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

STUDY TITLE
ACUTE EYE IRRITATION IN RABBITS

TEST SUBSTANCE
[redacted]

STUDY DIRECTOR
Stéphane de Jouffrey

STUDY COMPLETION DATE
28 January 1997

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

LABORATORY STUDY NUMBER
14888 TAL

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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 Joffrey Date: 28 January 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

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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	20 Aug. 96	23 Aug. 96	23 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: 28 January 1997
Doctor of Biochemistry
Head of Quality Assurance Unit
and Scientific Archives

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SUMMARY

At the request of Elf Atochem S.A., Paris-la-Défense, France, the potential of the test substance [redacted] to induce ocular irritation was evaluated in rabbits according to O.E.C.D. (No. 405, 24th February 1987) and E.C. (92/69/E.E.C., B_5, 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.

As no irritant effects were anticipated, a single dose of 0.1 ml of the test substance was instilled into the left conjunctival sac of three male New Zealand White rabbits. The right eye served as control.

The test substance was used in its original form.

The eyes were not rinsed after administration of the test substance.

Ocular reactions were observed approximately one hour, 24, 48 and 72 hours after the administration and then daily until reversibility of the ocular reactions.

The mean values of the scores recorded for each animal after 24, 48 and 72 hours were calculated.

Results

Very slight to moderate conjunctival reactions were noted in all animals: very slight to moderate chemosis, very slight to moderate conjunctival redness and clear to whitish purulent discharge were observed from day 1 up to day 4 (one animal) or 6 (two animals).
Slight iritis was observed on day 2 in two animals; it persisted for 24 hours in one of them.
Very slight corneal opacity was also noted in all animals on day 2; it persisted up to day 4.

Reversibility of ocular lesions was noted on day 5-(one animal) or 7.

The mean scores calculated for each animal over 24, 48 and 72 hours were 1.3, 2.0 and 2.0 for chemosis, 2.0, 2.3 and 2.3 for redness of the conjunctiva, 0.7, 0.0 and 0.3 for iris lesions and 1.0, 1.0 and 1.0 for corneal opacity.

Conclusion

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

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RESUME

A la demande de Elf Atochem S.A., Paris-la-Défense, France, l'irritation oculaire pouvant être induite par le produit, [redacted] a été évaluée chez le Lapin conformément aux lignes directrices de l'O.C.D.E. (No. 405, 24th February 1987) et de la C.E.E. (92/69/E.E.C., B_5, 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.

Aucun effet irritant n'étant supposé, une dose unique de 0,1 ml de produit a été instillée dans le cul de sac conjonctival de l'oeil gauche de 3 lapins mâles New Zealand White. L'oeil droit a servi de témoin.

Le produit a été utilisé tel quel.

Aucun rinçage des yeux n'a été réalisé après l'administration du produit.

Les réactions oculaires ont été observées environ 1 heure, 24, 48 et 72 heures après l'administration puis quotidiennement jusqu'à la réversibilité des lésions.

La moyenne des scores enregistrés après 24, 48 et 72 heures a été calculée pour chaque animal.

Résultats

Des réactions conjonctivales très légères à modérées sont observées chez tous les animaux : un chémosis très léger à modéré, une rougeur de la conjonctive très légère à modérée, et un larmoiement clair (ou purulent blanchâtre chez 1 animal) sont notés du jour 1 jusqu'au jour 4 (1 animal) ou 6 (2 animaux).
Un léger iritis est observé chez 2 animaux au jour 2 ; il persiste pendant 24 heures chez l'un d'entre eux.
Une opacité cornéenne très légère est également notée chez tous les animaux au jour 2 ; elle persiste jusqu'au jour 4.

La réversibilité des lésions oculaires est observée au jour 5 (1 animal) ou 7.

Les scores moyens individuels calculés après 24, 48 et 72 heures sont de 1,3 ; 2,0 et 2,0 pour le chémosis, 2,0 ; 2,3 et 2,3 pour la rougeur de la conjonctive, 0,7 ; 0,0 et 0,3 pour l'iritis et 1,0 ; 1,0 et 1,0 pour l'opacité cornéenne.

Conclusion

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

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1. INTRODUCTION

The objective of this study was to evaluate the potential of the test substance [redacted] to induce ocular irritation following a single administration in rabbits.

In the assessment of the toxic characteristics of a test substance, determination of the irritant effects on the eyes 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 exposure of the eyes, and associated mucous membranes, to the test substance.

This study was conducted in compliance with:
. O.E.C.D. guideline No. 405, 24th February 1987.
. E.C. Directive No. 92/69/E.E.C., B_5, 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 used in its original form.

