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CIT

SPONSOR
Elf Atochem S.A.
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, B. and Kligman, A.M.)

STUDY DIRECTOR
Xavier Manciaux

STUDY COMPLETION DATE
15 October 1999

PERFORMING LABORATORY
CIT
Centre International de Toxicologie
BP 563 - 27005 Evreux - France

LABORATORY STUDY NUMBER
18744 TSG

Company Sanitized. Does not contain TSCA CBI

CONTENTS

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° 90-206 du 7 mars 1990 concernant les Bonnes Pratiques de Laboratoire (Journal Officiel du 9 mars 1990), Ministère de l'Industrie et de l'Aménagement du Territoire.
- Council Directive 87/18/EEC of 18 December 1986 on the harmonization of laws, regulations or 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 15 of 17.1.87).

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: 15 October 1999
Study Director
Doctor of Pharmacy

OTHER SCIENTISTS INVOLVED IN THIS STUDY

For Pharmacy: P.O. Guillaumat
Doctor of Pharmacy

For Toxicology: C. Pelcot
Study Supervisor

STATEMENT OF QUALITY ASSURANCE UNIT

Type of inspections	Dates		
	Inspections	Reported to Study Director (*)	Reported to Management (*)
Protocol	4 June 1999	4 June 1999	4 June 1999
Report	29 September 1999	13 October 1999	13 October 1999

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: 15 October 1999
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 S.A., 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 (92/69/EEC, B.6, 31st July 1992) 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 1 (five males and five females) and a treated group 2 (ten males and ten females).

On day 1, intradermal injections of Freund's complete adjuvant mixed with the test substance (treated group) or the vehicle (control group) were performed in the interscapular region.

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 to the same test site which was then covered by an occlusive dressing for 48 hours.

On day 22, after a rest period of 12 days, 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: [redacted] at the concentration of 10% (w/w) in sterile isotonic saline solution (0.9% NaCl),
- topical application: [redacted] undiluted.

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

At the end of the study, animals were killed without examination of internal organs.
Skin samples were taken from the challenge application sites of all the animals.
No histological examination was performed.

The sensitivity of the guinea-pigs in CIT experimental conditions was checked with a positive sensitizer, 2,4-Dinitro Chlorobenzene (DNCB). During the induction period, the reference substance DNCB was applied at the concentrations of 0.1% (w/w) (day 1) and 1% (w/w) (day 8) in corn oil. For the challenge application, the reference substance DNCB was applied at the concentration of 1% (w/w) in corn oil.

Results

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

After the challenge application, no cutaneous reactions were observed in the animals of the control group.
In the treated group, at the 24-hour reading, a very slight, well-defined or moderate erythema was noted in 5/20, 13/20 and 1/20 animals, respectively. A slight oedema was recorded in 4/20 animals.
At the 48-hour reading, a very slight or well-defined erythema persisted in 9/20 and 7/20 animals, respectively. Dryness of the skin was observed in 16/20 animals.

The species and strain which were used showed a satisfactory sensitization response in 90% animals treated with DNCB.

Conclusion

Under our experimental conditions and according to the maximization method of Magnusson and Kligman, the test substance [redacted] induces delayed contact hypersensitivity in 14/20 (70%) 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 be considered as a skin sensitizer.

RESUME

A la demande de Elf Atochem S.A., Paris-la-Défense, France, le potentiel du produit [redacted] à 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 (92/69/EEC, B.6, 31 juillet 1992).
L'étude est réalisée conformément aux règles de Bonnes Pratiques de Laboratoire.

Méthodes

Trente cobayes sont répartis en 2 groupes : un groupe témoin 1 (5 mâles et 5 femelles) et un groupe traité 2 (10 mâles et 10 femelles).

Au jour 1, des injections intradermiques d'adjuvant de Freund mélangé avec le produit (groupe traité) ou le véhicule (groupe témoin) sont effectuées au niveau de la région interscapulaire.

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, après une période de repos de 12 jours, 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 : [redacted] à la concentration de 10 % (p/p) dans du NaCl à 0,9 %,
- application cutanée : [redacted] non dilué.

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

A la fin de l'étude, les animaux sont sacrifiés sans examen des organes internes.
Des prélèvement cutanés sont effectués au niveau des sites d'application déclenchante chez tous les animaux.
Aucun examen histologique n'est réalisé.

