CONFIDENTIAL

SPONSOR
Elf Atochem SA
Cours Michelet
La Défense 10
92091 Paris-la-Défense CEDEX
France

TEST SUBSTANCE
[redacted]

STUDY TITLE
SKIN SENSITIZATION TEST
IN GUINEA-PIGS
(Maximization method of Magnusson and Kligman)

STUDY DIRECTOR
Xavier Manciaux

STUDY COMPLETION DATE
28 August 2000

TEST FACILITY
CIT
Centre International de Toxicologie
BP 563 - 27005 Evreux - France

LABORATORY STUDY NUMBER
[redacted]

Company Sanitized. Does not contain TSCA CBI

CONTENTS

STATEMENT OF THE STUDY DIRECTOR 4

OTHER SCIENTIST INVOLVED IN THIS STUDY 4

STATEMENT OF QUALITY ASSURANCE UNIT 5

SUMMARY 6

RESUME 8

1. INTRODUCTION 10

2. MATERIALS AND METHODS 10

2.1 TEST SUBSTANCE AND OTHER SUBSTANCES 10
2.1.1 Identification of the test substance 10
2.1.2 Vehicle 10
2.1.3 Dosage form preparation 10
2.1.4 Other substances 11

2.2 TEST SYSTEM 11
2.2.1 Animals 11
2.2.2 Environmental conditions 11
2.2.3 Food and water 12

2.3 TREATMENT 12
2.3.1 Preliminary test 12
2.3.2 Main study 13
2.3.2.1 Preparation of the animals 13
2.3.2.2 Induction phase by intradermal and cutaneous routes 13
2.3.2.2.1 Intradermal route 13
2.3.2.2.2 Cutaneous route 13
2.3.2.3 Challenge phase 14

2.4 SUMMARY DIAGRAM 15
Figure 1: Treatment sites 15

2.5 SCORING OF CUTANEOUS REACTIONS 16

2.6 CLINICAL EXAMINATIONS 16

2.7 BODY WEIGHT 16

2.8 PATHOLOGY 16
2.8.1 Necropsy 16
2.8.2 Skin samples 16

2.9 DETERMINATION OF THE ALLERGENICITY LEVEL 16

2.10 CHRONOLOGY OF THE STUDY	17
2.11 PROTOCOL ADHERENCE	18
2.12 ARCHIVING	18
3. RESULTS	19
3.1 CHOICE OF THE VEHICLE	19
3.2 PRELIMINARY STUDY	19
3.2.1 Administration by intradermal route	19
3.2.2 Application by cutaneous route	20
3.3 MAIN STUDY	20
3.3.1 Clinical examinations	20
3.3.2 Body weight	20
3.3.3 Challenge phase - Scoring of cutaneous reactions	21
4. CONCLUSION	22
APPENDICES	23
1. Test article description and analytical certificate	24
2. Diet formula	27
3. Individual body weight values	29
4. Positive control to check the sensitivity of Dunkin-Hartley guinea-pigs	31 and 32

STATEMENT OF THE STUDY DIRECTOR

The study was performed in compliance with the principles of Good Laboratory Practice as described in:

- OECD Principles on Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.
- Décret N° 98-1312 du 31 décembre 1998 concernant les Bonnes Pratiques de Laboratoire (Journal Officiel du 1er janvier 1999), Ministère de l'Economie, des Finances et de l'Industrie.
- Commission Directive 1999/11/EC of 8 March 1999 adapting to technical progress the Principles of Good Laboratory Practice as specified in Council Directive 87/18/EEC on the harmonization of laws, regulations and administrative provisions relating to the application of the Principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (OJ No. L 77 of 23.3.1999).

I declare that this report constitutes a true and faithful record of the procedures undertaken and the results obtained during the performance of the study.

This study was performed at CIT, Centre International de Toxicologie, BP 563, 27005 Evreux, France.

Toxicology

X. Manciaux — Date: 28 August 2000
Study Director
Doctor of Pharmacy

OTHER SCIENTIST INVOLVED IN THIS STUDY

For Pharmacy: P.O. Guillaumat
Doctor of Pharmacy

STATEMENT OF QUALITY ASSURANCE UNIT

Type of inspections	Dates		
	Inspections	Reported to Study Director (*)	Reported to Management (*)
Protocol	14 December 1999	15 December 1999	13 December 1999
Report	24 July 2000	17 August 2000	17 August 2000

In addition to the above-mentioned inspections, at about the same time as the study described in the present report, "process-based" and routine facility inspections of critical procedures relevant to this study type were also made by the Quality Assurance Unit.
The findings of these inspections were reported to the Study Director and to CIT Management.

The inspections were performed in compliance with CIT Quality Assurance Unit procedures and the Good Laboratory Practice.

The reported methods and procedures were found to describe those used and the results to constitute an accurate and complete reflection of the study raw data.

