CONFIDENTIAL

SPONSOR

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

TEST SUBSTANCE

[redacted]

STUDY TITLE

ACUTE ORAL TOXICITY IN RATS

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

18745 TAR

CONTENTS

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	9 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 acute oral toxicity of the test substance [redacted] was evaluated in rats according to OECD (No. 401, 24th February 1987) and EC (92/69/EEC, 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 (gavage) to one group of ten fasted Sprague-Dawley rats (five males and five females).

The test substance was administered undiluted at the dose of 2000 mg/kg, taking into consideration that its specific gravity was 1.05 g/ml.

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

All animals were subjected to necropsy.

Results

No deaths occurred at 2000 mg/kg.

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

No apparent abnormalities were observed at necropsy in all animals.

Conclusion

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

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 does not present a significant acute toxic risk if swallowed.

RESUME

A la demande de Elf Atochem S.A., Paris-la-Défense, France, la toxicité aiguë du produit [redacted] après administration unique par voie orale chez le Rat a été évaluée conformément aux lignes directrices de l'OCDE (n° 401, 24 février 1987) et de la CEE (92/69/EEC, B.1, 31 juillet 1992).
L'étude a été réalisée conformément aux règles de Bonnes Pratiques de Laboratoire.

Méthode

Le produit a été administré par voie orale (gavage) 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 non dilué à la dose de 2000 mg/kg, en tenant compte de sa densité (d = 1,05 g/ml).

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

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

Aucun signe clinique n'est observé pendant l'étude.

L'évolution pondérale des animaux n'est pas influencée par le traitement.

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

Conclusion

Dans nos conditions expérimentales, la DL 0 orale du produit [redacted] est supérieure ou égale à 2000 mg/kg chez le Rat. Aucun signe de toxicité n'est observé à cette dose.

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 ne présente pas de risque toxique aigü significatif en cas d'ingestion.

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 following a short-term exposure by the oral route in humans.

The study was conducted in compliance with:
. OECD guideline No. 401, 24th February 1987,
. EC Directive No. 92/69/EEC, 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.

The test substance was identified as follows:
. name:
  - protocol and labelling: [redacted]
. batch number:
  - protocol and labelling: [redacted]
. Elf Atochem filing number: [redacted]
. description: [redacted]
. container: one plastic 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 an analytical certificate (presented in appendix 1) provided by the Sponsor.

2.1.2 Formulation procedure

The test substance was used undiluted.

2.2 TEST SYSTEM

2.2.1 Animals

Species, strain: rat, Sprague-Dawley ICO: OFA-SD (IOPS Caw).
Reason for this choice: rodent species generally accepted by regulatory authorities 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 6 weeks old, and had a mean body weight ± standard deviation of 177 ± 4 g for the males and 145 ± 7 g for the females.
Acclimatization: at least 5 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 throughout the study, the conditions in the animal room were set 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 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 are verified and calibrated at regular intervals.
The animals were housed in polycarbonate cages (48 cm x 27 cm x 20 cm). Each cage contained one 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 and chemical analyses of the sawdust, including the detection of possible contaminants (pesticides, heavy metals), are performed regularly by external laboratories.
The results of these analyses are archived at CIT.

2.2.3 Food and water

All the animals had free access to A04 C pelleted diet (UAR, 91360 Villemoisson-sur-Orge, France), except as noted in "2.3.1 Fasting of the animals".
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, drinking water or bedding material at levels which may be expected to have interfered with or prejudiced the outcome of the study.

2.3 TREATMENT

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

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).

The test substance was administered undiluted, taking into consideration its specific gravity (1.05 g/ml).

The administration was performed in a single dose by oral route using a metal gavage tube fitted to a 1 ml plastic syringe (0.01 ml graduations).

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

2.3.3 Chronology of the study

The single administration was performed on 13 July 1999 in the morning (day 1) and was followed by a 14-day observation period until 27 July 1999 (day 15).

2.4 CLINICAL EXAMINATIONS

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 until day 15.
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 that of CIT control animals with the same initial body weight.

2.5 NECROPSY

On day 15, all animals were killed by carbon dioxide asphyxiation 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 PROTOCOL ADHERENCE

The study was performed in accordance with Study Protocol No. 18745 TAR 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

3.1 CLINICAL EXAMINATIONS

3.1.1 Clinical signs and mortality (table 1)

No clinical signs and no deaths were observed during the study.

3.1.2 Body weight (figures 1 and 2, tables 2 and 3)

The body weight gain of the treated animals was similar to that of CIT historical control animals.

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] is equal to or higher than 2000 mg/kg in rats. No signs of toxicity were observed at this dose.

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 does not present a significant acute toxic risk if swallowed.

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Figure 1: Body weight of treated rats