La sensibilité de la souche de cobayes a été contrôlée dans un essai récent réalisé dans les mêmes conditions expérimentales avec un produit sensibilisant connu, le 2,4-Dinitro Chlorobenzene (DNCB). Pendant la phase d'induction, le produit DNCB est appliqué aux concentrations de 0,1 % (p/p) (jour 1) et 1 % (p/p) (jour 8) dans l'huile de germe de maïs. A l'application cutanée déclenchante, le produit DNCB est appliqué à la concentration de 1 % (p/p) dans l'huile de germe de maïs.

Résultats

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

Après l'application déclenchante, aucune réaction cutanée n'est observée chez les animaux du groupe témoin.
Dans le groupe traité, à la lecture 24 heures, un érythème très léger, bien défini ou modéré est noté chez 5/20, 13/20 et 1/20 animaux, respectivement. Un léger oedème est enregistré chez 4/20 animaux.
A la lecture 48 heures, un érythème très léger ou bien défini persiste chez 9/20 et 7/20 animaux, respectivement. Une sécheresse cutanée est observée chez 16/20 animaux.

Les résultats obtenus avec le produit de référence montrent des réactions positives d'hypersensibilité cutanée retardée chez 90 % des Cobayes traités avec le DNCB.

Conclusion

Dans nos conditions expérimentales et selon la méthode de maximisation de Magnusson et Kligman, le produit [redacted] induit des réactions cutanées attribuables à une hypersensibilisation cutanée retardée chez 14/20 (70 %) Cobayes.

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 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. 92/69/EEC, B.6, 31st July 1992.

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 Elf Atochem S.A.

The test substance was identified as follows:
. name:
  - protocol and labelling: [redacted]
. batch number:
  - protocol and labelling: [redacted]
. Elf Atochem filing number: [redacted]
. description: brown liquid
. quantity and container: one smoked glass flask
. date of receipt: 5 May 1999
. storage conditions: at room temperature and protected from light
. expiry date: May 2000.

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

At the finalisation of the study report, no analytical certificate was available. Characterisation of the test substance, which appropriately defines the tested batch, is under the responsibility of 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. LR82423 (Laboratoire Frésénius, 92316 Sèvres, France).

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

2.1.3 Formulation procedure

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

2.1.4 Other substances

The other substances used were Freund's complete adjuvant, batch No. 88H8804 (Sigma, 38297 Saint-Quentin-Fallavier, France); sodium lauryl sulfate, batch No. 107H006 (Sigma, 38297 Saint-Quentin-Fallavier, France) and vaseline, batch No. 9946 (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: . two males and two females 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 1 consisting of ten animals (five males and five females) and a treated group 2 consisting of 20 animals (ten males and ten females).
Weight: on day 1, the animals of the main test were approximately 3 months old and had a mean body weight ± standard deviation of 361 ± 17 g for the males and 358 ± 22 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:
- 24 hours before treatment, the dorsal region of the animals was clipped,
- intradermal administrations of the test substance formulation (0.1 ml) at different concentrations were performed in the interscapular region,
- cutaneous reactions were evaluated approximately 24, 48 hours and 6 days after the injections.

By cutaneous route:
- 24 hours before treatment, both flank regions of the animals were clipped,
- a volume of 0.5 ml of the undiluted test substance or test substance formulation at the chosen concentration(s) was placed on a dry gauze pad (approximately 4 cm^2) which was then applied to the skin and held in place by 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 and before each treatment, 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),
. clipped again on day 25 (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 sites	Treated group	Control group
Anterior	1: FCA diluted at 50% (v/v) with 0.9% NaCl	1: FCA diluted at 50% (v/v) with 0.9% NaCl
Middle	2: test substance at 10% (w/w) in 0.9% NaCl	2: vehicle
Posterior*	3: test substance at 10% (w/w) in a mixture FCA /0.9% NaCl 50/50 (v/v)	3: vehicle at 50% (w/w) in a mixture FCA /0.9% NaCl 50/50 (v/v)

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 cutaneous application to the interscapular region was performed as follows:

Control group
. application of 0.5 ml of the vehicle.

Treated group
. application of 0.5 ml of the undiluted test substance.

The test substance or the vehicle was placed on a dry gauze pad, which was then applied to the interscapular region.

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

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

The presence of cutaneous irritation was checked 1 hour after removal of the occlusive dressing.

