LABORATORIES & CLINICS GROUP
BIOSCIENCE

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"ACUTE ORAL TOXICITY STUDY IN RATS"

RBM EXP. No. 980429

EEC Guidelines (B.1)
OECD Guidelines (401)

Issued on October 16, 1998

SPONSOR

AUSIMONT
Viale S.Pietro, 50/A
20021 BOLLATE (Milano)
Italy

PERFORMING LABORATORY

Istituto di Ricerche Biomediche
"Antoine Marxer" RBM S.p.A.
Via Ribes, 1
10010 - COLLERETTO GIACOSA (Torino)
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RBM Exp. No. 980429

TITLE OF THE STUDY

"Acute oral toxicity study in rats treated with the test article [redacted]"

PURPOSE OF THE STUDY

The purpose of the study was to evaluate the acute oral toxicity of the test article [redacted].

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RBM Exp. No. 980429

FOREWORD

On behalf of **AUSIMONT Viale S.pietro, 50/A. 20021-BOLLATE-Milano-Italy**, Istituto di Ricerche Biomediche "Antoine Marxer" RBM S.p.A., authorized by the Italian Health Authorities (1-2) to conduct safety studies, has performed an acute toxicity study by oral route in Sprague Dawley Crl: CD(SD) BR rats (RBM-Experiment No. 980429), with the test article:

[redacted]

A sample of the substance used, along with pertinent documentation, is held in sufficient quantity in the RBM archives and is at the disposal of the Ministero della Sanità.

The undersigned declares that the experiment was conducted using the same batch of substance as that of the sample held on file.

For verification by the Ministero della Sanità, the undersigned moreover guarantees the identification and classification of all those materials, documents and recordings used in conducting the experiment, held on file for a period of at least 10 years from the date of this report. Following this time, they will be placed at the disposal of the Sponsor.

Dr. Roberto Maraschin

Scientific and Operative Director

Ivrea, October 16, 1998

(1): **Pharmaceuticals:**
Authorization dated March 12, 1976 in accordance with "Circolare 73", May 16, 1974

(2): **Chemicals:**
Authorization in accordance with DPR 927/81 (D.M. dated January 7, 1988 published in G.U. No. 12, dated January 16, 1988).

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RBM Exp. No. 980429

QUALITY ASSURANCE STATEMENT

RBM Experiment number: 980429

Study title:

"Acute oral toxicity study in rats treated with the test article [redacted]"

Studies of the type described in this report are conducted in a manner which involves frequent repetition of identical or similar procedures.

In compliance with the Principles of Good Laboratory Practice, at the time of this study, procedure-based inspections were made by the Q.A.U. of critical phases and procedures relevant to this type of study. For the inspection of any given procedure, studies were selected at random. All such inspections were reported promptly to the study director and to facility management.

This study was inspected on:

Dates of inspection/audit	Dates of report to Study Director and Management
May 29, 1998 October 13 – 14, 1998	May 29, 1998 October 14, 1998

This report has been audited by the Q.A.U. and was found to be an accurate description of such methods and procedures as were used during the conduct of the study and an accurate reflection of the raw data.

Date of final report audit: October 20 1998

Enrico Invernizzi
Head of Quality Assurance Unit

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RBM Exp. No. 980429

CERTIFICATION OF GLP COMPLIANCE

Study No. 980429 entitled :

"Acute oral toxicity study in rats treated with the test article [redacted] "

I hereby confirm that this study was conducted in accordance with the OECD [C(81) 30 (final)], Principles of Good Laboratory Practice (GLP).

The Sponsor is responsible for GLP compliance of any information supplied.

These principles were adopted by the EEC and incorpored into EEC Directive 88/320, that was legally enforced by the Italian Health Authority [D.M. dated June 26, 1986 as published in G.U. No. 198, dated August 27, 1986 and D.L. January 27, 1992, No. 120 as published in G.U. (Supplement) No. 40, February 18, 1992].

The final report fully and accurately reflects the raw data generated during the conduct of the study.

This report consists of 40 pages.

Study Director

Dr. Ping Yu

Ivrea, October 21, 1998

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RBM Exp. No. 980429

SCIENTISTS INVOLVED IN THE STUDY

Study No. 980429

"Acute oral toxicity study in rats treated with the test article [redacted] "

Study Director	Dr. Ping Yu
Senior Scientist for General Toxicology	Dr. Sergio Peano
Head of General Toxicology I Unit	Dr. Germano Oberto
Centralized Pharmacy Head	Dr. Rita Bussi
Pharmacy Service Head	Dr. Bruna Piccioli

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MATERIALS AND METHODS

EXPERIMENTAL DESIGN

RBM Experiment No.: 980429

Test article: [redacted]

Administration route: oral (by gavage)

Duration of treatment period: single administration

Duration of post-treatment observation period: 14 days

The test method was in accordance with European Economic Community Guidelines - Annex to Commission Directive 92/69/EEC of July 31, 1992 adapting to technical progress for the seventeenth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (B.1) and with Organization for Economic Cooperation and Development Guidelines (section 4, subpart 401, Paris 1981 and subsequent revisions).

TEST SYSTEM

Species, strain and substrain: Sprague Dawley Crl: CD (SD) BR rat

Justification for selection of the test system : the Sprague Dawley rat was chosen as rodent species since it is an appropriate experimental model widely accepted by Health Authorities, with documented susceptibility to a wide range of toxic substances

Number and sex of animals: 5 males/dose at the doses of 126 and 162 mg/kg
5 males and 5 females at the dose of 90 mg/kg

Supplier:	Charles River Italia S.p.A. Via Indipendenza, 11 22050 CALCO (Lecco) Shipping slips Nos. 03930 (May 29, 1998), 04317 (June 12, 1998) and 04635 (June 26, 1998)
Age (at randomization):	no more than three months
Body weight (at randomization):	Males: 282-324 g Females: 242-286 g
Acclimatization:	at least 5 days before the start of the test. Animals were observed daily to ascertain their fitness for the study.
Housing:	5 animals/sex/cage in air-conditioned room. - Temperature: 22°C ± 2 - Relative humidity: 55% ± 10 - Air changes: about 20 / hour filtered on HEPA 99.97% - Light: 12 hour cycle (7 a.m. - 7 p.m.) - Cage size: grill cages 40.5x38.5x18h cm with stainless steel feeder. The waste that dropped through the grill bottom onto removable paper was periodically disposed of.
Animal identification:	by appropriately coloring different areas of the limbs. Cage card gave experiment number, dosage group, sex and date of administration.
Diet:	GLP 4RF21 top certificate pelleted diet produced by Charles River Italia's feed licencee Mucedola S.r.l., Settimo Milanese. The declared contents on the label, on dry matter basis (moisture 12%), were:

crude protein	18.50%
crude fat	3.00%
crude fiber	6.00%
crude ash	7.00%

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The diet was supplemented by the Producer with vitamins and trace elements. The Producer supplies a certificate of analysis for nutrients and contaminants, the levels of which are within the limits proposed by EPA-TSCA (44FR:44053-44093, July 26, 1979).
RBM has the animal feed re-analyzed at least twice a year for bacterial contamination.
The diet was available "ad libitum" to the animals.

Water: from the municipal water main system.
Water is filtered and distributed "ad libitum" to the animals by an automatic valve system.
Periodically drinking water is analyzed for microbial count, heavy metals, other contaminants (e.g. solvents, pesticides) and other chemical and physicals characteristics. The accepted limits of quality of the drinking water were those defined in EEC directive 80/778

Contaminants that might interfere with the objectives of the study were not expected to be present in the diet or drinking water.

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TEST ARTICLE, CHARACTERIZATION

Identification:	[redacted]
Batch:	2/SPINETTA
Characteristics:	white powder
Purity:	> 99%
Manufacturing date:	March 30, 1998
Expiry date:	December 2000
Storage conditions:	at room temperature

VEHICLE CHARACTERIZATION

Deionized water

TEST ARTICLE FORMULATE PREPARATION

When required, an exact amount of test article was weighed in a suitable graduated container and made up to final volume with vehicle to obtain a solution in the concentration required.

The formulates were administered within one hour of the preparation.

TEST DESCRIPTION

Administration route: oral (by gavage)

Reason for selection of administration route: possible ingestion by humans

Experimental design:

Dose* mg/kg	Treated Animals	Treatment Date	Final killing
162	5 males	July 9, 1998	Found dead
126	5 males	August 12, 1998	September 2,1998
90	5 males	July 22, 1998	August 12, 1998
90	5 females	August 12, 1998	August 26, 1998

*The doses were defined on the basis of a preliminary study.

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Administration method:	The volume of administration was 10 ml/kg defined on the basis of the individual body weight. The administration was done by gavage to rats which had been fasted about 16 hours. Feed was returned to the rats about three hours after the test article administration.
Observation period:	14 or 21 *days after administration * for males in groups of 90 and 126 mg/kg due to the delayed clinical changes.
Observation of clinical signs and mortality:	at 30 minutes, 2, 4 and 6 hours on the first day after the administration (day 1) and then twice a day up to termination of the observation period
Body weight:	twice pre-trial (at randomization and on day 1 just before administration) and on days 3, 8 and 14. On day 1 the animals were weighed after a 16-hour fasting period. For the males in groups of 90 and 126 mg/kg body weights were also recorded on day 21.
Gross pathology:	on animals which died before the end of the study and on animals killed (fasted overnight) by excision of the femoral arteries, after i.p. overdosage anesthesia with 5% sodium pentobarbital, at the end of the observation period
Histology:	portions of abnormal entities found in the necropsied animals were collecred. The tissue samples were fixed and preserved in 10% buffered formalin. Histologic examination was not performed
LD_{50} and its statistical limits:	LD_{50} was calculated by the method of the Probit (Bliss – Finney) - A.P. Rosiello et al., J. Tox. and Env. Health, 3: 797-809, 1977.

