SPONSOR
Elf Atochem S.A.
La Défense 10 - Cédex 42
92091 Paris-la-Défense
France

STUDY TITLE
SKIN SENSITIZATION TEST IN GUINEA-PIGS (Maximization method of Magnusson, B. and Kligman, A.M.)

TEST SUBSTANCE
[redacted]

STUDY DIRECTOR
Stéphane de Jouffrey

STUDY COMPLETION DATE
24th March 1995

PERFORMING LABORATORY
Centre International de Toxicologie (C.I.T.)
Miserey - 27005 Evreux - France

LABORATORY STUDY NUMBER
12407 TSG

CONTENTS

STATEMENT OF THE STUDY DIRECTOR

The study was performed in compliance with the principles of Good Laboratory Practice Regulations:
. O.E.C.D. principles of Good Laboratory Practice, C(81)30(final) Annex 2. May 12, 1981.

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 the Centre International de Toxicologie (C.I.T.), Miserey, 27005 Evreux, France.

Toxicology

S. de Joutfrey Date: 24.3.95
Study Director
Doctor of Veterinary Medicine
Head of Short-term and Environmental Toxicology

OTHER SCIENTISTS INVOLVED IN THIS STUDY

For Pharmacy: J. Richard
Doctor of Pharmacy

For Toxicology: C. Pelcot
Study Supervisor

STATEMENT OF QUALITY ASSURANCE UNIT

1. Specific study inspections

Type of inspections	Dates (day/month/year)	
	Inspections	Report to Study Director / Management (*)
Protocol	24.10.94	26.10.94
Report	14.3.95	14.3.95

2. Routine inspections performed on other studies of the same type according to a frequency defined in Q.A.U. procedures

Inspected phase	Dates (day/month/year)	
	Inspections	Report to Study Director / Management (*)
Animals/housing	13.10.94	17.10.94
Treatment	15.11.94	16.11.94
Test substance/preparation	23.11.94	25.11.94

The inspections were performed in compliance with C.I.T. Quality Assurance Unit procedures and the Good Laboratory Practice Regulations.

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

M. Labiche Date: 24.3.95
Pharmacist
Head of Quality Assurance Unit
and Scientific Archives

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 following intradermal injection and cutaneous application was evaluated in guinea-pigs according to the maximization method of Magnusson and Kligman. The study was conducted in compliance with the principles of Good Laboratory Practice Regulations.

Methods

Thirty guinea-pigs (15 males and 15 females) were allocated to 2 groups: a control group 1 (5 males and 5 females) and a treated group 2 (10 males and 10 females).
The sensitization potential of the test substance was evaluated after a 10-day induction period during which time the animals were treated with sterile isotonic saline solution (0.9% NaCl) (control group) or the test substance (treated group). On day 1, in presence of Freund's complete adjuvant, 0.1 ml of the test substance at a concentration of 1% (w/w) in the vehicle was administered by intradermal route. On day 8, 0.5 ml of the test substance in its original form was applied by cutaneous route during 48 hours by means of an occlusive dressing. After a period of 12 days without treatment, a challenge cutaneous application of 0.5 ml of the vehicle (left flank) and 0.5 ml of the test substance in its original form (right flank) were administered to all animals. The test substance and the vehicle were prepared on a dry gauze pad then were applied to the skin and held in place for 24 hours by means of an occlusive dressing. Cutaneous reactions on the challenge application sites were then evaluated 24 and 48 hours after removal of the dressing.

After the final scoring period, the animals were killed. Due to the absence of cutaneous reactions, no skin samples were taken from the challenge application sites from all the animals.

The sensitivity of the guinea-pigs in C.I.T. experimental conditions were checked in a recent study with a positive sensitizer: Dinitro 2.4 Chlorobenzene. During induction period the test substance was applied at 0.1% (day 1) and 5% (day 8) concentrations. At cutaneous challenge application, 1% (w/w) was tested on the right flank.

Results

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

After 24 and 48 hours following removal of the dressing of the cutaneous challenge application of the test substance, no cutaneous reactions were recorded.

The guinea-pigs which were used in a recent study showed a satisfactory sensitization response in 95% animals using a positive sensitizer (appendix 5).

Conclusion

Under our experimental conditions and according to the maximization method established by Magnusson and Kligman, no cutaneous reactions attributable to the sensitization potential of the test substance [redacted] in its original form were observed in guinea-pigs.