2.3.2.3 Challenge phase

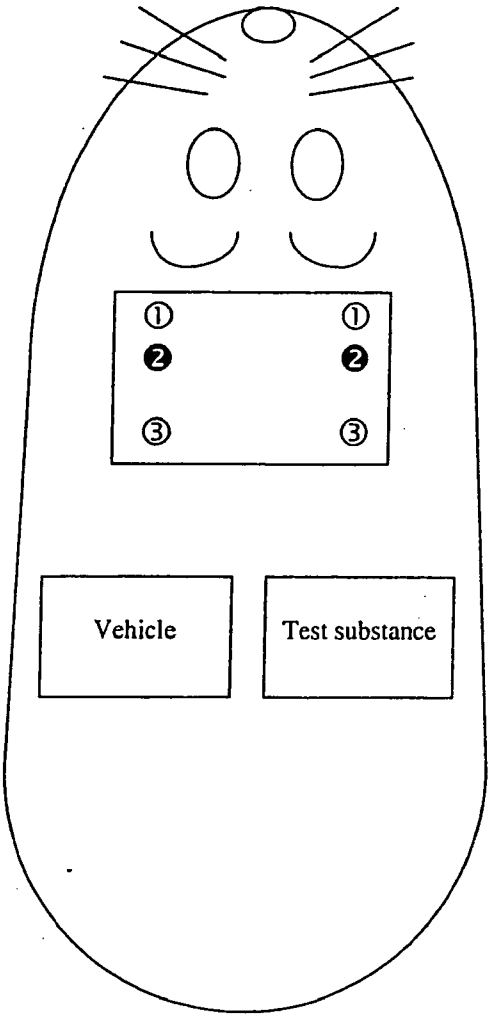
On day 22, the animals of both groups received an application of 0.5 ml of the undiluted test substance to the posterior right flank and 0.5 ml of the vehicle to the posterior left flank. This application was performed using a 1 ml plastic syringe (0.01 ml graduations). The test substance or the vehicle was placed on a dry gauze pad, which was then applied to a 4 cm^2 (2 cm x 2 cm) clipped area of the skin.

The pads were held in contact with the skin for 24 hours by means of an occlusive, hypoallergenic dressing and an adhesive anallergenic waterproof plaster.

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

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

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 50/50 (v/v) Freund's complete adjuvant / 0.9% NaCl

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:

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 slight eschar formation (injuries in depth) 4

Oedema formation

. No oedema 0
. Very slight oedema (barely perceptible) 1
. Slight oedema (visible swelling with well-defined edges) 2
. Moderate oedema (visible swelling raised more than 1 millimetre) 3
. Severe oedema (visible swelling raised more than 1 millimetre and extending beyond the area of exposure) 4

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), on days 8 and 15 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

At the end of the study, skin samples were taken from the posterior left and right flanks of all the animals. The samples were preserved in 10% buffered formalin.

2.8.3 Microscopic examination

No histological examination was performed.

2.9 DETERMINATION OF THE ALLERGENICITY LEVEL

The treated animals show a positive reaction if macroscopic cutaneous reactions are clearly visible (erythema and/or oedema ≥ 2) and if the treated animals have a greater intensity 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	very weak
9-28	II	weak
29-64	III	moderate
65-80	IV	strong
81-100	V	very strong

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.

2.10 CHRONOLOGY OF THE STUDY

The chronology of the main study is summarized as follows:

Procedure	Date	Day
Arrival of the animals	1 July 1999	-8
Weighing and allocation of the animals into groups	8 July 1999	-1
Weighing, induction by intradermal injection	9 July 1999	1
Lauryl sulfate application	15 July 1999	7
Weighing, induction by cutaneous route	16 July 1999	8
Removal of occlusive dressings	18 July 1999	10
Weighing	23 July 1999	15
Challenge cutaneous application	30 July 1999	22
Removal of occlusive dressings	31 July 1999	23
Scoring of cutaneous reactions after		
. 24 hours	1 August 1999	24
. 48 hours	2 August 1999	25
Weighing, sacrifice of the animals and skin samples	2 August 1999	25

2.11 PROTOCOL ADHERENCE

The study was performed in accordance with the Study Protocol No. 18744 TSG and subsequent amendments with the following deviation from the agreed Study Protocol:

- the positive control corresponding to the study design was performed more than 6 months ago.

This deviation was not considered to compromise the validity or integrity of the study.

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.
- histological specimens:
  - tissues in preservative.

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.