RECORD FILING

The protocol, a reserve sample of the batch of the test article used, the raw data bound in a register numbered 980429 /1, the specimens, the final report and all other documents pertinent to the conduct of this study, including records and reports of maintenance, cleaning, calibration and inspection of equipment, analysis of diet and water are filed at RBM premises for ten years from the issue date of this report and then sent to the Sponsor.

PROCEDURAL DETAILS

The study was conducted in accordance with the procedures described in the RBM Standard Operating Procedures (SOP's) collection.

Protection of animals used in the experiment is in accordance with Directive 86/609/EEC, enforced by the Italian D. L. No. 116 of January 27, 1992.

Physical facilities and equipment for accommodation and care of animals are in accordance with the provisions of EEC Council Directive 86/609.

The Institute is fully authorized by Competent Veterinary Health Authorities.

RESULTS

CLINICAL OBSERVATIONS

MORTALITY *(TABLE 1)*

The mortality which occurred at the various doses is given below:

Dose (mg/kg)	90	126	162
Treated animals	5M+5F	5M	5M
Mortality	0	3M	5M
Total (%)	0%	60%	100%

The deaths occurred 6-16 days after dosing, with the first case observed on day 6 after administration in the 162 mg/kg group.

No deaths occurred in the animals of either sex in the lowest dose group (90 mg/kg).

Even though the LD_{50} was not calculable with the Probit method, the approximate LD_{50} could be considered 120 mg/kg (with 0% mortity at 90 mg/kg and 100% mortity at 162 mg/kg)

CLINICAL SIGNS *(TABLE 2 AND APPENDIX 1)*

Hypoactivity, piloerection and hunched posture were observed in the males of the various dose groups, starting 5-6 days (162 mg/kg group) or 6-12 days (the lower doses) after dosing. Sedation was observed in rats of the highest dose group (162 mg/kg).

Piloerction was the only clinical change observed in the females received the test article at the lowest dose (7-12 days after treatment).

Complete or partial recovery was achieved at the end of the observation period in the surviving animals.

BODY WEIGHT *(APPENDIX 2)*

Decrease in body weight or retarded growth was found in animals given the various doses during the observation period.

POST-MORTEM EXAMINATION

GROSS PATHOLOGY *(TABLE 3 AND APPENDIX 3)*

At the necropsy of animals which died before the end of the observation period, the main macroscopic findings were marked or moderate liver paleness, decreased size of spleen, ulcer/erosion and congestion of stomach, congestion and catarrhal content of the intestine. Moreover, kidney and/or medulla congestion, pale spleen, congestion and/or decreased size of thymus and congestion of testes were seen in a few animals.

At the autopsy carried out at the end of the observation period, no appreciable macroscopic findings were evident in any rat.

SUMMARY AND CONCLUSIONS

Experimental data from a toxicity study in which Sprague Dawley Crl:CD(SD) BR rats received oral administration of the test article [redacted] are given in this report.

The test method was in accordance with European Economic Community Guidelines - Annex to Commission Directive 92/69/EEC of July 31, 1992 adapting to technical progress for the seventeenth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (B.1) and with Organization for Economic Cooperation and Development Guideline (section 4, subpart 401, Paris 1981 and subsequent revisions).

The test article was administered to the rats as a solution in deionized water at the dosages of 90, 126 and 162 mg/kg to groups of 5 males/dose and at the dose of 90 mg/kg to 5 females for confirmation in the other sex. All rats were treated after a 16-hour fasting period. The day of treatment was considered day 1 of the study. The animals were weighed twice before treatment (at randomization and on day 1 just before treatment) and on days 3, 8 and 14 (surviving males in the 90 and 126 mg/kg groups were also weighed on day 21). They were clinically observed for 14 or 21 days following the treatment. Macroscopic examinations were performed in the animals which died before the end of the study. At the end of the observation period the surviving rats were killed (fasted overnight) by excision of the femoral arteries after i.p. overdosage anesthesia with 5% sodium pentobarbital and were subjected to a thorough autopsy.

The mortality which occurred at the various doses is given below:

Dose (mg/kg)	90	126	162
Treated animals	5M+5F	5M	5M
Mortality	0	3M	5M
Total (%)	0%	60%	100%

The deaths occurred 6-16 days after dosing, with the first case observed on day 6 after administration in the 162 mg/kg group.

No deaths occurred in the animals of either sex in the lowest dose group (90 mg/kg).

Even though the LD_{50} was not calculable with the Probit method, the approximate LD_{50} could be considered 120 mg/kg (with 0% mortity at 90 mg/kg and 100% mortity at 162 mg/kg)

Hypoactivity, piloerection and hunched posture were observed in the males of the various dose groups, starting 5-6 days (162 mg/kg group) or 6-12 days (the lower doses) after dosing. Sedation was observed in rats of the highest dose group (162 mg/kg). Piloerection was the only clinical change observed in the females that received the test article at the lowest dose (7-12 days after treatment). Complete or partial recovery was achieved at the end of the observation period in the surviving animals. Moreover, decrease in body weight or retarded growth was found in animals given the various doses during the observation period.

At the necropsy of animals which died before the end of the observation period, the main macroscopic findings were marked or moderate liver paleness, decreased size of spleen, ulcer/erosion and congestion of stomach, congestion and catarrhal content of the intestine.

At the autopsy carried out at the end of the observation period, no appreciable macroscopic findings were evident in any rat.

In conclusion, the approximate **LD_{50}** of the test article [redacted], when administered to rats by oral route, was **120 mg/kg**. The compound induced delayed toxicity (liver and gastrointestinal system were mainly involved) in animals given the higher doses.

Dr. Ping Yu
Study Director
October 16. 1998

Dr. Sergio Peano
Senior Scientist for General Toxicology
Oct. 16, 1998

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RBM Exp. No. 980429

GROUP DATA

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980429

TABLE 1. - Mortality and LD50 calculation (p. 1)

Males - Females

Dose (mg/kg)	90	126	162
Treated animals	10	5	5
Day 6	0	0	1
7	0	0	1
9	0	0	3
16	0	3	0
Total no. (day 21)	0	3	5
Total (%)	0.0%	60.0%	100.0%

LD50 not calculable

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980429

TABLE 2. - Clinical signs (maximum daily frequency) (p. 1)
(no. of animals affected, from-to)

	Males		
Dose (mg/kg)	90	126	162
no. of treated animals	5	5	5
Death	-	3 16d	5 6d- 9d
Sedation	-	-	3 8d- 8d
Hypoactivity	3 8d-15d	5 12d-15d	4 6d- 7d
Piloerection	5 7d-19d	5 6d-16d	5 5d- 8d
Hunched posture	5 7d-15d	5 7d-15d	5 5d- 8d
Palpebral, partial closure	-	-	3 8d- 8d
Hypothermia	-	-	3 8d- 8d
Recovery	5 20d	2 17d	-

- (not observed) from-to (first-last observation in one or more animals)
Time : d (days)

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980429

TABLE 2. - Clinical signs (maximum daily frequency) (p. 2)
(no. of animals affected, from-to)

	Females
Dose (mg/kg)	90
no. of treated animals	5
Piloerection	5 7d-12d
Recovery	5 13d

from-to (first-last observation in one or more animals)
Time : d (days)

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980429

TABLE 3. - Gross pathology examination (p. 1)
(no. of cases, mean severity, %)

Dead or agonal sacrificed an. Males

Dose (mg/kg)	90	126	162
no. of animals	0	3	5
no. of animals without appreciable lesions	0	0	1
Intestine			
congestion	-	0	1(2.0) 20.00%
catarrhal content	-	0	3(2.0) 60.00%
Kidneys			
congestion	-	0	1(3.0) 20.00%
medulla, congestion	-	1(3.0) 33.33%	3(3.0) 60.00%
Liver			
pale	-	3(2.3) 100.00%	4(2.2) 80.00%

- (not examined)
Severity : 0(very slight) 1(slight) 2(moderate) 3(severe)

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980429

TABLE 3. - Gross pathology examination (p. 2)
(no. of cases, mean severity, %)

Dead or agonal sacrificed an. Males

Dose (mg/kg)	90	126	162
no. of animals	0	3	5
no. of animals without appreciable lesions	0	0	1
Spleen			
decreased size	-	3(2.3) 100.00%	3(2.0) 60.00%
pale	-	1(2.0) 33.33%	0
Stomach			
congestion	-	2(2.0) 66.67%	0
erosion	-	1(3.0) 33.33%	1(2.0) 20.00%
ulcer	-	1(3.0) 33.33%	0
Testes			
congestion	-	1(3.0) 33.33%	3(2.3) 60.00%

- (not examined)
Severity : 0(very slight) 1(slight) 2(moderate) 3(severe)

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980429

TABLE 3. - Gross pathology examination (p. 3)
(no. of cases, mean severity, %)

Dead or agonal sacrificed an. Males

Dose (mg/kg)	90	126	162
no. of animals	0	3	5
no. of animals without appreciable lesions	0	0	1
Thymus			
congestion	-	0	2(3.0) 40.00%
decreased size	-	0	1(3.0) 20.00%

- (not examined)
Severity : 0(very slight) 1(slight) 2(moderate) 3(severe)

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980429

TABLE 3. - Gross pathology examination (p. 4)
(no. of cases, mean severity, %)

Final killing — Males

Dose (mg/kg)	90	126	162
no. of animals	5	2	0
no. of animals without appreciable lesions	5	2	0

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980429

TABLE 3. - Gross pathology examination (p. 5)
(no. of cases, mean severity, %)

Final killing	Females
Dose (mg/kg)	90
no. of animals	5
no. of animals without appreciable lesions	5

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APPENDICES

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980429

APPENDIX 1. - Clinical signs incidence (p. 1)
(no. of animals affected)

Dose (mg/kg) 90

Cage # 7M	Day	1				2		3		4		5		6		7		8		9		10		11		12		13		14		15		16		17	
	Time	30m	2h	4h	6h	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A
No clinical signs		5	5	5	5	5	5	5	5	5	5	5	5	5	5															1	1	1	1	1	1	3	3
Hypoactivity																		2	2	3	3	3	3	3	3	3	3	3	3	2	2	1	1				
Piloerection																5	5	5	5	5	5	5	5	5	5	5	5	5	5	4	4	4	4	4	4	2	2
Hunched posture																2	2	5	5	5	5	5	5	3	3	3	3	3	3	2	2	1	1				