L. Valette-Talbi Date: 28 August 2000
Doctor of Biochemistry
Head of Quality Assurance Unit
and Scientific Archives

(*) The dates indicated correspond to the dates of signature of audit reports by Study Director and Management.

SUMMARY

At the request of Elf Atochem SA, Paris-la-Défense, France, the potential of the test substance [redacted] to induce delayed contact hypersensitivity was evaluated in guinea-pigs according to the maximization method of Magnusson and Kligman and to OECD (No. 406, 17th July 1992) and EC (96/54/EEC, B.6, 30 July 1996) guidelines.
The study was conducted in compliance with the principles of Good Laboratory Practice Regulations.

Methods

Thirty guinea-pigs were allocated to two groups: a control group of five males and five females and a treated group of ten males and ten females.

On day 1, three pairs of intradermal injections were performed in the interscapular region of all animals:
- Freund's complete adjuvant (FCA) diluted at 50% (v/v) with 0.9% NaCl (both groups),
- test substance at the chosen concentration in the chosen vehicle (treated group) or vehicle alone (control group),
- test substance at the chosen concentration in a mixture FCA/0.9% NaCl 50/50 (treated group) or vehicle at the concentration of 50% in a mixture FCA/0.9% NaCl 50/50 (control group).

On day 7, the same region received a topical application of sodium lauryl sulfate in vaseline (10%, w/w) in order to induce local irritation.

On day 8, the test substance (treated group) or the vehicle (control group) was applied topically to the same test site which was then covered by an occlusive dressing for 48 hours.

On day 22, all animals of the treated and control groups were challenged by a cutaneous application of the test substance to the right flank. The left flank served as control and received the vehicle only. Test substance and vehicle were maintained under an occlusive dressing for 24 hours.
Skin reactions were evaluated approximately 24 and 48 hours after removal of the dressing.

Test substance concentrations were as follows:

Induction (treated group)
- intradermal injections (day 1): [redacted] at the concentration of 25% (w/w) in sterile isotonic saline solution (0.9% NaCl),
- topical application (day 8): [redacted] undiluted.

Challenge (all groups)
- topical application (day 22): [redacted] undiluted.

At the end of the study, animals were killed without examination of internal organs.
No skin samples were taken from the challenge application sites.

Results

No clinical signs and no deaths were noted during the study.

No well-defined cutaneous reactions were observed after the challenge application.

Conclusion

Under our experimental conditions and according to the maximization method of Magnusson and Kligman, the test substance [redacted] does not induce delayed contact hypersensitivity in guinea-pigs.

According to the classification criteria laid down in Directive 93/21/EEC (27th April 1993) adapting to technical progress for the eighteenth time Council Directive 67/548/EEC, the test substance should not be considered as a skin sensitizer.

RESUME

A la demande de Elf Atochem SA, Paris-la-Défense, France, le potentiel du produit [redacted] induire une hypersensibilisation cutanée retardée est évalué chez le Cobaye selon la méthode de maximisation de Magnusson et Kligman et conformément aux lignes directrices de l'OCDE (n° 406, 17 juillet 1992) et de la CEE (96/54/EEC, B.6, 30 juillet 1996).
L'étude est réalisée conformément aux règles de Bonnes Pratiques de Laboratoire.

Méthodes

Trente cobayes sont répartis en 2 groupes : un groupe témoin de 5 mâles et 5 femelles et un groupe traité de 10 mâles et 10 femelles.

Au jour 1, trois paires d'injections intradermiques sont effectuées au niveau de la région interscapulaire de tous les animaux :
- Adjuvant complet de Freund (FCA) dilué à 50 % (v/v) dans du NaCl à 0,9 % (groupe traité et groupe témoin),
- produit à tester à la concentration choisie dans le véhicule (groupe traité) ou véhicule seul (groupe témoin),
- produit à tester à la concentration choisie dans une mixture FCA/ NaCl à 0,9 % 50/50 (groupe traité) ou véhicule à la concentration de 50 % dans une mixture FCA/ NaCl à 0,9 % 50/50 (groupe témoin).

Au jour 7, une application cutanée de laurylsulfate sodique à 10 % (p/p) dans de la vaseline est effectuée sur la même zone dans le but d'induire une irritation locale.

Au jour 8, le produit (groupe traité) ou le véhicule (groupe témoin) sont appliqués sur le même site, qui est ensuite recouvert d'un pansement occlusif pendant 48 heures.

Au jour 22, tous les animaux des groupes traité et témoin reçoivent une application cutanée déclenchante de produit sur le flanc droit. Le flanc gauche sert de témoin et reçoit le véhicule seul. Le produit et le véhicule sont maintenus sous pansement occlusif pendant 24 heures.
L'évaluation des réactions cutanées est effectuée environ 24 et 48 heures après l'enlèvement du pansement.