3. RESULTS

3.1 CHOICE OF THE VEHICLE

The vehicle chosen was 0.9% NaCl: a homogeneous solution was obtained whatever the proportion.
The test substance formulation 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

In order to determine the concentration to be used in the main study, the following concentrations were tested:

Animal number	Concentration of the test substance % (w/w)	Scoring after treatment 24 hours	48 hours	6 days
male 01	75 + FCA	N	N	-
	75	N	N	-
female 01	75 + FCA	N	N	-
	75	N	N	-
male 01	50 + FCA	N	N	-
	50	N	N	-
female 01	50 + FCA	N	N	-
	50	N	N	-
male 01	25 + FCA	N	N	-
	25	N	N	-
female 01	25 + FCA	N	N	-
	25	N	N	-
male 02	10 + FCA	I	I/Oe	A
	10	I	I/Oe	A
female 02	10 + FCA	I	I/Oe	A
	10	I	I/Oe	A
male 02	5 + FCA	I	I/Oe	A
	5	I	I/Oe	LI
female 02	5 + FCA	I	I/Oe	A
	5	I	I/Oe	LI
male 02	1 + FCA	I	I/Oe	A
	1	I	I/Oe	LI
female 02	1 + FCA	I	I/Oe	A
	1	I	I/Oe	LI

Oe : oedema
N : necrosis
I : irritant
LI : slightly irritant
A : crusts
- : cutaneous examinations not performed
FCA : Freund's Complete Adjuvant

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 10% (w/w).

3.2.2 Application by cutaneous route

Several concentrations were tested in order to determine the concentration(s) to be used in the main study.

Animal number	Concentration of the test substance %		Scoring after removal of the dressing 24 hours		48 hours	
			E	O	E	O
male 01	100	RF	0	0	0	0
	50 (w/w)	LF	0	0	0	0
female 01	100	RF	0	0	0	0
	50 (w/w)	LF	0	0	0	0

E : erythema
O : oedema
RF : right flank
LF : left flank

After 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 mortality were observed during the study.

From day 13, crusts associated with bleeding were observed on the site of intradermal injections in all animals of the treated group.

The body weight gain of the treated animals was similar to that of the control animals (figures 2 and 3, appendix 3).

3.3.2 Scoring of cutaneous reactions

3.3.2.1 End of the induction period

On day 10, after the cutaneous application of the induction period, signs of irritation were observed at the interscapular test site in the control and treated groups.

3.3.2.2 Challenge application

Scoring of skin reactions was as follows:

Sex	Animal number	Control group							
		24 hours				48 hours			
		Erythema		Oedema		Erythema		Oedema	
		LF	RF	LF	RF	LF	RF	LF	RF
Male	61	0	0	0	0	0	0	0	0
	62	0	0	0	0	0	0	0	0
	63	0	0	0	0	0	0	0	0
	64	0	0	0	0	0	0	0	0
	65	0	0	0	0	0	0	0	0
Female	76	0	0	0	0	0	0	0	0
	77	0	0	0	0	0	0	0	0
	78	0	0	0	0	0	0	0	0
	79	0	0	0	0	0	0	0	0
	80	0	0	0	0	0	0	0	0

Sex	Animal number	Treated group							
		24 hours				48 hours			
		Erythema		Oedema		Erythema		Oedema	
		LF	RF	LF	RF	LF	RF	LF	RF
Male	66	0	2	0	0	0	1/S	0	0
	67	0	2	0	0	0	2/S	0	0
	68	0	2	0	2	0	2/S	0	0
	69	0	1	0	0	0	1/S	0	0
	70	0	2	0	2	0	2/S	0	0
	71	0	1	0	0	0	0	0	0
	72	0	2	0	0	0	2/S	0	0
	73	0	3	0	2	0	2/S	0	0
	74	0	2	0	0	0	1/S	0	0
	75	0	2	0	0	0	1/S	0	0
Female	81	0	0	0	0	0	0	0	0
	82	0	2	0	0	0	1/S	0	0
	83	0	1	0	0	0	1/S	0	0
	84	0	1	0	0	0	0	0	0
	85	0	2	0	0	0	1/S	0	0
	86	0	2	0	0	0	2/S	0	0
	87	0	2	0	0	0	2/S	0	0
	88	0	2	0	0	0	1/S	0	0
	89	0	2	0	2	0	1/S	0	0
	90	0	1	0	0	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, at the 24-hour reading, a very slight, well-defined or moderate erythema (grade 1, 2 or 3) was noted in 5/20, 13/20 and 1/20 animals, respectively. A slight oedema (grade 2) was recorded in 4/20 animals.
At the 48-hour reading, a very slight or well-defined erythema (grade 1 or 2) persisted in 9/20 and 7/20 animals, respectively. Dryness of the skin was observed in 16/20 animals.