Cage # 7M (follows)	Day	18		19		20		21	
	Time	M	A	M	A	M	A	M	A
No clinical signs		3	3	4	4	5	5	5	5
Piloerection		2	2	1	1				

Cage # 8F	Day	1				2		3		4		5		6		7		8		9		10		11		12		13		14	
	Time	30m	2h	4h	6h	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A
No clinical signs		5	5	5	5	5	5	5	5	5	5	5	5	5	5					2	2	4	4	4	4	4	4	5	5	5	5
Piloerection																5	5	5	5	3	3	1	1	1	1	1	1				

Time: m (minutes) h (hours) M (morning) A (afternoon)

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980429

APPENDIX 1. - Clinical signs incidence (p. 2)
(no. of animals affected)

Dose (mg/kg) 126

Cage # 9M	Day	1				2		3		4		5		6		7		8		9		10		11		12		13		14		15		16		17	
	Time	30m	2h	4h	6h	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A
Death																																3			
No clinical signs		5	5	5	5	5	5	5	5	5	5	5	5																					2	2
Hypoactivity																										5	5	5	5	5	5	4	4		
Piloerection														5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	2	2		
Hunched posture																5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5				

Cage # 9M (follows)	Day	18		19		20		21	
	Time	M	A	M	A	M	A	M	A
No clinical signs		2	2	2	2	2	2	2	2

Time: m (minutes) h (hours) M (morning) A (afternoon)

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980429

APPENDIX 1. - Clinical signs incidence (p. 3)
(no. of animals affected)

Dose (mg/kg) 162

Cage # 5M	Day	1				2		3		4		5		6		7		8		9
	Time	30m	2h	4h	6h	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M
Death														1		1				3
No clinical signs		5	5	5	5	5	5	5	5	5	5									
Sedation																		3	3	
Hypoactivity														4	4	3	3			
Piloerection												5	5	4	4	3	3	3	3	
Hunched posture												5	5	4	4	3	3	3	3	
Palpebral, partial closure																		3	3	
Hypothermia																		3	3	

Time: m (minutes) h (hours) M (morning) A (afternoon)

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980429

APPENDIX 2. - Body weight (g) (p. 1)
(individual)

Dose (mg/kg) 90

Animal #		31M	32M	33M	34M	35M	36F	37F	38F	39F	40F
Week	day										
	0	282	295	299	298	310	260	280	256	286	242
1	1	258	271	278	276	288	242	263	239	264	226
1	3	253	269	269	271	276	252	270	244	277	231
2	8	248	218	210	257	238	272	295	269	289	252
2	14	298	172	216	269	189	288	307	277	295	269
3	21	331	214	318	303	204					

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980429

APPENDIX 2. - Body weight (g) (p. 2)
(individual)

Dose (mg/kg) 126

Animal #		41M	42M	43M	44M	45M
Week	day					
	0	315	320	314	320	320
1	1	295	298	293	301	308
1	3	290	292	290	296	294
2	8	242	258	212	248	268
2	14	233	245	207	230	254
3	21		339		331	

S2

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980429

APPENDIX 2. - Body weight (g) (p. 3)
(individual)

Dose (mg/kg) 162

Animal #		21M	22M	23M	24M	25M
Week	day					
	0	302	324	309	324	313
1	1	279	298	280	300	296
1	3	267	285	272	278	275
2	8		204	192	202	

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980429

APPENDIX 3. - Gross pathology examination (p. 1)
(individual)

Dead or agonal sacrificed an.

Dose (mg/kg) 126

An#	Death day/code#	TISSUE	Gross observations
41M	16 M2	Liver	pale, diffuse, moderate
		Spleen	decreased size, diffuse, moderate
		Stomach	congestion, diffuse, moderate erosion, focal, severe
43M	16 M2	Liver	pale, diffuse, moderate
		Spleen	decreased size, diffuse, severe
45M	16 M2	Kidneys	medulla, congestion, diffuse, severe
		Liver	pale, diffuse, severe
		Spleen	decreased size, diffuse, moderate pale, diffuse, moderate
		Stomach	congestion, diffuse, moderate ulcer, focal, severe
		Testes	congestion, diffuse, severe

Death code : M2(Natural death)

REDACTED AS TO TRADE NAMES

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980429

APPENDIX 3. - Gross pathology examination (p. 2)
(individual)

Dead or agonal sacrificed an.

Dose (mg/kg) 162

An#	Death day/code#	TISSUE	Gross observations
21M	7 M2	Liver	pale, multifocal, severe
22M	9 M2	Intestine	congestion, diffuse, moderate catarrhal content, diffuse, moderate
		Kidneys	medulla, congestion, diffuse, severe
		Liver	pale, diffuse, moderate
		Spleen	decreased size, diffuse, moderate
		Stomach	erosion, multifocal, moderate
		Testes	congestion, diffuse, moderate
		Thymus	congestion, diffuse, severe decreased size, diffuse, severe
23M	9 M2	Intestine	catarrhal content, diffuse, moderate
		Kidneys	medulla, congestion, diffuse, severe
		Liver	pale, diffuse, moderate
		Spleen	decreased size, diffuse, moderate

Death code : M2(Natural death)

REDACTED AS TO TRADE NAMES

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980429

APPENDIX 3. - Gross pathology examination (p. 3)
(individual)

Dead or agonal sacrificed an.

Dose (mg/kg) 162

An#	Death day/code#	TISSUE	Gross observations
23M	9 M2	Testes	congestion, diffuse, moderate
24M	9 M2	Intestine	catarrhal content, diffuse, moderate
		Kidneys	medulla, congestion, diffuse, severe congestion, diffuse, severe
		Liver	pale, diffuse, moderate
		Spleen	decreased size, diffuse, moderate
		Testes	congestion, diffuse, severe
		Thymus	congestion, diffuse, severe
25M	6 M2	General observation	no macroscopically appreciable lesions

Death code : M2(Natural death)

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980429

APPENDIX 3. - Gross pathology examination (p. 4)
(individual)

Final killing

Dose (mg/kg) 90

An#	Death day	T I S S U E	Gross observations
31M	22	General observation	no macroscopically appreciable lesions
32M	22	General observation	no macroscopically appreciable lesions
33M	22	General observation	no macroscopically appreciable lesions
34M	22	General observation	no macroscopically appreciable lesions
35M	22	General observation	no macroscopically appreciable lesions
36F	15	General observation	no macroscopically appreciable lesions
37F	15	General observation	no macroscopically appreciable lesions
38F	15	General observation	no macroscopically appreciable lesions
39F	15	General observation	no macroscopically appreciable lesions
40F	15	General observation	no macroscopically appreciable lesions

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980429

APPENDIX 3. - Gross pathology examination (p. 5)
(individual)

Final killing

Dose (mg/kg) 126

An#	Death day	T I S S U E	Gross observations
42M	22	General observation	no macroscopically appreciable lesions
44M	22	General observation	no macroscopically appreciable lesions

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LABORATORIES & CLINICS GROUP
BIOSCIENCE

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Italy

Tel: 0125 222111
Fax: 0125 222599

[redacted]

[redacted] $Ce(C_3F_6O)_3\text{-}CF_2COO^-NH_4^+$

"ACUTE ORAL TOXICITY STUDY IN RATS"

RBM EXP. No. 980431

EEC Guidelines (B.1)
OECD Guidelines (401)

Issued on October 16, 1998

SPONSOR

AUSIMONT
Viale S.Pietro, 50/A
20021 BOLLATE (Milano)
Italy

PERFORMING LABORATORY

Istituto di Ricerche Biomediche
"Antoine Marxer" RBM S.p.A.
Via Ribes, 1
10010 - COLLERETTO GIACOSA (Torino)
Italy

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RBM Exp. No. 980431

TITLE OF THE STUDY

"Acute oral toxicity study in rats treated with the test article [redacted] "

PURPOSE OF THE STUDY

The purpose of the study was to evaluate the acute oral toxicity of the test article [redacted].

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LABORATORIES & CLINICS GROUP
BIOSCIENCE

RBM Exp. No. 980431

FOREWORD

On behalf of **AUSIMONT Viale S.pietro, 50/A. 20021-BOLLATE-Milano-Italy**, Istituto di Ricerche Biomediche "Antoine Marxer" RBM S.p.A., authorized by the Italian Health Authorities (1-2) to conduct safety studies, has performed an acute toxicity study by oral route in Sprague Dawley Crl: CD(SD) BR rats (RBM-Experiment No. 980431), with the test article:

[redacted]

A sample of the substance used, along with pertinent documentation, is held in sufficient quantity in the RBM archives and is at the disposal of the Ministero della Sanità.

The undersigned declares that the experiment was conducted using the same batch of substance as that of the sample held on file.

For verification by the Ministero della Sanità, the undersigned moreover guarantees the identification and classification of all those materials, documents and recordings used in conducting the experiment, held on file for a period of at least 10 years from the date of this report. Following this time, they will be placed at the disposal of the Sponsor.

Dr. Roberto Maraschin
Scientific and Operative Director

Ivrea, October 16, 1998

(1): **Pharmaceuticals:**
Authorization dated March 12, 1976 in accordance with "Circolare 73", May 16, 1974

(2): **Chemicals:**
Authorization in accordance with DPR 927/81 (D.M. dated January 7, 1988 published in G.U. No. 12, dated January 16, 1988).

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RBM Exp. No. 980431

QUALITY ASSURANCE STATEMENT

RBM Experiment number: 980431

Study title:

"Acute oral toxicity study in rats treated with the test article [redacted]"

Studies of the type described in this report are conducted in a manner which involves frequent repetition of identical or similar procedures.

