CONFIDENTIAL

CIT

SPONSOR

Elf Atochem S.A.
Cours Michelet
La Défense 10
92091 Paris-la-Défense CEDEX
France

TEST SUBSTANCE

[redacted]

STUDY TITLE

ACUTE DERMAL IRRITATION IN RABBITS

STUDY DIRECTOR

Xavier Manciaux

STUDY COMPLETION DATE

13 December 1999

PERFORMING LABORATORY

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

LABORATORY STUDY NUMBER

18749 TAL

Company Sanitized. Does not contain TSCA CBI

IFM recherche
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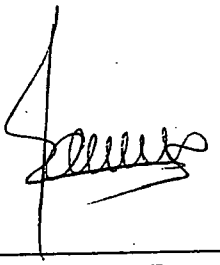
STATEMENT OF THE STUDY DIRECTOR

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: 13 December 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	10 November 1999	7 December 1999	10 December 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: 13 December 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 skin irritation was evaluated in rabbits according to OECD (No. 404, 17th July 1992) and EC (92/69/EEC, B.4, 31st July 1992) guidelines. The study was conducted in compliance with the principles of Good Laboratory Practice Regulations.

Methods

The study design was established according to available information on the test substance and the above guidelines.

In the first instance, the test substance was applied for periods of 3 minutes and 4 hours to a single male New Zealand White rabbit.
Since the test substance was not severely irritant on this first animal, it was then applied for 4 hours to two other animals.

A single dose of 0.5 ml of the undiluted test substance was applied to the closely-clipped skin of one flank.

The test substance was held in contact with the skin by means of a semi-occlusive dressing. Cutaneous reactions were observed approximately 1 hour, 24, 48 and 72 hours after removal of the dressing.
The mean values of the scores for erythema and oedema were calculated for each animal.

Results

After a 3-minute exposure (one animal):
No cutaneous reactions were observed.

After a 4-hour exposure (three animals):
A very slight erythema was noted in two animals on day 1; it persisted up to day 3 in one of them.
No other cutaneous reactions were observed during the study.

Mean scores over 24, 48 and 72 hours for each animal were 0.0, 0.7 and 0.0 for erythema and 0.0, 0.0 and 0.0 for oedema.

Conclusion

Under our experimental conditions, the test substance [redacted] is non-irritant when applied topically to rabbits.

According to the classification criteria laid down in Commission Directive 93/21/EEC (27th April 1993) adapting to technical progress for the eighteenth time Council Directive 67/548/EEC, the test substance was considered non-irritant.

RESUME

A la demande de Elf Atochem S.A., Paris-la-Défense, France, les propriétés irritantes du produit [redacted] après application cutanée ont été évaluées chez le Lapin selon les lignes directrices de l'OCDE (n° 404, 17 juillet 1992) et de la CEE (92/69/EEC, B.4, 31 juillet 1992).
L'étude a été réalisée conformément aux règles de Bonnes Pratiques de Laboratoire.

Méthode

L'étude a été réalisée selon les informations disponibles sur le produit et les lignes directrices mentionnées ci-dessus.

Dans un premier temps, le produit a été appliqué pendant 3 minutes et 4 heures sur 1 seul lapin mâle New Zealand White.
Le produit n'étant pas fortement irritant sur ce premier animal, il a ensuite été appliqué pendant 4 heures sur 2 autres animaux.

Une dose unique de 0,5 ml de produit non dilué a été appliquée sur une surface de peau tondue d'un des flancs.

Le produit a été maintenu en contact avec la peau au moyen d'un pansement semi-occlusif. Les réactions cutanées ont été observées environ 1 heure, 24, 48 et 72 heures après l'enlèvement du pansement.
La moyenne des scores a été calculée pour l'érythème et pour l'oedème pour chaque animal.

Résultats

Après une exposition de 3 minutes (1 animal) :
Aucune réaction cutanée n'est observée pendant l'étude.

Après une exposition de 4 heures (3 animaux) :
Un érythème très léger est noté chez 2 animaux au jour 1 ; il persiste jusqu'au jour 3 chez 1 animal.
Aucune autre réaction cutanée n'est observée durant l'étude.

Les scores moyens calculés pour chaque animal après 24, 48 et 72 heures sont de 0,0 ; 0,7 et 0,0 pour l'érythème et de 0,0 ; 0,0 et 0,0 pour l'oedème.

