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

Selection of concentrations of test substance for the main study

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

Induction intradermal injection - 5% v/v in water for irrigation

This was the highest concentration that caused irritation but did not cause necrosis or give signs of toxicity.

Induction topical application - As supplied

Topical challenge - As supplied and 50% v/v in water for irrigation

From preliminary investigations the neat material applied topically did not give rise to irritating effects.

Main study

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

Induction

Induction intradermal injections - test animals

A 4 × 6 cm area of dorsal skin on the scapular region of the guinea-pig was clipped free of hair with electric clippers. On Day 1, three pairs of intradermal injections (0.1 ml/site) were made into a 2 × 4 cm 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. [ ]5% v/v in water for irrigation.

3. [ ]5% v/v in a 50 : 50 mixture of Freund's Complete Adjuvant and water for irrigation.

Induction topical application - test animals

The preliminary investigations indicated that the neat material applied topically did not produce skin irritation. Therefore, the same 4 × 6 cm interscapular area was clipped and shaved free of hair on Day 6 and the site was pre-treated by gentle rubbing with 0.5 ml per site of 10% w/w sodium lauryl sulphate in petrolatum on Day 7. On Day 8, a 2 × 4 cm patch of Whatman No. 3 paper was saturated with approximately 0.4 ml of [ ], as supplied. The patch was placed over the injection sites on the skin of the interscapular region of the test animals and covered by a length of impermeable plastic adhesive tape (5 cm width "Blenderm"). This in turn was firmly secured by elastic adhesive bandage (5 cm width "Elastoplast") wound round the torso of the animal and fixed with an impervious plastic adhesive tape. The dressing was left in place for approximately 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 to the intradermal injections for control and test animals were recorded 24 hours following the injections and the reactions to the induction topical application were recorded on removal of the bandages.

Challenge

Challenge - control and test animals

The control and test animals were challenged topically two weeks after the topical induction application using [ ]as supplied and 50% v/v in water for irrigation.

Hair was removed by clipping and then shaving from an area on the left flank of each guinea-pig. A 2 × 2 cm patch of Whatman No. 3 paper was saturated with approximately 0.2 ml of [ ]as supplied and applied to an anterior site on the flank. [ ] [ ]50% v/v in water for irrigation was applied in a similar manner to the posterior site. The patches were sealed to the flank for 24 hours under strips of "Blenderm" (5 cm width) secured with "Elastoplast" (7.5 cm width) wound round the trunk and fixed with an impervious plastic adhesive tape.

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

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 following completion of the study prior to termination.

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
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to eschar formation preventing grading of erythema	4

Oedema formation:

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 and extending beyond the area of exposure)	4

Other lesions:

* Dryness and sloughing of the epidermis

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.

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 arising from the performance of this study at Huntingdon Life Sciences is the property of the Sponsor and is lodged together with a copy of the final report in the Huntingdon Life Sciences Archive.

Such records will be retained for a minimum period of five years from the date on which the Study Director signs the final report. At the end of the five year retention period the Sponsor will be contacted and advice sought on the return, disposal or further retention of the records.

Huntingdon Life Sciences will retain the Quality Assurance records relevant to this study and a copy of the final report in its archive indefinitely.

DEVIATIONS FROM PROTOCOL

There were no deviations from the protocol.

[ ]

RESULTS

MORTALITY AND CLINICAL SIGNS

There were no deaths and no signs of ill health or toxicity were observed on this study.

BODYWEIGHT (Appendix 1)

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

INDUCTION (Table 1)

Intradermal injections

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

No irritation was seen in test animals at sites receiving [ ]5% v/v in water for irrigation and no irritation was observed in control animals receiving water for irrigation.

Topical application

No erythema was observed in test animals following topical application with [ ] as supplied.

No erythema was seen in the control guinea-pigs.

CHALLENGE (Table 2)

Dermal reactions were noted for all test animals receiving the neat material and no dermal reactions were noted for test animals receiving 50% v/v in water for irrigation. As no reactions were observed for control animals the reactions seen in test animals at the site receiving the neat material are indicative of hypersensitivity and [ ]is considered to have the potential to cause skin sensitization.

[ ]

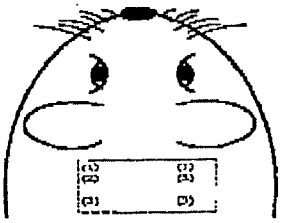
CONCLUSION

In this study [ ]produced evidence of skin sensitization (delayed contact hypersensitivity) in all of the ten test animals. [ ] is considered to have the potential to cause skin sensitization.

As all of the animals gave positive responses, [ ]requires labelling with the risk phrase R43 "May cause sensitization by skin contact" in accordance with Commission Directive 93/21/EEC.

FIGURE 1

Position of intradermal injections and topical induction application



A 4 × 6 cm area of dorsal skin on the scapular region of the guinea-pig was clipped free of hair and three pairs of intradermal injections were made into a 2 × 4 cm area within the clipped area as shown above. The topical induction application was made to the same 2 x 4 cm area 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 [ ] 5% v/v in water for irrigation.