In compliance with the Principles of Good Laboratory Practice, at the time of this study, procedure-based inspections were made by the Q.A.U. of critical phases and procedures relevant to this type of study. For the inspection of any given procedure, studies were selected at random. All such inspections were reported promptly to the study director and to facility management.

This study was inspected on:

Dates of inspection/audit	Dates of report to Study Director and Management
May 29, 1998 October 15 – 16, 1998	May 29, 1998 October 16, 1998

This report has been audited by the Q.A.U. and was found to be an accurate description of such methods and procedures as were used during the conduct of the study and an accurate reflection of the raw data.

Date of final report audit: October 20, 1998

Enrico Invernizzi
Head of Quality Assurance Unit

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BIOSCIENCE

RBM Exp. No. 980431

CERTIFICATION OF GLP COMPLIANCE

Study No. 980431 entitled :

"Acute oral toxicity study in rats treated with the test article [redacted] "

I hereby confirm that this study was conducted in accordance with the OECD [C(81) 30 (final)], Principles of Good Laboratory Practice (GLP).

The Sponsor is responsible for GLP compliance of any information supplied.

These principles were adopted by the EEC and incorporated into EEC Directive 88/320, that was legally enforced by the Italian Health Authority [D.M. dated June 26, 1986 as published in G.U. No. 198, dated August 27, 1986 and D.L. January 27, 1992, No. 120 as published in G.U. (Supplement) No. 40, February 18, 1992].

The final report fully and accurately reflects the raw data generated during the conduct of the study.

This report consists of 42 pages.

Study Director

Dr. Ping Yu

Ivrea, October 21, 1998

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RBM Exp. No. 980431

SCIENTISTS INVOLVED IN THE STUDY

Study No. 980431

"Acute oral toxicity study in rats treated with the test article [redacted]"

Study Director	Dr. Ping Yu
Senior Scientist for General Toxicology	Dr. Sergio Peano
Head of General Toxicology I Unit	Dr. Germano Oberto
Centralized Pharmacy Head	Dr. Rita Bussi
Pharmacy Service Head	Dr. Bruna Piccioli

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RBM Exp. No. 980431

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

RBM Experiment No.: 980431

Test article: [redacted]

Administration route: oral (by gavage)

Duration of treatment period: single administration

Duration of post-treatment observation period: 14 days

The test method was in accordance with European Economic Community Guidelines - Annex to Commission Directive 92/69/EEC of July 31, 1992 adapting to technical progress for the seventeenth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (B.1) and with Organization for Economic Cooperation and Development Guidelines (section 4, subpart 401, Paris 1981 and subsequent revisions).

TEST SYSTEM

Species, strain and substrain: Sprague Dawley Crl: CD (SD) BR rat

Justification for selection of the test system : the Sprague Dawley rat was chosen as rodent species since it is an appropriate experimental model widely accepted by Health Authorities, with documented susceptibility to a wide range of toxic substances

Number and sex of animals: 5 males/dose at the doses of 63, 81 and 145 mg/kg
5 males and 5 females at the dose of 45 mg/kg.

Supplier:	Charles River Italia S.p.A. Via Indipendenza, 11 22050 CALCO (Lecco) Shipping slips Nos. 03930 (May 29, 1998), 04317 (June 12, 1998), 04479 (June 19, 1998), 04635 (June 26, 1998) and 05128 (July 17, 1998)
Age (at randomization):	no more than three months
Body weight (at randomization):	Males: 242-302 g Females: 207-230 g
Acclimatization:	at least 5 days before the start of the test. Animals were observed daily to ascertain their fitness for the study.
Housing:	5 animals/sex/cage in air-conditioned room. - Temperature: 22°C ± 2 - Relative humidity: 55% ± 10 - Air changes: about 20 / hour filtered on HEPA 99.97% - Light: 12 hour cycle (7 a.m. - 7 p.m.) - Cage size: grill cages 40.5x38.5x18h cm with stainless steel feeder. The waste that dropped through the grill bottom onto removable paper was periodically disposed of.
Animal identification:	by appropriately coloring different areas of the limbs. Cage card gave experiment number, dosage group, sex and date of administration.
Diet:	GLP 4RF21 top certificate pelleted diet produced by Charles River Italia's feed licencee Mucedola S.r.l., Settimo Milanese. The declared contents on the label, on dry matter basis (moisture 12%), were:

crude protein	18.50%
crude fat	3.00%
crude fiber	6.00%
crude ash	7.00%

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The diet was supplemented by the Producer with vitamins and trace elements. The Producer supplies a certificate of analysis for nutrients and contaminants, the levels of which are within the limits proposed by EPA-TSCA (44FR:44053-44093, July 26, 1979).
RBM has the animal feed re-analyzed at least twice a year for bacterial contamination.
The diet was available "ad libitum" to the animals.

Water: from the municipal water main system.
Water is filtered and distributed "ad libitum" to the animals by an automatic valve system.
Periodically drinking water is analyzed for microbial count, heavy metals, other contaminants (e.g. solvents, pesticides) and other chemical and physicals characteristics. The accepted limits of quality of the drinking water were those defined in EEC directive 80/778

Contaminants that might interfere with the objectives of the study were not expected to be present in the diet or drinking water.

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RBM Exp. No. 980431

TEST ARTICLE, CHARACTERIZATION

Identification:	[redacted]
Batch:	4/SPINETTA
Characteristics:	white powder
Purity:	> 99%
Manufacturing date:	March 30, 1998
Expiry date:	December 2000
Storage conditions:	at room temperature

VEHICLE CHARACTERIZATION

Deionized water

TEST ARTICLE FORMULATE PREPARATION

When required, an exact amount of test article was weighed in a suitable graduated container and made up to final volume with vehicle to obtain the concentration required.

Magnetic stirring was used to obtain a homogeneous suspension. Formulates were kept magnetically stirred until the end of administration and were administered within one hour of the preparation.

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TEST DESCRIPTION

Administration route: oral (by gavage)

Reason for selection of administration route: possible ingestion by humans

Experimental design:

Dose* Mg/kg	Treated animals	Treatment Date	Final killing
145	5 males	July 9, 1998	Found dead
81	5 males	August 4, 1998	August 25,1998
63	5 males	August 20, 1998	September 10, 1998
45	5 males	July 22, 1998	August 5, 1998
45	5 females	August 4, 1998	August 25, 1998

* The doses were defined on the basis of a preliminary study.

Administration method: The volume of administration was 10 ml/kg defined on the basis of the individual body weight. The administration was done by gavage to rats which had been fasted about 16 hours. Feed was returned to the rats about three hours after the test article administration.

Observation period: 14 or 21 *days after administration

* for males in groups of 63 and 81 mg/kg and for females in group of 45 mg/kg due to the delayed clinical changes.

Observation of clinical signs and mortality: at 30 minutes, 2, 4 and 6 hours on the first day after the administration (day 1) and then twice a day up to termination of the observation period

Body weight: twice pre-trial (at randomization and on day 1 just before administration) and on days 3, 8 and 14. On day 1 the animals were weighed after a 16-hour fasting period. For males in groups of 63 and 81 mg/kg and for females in group of 45 mg/kg body weights were also recorded on day 21.

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Gross pathology:	on animals which died before the end of the study and on animals killed (fasted overnight) by excision of the femoral arteries, after i.p. overdosage anesthesia with 5% sodium pentobarbital, at the end of the observation period
Histology:	portions of abnormál entities found in the necropsied animals were collecred. The tissue samples were fixed and preserved in 10% buffered formalin. Histologic examination was not performed
LD_{50} and its statistical limits:	LD_{50} was calculated by the method of the Probit (Bliss - Finney) - A.P. Rosiello et al., J. Tox. and Env. Health, 3: 797-809, 1977.

RECORD FILING

The protocol, a reserve sample of the batch of the test article used, the raw data bound in a register numbered 980431 /1, the specimens, the final report and all other documents pertinent to the conduct of this study, including records and reports of maintenance, cleaning, calibration and inspection of equipment, analysis of diet and water are filed at RBM premises for ten years from the issue date of this report and then sent to the Sponsor.

PROCEDURAL DETAILS

The study was conducted in accordance with the procedures described in the RBM Standard Operating Procedures (SOP's) collection.

Protection of animals used in the experiment is in accordance with Directive 86/609/EEC, enforced by the Italian D. L. No. 116 of January 27, 1992.

Physical facilities and equipment for accommodation and care of animals are in accordance with the provisions of EEC Council Directive 86/609.

The Institute is fully authorized by Competent Veterinary Health Authorities.

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RBM Exp. No. 980431

RESULTS

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CLINICAL OBSERVATIONS

MORTALITY *(TABLE 1)*

The mortality which occurred at the various doses is given below:

Dose (mg/kg)	45	63	81	145
Treated animals	5M+5F	5M	5M	5M
Mortality	0	2M	4M	5M
Total (%)	0%	40%	80%	100%

The deaths occurred 4-9 days after dosing, with the first case observed on day 4 after administration in the 145 mg/kg group.

No deaths occurred in the animals of either sex in the lowest dose group (45 mg/kg).

The LD_{50} was calculated to be 67.7 mg/kg with 95% confidence limits of 58.5 – 78.3 mg/kg.

CLINICAL SIGNS *(TABLE 2 AND APPENDIX 1)*

Piloerection and hunched posture were observed in the animals of the various dose groups, starting 3-4 days (81 and 145 mg/kg groups) or 3-12 days (lower doses) after dosing. These changes were accompanied by hypoactivity in rats of the higher dose groups (81 and 145 mg/kg). Diarrhea was observed in two females of the 45mg/kg group 12-14 days after treatment.

Complete or partial recovery was achieved at the end of the observation period in the surviving animals.

BODY WEIGHT *(APPENDIX 2)*

Decrease in body weight or retarded growth was found in animals given the various doses during the observation period.

POST-MORTEM EXAMINATION

GROSS PATHOLOGY *(TABLE 3 AND APPENDIX 3)*

At the necropsy of animals which died before the end of the observation period, the main macroscopic findings were marked or moderate liver paleness and erosion and/or thinning walls of stomach. These changes were mainly confined to animals of the higher dose groups (81 and 145 mg/kg). Moreover, kidney medulla congestion and congestion of lungs or thymus were seen in a few animals.

