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
Xavier Manciaux

STUDY COMPLETION DATE
20 May 1998

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

LABORATORY STUDY NUMBER
16046 TAL

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:

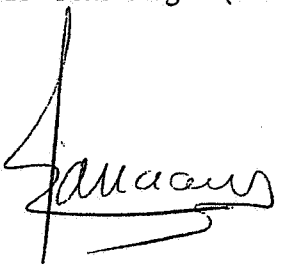
. O.E.C.D. principles of Good Laboratory Practice, Decision Concerning Mutual Acceptance of Data in the Assessment of Chemicals, C(81)30(final) Annex 2. May 12, 1981.

. 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.

. Council Directive 87/18/E.E.C. 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 (O.J. 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 the Centre International de Toxicologie (C.I.T.), Miserey, 27005 Evreux, France.



Toxicology

X. Manciaux Date: 20 May 1998
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	28 August 1997	2 September 1997	2 September 1997
Report	11 May 1998	12 May 1998	12 May 1998

At about the same time as the study described in this report, "process-based" and routine facility inspections of critical procedures relevant to this study type were made by the Quality Assurance Unit.
The findings of these inspections were reported to the Study Director and to C.I.T. Management.

The inspections were performed in compliance with C.I.T. 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: 20 May 1998
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 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 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 and then daily until the end of the observation period.

The mean values of the scores for erythema and oedema were calculated for each animal.

Results

Well-defined to severe cutaneous reactions were observed in all animals from day 1. These reactions persisted up to day 11 at the latest. Dryness of the skin was noted in all animals from day 5 or 6 up to day 13 or 15.

Mean scores over 24, 48 and 72 hours for each animal were 3.0, 2.0 and 2.0 for erythema and 4.0, 2.0 and 2.7 for oedema.

Conclusion

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

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

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'O.C.D.E. (No. 404, 17 juillet 1992) et de la C.E.E. (92/69/E.E.C., 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.

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 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 puis quotidiennement jusqu'à la fin de la période d'observation.

La moyenne des scores a été calculée pour l'érythème et pour l'oedème pour chaque animal.

Résultats

Des réactions cutanées bien définies à sévères sont observées chez tous les animaux à partir du jour 1. Ces réactions persistent jusqu'au jour 11 au plus. Une sécheresse cutanée est notée chez tous les animaux du jour 5 ou 6 au jour 13 ou 15.

Les scores moyens calculés pour chaque animal après 24, 48 et 72 heures sont de 3,0 ; 2,0 et 2,0 pour l'érythème et de 4,0 ; 2,0 et 2,7 pour l'oedème.

Conclusion

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

Selon les critères de classification décrits dans la Directive 93/21/C.E.E (27 avril 1993) portant dix-huitième adaptation au progrès technique de la Directive 67/548/C.E.E., le produit est considéré irritant pour la peau.

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:
. 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.

The test substance was identified as follows:
. name:
  - protocol and labelling: [redacted]
. batch number:
  - protocol and labelling [redacted]
. Elf Atochem filing number: [redacted]
. description:
. at receipt: [redacted]
. on the test article description: [redacted]
. container: one plastic flask
. date of receipt: 23 August 1997
. storage conditions: at room temperature and protected from light
. expiry date: August 1998.

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 finalization of the study report, an analytical certificate was not available. Characterization of the test substance, which appropriately defines the tested batch, is under the responsibility of the Sponsor.

The pH of the test substance was [redacted]

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.
Justification of the test system : species generally accepted by regulatory authorities 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 guidelines 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 of 2.5 ± 0.2 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 retained. 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 (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

During the study, the animals had free access to 112 C pelleted diet (U.A.R., 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 F.G. Millipore membrane (0.22 micron) was provided *ad libitum*.
Bacteriological and chemical analysis 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 C.I.T.

No contaminants are known to be present in the diet or drinking water at levels which may be expected to interfere with or prejudice the outcome of the study.

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(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 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 O.E.C.D. (No. 404) and E.C. (92/69/E.E.C., B_4) guidelines.