Les concentrations de produit sont les suivantes :

Induction (groupe traité)
- injections intradermiques (jour 1) : [redacted] p/p) dans du NaCl à 0,9 %,
- application cutanée (jour 8) : [redacted] non dilué.

Application déclenchante (tous les groupes)
- application cutanée (jour 22) : [redacted] non dilué.

A la fin de l'étude, les animaux sont sacrifiés sans examen des organes internes.
Aucun prélèvement cutané n'est effectué au niveau des sites d'application déclenchante.

Résultats

Aucun signe clinique ni aucune mortalité ne sont notés pendant l'étude.

Aucune réaction cutanée bien définie n'est observée après l'application déclenchante.

Conclusion

Dans nos conditions expérimentales et selon la méthode de maximisation de Magnusson et Kligman, le produit [redacted] n'induit pas de réaction cutanée attribuable à une hypersensibilisation cutanée retardée chez le Cobaye.

Selon les critères de classification décrits dans la Directive 93/21/CEE (27 avril 1993) portant dix-huitième adaptation au progrès technique de la Directive 67/548/CEE, le produit est considéré non sensibilisant par contact avec la peau.

1. INTRODUCTION

The objective of this study, performed according to the maximization method of Magnusson and Kligman (1), was to evaluate the potential of the test substance [redacted] to induce delayed contact hypersensitivity in guinea-pigs.

The results of the study are of value in predicting the contact sensitization potential of the test material in humans.

The study was conducted in compliance with:
. OECD guideline No. 406, 17th July 1992,
. EC Directive No. 96/54/EEC, B.6, 30 July 1996.

2. MATERIALS AND METHODS

2.1 TEST SUBSTANCE AND OTHER SUBSTANCES

2.1.1 Identification of the test substance

The test substance [redacted] used in the study was supplied by the Sponsor.

It was identified as follows:
. name:
- protocol and labelling: [redacted]
. batch number:
- protocol and labelling: [redacted]
. Elf Atochem filing number: [redacted]
. description: yellow liquid
. container: one plastic flask
. date of receipt: 16 December 1999
. storage conditions: at room temperature
. expiry date: November 2000.

Data relating to the characterization of the test substance are documented in a test article description and an analytical certificate (presented in appendix 1) provided by the Sponsor.

2.1.2 Vehicle

The choice of the vehicle was based on tests to check the homogeneity (visual check) of the preparation (for cutaneous application and intradermal injections) and its free passage through a needle (for intradermal injections). The highest concentrations which satisfied these criteria were called the maximal practicable concentrations.
The vehicle used was 0.9% NaCl, batch No. LR92011 (Laboratoire Fresenius, 92316 Sèvres, France).

2.1.3 Dosage form preparation

All dosage form preparations were made freshly on the morning of administration and any unused material was discarded that same day.

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(1) Magnusson B. and Kligman A.M.: The identification of contact allergens by animal assay. The guinea-pig maximization test. *J. Invest. Derm.*, 52: 268-276 (1969).

2.1.4 Other substances

The other substances used were Freund's complete adjuvant, batch No. 39H8926 (Sigma, 38297 Saint-Quentin-Fallavier, France); sodium lauryl sulfate, batch No. 107H0006 (Sigma, 38297 Saint-Quentin-Fallavier, France) and vaseline, batch No. 1144 (Coopérative Pharmaceutique Française, 77000 Melun, France).

2.2 TEST SYSTEM

2.2.1 Animals

Species and sex: male and female guinea-pigs.
Strain and sanitary status: Hartley Crl: (HA) BR, *Caesarian obtained, Barrier sustained - Virus Antibody Free (COBS - VAF®).*
Reason for this choice: species generally accepted by regulatory authorities for this type of study. The strain used has been shown to produce a satisfactory sensitization response using known sensitizers.
Breeder: Charles River France, 76410 Saint-Aubin-lès-Elbeuf, France.
Number: . one male and one female for the preliminary test,
. 30 animals (15 males and 15 females) for the main test.
Females were nulliparous and non-pregnant.
Allocation of the animals to the groups: on day -1, the animals were weighed and randomly allocated to two groups: a control group of ten animals (five males and five females) and a treated group of 20 animals (ten males and ten females).
Age/weight: on day 1, the animals of the main test were approximately 3 months old and had a mean body weight ± standard deviation of 364 ± 14 g for the males and 341 ± 16 g for the females.
Acclimatization: at least 5 days before the beginning of the study.
Identification of the animals: ear-tattoo.

