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
Xavier Manciaux

STUDY COMPLETION DATE
14 May 1998

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

LABORATORY STUDY NUMBER
16045 TAR

CONTENTS

[redacted] contain TSCA CBI

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. n° 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: 14 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.

p.o.

L. Valette-Talbi Date: 14 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 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 the dose of 2000 mg/kg, taking into consideration that its density was 0.848.

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

No death 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.

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/E.E.C. (27th April 1993) adapting to technical progress for the eighteenth time Council Directive 67/548/E.E.C., 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'O.C.D.E. (No. 401, 24 février 1987) et de la C.E.E. (92/69/E.E.C., 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 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 sa densité (d = 0,848).

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 DL0 orale du produit [redacted] est supérieure ou égale à 2000 mg/kg chez le Rat. Aucun signe clinique n'est observé à cette dose.

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 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 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.

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.

2.1.2 Formulation procedure

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 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 192 ± 6 g for the males and 144 ± 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 retained. 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 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 and chemical analysis 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 C.I.T.

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 by the supplier for composition and contaminant levels.
The diet formula is presented in appendix 1/2.

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, drinking water or bedding material at levels which may be expected to interfere with or prejudice 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 in its original form taking into consideration that its density was 0.848

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 16 October 1997 in the morning (day 1) and was followed by a 14-day observation period until 30 October 1997 (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 C.I.T. 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. 16045 TAR 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

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 (figures 1 and 2, tables 2 and 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] 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/E.E.C. (27th April 1993) adapting to technical progress for the eighteenth time Council Directive 67/548/E.E.C., the test substance does not present a significant acute toxic risk if swallowed.

Figure 1: Body weight of treated rats (g)

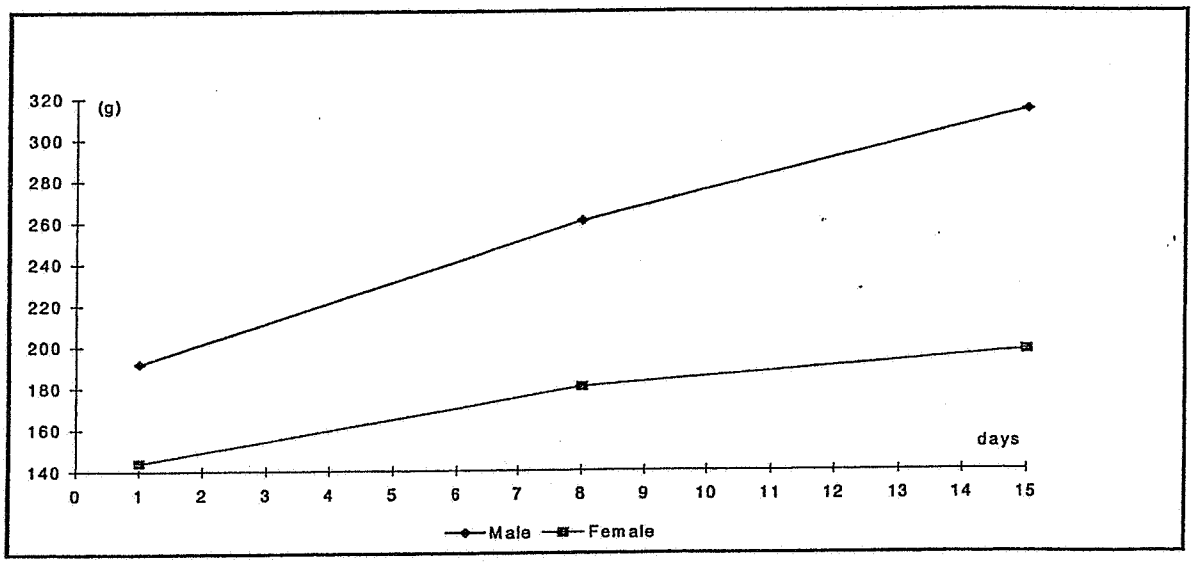
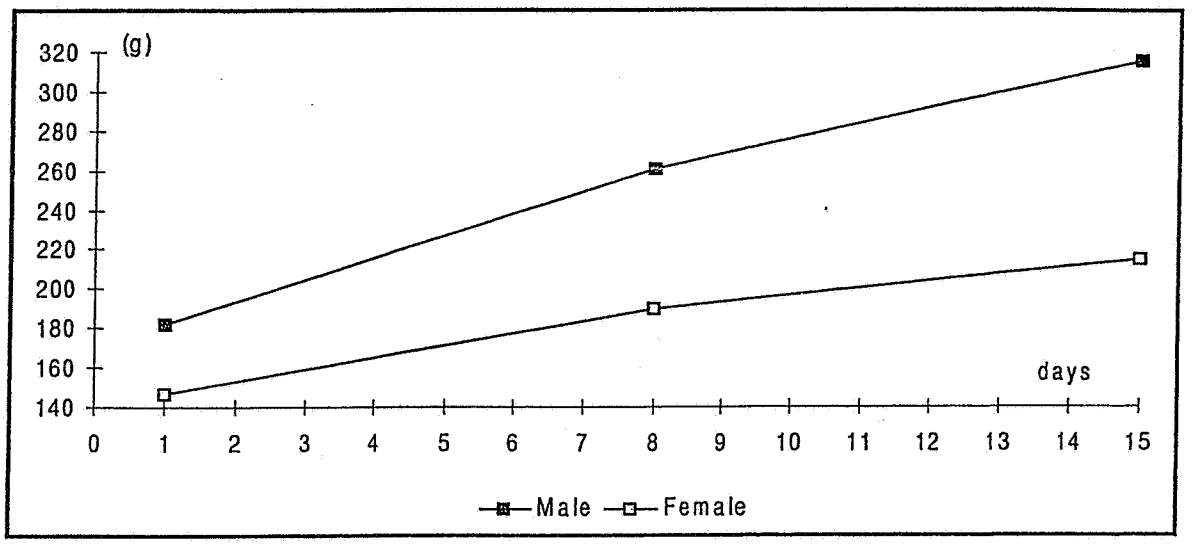


Figure 2: Body weight of C.I.T. historical control rats (g)



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

Table 1: Individual clinical signs and mortality

Dose (mg/kg)	Time	Animals		Mortality	Clinical signs
		Males	Females		
2000	30 min 1h - 2h - 4h 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	2.36	Male	01	199	69	268	55	323
			02	197	75	272	64	336
			03	184	67	251	50	301
			04	187	64	251	37	288
			05	193	67	260	60	320
			M	192	68	260	53	314
			SD	6	4	10	10	19
2000	2.36	Female	01	136	28	164	17	181
			02	152	37	189	16	205
			03	143	37	180	21	201
			04	151	31	182	17	199
			05	138	49	187	15	202
			M	144	36	180	17	198
			SD	7	8	10	2	10

(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	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.: C.I.T. 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	01-02-03-04-05	None

D: day

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: 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: 01.69.04.03.57 - Fax : 01.69.04.81.97
(Ref. Doc. UAR: 1992)