4. CONCLUSION

Under our experimental conditions and according to the maximization method of Magnusson and Kligman, the test substance [redacted] induces delayed contact hypersensitivity in 14/20 (70%) 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 be considered as a skin sensitizer.

Figure 2: Male body weight

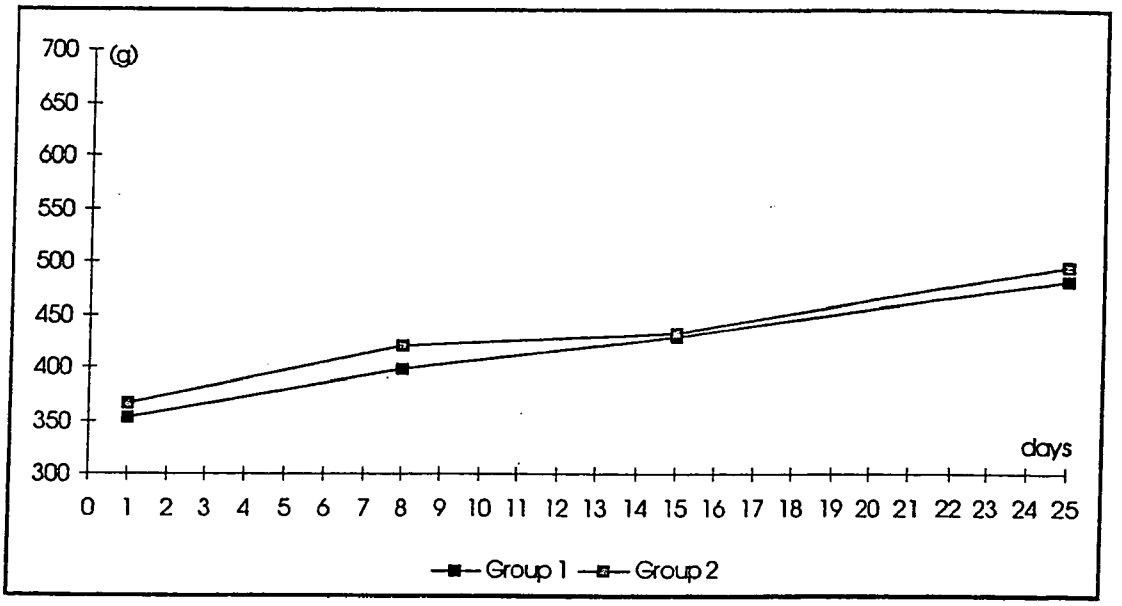
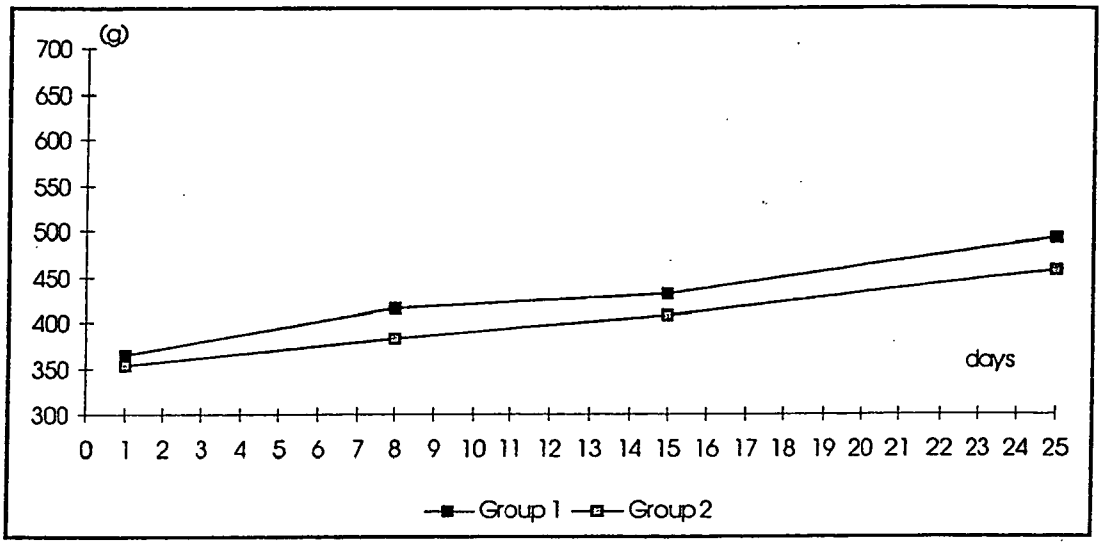