2.2.2 Environmental conditions

The conditions in the animal room were set as follows:
. temperature: 21 ± 2°C
. relative humidity: 30 to 70%
. light/dark cycle: 12 h/12 h
. ventilation: approximately 12 cycles/hour of filtered, non-recycled air.
The temperature and relative humidity were under continuous control and recording. The records were checked daily and filed. In addition to these daily checks, the housing conditions and corresponding instrumentation and equipment are verified and calibrated at regular intervals.
During the acclimatization period and throughout the study, the animals were housed individually in polycarbonate cages (48 cm x 27 cm x 20 cm) equipped with a polypropylene bottle.
Dust-free sawdust was provided as litter (SICSA, 94142 Alfortville, France).
Bacteriological and chemical analyses of the sawdust, including the detection of possible contaminants (pesticides, heavy metals), are performed regularly by external laboratories.
The results of these analyses are archived at CIT.

2.2.3 Food and water

During the study, the animals had free access to "106 pelleted diet" (UAR, 91360 Villemoisson-sur-Orge, France).
Food is analysed regularly by the supplier for composition and contaminant levels.
The diet formula is presented in appendix 2.

Drinking water filtered by a FG Millipore membrane (0.22 micron) was provided *ad libitum.*
Bacteriological and chemical analyses of the water and diet, including the detection of possible contaminants (pesticides, heavy metals and nitrosamines), are performed regularly by external laboratories.
The results of these analyses are archived at CIT.

No contaminants were known to have been present in the diet, drinking water or bedding material at levels which may be expected to have interfered with or prejudiced the outcome of the study.

2.3 TREATMENT

2.3.1 Preliminary test

A preliminary test was conducted in order to determine the concentrations to be tested in the main study.

By intradermal route (tested concentrations: 75%, 50% and 25% (w/w)):
- 24 hours before treatment, the dorsal region of the animals was clipped,
- intradermal administrations of the dosage form preparations (0.1 ml) were performed in the interscapular region,
- cutaneous reactions were evaluated approximately 24, 48 hours and 6 days after the injections.

By cutaneous route (tested concentrations: 100% and 50% (w/w)):
- 24 hours before treatment, both flank regions of the animals were clipped,
- the filter paper of a chamber (Finn Chamber®) was fully-loaded with the dosage form preparations. The chamber was then applied to the clipped area of the skin (one concentration per flank). The chamber was held in place by means of an occlusive dressing for 24 hours,
- cutaneous reactions were evaluated approximately 24 and 48 hours after removal of the dressings.

Criteria for selection of concentrations
The following criteria were used:
- the concentrations should be well-tolerated systemically and locally,
- intradermal injections should cause moderate irritant effects (no necrosis or ulceration of the skin),
- cutaneous application for the induction should cause at most weak or moderate skin reactions or be the maximal practicable concentration,
- cutaneous application for the challenge phase should be the highest concentration which does not cause irritant effects.

2.3.2 Main study

2.3.2.1 Preparation of the animals

For all animals, the application sites were:
. clipped on days -1 and 7 (interscapular region 4 cm x 2 cm),
. clipped and shaved on day 21 (each flank 2 cm x 2 cm).

2.3.2.2 Induction phase by intradermal and cutaneous routes

2.3.2.2.1 Intradermal route

On day 1, six injections were made deep into the dermis of a 4 cm x 2 cm clipped interscapular area, using a needle (diameter: 0.50 x 16 mm) mounted on a 1 ml plastic syringe (0.01 ml graduations).

Three injections of 0.1 ml were made into each side of this interscapular region (i.e. three pairs of sites), as follows:

Injection	Site	Treated group	Control group
1	Anterior	FCA at 50% (v/v) in 0.9% NaCl	FCA at 50% (v/v) in 0.9% NaCl
2	Middle	test substance at 25% (w/w) in 0.9% NaCl	0.9% NaCl
3	Posterior*	test substance at 25% (w/w) in a mixture FCA /0.9% NaCl 50/50	vehicle at 50% (w/v) in a mixture FCA /0.9% NaCl 50/50

FCA: Freund's complete adjuvant
* : The test substance was first dissolved in the aqueous phase prior to mixing with FCA. The final concentration of the test substance was equal to that used in injection 2.

The anterior and middle pairs of injections were performed close to each other and nearest the head, while the posterior pair was performed towards the caudal part of the test area.

2.3.2.2.2 Cutaneous route

On day 7, the interscapular area was clipped.
As the test substance was shown to be non-irritant during the preliminary test, the animals were treated with 0.5 ml of sodium lauryl sulfate at the concentration of 10% (w/w) in vaseline, in order to induce local irritation.

On day 8, a pad of filter paper (approximately 8 cm^2) was fully-loaded with the undiluted test substance and was then applied to the interscapular region of the animals of the treated group. The animals of the control group received an application of the vehicle alone under the same experimental conditions.

The pad was held in place for 48 hours by means of an adhesive hypoallergenic dressing and an adhesive anallergenic waterproof plaster.