At the autopsy carried out at the end of the observation period, no appreciable macroscopic findings were evident in any rat.

SUMMARY AND CONCLUSIONS

Experimental data from a toxicity study in which Sprague Dawley Crl:CD(SD) BR rats received oral administration of the test article [redacted] are given in this report.

The test method was in accordance with European Economic Community Guidelines - Annex to Commission Directive 92/69/EEC of July 31, 1992 adapting to technical progress for the seventeenth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (B.1) and with Organization for Economic Cooperation and Development Guideline (section 4, subpart 401, Paris 1981 and subsequent revisions).

The test article was administered to the rats as a suspension in deionized water at the dosages of 45, 63, 81 and 145 to groups of 5 males/dose and at the dose of 45 mg/kg to 5 females for confirmation in the other sex. All rats were treated after a 16-hour fasting period. The day of treatment was considered day 1 of the study. The animals were weighed twice before treatment (at randomization and on day 1 just before treatment) and on days 3, 8 and 14 (surviving males in the 63 and 81 mg/kg groups and females in the 45 mg/kg group were also weighed on day 21). They were clinically observed for 14 or 21 days following the treatment. Macroscopic examinations were performed in the animals which died before the end of the study. At the end of the observation period the surviving rats were killed (fasted overnight) by excision of the femoral arteries after i.p. overdosage anesthesia with 5% sodium pentobarbital and were subjected to a thorough autopsy.

The mortality which occurred at the various doses is given below:

Dose (mg/kg)	45	63	81	145
Treated animals	5M+5F	5M	5M	5M
Mortality	0	2M	4M	5M
Total (%)	0%	40%	80%	100%

The deaths occurred 4-9 days after dosing, with the first case observed on day 4 after administration in the 145 mg/kg group.

No deaths occurred in the animals of either sex in the lowest dose group (45 mg/kg).

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The LD_{50} was calculated to be 67.7 mg/kg with 95% confidence limits of 58.5 – 78.3 mg/kg.

Piloerection and hunched posture were observed in the animals of the various dose groups, starting 3-4 days (81 and 145 mg/kg groups) or 3-12 days (lower doses) after dosing. These changes were accompanied by hypoactivity in rats of the higher dose groups (81 and 145 mg/kg). Diarrhea was observed in two females of the 45mg/kg group 12-14 days after treatment. Complete or partial recovery was achieved at the end of the observation period in the surviving animals. Moreover, decrease in body weight or retarded growth was found in animals given the various doses during the observation period.

At the necropsy of animals which died before the end of the observation period, the main macroscopic findings were marked or moderate liver paleness and erosion and/or thinning walls of stomach. These changes were mainly confined to animals of the higher dose groups (81 and 145 mg/kg). At the autopsy carried out at the end of the observation period, no appreciable macroscopic findings were evident in any rat.

In conclusion, the **LD_{50}** of the test article [redacted], when administered to rats by oral route, was **67.7 mg/kg (95% confidence limits: 58.5-78.3 mg/kg)**. The compound induced delayed toxicity (liver and stomach were mainly involved) in animals given the higher doses.

Dr. Ping Yu
Study Director
October 16, 1998

Dr. Sergio Peano
Senior Scientist for General Toxicology
Oct. 16, 1998

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RBM Exp. No. 980431

GROUP DATA

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Test article: [redacted]

Title : Acute oral toxicity study in rats

RBM exp. : 980431

TABLE 1. - Mortality and LD50 calculation (p. 1)

Males - Females

Dose (mg/kg)		45	63	81	145
Treated animals		10	5	5	5
Day	4	0	0	0	1
	5	0	0	0	3
	6	0	0	0	1
	7	0	0	1	0
	8	0	2	2	0
	9	0	0	1	0
Total no.	(day 21)	0	2	4	5
Total (%)		0.0%	40.0%	80.0%	100.0%

Median lethal dose (LD50) = 67.72

95% confidence limits = 58.54 - 78.34

Slope (SE) = 5.15 1.58

Heterogeneity P = 0.963 NS

Linear regression y =-16.7295+5.1548x

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

TABLE 2. - Clinical signs (maximum daily frequency) (p. 1)
(no. of animals affected, from-to)

Males

Dose (mg/kg)	45	63	81	145
no. of treated animals	5	5	5	5
Death	-	2 8d	4 7d- 9d	5 4d- 6d
Hypoactivity	-	-	4 7d- 9d	4 4d- 5d
Piloerection	2 7d-12d	5 5d-18d	4 3d-16d	4 4d- 5d
Hunched posture	2 7d-12d	3 12d-16d	1 4d- 4d	4 4d- 5d
Recovery	5 13d	3 19d	1 17d	-

- (not observed) from-to (first-last observation in one or more animals)
Time : d (days)

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

TABLE 2. - Clinical signs (maximum daily frequency) (p. 2)
(no. of animals affected, from-to)

Females

Dose (mg/kg)	45
no. of treated animals	5
Piloerection	4 3d-16d
Hunched posture	2 12d-14d
Diarrhea	2 12d-14d
Recovery	5 17d

from-to (first-last observation in one or more animals)
Time : d (days)

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

TABLE 3. - Gross pathology examination (p. 1)
(no. of cases, mean severity, %)

Dead or agonal sacrificed an. Males

Dose (mg/kg)	45	63	81	145
no. of animals	0	2	4	5
no. of animals without appreciable lesions	0	1	0	0
General observation				
cannibalized	-	0	0	1 20.00%
Kidneys				
medulla, congestion	-	0	0	2(2.0) 40.00%
Liver				
pale	-	1(2.0) 50.00%	3(2.0) 75.00%	4(2.0) 80.00%
Lungs				
congestion	-	0	0	1(2.0) 20.00%

- (not examined)
Severity : 0(very slight) 1(slight) 2(moderate) 3(severe)

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

TABLE 3. - Gross pathology examination (p. 2)
(no. of cases, mean severity, %)

Dead or agonal sacrificed an. Males

Dose (mg/kg)	45	63	81	145
no. of animals	0	2	4	5
no. of animals without appreciable lesions	0	1	0	0
Stomach				
erosion	-	0	0	2(2.0) 40.00%
thinning walls	-	0	1(2.0) 25.00%	2(2.0) 40.00%
Thymus				
congestion	-	0	0	1(2.0) 20.00%

- (not examined)
Severity : 0(very slight) 1(slight) 2(moderate) 3(severe)

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

TABLE 3. - Gross pathology examination (p. 3)
(no. of cases, mean severity, %)

Final killing — Males

Dose (mg/kg)	45	63	81	145
no. of animals	5	3	1	0
no. of animals without appreciable lesions	5	3	1	0

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

TABLE 3. - Gross pathology examination (p. 4)
(no. of cases, mean severity, %)

Final killing	Females
Dose (mg/kg)	45
no. of animals	5
no. of animals without appreciable lesions	5

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APPENDICES

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

APPENDIX 1. - Clinical signs incidence (p. 1)
(no. of animals affected)

Dose (mg/kg) 45

Cage # 7M	Day	1				2	3	4	5	6	7	8	9	10	11	12	13	14
	Time	30m	2h	4h	6h	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
No clinical signs		5	5	5	5	5 5	5 5	5 5	5 5	5 5	3 3	3 3	3 3	3 3	3 3	3 3	5 5	5 5
Piloerection											2 2	2 2	2 2	2 2	2 2	2 2		
Hunched posture											1 1	2 2	2 2	2 2	2 2	2 2		

Cage # 8F	Day	1				2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	Time	30m	2h	4h	6h	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
No clinical signs		5	5	5	5	5 5	4 4	4 4	4 4	4 4	4 4	1 1	1 1	3 3	3 3	2 2	2 2	2 2	2 2	3 3	5 5
Piloerection							1 1	1 1	1 1	1 1	1 1	4 4	4 4	2 2	2 2	3 3	3 3	3 3	3 3	2 2	
Hunched posture																2 2	2 2	1 1			
Diarrhea																2 2	2 2	1 1			

Cage # 8F (follows)	Day	18	19	20	21
	Time	M A	M A	M A	M A
No clinical signs		5 5	5 5	5 5	5 5

Time: m (minutes) h (hours) M (morning) A (afternoon)

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

APPENDIX 1. - Clinical signs incidence (p. 2)
(no. of animals affected)

Dose (mg/kg) 63

Cage # 11M	Day	1				2		3		4		5		6		7		8		9		10		11		12		13		14		15		16		17	
	Time	30m		2h	4h	6h	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A
Death																			2																			
No clinical signs		5		5	5	5	5	5	5	5	5	5								1	1	1	1	1	1												
Piloerection													5	5	5	5	5	5	3	3	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
Hunched posture																											3	3	3	3	3	3	3	3				

Cage # 11M (follows)	Day	18		19		20		21	
	Time	M	A	M	A	M	A	M	A
No clinical signs				3	3	3	3	3	3
Piloerection		3	3						

Time: m (minutes) h (hours) M (morning) A (afternoon)

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

APPENDIX 1. - Clinical signs incidence (p. 3)
(no. of animals affected)

Dose (mg/kg) 81

Cage # 9M	Day	1				2		3		4		5		6		7		8		9		10		11		12		13		14		15		16		17	
	Time	30m	2h	4h	6h	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A
Death																1		2		1																
No clinical signs		5	5	5	5	5	5	4	4	4	4	5	5	5	5																			1	1
Hypoactivity																4	4	2	2	1	1														
Piloerection								1	1	1	1					4	4	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
Hunched posture										1	1																								

Cage # 9M (follows)	Day	18		19		20		21	
	Time	M	A	M	A	M	A	M	A
No clinical signs		1	1	1	1	1	1	1	1

Time: m (minutes) h (hours) M (morning) A (afternoon)

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

APPENDIX 1. - Clinical signs incidence (p. 4)
(no. of animals affected)