Figure 3: Female body weight



APPENDICES

1. Test article description

[redacted]

TOXICOLOGY DEPARTMENT
CONFIDENTIAL
April 99

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

TEST ARTICLE DESCRIPTION

[redacted]

IDENTITY

Test article name		[redacted]
Chemical name		
CAS number		
EINECS number		
Purity		
Origin and batch	:	Elf Atochem, VSP
Batch		[redacted]
Elf Atochem filing number	:	CAL 1393/99

PHYSICAL AND CHEMICAL PROPERTIES

Appearance	:	brownish liquid
Melting point	:	-22°C
Boiling point	:	95°C
Flash point	:	50°C
Solubility	:	water

TOXICOLOGICAL INFORMATIONS AND USE SAFETY

See safety data sheet

STORAGE AND DISPOSAL

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

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)	8	(1)	15	(1)	25
1	Male	61	314	328	52	380	46	426	54	480
		62	349	362	43	405	31	436	54	490
		63	359	373	55	428	0	428	35	463
		64	319	334	32	366	52	418	63	481
		65	347	366	45	411	23	434	58	492
		M	338	353	45	398	30	428	53	481
		SD	20	20	9	25	21	7	11	11
	Female	76	379	391	47	438	5	443	31	474
		77	348	354	46	400	45	445	84	529
		78	342	345	51	396	11	407	81	488
		79	338	355	49	404	12	416	53	469
		80	371	380	57	437	4	441	61	502
		M	356	365	50	415	15	430	62	492
		SD	18	20	4	21	17	18	22	24
2	Male	66	360	374	49	423	35	458	51	509
		67	363	375	44	419	35	454	65	519
		68	371	369	58	427	-5	422	69	491
		69	366	379	58	437	40	477	64	541
		70	324	340	135	475	-86	389	72	461
		71	337	336	21	357	10	367	43	410
		72	364	370	52	422	38	460	60	520
		73	362	366	41	407	20	427	60	487
		74	358	372	47	419	1	420	60	480
		75	367	376	47	423	27	450	75	525
		M	357	366	55	421	12	432	62	494
		SD	15	15	30	29	38	34	10	38
	Female	81	344	345	28	373	17	390	60	450
		82	332	328	-9	319	39	358	42	400
		83	344	355	44	399	18	417	43	460
		84	345	359	48	407	24	431	42	473
		85	336	344	21	365	24	389	57	446
		86	396	406	49	455	6	461	62	523
		87	322	329	27	356	26	382	36	418
		88	346	351	26	377	25	402	51	453
		89	337	342	33	375	38	413	59	472
		90	370	379	11	390	44	434	39	473
		M	347	354	28	382	26	408	49	457
		SD	21	24	18	36	12	30	10	33

(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	:	DNCB
CIT Study - Date	:	(CIT/Study No. 17335 TSG) - September 1998
Number of animals	:	1 control group of 5 animals and 1 treated group of 10 animals
Induction	:	0.1% (w/w) intradermal route day 1 1% (w/w) cutaneous route day 8
Challenge application:		1% (w/w)

Conclusion

Under our experimental conditions and according to the Magnusson and Kligman method, the test substance DNCB at the concentration of 1% (w/w) induced positive skin sensitization reactions in 90% guinea-pigs.

INDIVIDUAL REACTIONS: CHALLENGE PHASE
MACROSCOPIC FINDINGS

Groups	Sex	Animals	24-hour		48-hour		Conclusion
			Erythema	Oedema	Erythema	Oedema	
Control	Female	160	0	0	0	0	-
		161	0	0	0	0	-
		162	0	0	0	0	-
		163	0	0	0	0	-
		164	0	0	0	0	-
Treated	Female	165	2	2	2/S	2	+
		166	2	0	2/S	0	+
		167	2	2	2/S	2	+
		168	3	2	2/S/A	2	+
		169	3	2	2/S	0	+
		170	2	0	2/S	0	+
		171	2	0	2/S	0	+
		172	2	0	1/S	0	+
		173	1	0	1/S	0	-
		174	2	0	1/S	0	+

\- : negative
\+ : hypersensitizing reactions
S : dryness of the skin
A : crusts