The test substance was applied for 4 hours to three animals.

2.3.3 Application of the test substance

The test substance was used undiluted.

A single dose of 0.5 ml of the test substance was placed 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 (Coheban®), Laboratoires 3M Santé, 92245 Malakoff, France).

The untreated skin served as control.

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

2.3.4 Date of treatment

Animal number	Date of treatment (day 1)	End of the observation period
01	7 October 1997	20 October 1997
02	7 October 1997	20 October 1997
03	7 October 1997	21 October 1997

2.4 CUTANEOUS EXAMINATIONS

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

Following the O.E.C.D. and E.C. 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.

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 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 PROTOCOL ADHERENCE

The study was performed in accordance with Study Protocol No. 16046 TAL and subsequent amendments, with the following deviation from the agreed Study Protocol:
- the relative humidity recorded in the animal room was sometimes outside of the target ranges specified in the protocol.

This minor deviation was 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 C.I.T., 27005 Miserey, 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 C.I.T. for a further period.

3. RESULTS (table 1)

Well-defined to severe cutaneous reactions (well-defined or moderate erythema, grade 2 or 3, and slight or severe oedema, grade 2 or 4) were observed in all animals from day 1. These reactions persisted up to day 11 at the latest. Dryness of the skin was noted in all animals from day 5 or 6 up to day 13 or 15.

Mean scores over 24, 48 and 72 hours for each animal were 3.0, 2.0 and 2.0 for erythema and 4.0, 2.0 and 2.7 for oedema.

4. CONCLUSION

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

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

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

Rabbit number	Dermal Irritation	Scores				Mean irritation score (1)	Interpretation (+) (-)
		1h D1	24h D2	48h D3	72h D4		
01	Erythema	2	3	3	3	3.0	(+)
	Oedema	4	4	4	4	4.0	(+)
	Other	*	*	*	*		
02	Erythema	2	2	2	2	2.0	(+)
	Oedema	4	2	2	2	2.0	(+)
	Other	*	*	*	*		
03	Erythema	2	2	2	2	2.0	(+)
	Oedema	4	4	2	2	2.7	(+)
	Other	*	*	*	*		

(1) mean of scores on days 2, 3 and 4
h = hour
D = day
(+) = irritant according to E.E.C. criteria

Table 1 (continued)

Rabbit number	Dermal Irritation	Scores										
		D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15
01	Erythema	3	2	1	1	0	0	0	0	0	0	-
	Oedema	4	2	0	0	0	0	0	0	0	0	-
	Other	*	S	S	S	S	S	S	S	S	*	-
02	Erythema	1	1	1	1	0	0	0	0	0	0	-
	Oedema	0	0	0	0	0	0	0	0	0	0	-
	Other	S	S	S	S	S	S	S	S	S	*	-
03	Erythema	2	2	2	2	2	2	1	0	0	0	0
	Oedema	2	0	0	0	0	0	0	0	0	0	0
	Other	*	S	S	S	S	S	S	S	S	S	S

D = day
* = None
S = Dryness of the skin
- = Cutaneous examination not performed

APPENDICES

1. Test article description

TOXICOLOGY DEPARTMENT
CONFIDENTIAL
11 August 1997

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

TEST ARTICLE DESCRIPTION

[redacted]

IDENTITY

Test article name	:	[redacted]
CAS number	:	
EINECS number	:	
Purity	:	
Origin	:	Elf Atochem , CERDATO, Serquigny
Batch	:	[redacted]
Elf Atochem filing number	:	

PHYSICAL AND CHEMICAL PROPERTIES

Appearance	:	[redacted]
Specific gravity	:	
Boiling point	:	
Vapor pressure	:	
Flash point	:	
Solubility	:	

TOXICOLOGICAL INFORMATIONS AND USE SAFETY

see « fiche de données de sécurité »

STORAGE AND DISPOSAL

Storage	:	in dark and at room temperature
Expiry date	:	August 1998
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 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"

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