[redacted]

CONFIDENTIAL

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

STUDY TITLE
ACUTE DERMAL IRRITATION
IN RABBITS

TEST SUBSTANCE
[redacted]

STUDY DIRECTOR
Stéphane de Jouffrey

STUDY COMPLETION DATE
3 February 1997

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

LABORATORY STUDY NUMBER
14887 TAL

CONTENTS

STATEMENT OF THE STUDY DIRECTOR

The study was performed in compliance with the following Principles of Good Laboratory Practice Regulations:

. O.E.C.D. Principles of Good Laboratory Practice, Decision Concerning Mutual Acceptance of Data in the Assessment of Chemicals, C(81)30(final) Annex 2. May 12, 1981.

. 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.

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 Jourrey Date: 3 February 1997
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		
	Inspections	Report to Study Director (*)	Report to Management (*)
Protocol	21 Oct. 96	29 Oct. 96	29 Oct. 96
Report	20 Jan. 97	21 Jan. 97	21 Jan. 97

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

Inspected phase	Dates		
	Inspections	Report toReport to Study Director (*)	Management (*)
Treatment/test substance	13 Aug. 96	22 Aug. 96	22 Aug. 96
Preparation/test substance	25 Sept. 96	25 Sept. 96	25 Sept. 96

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 and Management.

L. Valette-Talbi · Date: 3 February 1997
Doctor of Biochemistry
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 dermal irritation was evaluated in rabbits according to O.E.C.D. (No. 404, 17th July 1992) and E.C. (92/69/E.E.C., 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.

The test substance was applied for 4 hours to three male New Zealand White rabbits.

A single dose of 0.5 ml of the test substance in its original form was applied to the closely-clipped skin of the flank.

The test substance was held in contact with the skin by means of a semi-occlusive dressing. Cutaneous reactions were observed approximately one 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

Very slight or well-defined erythema was noted in all animals at the 1-hour reading. It persisted for 24 hours in one animal, and for 48 hours in the two other animals. No oedema was observed.

Mean scores over 24, 48 and 72 hours for individual animal were 0.3, 1.0 and 0.7 for erythema and 0.0 for oedema.

Conclusion

Under our experimental conditions, the test substance [redacted] was considered non-irritant when administered by cutaneous route in rabbits.

RESUME

A la demande de Elf Atochem S.A., Paris-la-Défense, France, l'irritation cutanée pouvant être induite par le produit [redacted] a été évaluée chez le Lapin conformément aux lignes directrices de l'O.C.D.E. (No. 404, 17th July 1992) et de la C.E.E. (92/69/E.E.C., B_4, 31st July 1992). L'étude a été réalisée conformément aux règles de Bonnes Pratiques de Laboratoire.

Méthodes

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

Le produit a été appliqué pendant 4 heures sur 3 lapins mâles New Zealand White.

Une dose unique de 0,5 ml de produit tel quel a été appliquée sur une surface de peau tondue sur le flanc.

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 chaque animal pour l'érythème et pour l'oedème.

Résultats

Un érythème très léger ou bien défini est observé chez tous les animaux à la lecture 1 heure. Il persiste pendant 24 heures chez 1 animal, et pendant 48 heures chez les 2 autres. Aucun oedème n'est noté.

La moyenne des scores sur les temps 24, 48 et 72 heures pour chaque animal est de 0,3 ; 1,0 et 0,7 pour l'érythème et de 0,0 pour l'oedème.

Conclusion

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

1. INTRODUCTION

The objective of this study was to evaluate the potential of the test substance [redacted] to induce dermal irritation following a single administration in 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 existence of hazards to Man likely to arise from cutaneous exposure to the test substance.

This study was conducted in compliance with:
. O.E.C.D. guideline No. 404, 17th July 1992.
. E.C. Directive No. 92/69/E.E.C., 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.

Documentation supplied by the Sponsor identified the test substance as follows:
. name:
- protocol and labelling: [redacted]
. batch number:
- protocol and labelling: [redacted]
. Elf Atochem filing number: [redacted]
. description: dark brown liquid
. container: one plastic flask
. date of receipt: 11 October 1996
. storage conditions: at room temperature and protected from light.

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 beginning of the study, the analytical certificate was not available.

The pH of the test substance as mentioned in the safety data sheet was 8.5.

2.1.2 Preparation

The test substance was applied in its original form.

2.2. TEST SYSTEM

2.2.1 Animals

Sex, species, strain: male New Zealand White rabbits.
Reason for this choice: species commonly requested by the international regulations for this type of study.
Breeder: Elevage Cunicole de Val de Selle, 80160 Prouzel, France.
Number of animals and identification: three animals were used, as recommended by the international regulations and taking into account that a good correlation of results can be obtained with either three or six animals (1). The animals were identified individually with a metal tag in the ear.
Weight: on the day of treatment, the animals had a mean body weight ± standard deviation of 2.2 ± 0.2 kg.
Acclimatization: at least five days before the beginning of the study.