1. INTRODUCTION

The objective of this study, performed according to maximization method established by 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 Man.

During the induction period, the test substance was administered by intradermal route (together with an adjuvant to maximise potential reactions) and cutaneous route. After a rest period of 12 days, a challenge application with the test substance was performed in order to provoke a cutaneous sensitization reaction.

The study was conducted in compliance with:
. O.E.C.D. guideline No. 406, 17th July 1992.
. E.C. Directive No. 92/69/E.E.C., B_6, 31st July 1992.

2. MATERIALS AND METHODS

2.1. TEST AND CONTROL SUBSTANCES

2.1.1 Test substance

The test substance [redacted] used in the study was supplied by Elf Atochem S.A.

Documentation supplied by the Sponsor identified the test substance as follows:
. denomination:
  - protocol: [redacted]
  - labelling: [redacted]
. batch number:
  - protocol: [redacted]
  - labelling: [redacted]
. description: brownish liquid
. container: 1 plastic flask
. date of receipt: 28.11.94
. storage conditions: at room temperature.

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

2.1.2 Vehicle

The vehicle used was sterile isotonic saline solution (0.9% NaCl), batch No. 3126 (Biosédra, 92240 Malakoff, France).

(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 Preparation

The test substance was prepared in an appropriate vehicle.

2.1.4 Other substances

The other substances used were Freund's complete adjuvant, batch No. 063H8800 (Sigma, 38297 Saint-Quentin-Fallavier, France); sodium laurylsulphate, batch No. 83H0238 (Sigma, 38297 Saint-Quentin-Fallavier, France) and vaseline, batch No. 0015 (Coopérative Pharmaceutique Française, 77000 Melun, France).

2.2. TEST SYSTEM

2.2.1 Animals

Species and strain: Dunkin-Hartley guinea-pigs.
Reason for this choice: species recommended by the international regulations for sensitization studies. The strain used has been shown to produce a satisfactory sensitization response using known positive sensitizers.
Breeder: Centre d'Elevage Lebeau, 78950 Gambais, France.
Number: 30 animals (15 males and 15 females).
Allocation of the animals to the groups: on day -1, the animals were weighed and randomly allocated to 2 groups: a control group 1 consisting of 10 animals (5 males and 5 females) and a treated group 2 consisting of 20 animals (10 males and 10 females).
Weight: on day -1, the animals had a mean body weight of 359 ± 17 g for the males and 345 ± 26 g for the females.
Acclimatization: at least 5 days before the beginning of the study.
Identification of the animals: the animals were identified individually by an ear-tattoo.

2.2.2 Environmental conditions

During the acclimatization period and throughout the study, the conditions in the animal room were as follows:
. temperature: 21 ± 2°C
. relative humidity: 30 to 70%
. light/dark cycle: 12 h/12 h
. ventilation: about 12 cycles/hour of filtered, non-recycled air.
During the acclimatization period and throughout the study, the animals were housed individually in polycarbonate cages (48 x 27 x 20 cm) equipped with a polypropylene bottle. Calibrated and dust-free sawdust was provided as litter (SICSA, 92142 Alfortville, France). An analysis of potential residues and major contaminants is performed periodically (Laboratoire Wolff, 92110 Clichy, France).

2.2.3 Food and water

During the study, the animals had free access to 106 diet" (U.A.R., 91360 Villemoisson-sur-Orge, France).
Food was periodically analysed (composition and contaminants) by the supplier.
The diet formula is presented in appendix 2.

Animals had free access to drinking water, filtered by a F.G. Millipore membrane (0.22 micron).
Bacteriological and chemical analysis of the water and detection of possible contaminants (pesticides, heavy metals and nitrosamines) are performed periodically.
Results are archived at C.I.T.

There were no contaminants in the diet, water or sawdust at levels likely to have influenced the outcome of the study.

2.3. TREATMENT

2.3.1 Preliminary test

A preliminary test was performed to define the concentration to be tested in the main study.

By intradermal route

Determination of the Minimum Irritant Concentration (M.I.C.):

. 24 hours before treatment, the dorsal region of the animals was clipped,
. the test substance was prepared in an appropriate vehicle,
. intradermal administration of the test substance (volume 0.1 ml) at increasing concentrations was performed in order to determine the minimum concentration which causes an irritation,
. evaluation of the potential cutaneous reactions, 24 and 48 hours after injection.