Dose (mg/kg) 145

Cage # 5M	Day	1				2		3		4		5		6
	Time	30m	2h	4h	6h	M	A	M	A	M	A	M	A	M
Death										1		3		1
No clinical signs		5	5	5	5	5	5	5	5					
Hypoactivity										4	4	1	1	
Piloerection										4	4	1	1	
Hunched posture										4	4	1	1	

Time: m (minutes) h (hours) M (morning) A (afternoon)

REDACTED AS TO TRADE NAMES

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

APPENDIX 2. - Body weight (g) (p. 1)
(individual)

Dose (mg/kg) 45

Animal #		31M	32M	33M	34M	35M	36F	37F	38F	39F	40F
Week	day										
	0	297	242	269	285	281	230	219	222	223	207
1	1	271	222	245	265	257	217	207	208	210	194
1	3	288	218	269	277	278	224	221	190	221	196
2	8	290	211	278	278	279	187	208	161	230	167
2	14	318	223	311	290	299	179	210	160	240	160
3	21						200	233	197	269	190

REDACTED AS TO TRADE NAMES

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

APPENDIX 2. - Body weight (g) (p. 2)
(individual)

Dose (mg/kg) 63

Animal #		51M	52M	53M	54M	55M
Week	day					
	0	300	268	257	278	287
1	1	284	254	232	248	267
1	3	306	276	258	285	289
2	8	238	210			250
2	14	191	172			193
3	21	256	249			276

REDACTED AS TO TRADE NAMES

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

APPENDIX 2. - Body weight (g) (p. 3)
(individual)

Dose (mg/kg) 81

Animal #		41M	42M	43M	44M	45M
Week	day					
	0	299	270	300	302	267
1	1	280	250	278	279	244
1	3	271	258	285	283	247
2	8	220		224		
2	14	219				
3	21	254				

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

APPENDIX 2. - Body weight (g) (p. 4)
(individual)

Dose (mg/kg) 145

Animal #		21M	22M	23M	24M	25M
Week	day					
	0	273	260	247	277	280
1	1	256	245	227	255	260
1	3	251	241	222	260	263

REDACTED AS TO TRADE NAMES

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

APPENDIX 3. - Gross pathology examination (p. 1)
(individual)

Dead or agonal sacrificed an.

Dose (mg/kg) 63

An#	Death day/code#	TISSUE	Gross observations
53M	8 M2	General observation	no macroscopically appreciable lesions
54M	8 M2	Liver	pale, diffuse, moderate

Death code : M2(Natural death)

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

APPENDIX 3. - Gross pathology examination (p. 2)
(individual)

Dead or agonal sacrificed an.

Dose (mg/kg) 81

An#	Death day/code#	TISSUE	Gross observations
42M	8 M2	Liver	pale, diffuse, moderate
43M	9 M2	Liver	pale, diffuse, moderate
44M	8 M2	Liver	pale, diffuse, moderate
		Stomach	thinning walls, diffuse, moderate

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Death code : M2(Natural death)

REDACTED AS TO TRADE NAMES

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

APPENDIX 3. - Gross pathology examination (p. 3)
(individual)

Dead or agonal sacrificed an.

Dose (mg/kg) 145

An#	Death day	code#	TISSUE	Gross observations
21M	5	M2	Kidneys	medulla, congestion, diffuse, moderate
			Liver	pale, diffuse, moderate
			Stomach	erosion, multifocal, moderate thinning walls, diffuse, moderate
22M	6	M2	Kidneys	medulla, congestion, diffuse, moderate
			Liver	pale, diffuse, moderate
			Thymus	congestion, diffuse, moderate
23M	5	M2	General observation	cannibalized
24M	5	M2	Liver	pale, diffuse, moderate
			Stomach	erosion, multifocal, moderate thinning walls, diffuse, moderate
25M	4	M2	Liver	pale, diffuse, moderate
			Lungs	congestion, diffuse, moderate

Death code : M2(Natural death)

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

APPENDIX 3. - Gross pathology examination (p. 4)
(individual)

Final killing

Dose (mg/kg) 45

An#	Death day	T I S S U E	Gross observations
31M	15	General observation	no macroscopically appreciable lesions
32M	15	General observation	no macroscopically appreciable lesions
33M	15	General observation	no macroscopically appreciable lesions
34M	15	General observation	no macroscopically appreciable lesions
35M	15	General observation	no macroscopically appreciable lesions
36F	22	General observation	no macroscopically appreciable lesions
37F	22	General observation	no macroscopically appreciable lesions
38F	22	General observation	no macroscopically appreciable lesions
39F	22	General observation	no macroscopically appreciable lesions
40F	22	General observation	no macroscopically appreciable lesions

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

APPENDIX 3. - Gross pathology examination (p. 5)
(individual)

Final killing

Dose (mg/kg) 63

An#	Death day	T I S S U E	Gross observations
51M	22	General observation	no macroscopically appreciable lesions
52M	22	General observation	no macroscopically appreciable lesions
55M	22	General observation	no macroscopically appreciable lesions

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980431

APPENDIX 3. - Gross pathology examination (p. 6)
(individual)

Final killing

Dose (mg/kg) 81

An#	Death day	T I S S U E	Gross observations
41M	22	General observation	no macroscopically appreciable lesions

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[redacted]

$ce(C_3F_6O)_{2,5}-CF_2COO^-NH_4$

"ACUTE ORAL TOXICITY STUDY IN RATS"

RBM EXP. No. 970592

Issued on March 25, 1998

SPONSOR

AUSIMONT
Viale S.Pietro, 50/A
20021 BOLLATE (Milano)
Italy

PERFORMING LABORATORY

Istituto di Ricerche Biomediche
"Antoine Marxer" RBM S.p.A.
Via Ribes, 1
10010 - COLLERETTO GIACOSA (Torino)
Italy

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RBM Exp. No. 970592

TITLE OF THE STUDY

"Acute oral toxicity study in rats treated with the test article [redacted]"

PURPOSE OF THE STUDY

The purpose of the study was to evaluate the oral acute toxicity of the test article [redacted].

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LABORATORIES & CLINICS GROUP BIOSCIENCE

RBM Exp. No. 970592

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This report consists of 36 pages.

Ivrea, March 25, 1998

Dr. Ping Yu
RBM Study Director

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LABORATORIES & CLINICS GROUP
BIOSCIENCE

RBM Exp. No. 970592

FOREWORD

On behalf of **AUSIMONT Viale S.pietro, 50/A. 20021-BOLLATE-Milano-Italy** - Ricerche Biomediche "Antoine Marxer" RBM S.p.A., authorized by the Italian Health Authorities (1-2) to conduct safety studies, has performed an acute toxicity study by oral route in Sprague Dawley Crl: CD(SD) BR rat (RBM- Experiment No. 970592), with the test article:

[redacted]

A sample of the substance used, along with pertinent documentation, is held in sufficient quantity in the RBM archives and is at the disposal of the Ministero della Sanità.

The undersigned declare that the experiment was conducted using the same batch of substance as that of the sample held on file.

For verification by the Ministero della Sanità, the undersigned moreover guarantee the identification and classification of all those materials, documents and recordings used in conducting the experiment, held on file for a period of at least 10 years from the date of this report. Following this time, they will be placed at the disposal of the Sponsor.

Dr. Roberto Maraschin

Scientific Director Recognized by the Italian Health Authorities as Responsible for General Toxicology Experimentation

Dr. Angelo Conz

General Manager of the Istituto di Ricerche Biomediche "Antoine Marxer" , RBM S.p.A.

Ivrea, March 25, 1998

(1): **Pharmaceuticals:**
Authorization dated March 12, 1976 in accordance with "Circolare 73", May 16, 1974

(2): **Chemicals:**
Authorization in accordance with DPR 927/81 (D.M. dated January 7, 1988 published in G.U. No. 12, dated January 16, 1988).

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RBM Exp. No. 970592

QUALITY ASSURANCE STATEMENT

RBM Experiment number: 970592

Study title:

"Acute oral toxicity study in rats treated with the test article [redacted]".

Studies of the type described in this report are conducted in a manner which involves frequent repetition of identical or similar procedures.

In compliance with the Principles of Good Laboratory Practice, at the time of this study, procedure-based inspections were made by the Q.A.U. of critical phases and procedures relevant to this type of study. For the inspection of any given procedure, studies were selected at random. All such inspections were reported promptly to the study director and to facility management.

This study was inspected on:

Dates of inspection/audit	Dates of report to Study Director and Management
January 12, 1998	January 13, 1998
March 24, 1998	March 24, 1998

This report has been audited by the Q.A.U. and was found to be an accurate description of such methods and procedures as were used during the conduct of the study and an accurate reflection of the raw data.

Date of final report audit: March 27, 1998

Enrico Invernizzi
Head of Quality Assurance Unit

Date : March 27, 1998

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BIOSCIENCE

RBM Exp. No. 970592

RBM MANAGEMENT DECLARATION OF GLP COMPLIANCE

Study No. 970592 entitled :

"Acute oral toxicity study in rats treated with the test article [redacted]"

was performed in compliance with the OECD-GLP in the testing of chemicals, [C(81) 30 (final)], regulations also enforced by the Italian Health Authority [D.M. dated June 26, 1986 as published in G.U. No. 198, dated August 27, 1986 and D.L. January 27, 1992, No. 120 as published in G.U. (Supplement) No. 40, February 18, 1992].

Dr. Ping Yu
RBM Study Director

Dr. Angelo Conz
General Manager of the Istituto di Ricerche Biomediche "Antoine Marxer", RBM S.p.A.