Conclusion

Dans nos conditions expérimentales, le produit [redacted] est considéré non irritant par voie cutanée chez le Lapin.

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 irritant.

1. INTRODUCTION

The objective of this study was to evaluate the potential of the test substance [redacted] to induce skin irritation following a single topical application to rabbits.

In the assessment of the toxic characteristics of a test substance, determination of the irritant and/or corrosive effects on the skin of mammals is an important initial step. Information derived from this test serves to indicate the possible hazards likely to arise from exposure of the skin to the test substance.

This study was conducted in compliance with:
. OECD guideline No. 404, 17th July 1992,
. EC Directive No. 92/69/EEC, B.4, 31st July 1992.

2. MATERIALS AND METHODS

2.1 TEST SUBSTANCE

2.1.1 Identification

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: 1392/99
. description: amber liquid
. 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 and in analytical certificate (presented in appendix 1) provided by the Sponsor.

The pH of the test substance, as mentioned on the test article description, was 5 ± 1.

2.1.2 Formulation procedure

The test substance was used undiluted.

2.2 TEST SYSTEM

2.2.1 Animals

Sex, species, strain: male New Zealand White rabbits.
Reason for this choice: species generally accepted by regulatory authorities for this type of study.
Breeder: Elevage des Dombes, 01400 Châtillon-sur-Chalaronne, France.
Number of animals: three animals were used, as recommended by the international guidelines.
Identification: the animals were identified individually with a metal ear tag.
Weight: on the day of treatment, the animals had a mean body weight ± standard deviation of 2.8 ± 0.1 kg.
Acclimatization: at least 5 days before the beginning of the study.

2.2.2 Environmental conditions

The conditions in the animal room were set as follows:
. temperature: 18 ± 3°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 were verified and calibrated at regular intervals.
The animals were housed individually in polystyrene cages (48.2 cm x 58 cm x 36.5 cm).
Each cage was equipped with a food container and a water bottle.

2.2.3 Food and water

During the study, the animals had free access to 112 C pelleted diet (UAR, 91360 Villemoisson-sur-Orge, France).
Each batch of food was analysed 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 or drinking water at levels which may be expected to have interfered with or prejudiced the outcome of the study.

2.3 TREATMENT

2.3.1 Preparation and selection of the animals

The day before treatment, both flanks of each animal were clipped using electric clippers and the skin of each animal was examined. Only animals with healthy intact skin were used.

2.3.2 Study design

The study design was established according to available information on the test substance and the OECD (No. 404) and EC (92/69/EEC, B.4) guidelines.

As possible irritant effects were anticipated, the test substance was evaluated on a single animal (No. 900) in the first instance. The duration of exposure was 3 minutes on one flank and 4 hours on the other flank.
Since the test substance was not severely irritant on this first animal, it was then applied for 4 hours to two other animals (Nos. 47 and 48).

2.3.3 Application of the test substance

Doses of 0.5 ml of the undiluted test substance were placed on a dry gauze pad, which was then applied to the right flank (application for 4 hours) or the left flank (application for 3 minutes) of the animals.

The test substance and the gauze pad were held in contact with the skin by means of an adhesive hypoallergenic aerated semi-occlusive dressing and a restraining bandage.

The untreated skin served as control.

No residual test substance was observed on removal of the dressing.

2.3.4 Chronology of the study

Animal number	Date of treatment (day 1)	End of the observation period
900	6 July 1999 (application for 3 minutes and 4 hours)	9 July 1999
47	8 July 1999	11 July 1999
48	8 July 1999	11 July 1999

2.4 CUTANEOUS EXAMINATIONS

The skin was examined approximately 1 hour, 24, 48 and 72 hours after removal of the dressing.

Following the OECD and EC guidelines:
. when there was no evidence of dermal irritation after 72 hours, the study was ended.
. when there was persistent cutaneous irritation after 72 hours, the observation period was extended to a maximum of 14 days (until day 15) in order to determine the progress of the lesions and their reversibility.
. when severe irritant effects were observed, the animals were killed on humane grounds.

Any change in the animals' behaviour was noted.

2.5 DESCRIPTION AND EVALUATION OF CUTANEOUS REACTIONS

Dermal irritation was evaluated for each animal according to the following scoring 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 (edges of area well-defined by definite raising)	2
. moderate oedema (raised approximately 1 millimetre)	3
. severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Any other lesions were noted.

