[redacted] CONFIDENTIAL

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

STUDY TITLE
ACUTE ~~ORAL~~ TOXICITY IN RATS

TEST SUBSTANCE
[redacted]

STUDY DIRECTOR
Stéphane de Jouffrey

STUDY COMPLETION DATE
5 February 1997

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

LABORATORY STUDY NUMBER
14886 TAR

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 Jouffrey Date: 5 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	20 Jan. 97	20 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 to Study Director (*)	Report to Management (*)
Treatment/test substance	14 Aug. 96	20 Aug. 96	20 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: 5 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 acute oral toxicity of the test substance [redacted] was evaluated in rats according to O.E.C.D. (No. 401, 24th February 1987) and E.C. (92/69/E.E.C., B_1, 31st July 1992) guidelines. The study was conducted in compliance with the Principles of Good Laboratory Practice Regulations.

Methods

The test substance was administered by oral route to one group of ten fasted Sprague-Dawley rats (five males and five females).

The test substance was administered in its original form, by gavage, at a dose of 2000 mg/kg, taking into consideration that its density was 1.07.

Clinical signs, mortality and body weight gain were checked for a period of 14 days following the single administration of the test substance.

All animals were subjected to necropsy.

Results

The general behaviour and body weight gain of the animals were not affected by treatment with the test substance.

No death occurred at 2000 mg/kg.

No abnormalities were observed at necropsy.

Conclusion

Under our experimental conditions, the oral LD_0 of the test substance [redacted] was higher than or equal to 2000 mg/kg in rats. No signs of toxicity were observed at this dose.

RESUME

A la demande de Elf Atochem S.A., Paris-la-Défense, France, la toxicité aiguë du produit [redacted] a été évaluée par voie orale chez le Rat conformément aux lignes directrices de l'O.C.D.E. (No. 401, 24th February 1987) et de la C.E.E. (92/69/C.E.E., B_1, 31st July 1992). L'étude a été réalisée conformément aux règles de Bonnes Pratiques de Laboratoire.

Méthodes

Le produit a été administré par voie orale à un groupe de 10 rats Sprague-Dawley (5 mâles et 5 femelles) mis à la diète hydrique.

L'administration a été effectuée avec le produit tel quel, par gavage, à la dose de 2000 mg/kg, en tenant compte de la densité du produit (d = 1,07).

Les signes cliniques, la mortalité et l'évolution pondérale des animaux ont été suivis pendant une période de 14 jours après l'administration unique du produit.

Un examen anatomopathologique a été effectué sur tous les animaux.

Résultats

Le comportement général et l'évolution pondérale des animaux ne sont pas influencés par le traitement.

La mortalité est nulle à la dose de 2000 mg/kg.

L'autopsie des animaux ne met en évidence aucune anomalie macroscopique.

Conclusion

Dans nos conditions expérimentales, la DL_0 du produit [redacted] administré par voie orale chez le Rat est supérieure ou égale à 2000 mg/kg. Aucun signe de toxicité n'est observé à cette dose.

1. INTRODUCTION

The objective of this study was to evaluate the toxicity of the test substance [redacted] following a single oral administration in rats.

In the assessment of the toxic characteristics of a test substance, determination of acute oral toxicity is an initial step. It provides information on health hazards likely to arise from a short-term exposure by the oral route in Man.

The study was conducted in compliance with:
. O.E.C.D. guideline No. 401, 24th February 1987.
. E.C. Directive No. 92/69/E.E.C., B_1, 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.

2.1.2 Preparation

The test substance was administered in its original form.

2.2. TEST SYSTEM

2.2.1 Animals

Species, strain: rat, Sprague-Dawley ICO: OFA-SD (IOPS Caw).
Reason for this choice: rodent species commonly requested by the international regulations for this type of study.
Breeder: Iffa Crédo, 69210 L'Arbresle, France.
Number and sex: one group of ten animals (five males and five females).
Age/weight: on the day of treatment, the animals were approximately six weeks old, and had a mean body weight ± standard deviation of 175 ± 4 g for the males and 142 ± 5 g for the females.
Acclimatization: at least five days before the beginning of the study.
Identification of the animals: the animals were identified individually by earmarks or earnotches.

2.2.2 Environmental conditions

During the acclimatization period and during the main test, 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: 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 in polycarbonate cages (48 cm x 27 cm x 20 cm). Each cage contained four to seven animals of the same sex during the acclimatization period and five rats of the same sex during the treatment period. Each cage contained dust-free sawdust (SICSA, 94142 Alfortville, France).
Bacteriological analysis of the sawdust and detection of possible contaminants (pesticides, heavy metals) are performed periodically.