Ivrea, March 27, 1998

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SCIENTISTS INVOLVED IN THE STUDY

STUDY No. 970592

"Acute oral toxicity study in rats treated with the test article [redacted]"

RBM Study Director	Dr. Ping Yu
Scientific Director Toxicology	Dr. Roberto Maraschin
Head of General Toxicology I Unit	Dr. Germano Oberto
Centralized Pharmacy Head	Dr. Rita Bussi
Pharmacy Service Head	Dr. Bruna Piccioli

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BIOSCIENCE

RBM Exp. No. 970592

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

RBM Experiment No.: 970592

Test article: [redacted]

Administration route: oral (by gavage)

Duration of treatment period: single administration

Duration of post-treatment observation period: 14 days

The test method was in accordance with European Economic Community Guidelines - Annex to Commission Directive 92/69/EEC of July 31, 1992 adapting to technical progress for the seventeenth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (B.1) and with Organization for Economic Cooperation and Development Guidelines (section 4, subpart 401, Paris 1981 and subsequent revisions).

TEST SYSTEM

Species, strain and substrain: Sprague Dawley Crl: CD (SD) BR rat

Justification for selection of the test system : the Sprague Dawley rat was chosen as rodent species since it is an appropriate experimental model widely accepted by Health Authorities, with documented susceptibility to a wide range of toxic substances

Number and sex of animals: 5 males /dose at the doses of 53, 82 and 128 mg/kg
5 females at the dose of 53 mg/kg

Supplier:	Charles River Italia S.p.A. Via Indipendenza, 11 22050 CALCO (Lecco) Shipping slips No.s 0014 (January 2, 1998), 597 (January 23, 1998) and 793 (January 30, 1998).
Body weight (at randomization)	Males: 246 - 334 g Females: 199 - 214 g The weight variation of the animals used for the study did not exceed ± 20% of the mean body weight for each sex.
Age (at randomization)	males and females <3 months
Acclimatization:	at least 5 days before the start of the test. Animals were observed daily to ascertain their fitness for the study.
Housing:	5 animals/sex/cage in air-conditioned room. - Temperature: 22°C ± 2 - Relative humidity: 55% ± 10 - Air changes: about 20 / hour filtered on HEPA 99.97% - Light: 12 hour cycle (7 a.m. - 7 p.m.) - Cage size: grill cages 40.5x38.5x18h cm with stainless steel feeder. The waste that dropped through the grill bottom onto removable paper was periodically disposed of.
Animal identification:	by appropriately coloring different areas of the limbs. Cage card gave experiment number, dosage group, sex and date of administration.
Diet:	GLP 4RF21 top certificate pelleted diet produced by Charles River Italia's feed licencee Mucedola S.r.l., Settimo Milanese. The declared contents, on the label, on dry matter basis (moisture 12%), were: crude protein 18.50% crude fat 3.00% crude fiber 6.00% crude ash 7.00%

The diet was supplemented by the Producer with vitamins and trace elements. The Producer supplies a certificate of analysis for nutrients and contaminants, the levels of which are within the limits proposed by EPA-TSCA (44FR:44053-44093, July 26, 1979).
RBM has the animal feed re-analyzed at least twice a year for bacterial contamination.
The diet was available "ad libitum" to the animals.

Water: from the municipal water main system.
Water is filtered and distributed "ad libitum" to the animals by an automatic valve system.
Periodically drinking water is analyzed for microbial count, heavy metals, other contaminants (e.g. solvents, pesticides) and other chemical and physicals characteristics. The accepted limits of quality of the drinking water were those defined in EEC directive 80/778

Contaminants that might interfere with the objectives of the study were not expected to be present in diet or drinking water.

TEST ARTICLE CHARACTERIZATION

Identification:	[redacted]
Batch:	19387/20
Characteristics:	white solid
Manufacturing date:	December, 1997
Expiry date:	December, 2000
Storage conditions:	at room temperature

VEHICLE CHARACTERIZATION

Deionized water

TEST ARTICLE FORMULATE PREPARATION

When necessary, an exact amount of test article was weighed in a suitable graduated container and was made up to final volume with vehicle to obtain the concentration required.

Formulates were given to rats within two hours of the preparation.

TEST DESCRIPTION

Administration route: oral (by gavage)

Reason for selection of administration route: possible ingestion by humans

Experimental design:

Dose* mg/kg	Treated animals	Treatment date	Final killing
128	5 males	February 3, 1998	Found dead
82	5 males	February 17, 1998	March 3, 1998
53	5 males	February 27, 1998	March 13, 1998
53	5 females	March 4, 1998	March 18, 1998

* The dose levels were defined on the basis of a preliminary study.

Administration method: The volumes to be administered were 10 ml/kg on the basis of body weight taken just before treatment. The administration was done by gavage to rats which had been fasted about 16 hours. Feed was returned to the rats about three hours after the test article administration.

Observation period: 14 days after administration

Observation of clinical signs and mortality: at 15 and 30 minutes, 2, 4 and 6 hours on the first day after the administration (day 1) and then twice a day up to termination of the observation period

Body weight: twice pre-trial (at randomization and on day 1 just before administration) and on days 3, 8 and 14. On day 1 the animals were weighed after a 16-hour fasting period.

Gross pathology:	on all animals which died during the observation period and on animals killed (fasted overnight) by excision of the femoral arteries, after i.p. overdosage anesthesia with 5% sodium pentobarbital, at the end of the observation period
Histology:	portions of any abnormal entities found in any of the necropsied animals were collected. The tissue samples were fixed and preserved in 10% buffered formalin. Histologic examination was not performed.
LD_{50} and its statistical limits:	LD_{50} was calculated by the method of the Probit (Bliss - Finney) - A.P. Rosiello et al., J. Tox. and Env. Health, 3: 797-809, 1977.

RECORD FILING

The protocol, a reserve sample of the batch of the test article used, the raw data bound in a register numbered 970592/1, the specimens, the final report and all other documents pertinent to the conduct of this study, including records and reports of maintenance, cleaning, calibration and inspection of equipment, analysis of diet and water are filed at RBM premises for ten years from the issue date of this report and then sent to the Sponsor.

PROCEDURAL DETAILS

The study was conducted in accordance with the procedures described in the RBM Standard Operating Procedures (SOP's) collection.

Protection of animals used in the experiment is in accordance with Directive 86/609/EEC, enforced by the Italian D. L. No. 116 of January 27, 1992.

Physical facilities and equipment for accommodation and care of animals are in accordance with the provisions of EEC Council Directive 86/609.

The Institute is fully authorized by Competent Veterinary Health Authorities.

RESULTS

CLINICAL OBSERVATIONS

MORTALITY *(TABLE 1)*

The mortality which occurred at the various doses is given below:

Dose (mg/kg)	53	82	128
Treated animals	5M+5F	5M	5M
Mortality	0	3M	5M
Total (%)	0%	60%	100%

The deaths occurred within 9 days of dosing, with the first case observed on day 6 after administration in the 128 mg/kg group.

The LD_{50} was calculated to be 82.8 mg/kg with 95% confidence limits of 68.9 - 99.5 mg/kg.

CLINICAL SIGNS *(TABLE 2 AND APPENDIX 1)*

At the higher doses tested (82 and 128 mg/kg) the compound induced delayed clinical changes including: sedation or hypoactivity, piloerection and hunched posture. These changes were detected starting days 6-8 after dosing.

Recovery was achieved at the end of the observation period in the surviving animals.

No changes of note were seen in animals of the lowest dose group (53 mg/kg).

BODY WEIGHT *(APPENDIX 2)*

Decrease in body weight or retarted growth was found in animals given the two higher doses (82 and 128 mg/kg) mainly during the first week of the observation period.

No effects on the body weight growth was observed in animals of the 53 mg/kg group.

POST-MORTEM EXAMINATION

GROSS PATHOLOGY *(TABLE 3 AND APPENDIX 3)*

At the necropsy of animals which died before the end of the observation period, the main macroscopic finding was marked or moderate liver paleness in all animals. Moreover, stomach congestion, kidney medulla congestion and decreased size of spleen were seen in some animals.

No appreciable findings were detected at the gross examination in animals which were sacrificed at the end of the observation period.

SUMMARY AND CONCLUSIONS

Experimental data from a toxicity study in which Sprague Dawley Crl:CD(SD) BR rats were treated by oral route with the test article [redacted] are given in this report.

The test method was in accordance with European Economic Community Guidelines - Annex to Commission Directive 92/69/EEC of July 31, 1992 adapting to technical progress for the seventeenth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (B.1) and with Organization for Economic Cooperation and Development Guideline (section 4, subpart 401, Paris 1981 and subsequent revisions).

The test article was administered as a solution in deionized water at the doses of 53, 82 and 128 mg/kg to groups of 5 males/dose and at the dose of 53 mg/kg also to 5 females for confirmation in the other sex . The volume of administration was 10 ml/kg.

All rats were treated after a 16-hour fasting period. The day of treatment was considered day 1 of the study. The animals were weighed twice before treatment (at randomization and on day 1 just before treatment) and on days 3, 8 and 14. They were clinically observed for 14 days following the treatment. Macroscopic examination was performed on all animals which died before the end of the study. On day 15 the surviving rats were killed (fasted overnight) by excision of the femoral arteries after i.p. overdosage anesthesia with 5% sodium pentobarbital and were subjected to a thorough autopsy.

The mortality which occurred at the various doses is given below:

Dose (mg/kg)	53	82	128
Treated animals	5M+5F	5M	5M
Mortality Total (%)	0 0%	3M 60%	5M 100%

Deaths occurred within 9 days of dosing, with the first case observed on day 6 after administration in the 128 mg/kg group.

The LD_{50} was calculated to be 82.8 mg/kg with 95% confidence limits of 68.9 - 99.5 mg/kg.

At the higher doses tested (82 and 128 mg/kg) the compound induced delayed clinical changes including sedation or hypoactivity, piloerection and hunched posture. These changes were detected starting days 6-8 after dosing. Recovery was achieved by the end of the observation period in the surviving animals.

No changes of note were seen in animals of the lowest dose group (53 mg/kg).

Depression in body weight growth was found in animals given the two higher doses (82 and 128 mg/kg) mainly during the first week of the observation period.

No effects on the body weight growth was observed in animals of the 53 mg/kg group.

At the necropsy of animals which died before the end of the observation period, the main macroscopic finding was marked or moderate liver paleness.

No appreciable findings were found in animals at the final killing.