2.6 INTERPRETATION OF RESULTS AND CLASSIFICATION OF SUBSTANCES

The results obtained were evaluated in conjunction with the nature and the reversibility of the findings observed.
Classification of the test substance is based on the criteria laid down in Commission Directive 93/21/EEC of 27 April 1993 adapting to technical progress for the eighteenth time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.

2.6.1 Interpretation of the results

2.6.1.1 Criteria for irritation

A substance or a preparation is considered to be irritating to the skin if, when it is applied to healthy intact animal skin for up to 4 hours, significant inflammation is caused and which persists for 24 hours or more after the end of the exposure period.

2.6.1.2 Criteria for corrosion

A substance or a preparation is considered to be corrosive if, when it is applied to healthy intact animal skin, it produces full thickness destruction of skin tissue on at least one animal during the test for skin irritation, or if the result can be predicted, for example: from strongly acid or alkaline reactions.

2.6.2 Classification of the test substances

2.6.2.1 Irritant substances

- symbol Xi, indication of danger "irritant",

- phrases indicating the nature of special risks: R 38: "Irritating to skin"

Inflammation is significant if:

. the mean value of the scores is two or more for either erythema and eschar formation or oedema formation. The same will be the case where the test has been completed using three animals if the score for either erythema and eschar formation or oedema formation observed in two or more animals is equivalent to the value of two or more,

. it persists in at least two animals at the end of the observation period. Specific effects such as hyperplasia, desquamation, discolouration, fissures, eschar and alopecia should be taken into account.

All scores obtained at each reading time (24, 48 and 72 hours) for an effect are used for calculating the respective mean values.

2.6.2.2 Corrosive substances

- symbol C, indication of danger: "corrosive",

- phrases indicating the nature of special risks:

. R 34: "Causes burns"
If, when applied to healthy intact animal skin, full-thickness destruction of skin tissue occurs as a result of up to 4 hours exposure, or if this result can be predicted.

. R 35: "Causes severe burns"
If, when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of up to 3 minutes exposure, or if this result can be predicted.

2.7 PROTOCOL ADHERENCE

The study was performed in accordance with Study Protocol No. 18749 TAL and subsequent amendments, with the following deviations from the agreed Study Protocol:

. the temperature and relative humidity recorded in the animal room were sometimes outside of the target ranges specified in the protocol.

These minor deviations were not considered to compromise the validity or integrity of the study.

2.8 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.

3. RESULTS

The observations recorded during the study are presented in tables 1 and 2.

After a 3-minute exposure (one animal):
No cutaneous reactions were observed.

After a 4-hour exposure (three animals):
A very slight erythema (grade 1) was noted in two animals on day 1; it persisted up to day 3 in one of them.
No other cutaneous reactions were observed during the study.

Mean scores over 24, 48 and 72 hours for each animal were 0.0, 0.7 and 0.0 for erythema and 0.0, 0.0 and 0.0 for oedema.

4. CONCLUSION

Under our experimental conditions, the test substance [redacted] is non-irritant when applied topically to rabbits.

According to the classification criteria laid down in Commission Directive 93/21/EEC (27th April 1993) adapting to technical progress for the eighteenth time Council Directive 67/548/EEC, the test substance was considered non-irritant.

Table 1: Individual cutaneous examinations and mean values of the scores recorded at each reading (24, 48 and 72 hours) for the first animal (application for 3 minutes)

Rabbit number	Dermal Irritation	Scores				Mean irritation score (1)	Interpretation (+) (-)
		1h D1	24h D2	48h D3	72h D4		
900	Erythema	0	0	0	0	0.0	(-)
	Oedema	0	0	0	0	0.0	(-)
	Other	*	*	*	*		

(1) mean of scores on days 2, 3 and 4
h = hour
D = day
(+) = irritant according to E.E.C. criteria
(-) = non-irritant according to E.E.C. criteria
* = None

Table 2: Individual cutaneous examinations and mean values of the scores recorded at each reading (24, 48 and 72 hours) for each animal (application for 4 hours)

Rabbit number	Dermal Irritation	Scores				Mean irritation score (1)	Interpretation (+) (-)
		1h D1	24h D2	48h D3	72h D4		
900	Erythema	0	0	0	0	0.0	(-)
	Oedema	0	0	0	0	0.0	(-)
	Other	*	*	*	*		
47	Erythema	1	1	1	0	0.7	(-)
	Oedema	0	0	0	0	0.0	(-)
	Other	*	*	*	*		
48	Erythema	1	0	0	0	0.0	(-)
	Oedema	0	0	0	0	0.0	(-)
	Other	*	*	*	*		