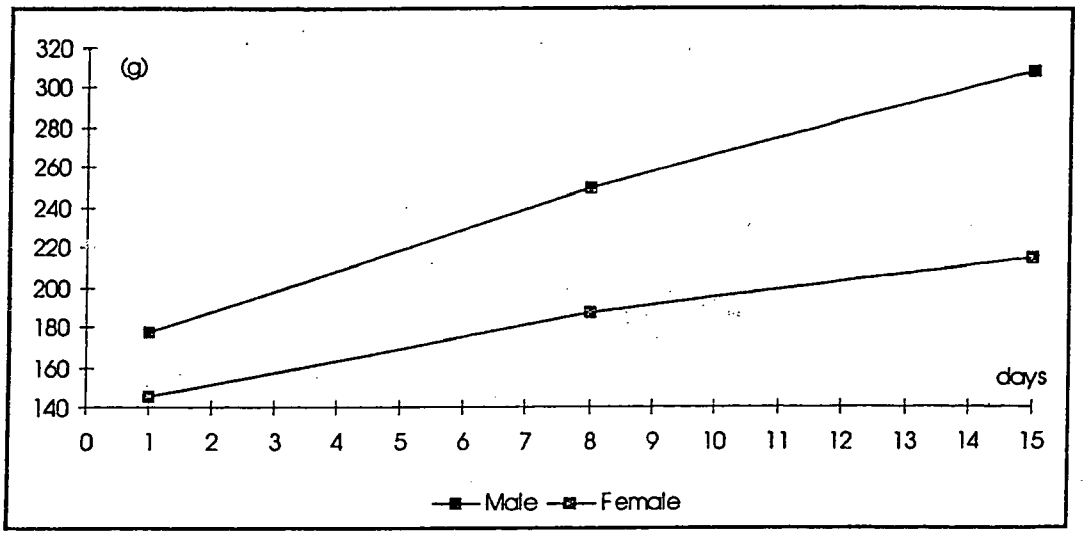
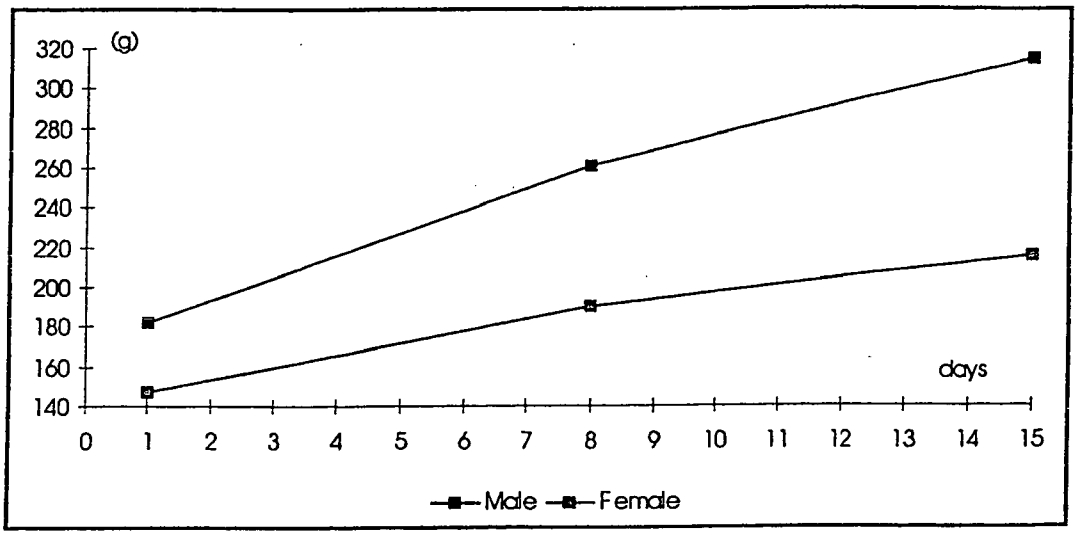


Figure 2: Body weight of CIT historical control rats



e.g.: CIT Historical data of animals dosed by the oral route: results of control animals from October 1995 to December 1997.

Table 1: Individual clinical signs and mortality

Dose (mg/kg)	Time	Animals		Mortality	Clinical signs
		Males	Females		
2000	1h - 2h - 4h D 2 to D 15	01-02-03-04-05	06-07-08-09-10	No	None

min : minutes
h : hour
D : day

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

Dose	Volume	Sex	Animals	Days				
mg/kg	ml/kg			1	(1)	8	(1)	15
2000	1.91	Male	01	174	64	238	62	300
			02	173	80	253	69	322
			03	177	73	250	56	306
			04	180	74	254	58	312
			05	182	72	254	48	302
			M	177	73	250	59	308
			SD	4	6	7	8	9
2000	1.91	Female	06	144	39	183	8	191
			07	136	51	187	14	201
			08	151	43	194	37	231
			09	142	32	174	46	220
			10	154	44	198	33	231
			M	145	42	187	28	215
			SD	7	7	9	16	18

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

Table 3: Mean body weight and weekly body weight change of CIT historical control rats

BODY WEIGHT OF CONTROL RATS
(g)

Dose mg/kg	Volume ml/kg	Sex		Days 1	8	15
0	10	Male	M	182	261	315
			SD	10	12	21
			n	45	45	45
0	10	Female	M	147	190	215
			SD	10	15	17
			n	45	45	45

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	79	54
			SD	7	13
0	10	Female	M	43	25
			SD	8	7

M : mean
SD : standard deviation

e.g.: CIT Historical data of animals dosed by the oral route: results of control animals from October 1995 to December 1997.

Table 4: Individual macroscopic examinations at necropsy

Dose mg/kg	Time	Animals		Macroscopic abnormalities
		Males	Females	
2000	D 15	01-02-03-04-05	06-07-08-09-10	No apparent abnormalities

D: day

APPENDICES

1. Test article description and analytical certificate

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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	:
Elf Atochem filing number	:

PHYSICAL AND CHEMICAL PROPERTIES	
Appearance	: [redacted]
Melting point	:
Flash point	:
Solubility	:

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	%	[redacted]	[redacted]	LCU 622
Point éclair	°C	[redacted]	[redacted]	LCU 057
pH		[redacted]	[redacted]	LCU 642
Taille des Particules	nm	[redacted]	[redacted]	LCU 672
Test d'application Cuir oléophobie et hydrophobie		[redacted]	[redacted]	LCU 674

Le Chef de Service du Laboratoire
SIGNATURE :

NOM : Robert Gruppo

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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 byproducts	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 "

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