By cutaneous route

Determination of the Minimum Irritant Concentration (M.I.C.) and Maximum Non-Irritant Concentration (M.N.I.C.):

. 24 hours before treatment, the dorsal region of the animals was clipped,
. 0.5 ml of the test substance in its original form was applied to a dry gauze pad of approximately 4 cm^2 and then held in place by an occlusive dressing for 24 hours,
. potential cutaneous reactions were evaluated 24 and 48 hours after removal of the gauze pads.

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 (scapular area 4 cm x 2 cm),
. clipped and shaved on day 21 (each flank 2 cm x 2 cm).

2.3.3 Induction phase by intradermal and cutaneous routes

2.3.3.1 Intradermal route

On day 1, 6 intradermal injections were made into a clipped area (4 cm x 2 cm) in the scapular region, using a needle (diameter: 0.50 x 16 mm, Térumo: C.M.L., 77140 Nemours, France) mounted on a 1 ml glass syringe (0.01 ml graduations, Record: Carrieri, 75005 Paris, France). Three injections of 0.1 ml were injected into each side of the animal, as follows:

Control group (figure 1)

. Freund's complete adjuvant diluted to 50% (v/v) with a sterile isotonic saline solution (0.9% NaCl),
. vehicle,
. a mixture of 50/50 (w/v) Freund's complete adjuvant diluted to 50% (v/v) with a sterile isotonic aqueous NaCl solution and the vehicle.

Treated group (figure 2)
. Freund's complete adjuvant diluted to 50% (v/v) with a sterile isotonic saline solution (0.9% NaCl),
. test substance at a concentration of 1% (w/w) in the vehicle,
. a mixture 50/50 (w/v) of Freund's complete adjuvant diluted to 50% (v/v) with a sterile isotonic saline solution (0.9% NaCl), and, the test substance at a concentration of 1% (w/w) in the vehicle.

2.3.3.2 Cutaneous route

On day 7, the scapular area was clipped. As the test substance is shown to be non-irritant after occlusive cutaneous treatment during preliminary test, the animals were treated with 0.5 ml of sodium laurylsulphate (10%) in vaseline to provoke local irritation.

On day 8, a cutaneous application on the 6 injection areas (4 cm x 2 cm) of the scapular region was performed.

Control group
. application of 0.5 ml of the vehicle.

Treated group
. application of 0.5 ml of a non-irritant concentration of the test substance i.e. in its original form.

The test substance and the vehicle were prepared on a dry gauze pad (Semes France, 54183 Heillecourt, France), which was then applied to the scapular region and held in place for 48 hours by means of an adhesive hypoallergenic dressing (Laboratoires de Pansements et d'Hygiène, 21300 Chenove, France) and an adhesive anallergenic waterproof plaster (Laboratoire des Professions Médicales, 92240 Malakoff, France).
No residual test substance was observed at removal of the dressing.
One hour after removal of the occlusive dressing, cutaneous reactions were recorded.

2.3.3.3 Challenge phase

At the end of the rest period on day 22, the test substance was applied at the Maximum Non-Irritant Concentration (M.N.I.C.) i.e. in its original form.

On day 22, the animals from both groups received an application of 0.5 ml of the M.N.I.C. of the test substance on the posterior right flank, and 0.5 ml of the vehicle on the posterior left flank. This application was performed using a 1 ml plastic syringe (0.01 ml graduations, Térumo: C.M.L., 77140 Nemours, France). The test substance and vehicle were prepared on a dry gauze pad (Semes France, 54183 Heillecourt, France), then applied to a 4 cm^2 (2 cm x 2 cm) clipped area of the skin. The gauze pad was held in contact with the skin for 24 hours by means of an occlusive, hypoallergenic dressing (Laboratoires de Pansements et d'Hygiène, 21300 Chenove, France) and an adhesive anallergenic waterproof plaster (Laboratoire des Professions Médicales, 92240 Malakoff, France).
No residual test substance was observed at removal of the dressing.

2.4. SCORING OF CUTANEOUS REACTIONS

Twenty-four and 48 hours after removal of the dressing from the challenge application site, the 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.