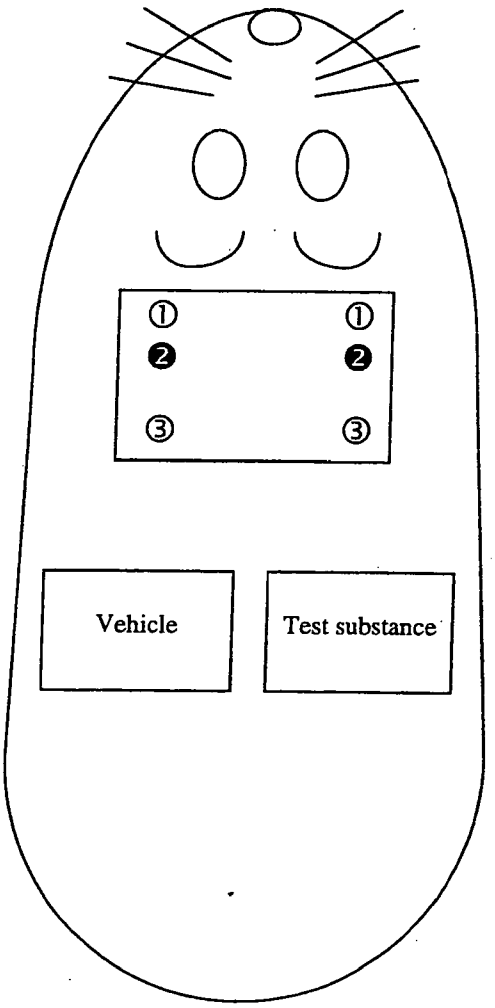
2.3.2.3 Challenge phase

On day 22, the animals of treated and control groups received an application of the test substance and vehicle. The filter paper of a chamber (Finn Chamber®) was fully-loaded with the undiluted test substance and was then applied to a clipped area of the skin of the posterior right flank of all animals.
The vehicle was applied under the same experimental conditions to the skin of the posterior left flank.

The chambers were held in contact with the skin for 24 hours by means of an adhesive anallergenic waterproof plaster.

2.4 SUMMARY DIAGRAM

Figure 1: Treatment sites



Induction site

Intradermal injections (day 1)*

Cutaneous application (day 7):

Sodium lauryl sulfate 10% in vaseline

Cutaneous application (day 8):
vehicle (control group)
or test substance at the chosen concentration (treated group)

Challenge application sites

Cutaneous application (day 22)

* Intradermal injections:

① 50% Freund's complete adjuvant and sterile isotonic solution (0.9% NaCl)

❷ vehicle (control group) or test substance at the chosen concentration in the vehicle (treated group)

③ vehicle at 50% (control group) or test substance at the chosen concentration (treated group) in a mixture of Freund's complete adjuvant / 0.9% NaCl 50/50

2.5 SCORING OF CUTANEOUS REACTIONS

Twenty-four and 48 hours after removal of the dressing of the challenge application, both flanks of the treated and control animals were observed in order to evaluate cutaneous reactions, according to the following scale:

. no visible change 0
. discrete or patchy erythema 1
. moderate and confluent erythema 2
. intense erythema 3

Any observed oedema was recorded.
Any other lesions were noted.

2.6 CLINICAL EXAMINATIONS

The animals were observed at least once a day during the study in order to check for clinical signs and mortality.

2.7 BODY WEIGHT

The animals were weighed individually on the day of allocation into the groups, on the first day of the study (day 1) and on the last day of the study (day 25).

2.8 PATHOLOGY

2.8.1 Necropsy

At the end of the study, all the animals were killed by carbon dioxide asphyxiation. No necropsy was performed.

2.8.2 Skin samples

No skin samples were taken.

2.9 DETERMINATION OF THE ALLERGENICITY LEVEL

The animals of the treated group show a positive reaction if macroscopic cutaneous reactions are clearly visible (score ≥ 1) and are of greater intensity and/or duration of response than the maximum reaction seen in control animals, or if macroscopic reactions are confirmed at microscopic examination as being due to the sensitization process.

Determination of the allergenicity level
The allergenicity level of the test substance is calculated by comparing the number of animals showing positive reactions with the number of surviving treated animals at the end of the study.

% of animals showing a reaction	Allergenicity level	Classification
0 - 8	I	weak
9 - 28	II	mild
29 - 64	III	moderate
65 - 80	IV	strong
81 - 100	V	extreme

According to the Commission Directive 93/21/EEC, when the reactions are positive in at least 30% of the treated animals, the test substance has sensitization properties and the symbol Xi, the indication of danger "Irritant" and the sentence "R 43: May cause sensitization by skin contact" must be applied.

The sensitivity of the experimental technique is regularly assessed using a known moderate sensitizer, MERCAPTOBENZOTHIAZOLE. In a recent study performed under CIT experimental conditions, the strain of guinea-pigs used showed a satisfactory sensitization response in 80% animals (see appendix 4).