(3) 0.1 ml of [ ] 5% 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			Topical application
		Site number			
		1	2	3	
Control	180	N	0	N	0
	181	N	0	N	0
	182	N	0	N	0
	183	N	0	N	0
	184	N	0	N	0
Test	185	N	0	N	0
	186	N	0	N	0
	187	N	0	N	0
	188	N	0	N	0
	189	N	0	N	0
	190	N	0	N	0
	191	N	0	N	0
	192	N	0	N	0
	193	N	0	N	0
	194	N	0	N	0

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: water for irrigation
Test animals: [ ] 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 [ ]

Freund's treated controls

Guinea-pig number	E = Erythema O = Oedema	Score			
		24 Hours		48 Hours	
		A	P	A	P
180	E	0	0	0	0
	O	0	0	0	0
181	E	0	0	0	0
	O	0	0	0	0
182	E	0	0	0	0
	O	0	0	0	0
183	E	0	0	0	0
	O	0	0	0	0
184	E	0	0	0	0
	O	0	0	0	0

A Anterior site, exposed to [ ] as supplied
P Posterior site, exposed to [ ] 50% v/v in water for irrigation

TABLE 2

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

Test animals

Guinea-pig number	E = Erythema O = Oedema	Score				Results Positive (+) Negative (-) Inconclusive (±)
		24 Hours		48 Hours		
		A	P	A	P	
185	E	2	0	2*	0	+
	O	1	0	1	0	
186	E	1	0	1	0	+
	O	1	0	1	0	
187	E	2	0	2*	0	+
	O	1	0	1	0	
188	E	2	0	2	0	+
	O	1	0	1	0	
189	E	1	0	1	0	+
	O	1	0	1	0	
190	E	2	0	2	0	+
	O	1	0	1	0	
191	E	2	0	2	0	+
	O	2	0	1	0	
192	E	2	0	2*	0	+
	O	2	0	1	0	
193	E	1	0	1	0	+
	O	0	0	0	0	
194	E	2	0	1	0	+
	O	1	0	1	0	

* Dryness and sloughing of the epidermis

A Anterior site, exposed to [ ] as supplied

P Posterior site, exposed to [ ] 50% v/v in water for irrigation

APPENDIX 1

Individual bodyweights (g)

Group	Guinea-pig number	Day 1	Pre-terminal
Control	180	434	735
	181	414	607
	182	416	607
	183	391	687
	184	401	686
Test	185	388	711
	186	449	697
	187	371	609
	188	388	652
	189	439	791
	190	401	640
	191	416	709
	192	399	671
	193	431	611
	194	411	589

APPENDIX 2

Results of preliminary investigations with [ ]

Intradermal injections

Vehicle: water for irrigation

Guinea-pig number	Concentration % v/v	Score		
		Hours	24	72
1054	10.0	D	6	5
		E	N	N
		O	2	2
	7.5	D	6	5
		E	N	N
		O	2	2
	5.0	D	0	0
		E	0	0
		O	0	0
	2.5	D	0	0
		E	0	0
		O	0	0
	1.0	D	0	0
		E	0	0
		O	0	0
	0.5	D	0	0
		E	0	0
		O	0	0
	0.25	D	0	0
		E	0	0
		O	0	0
	0.1	D	0	0
		E	0	0
		O	0	0
	Vehicle control	D	0	0
		E	0	0
		O	0	0

Guinea-pig number	Concentration % v/v	Score		
		Hours	24	72
1055	10.0	D	6	5
		E	N	N
		O	2	2
	7.5	D	5	5
		E	N	N
		O	1	2
	5.0	D	4	0
		E	1	0
		O	1	0
	2.5	D	0	0
		E	0	0
		O	0	0
	1.0	D	0	0
		E	0	0
		O	0	0
	0.5	D	0	0
		E	0	0
		O	0	0
	0.25	D	0	0
		E	0	0
		O	0	0
	0.1	D	0	0
		E	0	0
		O	0	0
	Vehicle control	D	0	0
		E	0	0
		O	0	0

Key:

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

APPENDIX 2

Results of preliminary investigations with [ ] (continued)

Topical application

Vehicle: water for irrigation

Guinea-pig number	Concentration % v/v	Score					
		0 Hours		24 Hours		48 Hours	
		E	O	E	O	E	O
1056	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
1057	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
1058	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
1059	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)

APPENDIX 3

Skin sensitization positive control study with hexyl cinnamic aldehyde (HCA) to the Magnusson & Kligman method (Sch. No. HLS/132)

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 9 January to 2 February 2001 using 15 guinea-pigs of the Dunkin Hartley strain supplied by D. Hall, Newchurch, Staffordshire, England.

Based on historical data, 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

Necrosis was recorded at all sites receiving Freund's Complete Adjuvant.

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 to moderate erythema was observed in test animals following topical application with HCA, as supplied. No erythema was seen in the control animals receiving a dry patch.

CHALLENGE

Slight to well-defined dermal reactions were observed for all of the ten test animals compared to no dermal reactions in the control animals. Therefore the reactions in the test animals represented hypersensitivity and all ten test animals gave positive sensitization responses.

CONCLUSION

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

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
30	E	0	0	0	0
	O	0	0	0	0
31	E	0	0	0	0
	O	0	0	0	0
32	E	0	0	0	0
	O	0	0	0	0
33	E	0	0	0	0
	O	0	0	0	0
34	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	
35	E	1	1	1*	0*	+
	O	1	0	1	0	
36	E	1	0	1*	0*	+
	O	0	0	0	0	
37	E	2	1	2*	1*	+
	O	1	0	1	0	
38	E	1	1	1*	0*	+
	O	1	0	0	0	
39	E	1	1	1*	0*	+
	O	0	0	0	0	
40	E	2	1	1*	1*	+
	O	1	1	1	0	
41	E	2*	1*	2*	1*	+
	O	1	1	1	1	
42	E	1	1	1*	0*	+
	O	1	0	1	0	
43	E	2*	1*	1*	1*	+
	O	1	1	1	0	
44	E	2	1	2*	1*	+
	O	1	0	1	0	

* 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