2.5. CLINICAL EXAMINATIONS

The animals were observed twice a day during the study in order to record clinical signs and to check for mortality.

2.6. BODY WEIGHT

The animals were weighed individually on the day of allocation into the groups, on the first day of the study (day 1), then on days 8, 15 and 25.

2.7. PATHOLOGY

2.7.1 Necropsy

On day 25, after the 48-hour observation period, the animals were killed by CO_2 inhalation in excess.

2.7.2 Cutaneous samples

On day 25, no skin samples were taken.

2.7.3 Microscopic examination

No histological examinations were performed.

2.8. 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 more marked than the most severe reactions of the control animals, or, if "doubtful" macroscopic reactions are confirmed at microscopic examination as being due to the sensitization process. Sensitization reactions are characterized at microscopic examination by basal spongiosis, reactional acanthosis of the epidermis and infiltration of mononucleated cells into the dermis (1).

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 E.E.C. directive 93/21/E.E.C. published in the Journal Officiel des Communautés Européennes, when the reactions are positive in at least 30% of the treated animals, the test substance has sensitization properties and the sentence "R 43: May cause sensitization by skin contact" must be applied.

(1) Duprat, P. ; Delsaut. L. ; Gradiski, D. ; Lepage, M. : Investigations histo–pathologiques et cytologiques lors de la mise en évidence, chez le cobaye, d'une allergie cutanée de type retardé. Revue Méd. Vét. 127: 7, 1083-1101 (1976).

2.9. SUMMARY DIAGRAMS

Figure 1: control group

Chronology

Day -1 Clipping of the scapular region

Day 1 Intradermal injection

Day 7 Clipping + Sodium laurylsulphate

Day 8 Application covered by an occlusive dressing

Day 10 Removal of dressing and scoring after one hour

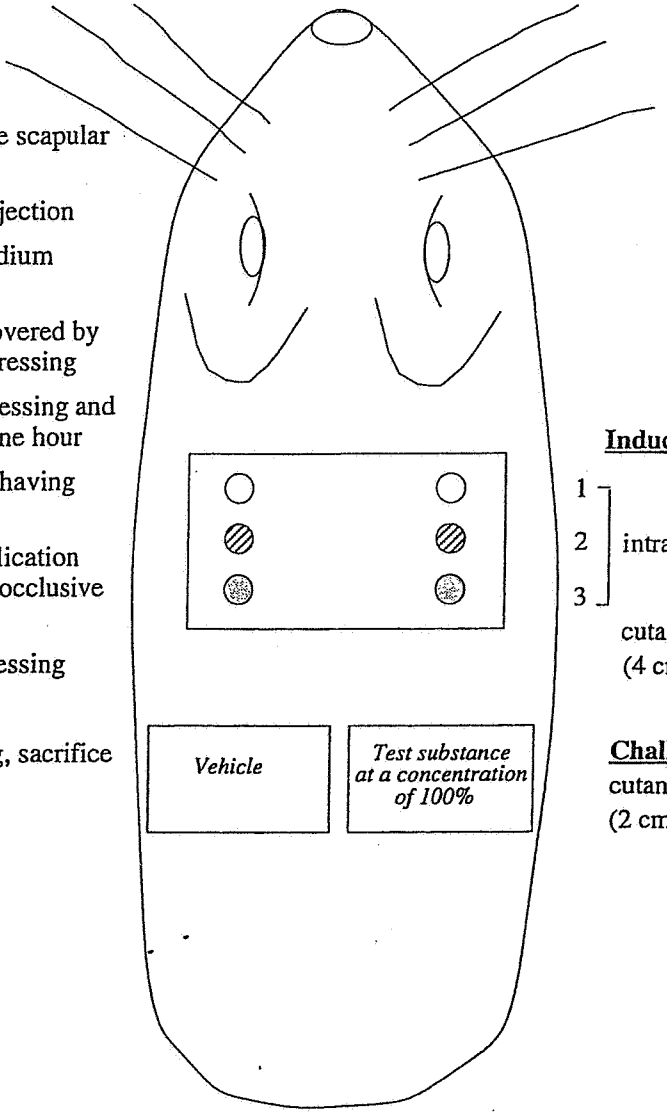
Day 21 Clipping and shaving of the flanks