2.10 CHRONOLOGY OF THE STUDY

The chronology of the main test is summarized as follows:

Procedure	Date	Day
Arrival of the animals	27 January 2000	-5
Weighing and allocation of the animals into groups	31 January 2000	-1
Weighing, induction by intradermal injection	1 February 2000	1
Sodium lauryl sulfate application	7 February 2000	7
Induction by cutaneous route	8 February 2000	8
Removal of occlusive dressings	10 February 2000	10
Challenge cutaneous application	22 February 2000	22
Removal of occlusive dressings	23 February 2000	23
Scoring of cutaneous reactions after		
. 24 hours	24 February 2000	24
. 48 hours	25 February 2000	25
Weighing, sacrifice of the animals	25 February 2000	25

2.11 PROTOCOL ADHERENCE

The study was performed in accordance with the Study Protocol No. [redacted]nd subsequent amendments. There were no deviations from the agreed Study Protocol.

2.12 ARCHIVING

The study documentation and specimens generated during the course of the study are archived at CIT, 27005 Evreux, France, for 10 years after the end of the *in vivo* phase of the study.

The archived study materials include:
. protocol and possible amendments,
. raw data,
. correspondence,
. final report and possible amendments.

On completion of this period, the archived study materials will be returned to the Sponsor, or may be archived at CIT for a further period.
In addition, raw data not specific to the study including, but not limited to, certificates of analyses for food, water and bedding (if applicable) and records of environmental data and equipment calibration, are also archived at CIT and retained for at least 30 years.

3. RESULTS

3.1 CHOICE OF THE VEHICLE

The vehicle chosen was 0.9% NaCl: a homogeneous dosage form preparation was obtained whatever the proportion.
The dosage form preparation at the concentration of 75% (w/w) passed freely through a needle and into the dermis.

3.2 PRELIMINARY STUDY

3.2.1 Administration by intradermal route
Results were as follows:

Animal number	Concentration of the test substance % (w/w)	Scoring after treatment 24 hours	48 hours	6 days
male 301	75 + FCA	I	N	A
	75	I	N	A
	50 + FCA	I	N	A
	50	I	I	A
	25 + FCA	I	I	A
	25	I	LI	LI
female 302	75 + FCA	I	N	A
	75	I	N	A
	50 + FCA	I	N	A
	50	I	I	A
	25 + FCA	I	I	A
	25	I	LI	LI

FCA : mixture Freund's Complete Adjuvant/0.9% NaCl 50/50 (v/v)
N : necrosis
I : irritation
LI : slight irritation
A : crusts

In order to respect the criteria for the selection of concentrations (the concentration should be well-tolerated systemically and locally, intradermal injections should cause moderate irritant effect but no necrosis or ulceration of the skin), concentration chosen for the main study was 25% (w/w).

3.2.2 Application by cutaneous route

Results were as follows:

Animal number	Concentration of the test substance %		Scoring after removal of the dressing 24 hours	48 hours
male 301	100	RF	0	0
	50 (w/w)	LF	0	0
female 302	100	RF	0	0
	50 (w/w)	LF	0	0

RF : right flank
LF : left flank

On removal of the dressing, no residual test substance was observed.

In order to respect the criteria for the selection of concentrations (the concentrations should be well-tolerated systemically and locally, cutaneous application for the induction should cause at most weak or moderate skin reactions or be the maximal practicable concentration, cutaneous application for the challenge phase should be the highest concentration which does not cause irritant effect), concentration chosen for the topical application of the induction phase (day 8) and for the challenge application (day 22) was 100%.

3.3 MAIN STUDY

3.3.1 Clinical examinations

No clinical signs and no deaths were observed during the study.

3.3.2 Body weight

The body weight gain of the treated animals was similar to that of the control animals (appendix 3).

3.3.3 Challenge phase - Scoring of cutaneous reactions
On removal of the dressing, no residual test substance was observed.

Scoring of skin reactions was as follows:

Sex	Animal number	Control group			
		24 hours		48 hours	
		LF	RF	LF	RF
Male	121	0	0	0	0
	122	0	0	0	0
	123	0	0	0	0
	124	0	0	0	0
	125	0	0	0	0
Female	136	0	0	0	0
	137	0	0	0	0
	138	0	0	0	0
	139	0	0	0	0
	140	0	0	0	0

Sex	Animal number	Treated group			
		24 hours		48 hours	
		LF	RF	LF	RF
Male	126	0	0	0	0
	127	0	0	0	0
	128	0	0	0	0
	129	0	0	0	0
	130	0	0	0	0/S
	131	0	0	0	0
	132	0	0	0	0
	133	0	0	0	0
	134	0	0	0	0
	135	0	0	0	0
Female	141	0	0	0	0
	142	0	0	0	0
	143	0	0	0	0
	144	0	0	0/S	0
	145	0	0	0	0
	146	0	0	0	0
	147	0	1	0	0/S
	148	0	0	0	0
	149	0	0	0	0
	150	0	0	0	0

LF : left flank (vehicle)
RF : right flank (undiluted test substance)
S : dryness of the skin

No cutaneous reactions were observed in the animals of the control group.
In the treated group, only a discrete erythema (grade 1) was noted in 1/20 animals at the 24-hour reading. Dryness of the skin was recorded in 3/20 animals at the 48-hour reading.
No other cutaneous reactions were observed.