2.2.3 Food and water

All the animals had free access to A04 C pelleted diet (U.A.R., 91360 Villemoisson-sur-Orge, France), except as noted in "2.3.1 Fasting of the animals".
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.

2.3. TREATMENT

As the test substance was anticipated to be non-toxic at 2000 mg/kg, a limit test was performed by administering 2000 mg/kg of the test substance to one group of ten animals (five males and five females).

2.3.1 Fasting of the animals

The animals were fasted for an overnight period of approximately 18 hours before dosing, but had free access to water.
Food was given back approximately 4 hours after administration of the test substance.

2.3.2 Administration of the test substance

The test substance was administered in its original form, taking into consideration that its density was 1.07.

The administration was performed in a single dose by oral route using a stainless steel round-tipped probe (diameter: 18 G.2", Perfektum: Poffer & Sons Inc., New Hyde Park, New York 11040, U.S.A.) fitted to a 1 ml glass syringe (0.01 ml graduations, Record: Carrieri, 75005 Paris, France).

The volume administered to each animal was adjusted according to body weight determined on the day of treatment.

2.3.3 Date of treatment and duration of the study

The single administration was performed on 5 November 1996 in the morning (day 1) and was followed by a 14-day observation period until 19 November 1996 (day 15).

2.4. CLINICAL EXAMINATIONS

The single administration was performed in the morning of day 1; it was followed by a 14-day observation period until day 15.

2.4.1 Clinical signs and mortality

The animals were observed frequently during the hours following administration of the test substance, for detection of possible treatment-related clinical signs. Thereafter, observation of the animals was made at least once a day. Type, time of onset and duration of clinical signs were recorded for each animal individually.

Time of death was recorded individually, in terms of the number of hours or days after dosing.

2.4.2 Body weight

The animals were weighed individually just before administration of the test substance on day 1 and then on days 8 and 15.

The body weight gain of the treated animals was compared to a reference curve of C.I.T. control animals with the same initial body weight.

2.5. NECROPSY

On day 15, all animals were killed by CO_2 inhalation in excess and a macroscopic examination was performed.

After opening the thoracic and abdominal cavities, a macroscopic examination of the main organs (digestive tract, heart, kidneys, liver, lungs, pancreas, spleen and any other organs with obvious abnormalities) was performed.
In case of macroscopic lesions, organ samples were taken and preserved in 10% buffered formalin.

No microscopic examination was performed.

2.6. DATA EVALUATION

Evaluation of the toxicity of the test substance following a single oral administration in rats should include the relationship, if any, between the animals' exposure to the test substance and the incidence and severity of all abnormalities including behavioural and clinical abnormalities, macroscopic lesions, body weight changes, mortality and any other toxic effects.

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

3.1. CLINICAL EXAMINATIONS

3.1.1 Clinical signs (table 1)

No clinical signs were observed during the study.

3.1.2 Mortality (table 1)

No death occurred during the observation period.

3.1.3 Body weight (treated animals: figure 1, table 2)
(historical control animals: figure 2, table 3)

The body weight gain of the animals was not influenced by treatment.

3.2. PATHOLOGY (table 4)

Macroscopic examination of the main organs of the animals revealed no apparent abnormalities.

4. CONCLUSION

Under our experimental conditions, the oral LD_0 of the test substance [redacted] was higher than or equal to 2000 mg/kg in rats. No signs of toxicity were observed at this dose.

Figure 1: Body weight of treated rats (g)