Day 22 Challenge application covered by an occlusive dressing

Day 23 Removal of dressing

Day 24 First scoring

Day 25 Second scoring, sacrifice of the animals



Induction site

1, 2, 3 intradermal injections

cutaneous application (4 cm x 2 cm)

Challenge application

cutaneous application (2 cm x 2 cm)

Intradermal injections

○ 1 – 50% Freund's complete adjuvant and NaCl at 0.9% solution

▨ 2 – vehicle

○ 3 – 1 + 2, 50/50 (w/v)

Figure 2: treated group

Chronology

Day -1 Clipping of the scapular region

Day 1 Intradermal injection

Day 7 Clipping + Sodium laurylsulphate

Day 8 Application covered by an occlusive dressing

Day 10 Removal of dressing and scoring after one hour

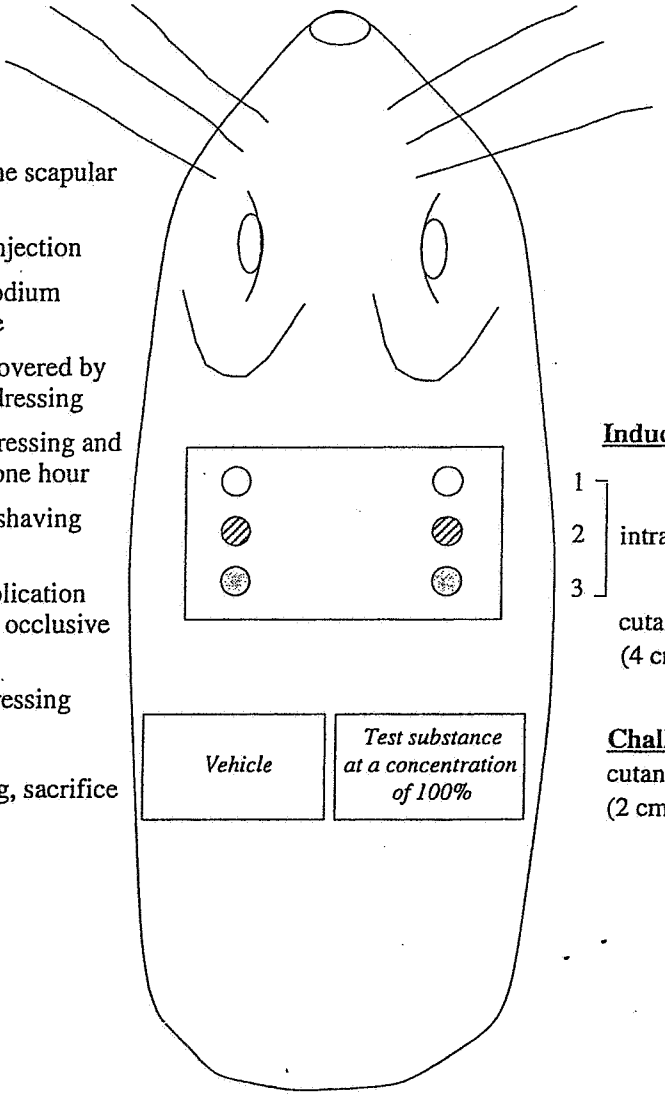
Day 21 Clipping and shaving of the flanks

Day 22 Challenge application covered by an occlusive dressing

Day 23 Removal of dressing

Day 24 First scoring

Day 25 Second scoring, sacrifice of the animals



Induction site

1, 2, 3 intradermal injections

cutaneous application (4 cm x 2 cm)

Challenge application

cutaneous application (2 cm x 2 cm)

Intradermal injections

○ 1 50% Freund's complete adjuvant and NaCl at 0.9% solution

▨ 2 test substance at a concentration of 1% (w/w) in the vehicle

○ 3 1 + 2, 50/50 (w/v)

2.10. CHRONOLOGY OF THE STUDY

The chronology of the study is summarized as follows:

Procedure	Date	Day
Arrival of the animals	1.12.94	-8
Allocation of the animals into groups	8.12.94	-1
Weighing, induction by intradermal injection	9.12.94	1
Laurylsulfate application	15.12.94	7
Weighing, induction by cutaneous route	16.12.94	8
Removal of occlusive dressings and scoring of local reactions after 1 hour	18.12.94	10
Weighing	23.12.94	15
Challenge cutaneous application	30.12.94	22
Removal of occlusive dressings	31.12.94	23
Scoring of cutaneous reactions after		
. 24 hours	1.1.95	24
. 48 hours	2.1.95	25
Weighing, sacrifice of the animals	2.1.95	25

2.11. ARCHIVES

The study archives:
. protocol and possible amendments,
. raw data,
. correspondence,
. final study report and possible amendments,

are stored on the premises of C.I.T., Miserey, 27005 Evreux, France, for 5 years after the end of the *in vivo* study. At the end of this period, the study archives will be returned to the Sponsor.