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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 2.4 ± 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) Talsma, D.M.; Leach, C.L.; Hatoum, N.S.; Gibbons, R.D.; Roger, J.C.; Garvin, J.P.: Reducing the number of rabbits in the Draize eye irritancy test: A statistical analysis of 155 studies conducted over 6 years. Fundamental and Applied Toxicology. 10: 1, 146-153 (1988).

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2.3. TREATMENT

2.3.1 Selection of the animals

The day before treatment, the eyes of each animal were examined in order to use only animals without any signs of ocular irritation. Animals showing signs of ocular irritation, ocular defects or pre-existing corneal injury were not used.

2.3.2 Study design

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

As no irritant effects were anticipated, the test substance was evaluated in three animals.

2.3.3 Administration of the test substance

The test substance was used in its original form.

A single dose of 0.1 ml of the test substance was instilled into the conjunctival sac of the left eye after gently pulling the lower lid away from the eyeball.

The lower and upper eyelids were held together for about one second to avoid any loss of test substance. The right eye, which remained untreated, served as a control.

The eyes were not rinsed after administration of the test substance.

2.3.4 Date of treatment

Animal number	Date of treatment (day 1)	End of the observation period
01	12 November 1996	16 November 1996
02	12 November 1996	18 November 1996
03	12 November 1996	18 November 1996

2.4. OCULAR EXAMINATIONS

The eyes were examined approximately one hour, 24, 48 and 72 hours after administration of the test substance.

Following the O.E.C.D. and E.C. guidelines:
- when there is no evidence of irritation after 72 hours, the study is ended.
- when there is persistent ocular irritation after 72 hours, the observation period is extended to a maximum of 21 days (until day 22) 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.

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2.5. DESCRIPTION AND EVALUATION OF OCULAR REACTIONS

Ocular reactions were evaluated for each animal according to the following numerical scale:

2.5.1 Conjunctival lesions and discharge

Chemosis (lids and/or nictitating membranes)

. no swelling	0
. any swelling above normal (includes nictitating membranes)	1
. obvious swelling with partial eversion of lids	2*
. swelling with lids about half-closed	3*
. swelling with lids more than half-closed	4*

Redness (refers to palpebral and bulbar conjunctivae, cornea and iris)

. blood vessels normal	0
. a number of blood vessels definitely hyperaemic (injected)	1
. diffuse, crimson colour, individual vessels not easily discernible	2*
. diffuse, beefy red	3*

Discharge

. absence of discharge	0
. slight discharge (does not include small amounts normally found in inner canthus)	1
. discharge with moistening of lids and hairs adjacent to lids	2
. discharge with moistening of lids and hairs on wide area around the eye	3

2.5.2 Iris lesions

. normal	0
. markedly deepened rugae, congestion, swelling, moderate circum-corneal hyperaemia, or injection, any of these or combination of any thereof, iris still reacting to light (sluggish reaction is positive)	1*
. no reaction to light, haemorrhage, gross destruction (any or all of these)	2*

2.5.3 Corneal lesions

Cornea (direct examination or, if necessary, with an Ultra-Violet lamp)
To determine the presence or absence of corneal opacification and to evaluate the affected area, one or two drops of 0.5% sodium fluorescein solution can be instilled into the eye (however, this must be performed before the 24-hour reading).
If corneal opacification is difficult to determine, the eye can be examined under a U.V. lamp (a clear fluorescence is visible in the areas of opacification).

Opacity (degree of intensity: area most dense taken for reading)

. no ulceration or opacity	0
. scattered or diffuse areas of opacity (other than slight dulling or normal lustre), details of iris clearly visible	1*
. easily discernible translucent area, details of iris slightly obscured	2*
. nacrous areas, no details of iris visible, size of pupil barely discernible	3*
. opaque cornea, iris not discernible through the opacity	4*

* indicates positive effect

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Area of opacity
. one quarter (or less) but not zero 1
. greater than one quarter but less than a half 2
. greater than one half but less than three quarters 3
. greater than three quarters up to whole area 4

Any other lesions observed 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 scores observed, whilst taking into account all the reactions of the treated animals.

2.6.1 Interpretation of the results

Criteria for irritation

A substance or a preparation is considered irritant for the eyes if, when applied to the eye of the animal, significant severe ocular lesions are caused within 72 hours after exposure and which persist for 24 hours or more after treatment with the test substance.