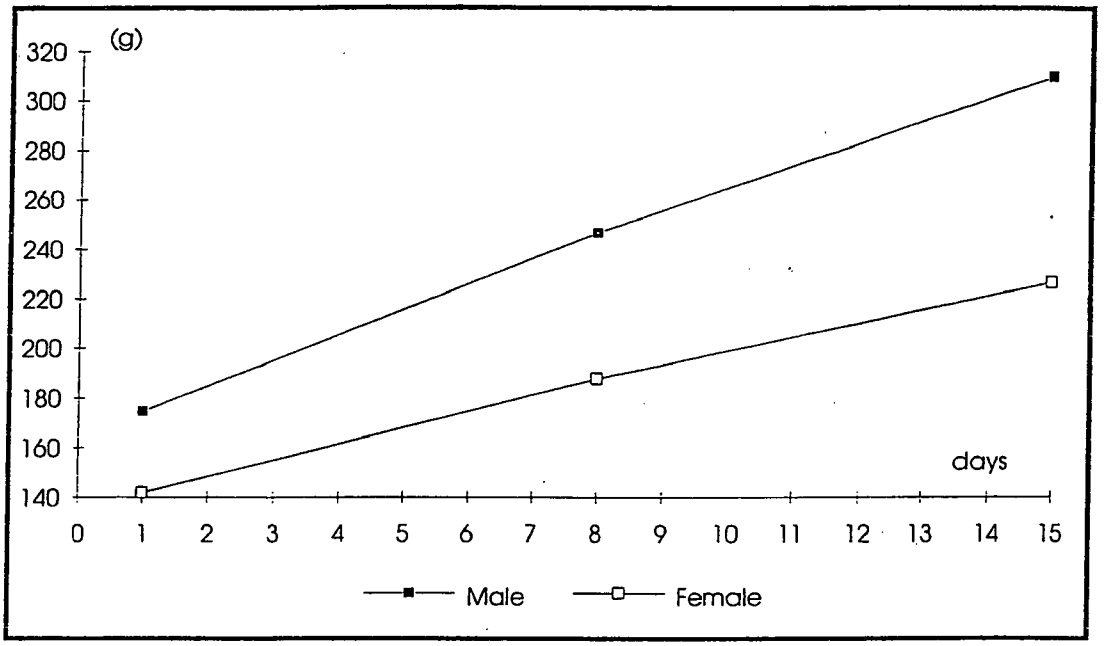
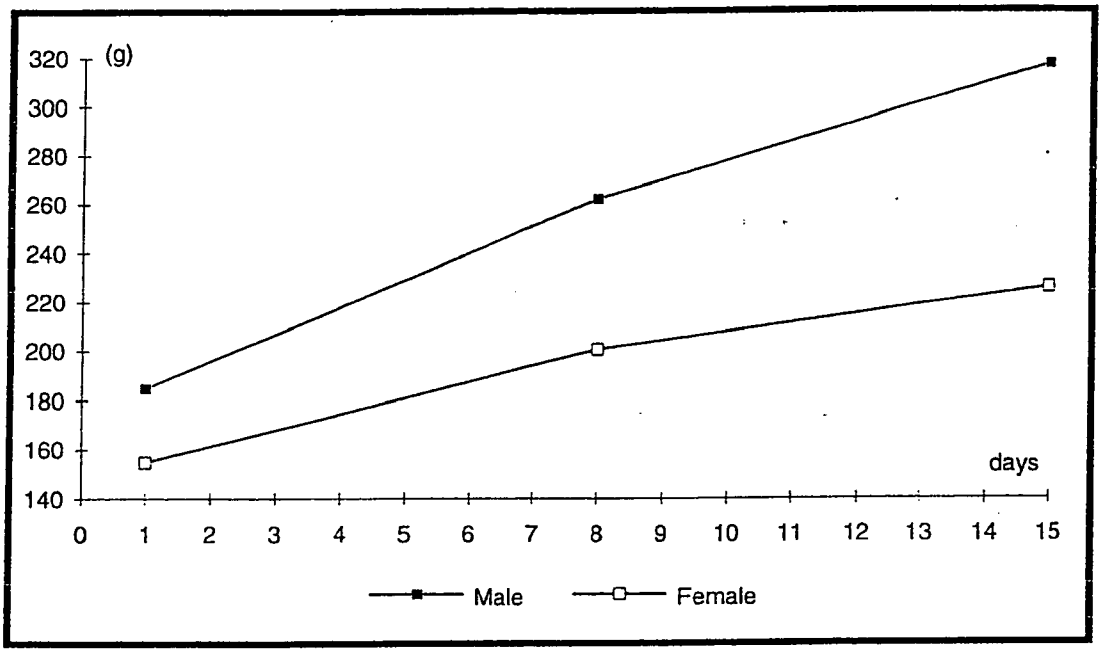


Figure 2: Body weight of C.I.T. historical control rats (g)



e.g.: C.I.T. Historical data of animals dosed by the oral route: results of control animals from October 1990 to January 1996.