3. RESULTS

3.1. PRELIMINARY STUDY

3.1.1 Administration by intradermal route

Several tests were performed to determine the minimal irritant concentration which did not provoke necrosis or ulceration.

Animal number	Concentration of the test substance % (w/w)	Scoring after treatment 24 hours	48 hours
Male No. 01	5	irritation	irritation
	1	irritation	irritation
	0.1	irritation	irritation
Female No. 01	5	irritation	irritation
	1	irritation	irritation
	0.1	irritation	irritation

Concentration used in the main study was 1% (w/w).

3.1.2 Application by cutaneous route

Animal number	Concentration of the test substance %		Scoring 24 hours after removal of the dressing (1) E	O	Scoring 48 hours E	O
Male No. 01	100	RF	0	0	0	0
		LF	0	0	0	0
Female No. 01	100	RF	0	0	0	0
		LF	0	0	0	0

M.N.I.C. is 100% of the test substance.
E : erythema
O : oedema
RF: right flank
LF: left flank

(1) no residual test substance was observed.

3.2. MAIN STUDY

3.2.1 Clinical examinations

No clinical signs or mortalities were observed during the study.

The body weight gain of the treated animals was normal when compared to that of the control animals (figures 3 and 4, appendix 3).

3.2.2 Scoring of cutaneous reactions

Observations of cutaneous reactions are presented in appendix 4.

3.2.2.1 End of the induction period

On day 10, after removal of the dressing, signs of irritation in control and treated groups were observed at the intradermal injection sites.

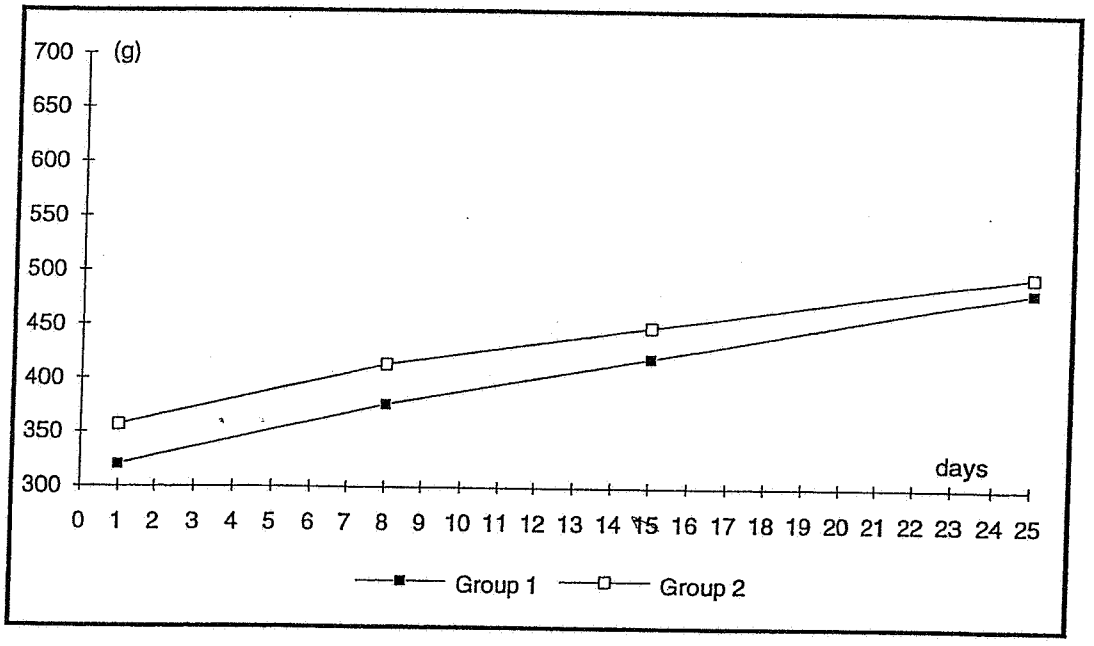
3.2.2.2 Challenge application

No cutaneous reactions were observed 24 and 48 hours after removal of the dressing of the challenge cutaneous application of the test substance.