2.2.2 Environmental conditions

During the acclimatization period and during the main test, the environmental 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 recorded continuously and records retained.
The housing conditions (temperature, relative humidity and ventilation) were checked monthly.
The animals were housed individually in polystyrene cages (35 cm x 55 cm x 32 cm or 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

All the animals had free access to 112 C pelleted diet (U.A.R., 91360 Villemoisson-sur-Orge, France). Each batch of food was analysed (composition and contaminants) by the supplier.
The diet formula is presented in appendix 2.

Drinking water filtered by a F.G. Millipore membrane (0.22 micron) was provided *ad libitum*.
Bacteriological and chemical analysis of the water and diet and detection of possible contaminants (pesticides, heavy metals and nitrosamines) are performed periodically.
Results are archived at C.I.T.

It was verified that no contaminants in the diet or water at levels likely to influence the outcome of the study were present.

(1) Hatoum, N.S.; Leach, C.L.; Talsma, D.M.; Gibbons, R.D.; Garvin, P.J.: A statistical basis for using fewer Rabbits in dermal irritation testing. Journal of the American College of Toxicology. 9: 49-60 (1990).

2.3. TREATMENT

2.3.1 Preparation and selection of the animals

The day before treatment, the flanks of each animal were clipped using electric clippers. The skin of each animal was examined in order to use only animals without any signs of cutaneous irritation. Animals showing signs of cutaneous irritation, cutaneous defects or pre-existing dermal injury were not used.

2.3.2 Study design

The study design was established according to available information on the test substance and following the O.E.C.D. and E.C. guidelines.

As no irritant effects were anticipated, the test substance was applied for 4 hours to three animals.

2.3.3 Application of the test substance

The test substance was used in its original form.

A single dose of 0.5 ml of the test substance was prepared on a 6 cm^2 dry gauze pad (Coopérative Pharmaceutique Française, 77000 Melun, France) which was then applied to the right flank of the animals for 4 hours.

The test substance and the gauze pad were held in contact with the skin by means of an adhesive hypoallergenic aerated semi-occlusive dressing (Laboratoires de Pansements et d'Hygiène, 21300 Chenove, France) and a restraining bandage (Laboratoires 3M Santé, 92245 Malakoff, France).

The untreated skin served as control.

No residual test substance was noted at removal of the dressing.

2.3.4 Date of treatment

Animal number	Date of treatment (day 1)	End of the observation period
01	5 November 1996	8 November 1996
02	5 November 1996	8 November 1996
03	5 November 1996	8 November 1996

2.4. CUTANEOUS EXAMINATIONS

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

Following the O.E.C.D. and E.C. guidelines:
- when there is no evidence of dermal irritation after 72 hours, the study is ended.
- when there is persistent cutaneous irritation after 72 hours, the observation period is 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 are observed, the animals are killed on humane ground.

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 taking into consideration the nature and the reversibility of the findings observed.

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 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 substance

2.6.2.1 Irritant substances

- symbol Xi: indication of danger: "irritant",

- phrase 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, fisures, 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 by 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. ARCHIVES

The study documentation and materials, namely:

. protocol and possible amendments,
. raw data,
. correspondence,
. final report and possible amendments,

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

3. RESULTS (table 1)

Very slight or well-defined erythema (grade 1 or 2) was noted in all animals at the 1-hour reading. It persisted for 24 hours in one animal, and for 48 hours in the two other animals. No oedema was observed.

Mean scores over 24, 48 and 72 hours for individual animal were 0.3, 1.0 and 0.7 for erythema and 0.0 for oedema.

4. CONCLUSION

Under our experimental conditions, the test substance FORAFAC 1203 (batch No. 25-28) was considered non-irritant when administered by cutaneous route in rabbits.

Table 1: Individual cutaneous examinations and mean values of the scores recorded at each reading (24, 48 and 72 hours) for each animal (4-hour exposure)

Rabbit number	Dermal Irritation	Scores				Mean irritation score (1)	Interpretation (+) (-)
		1h D1	24h D2	48h D3	72h D4		
01	Erythema	1	1	0	0	0,3	(-)
	Oedema	0	0	0	0	0,0	(-)
	Other	*	*	*	*		
02	Erythema	2	2	1	0	1,0	(-)
	Oedema	0	0	0	0	0,0	(-)
	Other	*	*	*	*		
03	Erythema	2	1	1	0	0,7	(-)
	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

[redacted]

APPENDICES

1. Test article description

[redacted]

TOXICOLOGY DEPARTMENT
CONFIDENTIAL
10 October 1996

elf atochem s.a.
La défense 10, cedex 42
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	:	
Elf Atochem filing number	:	[redacted]

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	:	December 1997
Disposal	:	incineration

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 biproducts and legumes	49
Vegetable proteins (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"

U.A.R., 7 rue Galliéni, 91360 Villemoisson - Tel: 69.04.03.57 - Fax : 69.04.81.97
(Ref. Doc. UAR: 1992)