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

2.6.2 Classification of the test substances

- Xi symbol, indication of danger "irritant",

- phrases indicating the nature of special risks:

R 36: "Irritating to eyes"

Ocular lesions are significant if the mean score has any of the following values:
. opacity of the cornea ≥ 2, but < 3,
. lesion of the iris ≥ 1, but ≤ 1.5,
. redness of the conjunctivae ≥ 2.5,
. oedema of the conjunctivae (chemosis) ≥ 2.

Or else, if the test is performed on three animals, if at least two of them show lesions equal to one of the following values:
. opacity of the cornea ≥ 2, but < 3,
. lesion of the iris ≥ 1, but ≤ 2,
. redness of the conjunctivae ≥ 2.5,
. oedema of the conjunctivae (chemosis) ≥ 2.

R 41: "Risk of serious damage to eyes"

Ocular lesions are severe:
if the mean score has any of the following values:
. opacity of the cornea ≥ 3,
. lesion of the iris > 1.5.

Or else, if the test is performed on three animals, if at least two of them show lesions equal to one of the following values:
. opacity of the cornea ≥ 3,
. lesion of the iris = 2.

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Or if they persist at the end of the observation period.

If the test substance or preparation induces irreversible colouration of the eyes, the phrase R 41 should also be applied.

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.

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3. RESULTS (table 1)

Very slight to moderate conjunctival reactions were noted in all animals: very slight to moderate chemosis (grades 1 to 3), very slight to moderate conjunctival redness (grades 1 to 3) and clear to whitish purulent discharge were observed from day 1 up to day 4 (1 animal) or 6 (two animals).
Slight iritis (grade 1) was observed on day 2 in two animals; it persisted for 24 hours in one of them.
Very slight corneal opacity (grade 1) was also noted in all animals on day 2; it persisted up to day 4.

Reversibility of ocular lesions was noted on day 5 (one animal) or 7.

The mean scores calculated for each animal over 24, 48 and 72 hours were 1.3, 2.0 and 2.0 for chemosis, 2.0, 2.3 and 2.3 for redness of the conjunctiva, 0.7, 0.0 and 0.3 for iris lesions and 1.0, 1.0 and 1.0 for corneal opacity.

4. CONCLUSION

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

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

Rabbit number	Region of eye	Description of ocular reactions	Scores							Mean irritation score (1)	Interpretation (+) (-)
			1h D1	24h D2	48h D3	72h D4	D5	D6	D7		
01	Conjunctivae	Chemosis	2	2	1	1	0	-	-	1,3	(-)
		Redness	1	3	2	1	0	-	-	2,0	(-)
		Discharge	E	2	0	0	0	-	-	0,7	
	Iris		0	1	1	0	0	-	-	0,7	(-)
	Corneal opacity	Intensity	0	1	1	1	0	-	-	1,0	(-)
		Area	0	3	2	1	0	-	-	2,0	
	Other		*	*	*	*	*	-	-		
	Fluorescein		/	U	U	U	U	-	-		
02	Conjunctivae	Chemosis	3	3	2	1	1	1	0	2,0	(+)
		Redness	2	3	2	2	1	1	0	2,3	(-)
		Discharge	E	S	S	0	0	0	0	(2)	
	Iris		0	0	0	0	0	0	0	0,0	(-)
	Corneal opacity	Intensity	0	1	1	1	0	0	0	1,0	(-)
		Area	0	3	2	1	0	0	0	2,0	
	Other		*	*	*	*	*	*	*		
	Fluorescein		/	U	U	U	U	/	/		
03	Conjunctivae	Chemosis	3	3	2	1	1	1	0	2,0	(+)
		Redness	2	3	2	2	1	1	0	2,3	(-)
		Discharge	E	2	0	0	0	0	0	0,7	
	Iris		0	1	0	0	0	0	0	0,3	(-)
	Corneal opacity	Intensity	0	1	1	1	0	0	0	1,0	(-)
		Area	0	3	2	1	0	0	0	2,0	
	Other		*	*	*	*	*	*	*		
	Fluorescein		/	U	U	U	U	/	/		

(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
(2) = not calculated
U = Fluorescein batch No. 5253
/ = Fluorescein not used
E = Scoring obscured by residual test substance
S = Whitish purulent discharge
- = Ocular examination not performed

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[redacted]

APPENDICES

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1. Test article description

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[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	:	[redacted]
CAS number	:	[redacted]
EINECS number	:	[redacted]
Purity	:	[redacted]
Origin and batch	:	Elf Atochem, VSP
Batch	:	25-28
Elf Atochem filing number	:	CAL 7443/96

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

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2. Diet formula

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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)

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