4. CONCLUSION

Under our experimental conditions and according to the maximization method established by Magnusson and Kligman, no cutaneous reactions attributable to the sensitization potential of the test substance [redacted] in its original form were observed in guinea-pigs.

Figure 4: Female body weight gain (g)



APPENDICES

1. Analytical certificate

Controle laboratoire N° 57/176.

Elf-Atochem.
ATELIER FORAFAC DE OISSEL
LE 29/7/94.

A l'attention de MME VAESKEN. (D.C.F.S)
de MR DELLE CASE. (V.S.P)

Les controles analytiques et les tests applicatifs éffectués sur l'opération 176 de [redacted] ont donné les résultats suivants:

* MATIERE SECHE	:	27.0 %	(Norme : de 26.5% à 27%)
* TAUX D'ETHANOL	:	34.0 %	(Norme : de 32.0% à 35.0%)
* TAUX D'EAU	:	39.0 %	(Norme : de 38.0% à 41.5%)
* TENSION SUPERFICIELLE à 1000 ppm en eau bidistillée aprés 30 minutes d'équilibre à 25°C	:	15.35 mN/m	(Norme : < 16 mN/m)

* POUVOIR MOUSSANT à 25°C:
en eau de mer.

après 30 secondes:		290 ml	(Norme : > 150 ml)
aprés 3 minutes	:	280 ml	(Norme : > 150 ml)
aprés 5 minutes	:	280 ml	(Norme : > 150 ml)

* MASSE VOLUMIQUE à 25°C	:	1.039 kg/m3	(Norme : 1.038+/-0.010 kg/m3

Les valeurs données par les tests applicatifs (y compris les tests d'étalement) sont conformes aux normes.

L'opération 176 de [redacted] est commercialisable.

Salutations.

MOREAU Jean-Francois.

Copie à MR GARCIA G. (CAL)
à MR GUILLAUD JL/MR FREMY
à MR DURUAL P.

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 biproducts 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.

U.A.R., 7 rue Galliéni, 91360 Villemoisson - Tel: 69.04.03.57 - Fax : 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	111	342	351	61	412	64	476	62	538
		112	375	382	83	465	63	528	82	610
		113	370	376	38	414	70	484	76	560
		114	369	385	67	452	60	512	72	584
		115	335	346	59	405	37	442	70	512
		M	358	368	62	430	59	488	72	561
		SD	18	18	16	27	13	33	7	38
	Female	126	334	331	50	381	41	422	54	476
		127	301	317	72	389	34	423	73	496
		128	307	308	57	365	50	415	79	494
		129	328	325	44	369	29	398	54	452
		130	316	320	57	377	61	438	53	491
		M	317	320	56	376	43	419	63	482
		SD	14	9	10	10	13	15	12	18
2	Male	116	358	369	43	412	33	445	41	486
		117	344	364	43	407	56	463	59	522
		118	360	371	64	435	37	472	49	521
		119	367	372	49	421	63	484	64	548
		120	317	337	63	400	62	462	111	573
		121	348	343	50	393	47	440	51	491
		122	336	338	67	405	44	449	68	517
		123	336	337	73	410	45	455	56	511
		124	341	350	76	426	52	478	85	563
		125	347	359	62	421	36	457	81	538
		M	345	354	59	413	48	461	67	527
		SD	14	15	12	13	11	14	21	29
	Female	131	317	321	40	361	54	415	70	485
		132	364	363	70	433	50	483	26	509
		133	393	399	48	447	69	516	68	584
		134	367	368	36	404	38	442	47	489
		135	349	342	70	412	30	442	48	490
		136	352	354	56	410	33	443	61	504
		137	345	344	85	429	15	444	49	493
		138	368	370	68	438	18	456	32	488
		139	339	329	31	360	12	372	35	407
		140	385	378	62	440	27	467	52	519
		M	358	357	57	413	35	448	49	497
		SD	22	24	17	31	18	38	15	43