4. CONCLUSION

Under our experimental conditions and according to the maximization method of Magnusson and Kligman, the test substance [redacted] does not induce delayed contact hypersensitivity in guinea-pigs.

According to the classification criteria laid down in Directive 93/21/EEC (27th April 1993) adapting to technical progress for the eighteenth time Council Directive 67/548/EEC, the test substance should not be considered as a skin sensitizer.

APPENDICES

1. Test article description and analytical certificate

TOXICOLOGY DEPARTMENT
CONFIDENTIAL
DTI 421
2nd December 1999

elf atochem s.a.
La défense 10, Cours Michelet
92091 Paris-la-Défense cedex, France

TEST ARTICLE DESCRIPTION

[redacted]

IDENTITY

Test article name	:	[redacted]
Purity	:	[redacted]
Origin	:	Elf Atochem Villers-Saint-Paul
Batch	:	[redacted]
Elf Atochem filing number	:	[redacted]

PHYSICAL AND CHEMICAL PROPERTIES

Appearance	:	Liquid
Specific gravity	:	1070 kg/m3 at 20°C (liquid)
Boiling point	:	80°C
Flash point	:	25°C (closed cup)
Solubility	:	Partially soluble in water (20°C)
	:	Soluble in : Methanol, Ethanol (400 g/l approximately)
	:	Insoluble in : Hydrocarbons, DMSO

TOXICOLOGICAL INFORMATION AND USE SAFETY

See Material and Safety Data Sheet

STORAGE AND DISPOSAL

Storage	:	in dark and at room temperature
Expiry date	:	November 2000
Disposal	:	incineration

[redacted]

elf atochem

ATO

Villers Saint Paul, le 18 Novembre 1999

CERTIFICAT D'ANALYSE

[redacted]

Lot N° [redacted]	Unité	Résultat	Spécification de fabrication	Méthode d'analyse
Extrait Sec	%	39,5	39,5 à 40,5	LCU 622
pH		7,3	7 à 8	LCU 642
Pouvoir moussant	ml	80	60 à 100	LCU 664
Tension superficielle	Mn/m	15,2	0 à 16	LCU 663
Taux de 1145	%	6,4	0 à 7	LCU 639
H2O2	%	<0,01	0 à 0,2	LCU637
Pouvoir filmogène Sur cyclo hexane sur eau de ville	Sec.	11	0 à 20	LCU 668
Pouvoir filmogène Sur cyclo hexane sur eau de mer	Sec.	14	0 à 60	LCU 668

Le Chef de Service du Laboratoire
SIGNATURE :

NOM : Robert Gruppo

Company Sanitized. Does not contain TSCA CBI

2. Diet formula

Ref: 106

COMPLETE DIET

GUINEA-PIG MAINTENANCE DIET

Appearance: 4.5 mm diameter granules

Conditioning: bags of 25 kgs

Daily portion: Guinea-pigs 35-50 g, water *ad libitum*.

FORMULA %	
Cereals	42
Grain byproducts and legumes	46
Vegetable protein (soya bean meal, yeast)	9
Vitamin and mineral mixture	3

AVERAGE ANALYSIS %	
Calorific value (Kcal/kg)	2600
Moisture	10
Proteins	17
Lipids	3
Carbohydrates (N.F.E.)	49
Fibre	13
Minerals (ash)	8

AMINO ACID VALUES (calculated in mg/kg)	
Arginine	8500
Cystine	2500
Lysine	7200
Methionine	2100
Tryptophan	2000
Glycine	6000

FATTY ACID VALUES (calculated in mg/kg)	
Palmitic acid	3600
Palmitoleic acid	0
Stearic acid	700
Oleic acid	5900
Linoleic acid	11200
Linolenic acid	3000

MINERALS (calculated in mg/kg)

	Nat. val.	CMV val.	Total
P	7400	1400	8800
Ca	5400	5600	11000
K	12000	0	12000
Na	1300	1950	3250
Mg	3270	130	3400
Mn	60	40	100
Fe	170	150	320
Cu	10	15	25
Zn	40	45	85
Co	0.1	1.5	1.6
I	0	0	0
Cl	0	0	0

VITAMINS (calculated per kg)