(1) mean of scores on days 2, 3 and 4
h = hour
D = day
(+) = irritant according to E.E.C. criteria
(-) = non-irritant according to E.E.C. criteria
* = None

APPENDICES

1. Test article description and analytical certificate

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 1392/99

PHYSICAL AND CHEMICAL PROPERTIES

Appearance	:	amber liquid
pH	:	5 ± 1
Density	:	1100 kg/m^3 at 20°C
Boiling point	:	98-100 °C
Flash point	:	no flash point
Solubility	:	water : insoluble soluble in alcohols, aromatic hydrocarbons

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

elf atochem
ATO

Elf Atochem S.A.
Usine de Villers-Saint-Paul
ZA Villers Saint Paul - Rieux
B.P. 20 - 60870 Rieux (France)
Tél : 44.74.44.74 - Fax : 44.74.42.07 - Télex : 140 429 ATO VSP

Villers Saint Paul, le 9 Novembre 1999

CERTIFICAT D'ANALYSE [redacted]

Lot N° [redacted]	Unité	Résultat	Spécification de fabrication	Méthode d'analyse
Extrait Sec	%	30,3	29 à 31	LCU 622
Tertio Butanol	%	0,95	0 à 1	LCU 653
Acrylamide	%	0,07	0 à 0,1	LCU 658
Acide acrylique	%	0,1	0 à 2	LCU 658
Test d'extinction	/	positif	positif	SAI 021

Le Chef de Service du Laboratoire
SIGNATURE :

NOM : Robert Gruppo

2. Diet formula

Ref: 112
COMPLETE DIET
RABBIT MAINTENANCE DIET
Appearance: 4.5 mm diameter granules
Conditioning: bags of 25 kgs

Daily portion: in accordance with race and body weight, Rabbits 100-150 g, water *ad libitum*.

FORMULA %

Cereals	43.8
Grain byproducts and legumes	49
Vegetable protein (soya bean meal, yeast)	4.2
Vitamin and mineral mixture	3

AVERAGE ANALYSIS %

Calorific value (Kcal/kg)	2200
Moisture	10
Proteins	13
Lipids	2.7
Carbohydrates (N.F.E.)	49.3
Fibre	17
Minerals (ash)	8

AMINO ACID VALUES
(calculated in mg/kg)

Arginine	6800
Cystine	2100
Lysine	4600
Methionine	1600
Tryptophan	1400
Glycine	5200

FATTY ACID VALUES
(calculated in mg/kg)

Palmitic acid	6400
Palmitoleic acid	0
Stearic acid	600
Oleic acid	6400
Linoleic acid	12100
Linolenic acid	2400

MINERALS (calculated in mg/kg)

	Nat. val.	CMV val.	Total
P	3500	3500	7000
Ca	4500	4500	9000
K	11600	0	11600
Na	400	1600	2000
Mg	2100	100	2200
Mn	40	40	80
Fe	160	140	300
Cu	12	15	27
Zn	30	45	75
Co	0.1	1.5	1.6
I	0	0	0
Cl	500	3000	3500

VITAMINS (calculated per kg)

	Nat. val.	CMV val.	Total
Vitamin A	2850 IU	6500 IU	9350 IU
Vitamin D3	30 IU	1000 IU	1030 IU
Vitamin B1	4.3 mg	0 mg	4.3 mg
Vitamin B2	3.8 mg	0 mg	3.8 mg
Vitamin B3	16 mg	0 mg	16 mg
Vitamin B6	1 mg	1 mg	2 mg
Vitamin B12	0 mg	0 mg	0 mg
Vitamin E	16 mg	10 mg	26 mg
Vitamin K3	6 mg	1 mg	7 mg
Vitamin PP	55 mg	5 mg	60 mg
Folic acid	0 mg	0 mg	0 mg
Biotin	0 mg	0 mg	0 mg
Choline	850 mg	200 mg	1050 mg
Meso-Inositol	0 mg	0 mg	0 mg

Available under quality "Control Ref.: 112 C"

UAR, 7 rue Galliéni, 91360 Villemoisson - Tel : 01.69.04.03.57 - Fax : 01.69.04.81.97
(Ref. Doc. UAR : 1992)