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

4. Individual observation of cutaneous reactions

MACROSCOPIC EXAMINATION OF CUTANEOUS REACTIONS

Challenge application

Group	Sex	Animals	Day 24 scoring period (after 24 hours)				Day 25 scoring period (after 48 hours)			
			Erythema		Oedema		Erythema		Oedema	
			LF	RF	LF	RF	LF	RF	LF	RF
Control 1	Male	111	0	0	0	0	0	0	0	0
		112	0	0	0	0	0	0	0	0
		113	0	0	0	0	0	0	0	0
		114	0	0	0	0	0	0	0	0
		115	0	0	0	0	0	0	0	0
	Female	126	0	0	0	0	0	0	0	0
		127	0	0	0	0	0	0	0	0
		128	0	0	0	0	0	0	0	0
		129	0	0	0	0	0	0	0	0
		130	0	0	0	0	0	0	0	0
Treated 2	Male	116	0	0	0	0	0	0	0	0
		117	0	0	0	0	0	0	0	0
		118	0	0	0	0	0	0	0	0
		119	0	0	0	0	0	0	0	0
		120	0	0	0	0	0	0	0	0
		121	0	0	0	0	0	0	0	0
		122	0	0	0	0	0	0	0	0
		123	0	0	0	0	0	0	0	0
		124	0	0	0	0	0	0	0	0
		125	0	0	0	0	0	0	0	0
	Female	131	0	0	0	0	0	0	0	0
		132	0	0	0	0	0	0	0	0
		133	0	0	0	0	0	0	0	0
		134	0	0	0	0	0	0	0	0
		135	0	0	0	0	0	0	0	0
		136	0	0	0	0	0	0	0	0
		137	0	0	0	0	0	0	0	0
		138	0	0	0	0	0	0	0	0
		139	0	0	0	0	0	0	0	0
		140	0	0	0	0	0	0	0	0

LF: left flank (control)
RF: right flank (treated)

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

Purpose: check the sensitivity of Dunkin-Hartley Guinea-pigs (Centre d'élevage Lebeau) to a positive control test article

Method	:	Magnusson and Kligman
Test substance	:	DINITRO 2.4 CHLOROBENZENE
C.I.T. Study - Date	:	December 1994 (CIT/Study No. 12437 TSG)
Number of animals	:	20 females
Induction	:	0.1% intradermal route day 1 5% cutaneous route day 8
Challenge application	:	1% right flank paraffin oil left flank

Conclusion

Under our experimental conditions and according to the Magnusson and Kligman method, DINITRO 2.4 CHLOROBENZENE at a concentration of 1% (w/w) induced positive skin sensitization reactions in 95% of the guinea-pigs.

INDIVIDUAL REACTIONS: CHALLENGE PHASE
MACROSCOPIC FINDINGS

Group	Sex	Animals	24-hour scoring period				48-hour scoring period				Conclusion	
			Erythema		Oedema		Erythema		Oedema			
			LF	RF	LF	RF	LF	RF	LF	RF	LF	RF
Treated	Female	11	0	2	0	0	0	2/S	0	0	-	+
		12	0	2	0	0	0	2/S/A	0	0	-	+
		13	0	1	0	0	0	1/S	0	0	-	+/-
		14	0	3	0	2	0	3/S	0	0	-	+
		15	0	2	0	2	0	2/S	0	0	-	+
		16	0	4	0	2	0	4	0	0	-	+
		17	0	3	0	2	0	1/S	0	0	-	+
		18	0	2	0	0	0	2/S	0	0	-	+
		19	0	2	0	0	0	2/S	0	0	-	+
		20	0	3	0	2	0	3/S	0	2	-	+
		21	0	3	0	2	0	3/S	0	0	-	+
		22	0	3	0	2	0	3/S	0	0	-	+
		23	0	2	0	0	0	1/S	0	0	-	+
		24	0	3	0	2	0	2/S	0	0	-	+
		25	0	2	0	0	0	2/S	0	0	-	+
		26	0	3	0	2	0	2	0	0	-	+
		27	0	2	0	0	0	1/S	0	0	-	+
		28	0	3	0	2	0	2	0	0	-	+
		29	0	3	0	2	0	3/S	0	2	-	+
		30	0	3	0	2	0	3/S	0	2	-	+

- : negative
+ : hypersensitizing reaction
+/- : borderline reactions
S : dryness of the skin
A : crust
LF : left flank
RF : right flank