	Nat. val.	CMV val.	Total
Vitamin A	3500 IU	7500 IU	11000 IU
Vitamin D3	30 IU	2000 IU	2030 IU
Vitamin B1	6 mg	6.4 mg	12.4 mg
Vitamin B2	5 mg	6.4 mg	11.4 mg
Vitamin B3	22 mg	26 mg	48 mg
Vitamin B6	0.7 mg	2.7 mg	3.4 mg
Vitamin B12	0.003 mg	0.012 mg	0.015 mg
Vitamin C	0 mg	400 mg	400 mg
Vitamin E	15 mg	60 mg	75 mg
Vitamin K3	5 mg	12.6 mg	17.6 mg
Vitamin PP	97 mg	14.5 mg	111.5 mg
Folic acid	2.2 mg	1.3 mg	3.5 mg
P.A.B. acid	0 mg	2.5 mg	2.5 mg
Biotin	0.02 mg	0.06 mg	0.08 mg
Choline	1010 mg	60 mg	1070 mg
Meso-Inositol	0 mg	62.5 mg	62.5 mg

This food is supplemented with stabilized coated vitamin C, avoiding the need of other food substances (greenery, ascorbic acid) if used within 4 months of date of manufacture.

UAR, 7 rue Galliéni, 91360 Villemoisson - Tel : 01.69.04.03.57 - Fax : 01.69.04.81.97

(Ref. Doc. UAR : 1992)

3. Individual body weight values

INDIVIDUAL BODY WEIGHT VALUES (g)

Groups	Sex	Animals	Days			
			-1	1	(1)	25
1	Male	121	344	349	143	492
		122	376	383	136	519
		123	362	355	84	439
		124	377	384	107	491
		125	360	364	113	477
		M	364	367	117	484
		SD	14	16	24	29
	Female	136	354	355	111	466
		137	333	330	105	435
		138	353	347	72	419
		139	331	339	111	450
		140	330	352	112	464
		M	340	345	102	447
		SD	12	10	17	20
2	Male	126	337	346	156	502
		127	350	349	138	487
		128	366	371	158	529
		129	393	387	105	492
		130	360	372	97	469
		131	341	346	193	539
		132	360	358	103	461
		133	350	357	163	520
		134	375	376	143	519
		135	353	361	118	479
		M	359	362	137	500
		SD	17	14	31	26
	Female	141	333	330	142	472
		142	340	346	169	515
		143	340	337	95	432
		144	373	366	40	406
		145	347	350	131	481
		146	343	319	59	378
		147	325	314	99	413
		148	332	328	132	460
		149	368	371	134	505
		150	340	331	99	430
		M	344	339	110	449
		SD	15	19	39	45

(1) = Body weight gain
M = Mean
SD = Standard Deviation

4. Positive control to check the sensitivity of Dunkin-Hartley guinea-pigs

Purpose: check the sensitivity of Dunkin-Hartley Guinea-pigs (Breeder: Charles River France) to a positive control test article

Method	:	Magnusson and Kligman
Test substance	:	MERCAPTOBENZOTHIAZOLE
CIT Study - Date	:	CIT/Study No. 19306 TSG - December 1999
Number of animals	:	one control group of 5 animals and one treated group of 10 animals
Induction	:	1% (w/w) intradermal route day 1 20% (w/w) cutaneous route day 8
Challenge application	:	20% (w/w) cutaneous route day 22

Conclusion

Under our experimental conditions and according to the Magnusson and Kligman method, the test substance MERCAPTOBENZOTHIAZOLE at the concentration of 20% (w/w) induced positive skin sensitization reactions in 80% guinea-pigs.

INDIVIDUAL REACTIONS: CHALLENGE PHASE
MACROSCOPIC FINDINGS

Groups	Sex	Animals	24-hour		48-hour		Conclusion
			LF	RF	LF	RF	
Control	Female	16	0	0/C	0	0/S/C	-
		17	0	0/C	0	0/S/C	-
		18	0	0/C	0	0/S/C	-
		19	0	0/C	0	0/S/C	-
		20	0	0/C	0	0/S/C	-
Treated	Female	21	0	2/C	1/S	2/C/S	+
		22	0	2/C	0	2/C/S	+
		23	0	2/C	0	2/C/S	+
		24	0	3/C	0	LS/C	+
		25	0	1/C	0	0/S	-
		26	0	2/C	0	LS/C	+
		27	0	3/C	0	LS/C	+
		28	0	1/C	0	LS/C	-
		29	0	2/C	0	LS/C	+
		30	0	2/C	0	0/S/C	+

LF : left flank (vehicle)
RF : right flank (test substance at the concentration of 20% (w/w))
S : dryness of the skin
LS : scoring masked by dryness of the skin
C : yellow coloration of the skin
- : negative
+ : hypersensitizing reactions