Table 1: Individual clinical signs and mortality

Dose (mg/kg)	Time	Animals Males	Animals Females	Mortality	Clinical signs
2000	30 min 1-2-4 h D 2 to D 15	01-02-03-04-05	01-02-03-04-05	No	None

min: minutes
h : hour
D : day

Table 2: Individual and mean body weight and weekly body weight change of treated rats (g)

Dose	Volume	Sex	Animals	Days				
mg/kg	ml/kg			1	(1)	8	(1)	15
2000	1.87	Male	01	178	68	246	64	310
			02	175	71	246	61	307
			03	170	84	254	63	317
			04	172	75	247	62	309
			05	178	62	240	64	304
			M	175	72	247	63	309
			SD	4	8	5	1	5
2000	1.87	Female	01	143	39	182	42	224
			02	136	48	184	36	220
			03	150	33	183	45	228
			04	141	61	202	44	246
			05	139	48	187	25	212
			M	142	46	188	38	226
			SD	5	11	8	8	13

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

Table 3: Mean body weight and weekly body weight change of C.I.T. historical control rats (g)

BODY WEIGHT OF CONTROL RATS

(g)

Dose mg/kg	Volume ml/kg	Sex		Days 1	8	15
0	10	Male	M	185	262	317
			SD	8	13	20
			n	90	90	90
0	10	Female	M	155	201	225
			SD	9	15	19
			n	90	90	90

M : mean
SD: standard deviation
n : number of animals

BODY WEIGHT CHANGE OF CONTROL RATS

(g)

Dose mg/kg	Volume ml/kg	Sex		Days 1 to 8	8 to 15
0	10	Male	M	77	55
			SD	9	13
0	10	Female	M	46	25
			SD	10	11

M : mean
SD: standard deviation

e.g.: C.I.T. Historical data of animals dosed by the oral route: results of control animals from October 1990 to January 1996.

Table 4: Individual macroscopic examinations at necropsy

Dose mg/kg	Time	Animals		Macroscopic abnormalities
		Males	Females	
2000	D 15	01-02-03-04-05	01-02-03-04-05	None

D : day

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	:	[redacted]
CAS number	:	not applicable
EINECS number	:	not applicable
Purity	:	[redacted]
Origin and batch	:	Elf Atochem, VSP
Batch	:	[redacted]
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: A04

COMPLETE DIET

RAT AND MOUSE MAINTENANCE DIET

Appearance: 15 mm diameter pellets or powder

Conditioning: 25 kg double paper bag with aluminium on the outside

Daily portion: Rat 18-25 g, Mouse 5-10 g, water *ad libitum*.

FORMULA %

Cereals and cereal biproducts	88
Vegetable protein (soya bean meal, yeast)	7
Animal protein (fish)	2
Vitamin and mineral mixture	3

AVERAGE ANALYSIS %

Calorific value (KCal/kg)	2900
Moisture	12
Proteins	17
Lipids	3
Carbohydrates (N.F.E.)	58.7
Fibre	4
Minerals (ash)	5

AMINO ACID VALUES
(calculated in mg/kg)

Arginine	9800
Cystine	2300
Lysine	8500
Methionine	3200
Tryptophan	1900
Glycine	8100

FATTY ACID VALUES
(calculated in mg/kg)

Palmitic acid	2600
Palmitoleic acid	Traces
Stearic acid	500
Oleic acid	8000
Linoleic acid	14500
Linolenic acid	Traces

MINERALS (calculated in mg/kg)

	Nat val.	CMV val.	Total
P	5900	0	5900
Ca	3300	5000	8300
K	6700	0	6700
Na	300	1600	1900
Mg	1900	100	2000
Mn	50	40	90
Fe	90	150	240
Cu	15	15	30
Zn	40	45	85
Co	T	1.5	1.5
I	0.3	0	0.3

VITAMINS (calculated per kg)

	Nat val.	CMV val.	Total
Vitamin A	Traces	7500 IU	7500 IU
Vitamin D3	Traces	1500 IU	1500 IU
Vitamin B1	6 mg	1 mg	7 mg
Vitamin B2	2 mg	4.5 mg	6.5 mg
Vitamin B3	10 mg	6.5 mg	16.5 mg
Vitamin B6	1.3 mg	1.3 mg	2.6 mg
Vitamin B12	0.01 mg	0.01 mg	0.02 mg
Vitamin E	15 mg	15 mg	30 mg
Vitamin K3	0.25 mg	2.25 mg	2.5 mg
Vitamin PP	60 mg	15 mg	75 mg
Folic acid	0.5 mg	0 mg	0.5 mg
Biotin	0.04 mg	0 mg	0.04 mg
Choline	1200 mg	400 mg	1600 mg

Available under quality "Control Ref.: A04 C "

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