In conclusion, the **LD_{50}** of the test article [redacted], when administered to rats as a single dose by oral route, was **82.8 mg/kg (95% confidence limits: 68.9-99.5 mg/kg)**. The compound induced delayed toxicity (liver was mainly involved) in animals given the higher doses.

Dr. Ping Yu

RBM Study Director

March 25, 1998

Dr. Roberto Maraschin

Scientific Director Recognized by the Italian Health Authorities as Responsible for General Toxicology Experimentation

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GROUP DATA

Test article: [redacted]
Title : Acuate oral toxicity study in rats
RBM exp. : 970592

TABLE 1. - Mortality and LD50 calculation (p. 1)

Males - Females

Dose (mg/kg)		53	82	128
Treated animals		10	5	5
Day	6	0	0	1
	7	0	0	2
	8	0	0	1
	9	0	3	1
Total no.	(day 14)	0	3	5
Total (%)		.0%	60.0%	100.0%

Median lethal dose (LD50) = 82.79

95% confidence limits = 68.92 - 99.46

Slope (SE) = 3.19 .78

Heterogeneity P = .557 NS

Linear regression y =-9.1040+3.1936x

REDACTED AS TO TRADE NAMES

Test article: [redacted]
Title : Acuate oral toxicity study in rats
RBM exp. : 970592

TABLE 2. - Clinical signs (maximum daily frequency) (p. 1)
(no. of animals affected, from-to)

	Males		
Dose (mg/kg)	53	82	128
no. of treated animals	5	5	5
Death	-	3 9d	5 6d- 9d
Sedation	-	1 8d- 8d	2 6d- 6d
Hypoactivity	-	-	2 7d- 8d
Piloerection	-	3 8d-12d	4 6d- 8d
Hunched posture	-	3 8d-12d	3 6d- 8d
Recovery	-	2 13d	-

- (not observed) from-to (first-last observation in one or more animals)
Time : d (days)

Test article: [redacted]
Title : Acuate oral toxicity study in rats
RBM exp. : 970592

TABLE 2. - Clinical signs (maximum daily frequency) (p. 2)
(no. of animals affected, from-to)

	Females
Dose (mg/kg)	53
no. of treated animals	5
No clinical signs	5 30m-14d

from-to (first-last observation in one or more animals)
Time : m (minutes) d (days)

Test article: [redacted]
Title : Acuate oral toxicity study in rats
RBM exp. : 970592

TABLE 3. - Gross pathology examination (p. 1)
(no. of cases, mean severity, %)

Dead or agonal sacrificed an. Males

Dose (mg/kg)	53	82	128
no. of animals	0	3	5
no. of animals without appreciable lesions	0	0	0
Kidneys			
medulla, congestion	-	3(2.0) 100.00%	3(2.0) 60.00%
Liver			
pale	-	3(2.0) 100.00%	5(2.8) 100.00%
Spleen			
decreased size	-	2(2.0) 66.67%	3(2.0) 60.00%
Stomach			
congestion	-	3(2.0) 100.00%	2(2.0) 40.00%

- (not examined)
Severity : 0(very slight) 1(slight) 2(moderate) 3(severe)

12 7

REDACTED AS TO TRADE NAMES

BIOSCIENCE LABORATORIES & CLINICS GROUP

Test article: [redacted]
Title : Acuate oral toxicity study in rats
RBM exp. : 970592

TABLE 3. - Gross pathology examination (p. 2)
(no. of cases, mean severity, %)

Final killing	Males		
Dose (mg/kg)	53	82	128
no. of animals	5	2	0
no. of animals without appreciable lesions	5	2	0

Test article: [redacted]
Title : Acuate oral toxicity study in rats
RBM exp. : 970592

TABLE 3. - Gross pathology examination (p. 3)
(no. of cases, mean severity, %)

Final killing	Females
Dose (mg/kg)	53
no. of animals	5
no. of animals without appreciable lesions	5

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APPENDICES

Test article: [redacted]
Title : Acuate oral toxicity study in rats
RBM exp. : 970592

APPENDIX 1. - Clinical signs incidence (p. 1)
(no. of animals affected)

Dose (mg/kg) 53

Cage # 9M	Day	1				2		3		4		5		6		7		8		9		10		11		12		13		14	
	Time	30m	2h	4h	6h	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A
No clinical signs		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Cage # 10F	Day	1				2		3		4		5		6		7		8		9		10		11		12		13		14	
	Time	30m	2h	4h	6h	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A
No clinical signs		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Time: m (minutes) h (hours) M (morning) A (afternoon)

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REDACTED AS TO TRADE NAMES

BOSCIENCE LABORATORIES & CLINICS GROUP

Test article: [redacted]
Title : Acuate oral toxicity study in rats
RBM exp. : 970592

APPENDIX 1. - Clinical signs incidence (p. 2)
(no. of animals affected)

Dose (mg/kg) 82

Cage # 7M	Day	1				2		3		4		5		6		7		8		9		10		11		12		13		14	
	Time	30m	2h	4h	6h	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A
Death																				3											
No clinical signs		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	1	1									2	2	2	2
Sedation																		1	1												
Piloerection																		3	3	2	2	2	2	2	2	2	2				
Hunched posture																		3	3	2	2	2	2	2	2	2	2				

Time: m (minutes) h (hours) M (morning) A (afternoon)

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Test article: [redacted]
Title : Acuate oral toxicity study in rats
RBM exp. : 970592

APPENDIX 1. - Clinical signs incidence (p. 3)
(no. of animals affected)

Dose (mg/kg) 128

Cage # 5M	Day	1				2		3		4		5		6		7		8		9
	Time	30m	2h	4h	6h	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M
Death														1		2		1		1
No clinical signs		5	5	5	5	5	5	5	5	5	5	5	5							
Sedation														2	2					
Hypoactivity																2	2	1	1	
Piloerection														4	4	2	2	1	1	
Hunched posture														3	3	2	2	1	1	

Time: m (minutes) h (hours) M (morning) A (afternoon)

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BIOSCIENCE LABORATORIES & CLINICS GROUP

Test article: [redacted]
Title : Acuate oral toxicity study in rats
RBM exp. : 970592

APPENDIX 2. - Body weight (g) (p. 1)
(individual)

Dose (mg/kg) 53

Animal #		41M	42M	43M	44M	45M	46F	47F	48F	49F	50F
Week	day										
	0	246	247	246	246	248	200	214	204	199	199
1	1	223	225	222	220	220	189	194	189	187	186
1	3	240	243	240	237	236	203	229	205	202	203
2	8	268	266	250	268	245	217	233	211	214	211
2	14	359	347	333	350	309	236	258	233	238	234

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REDACTED AS TO TRADE NAMES

Test article: [redacted]
Title : Acuate oral toxicity study in rats
RBM exp. : 970592

APPENDIX 2. - Body weight (g) (p. 2)
(individual)

Dose (mg/kg) 82

Animal #		31M	32M	33M	34M	35M
Week	day					
	0	334	334	300	334	334
1	1	318	309	271	324	322
1	3	310	310	261	346	312
2	8	232	255	208	280	266
2	14		343			369

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Test article: [redacted]
Title : Acuate oral toxicity study in rats
RBM exp. : 970592

APPENDIX 2. - Body weight (g) (p. 3)
(individual)

Dose (mg/kg) 128

Animal #		21M	22M	23M	24M	25M
Week	day					
	0	289	270	250	281	299
1	1	265	248	229	258	267
1	3	250	245	222	256	265
2	8		169			

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Test article: [redacted]
Title : Acuate oral toxicity study in rats
RBM exp. : 970592

APPENDIX 3. - Gross pathology examination (p. 1)
(individual)

Dead or agonal sacrificed an.

Dose (mg/kg) 82

An#	Death day/code#	TISSUE	Gross observations
31M	9 M2	Kidneys	medulla, congestion, diffuse, moderate
		Liver	pale, diffuse, moderate
		Spleen	decreased size, diffuse, moderate
		Stomach	congestion, diffuse, moderate
33M	9 M2	Kidneys	medulla, congestion, diffuse, moderate
		Liver	pale, diffuse, moderate
		Stomach	congestion, diffuse, moderate
34M	9 M2	Kidneys	medulla, congestion, diffuse, moderate
		Liver	pale, diffuse, moderate
		Spleen	decreased size, diffuse, moderate
		Stomach	congestion, multifocal, moderate

Death code : M2(Natural death)

Test article: [redacted]
Title : Acuate oral toxicity study in rats
RBM exp. : 970592

APPENDIX 3. - Gross pathology examination (p. 2)
(individual)

Dead or agonal sacrificed an.

Dose (mg/kg) 128

An#	Death day/code#	TISSUE	Gross observations
21M	7 M2	Liver	pale, diffuse, severe
		Spleen	decreased size, diffuse, moderate
22M	9 M2	Kidneys	medulla, congestion, diffuse, moderate
		Liver	pale, diffuse, moderate
		Stomach	congestion, diffuse, moderate
23M	8 M2	Liver	pale, diffuse, severe
		Spleen	decreased size, diffuse, moderate
		Stomach	congestion, diffuse, moderate
24M	6 M2	Kidneys	medulla, congestion, diffuse, moderate
		Liver	pale, diffuse, severe
		Spleen	decreased size, diffuse, moderate
25M	7 M2	Kidneys	medulla, congestion, diffuse, moderate
		Liver	pale, diffuse, severe

Death code : M2(Natural death)

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Test article: [redacted]
Title : Acuate oral toxicity study in rats
RBM exp. : 970592

APPENDIX 3. - Gross pathology examination (p. 3)
(individual)

Final killing

Dose (mg/kg) 53

An#	Death day	T I S S U E	Gross observations
41M	15	General observation	no macroscopically appreciable lesions
42M	15	General observation	no macroscopically appreciable lesions
43M	15	General observation	no macroscopically appreciable lesions
44M	15	General observation	no macroscopically appreciable lesions
45M	15	General observation	no macroscopically appreciable lesions
46F	15	General observation	no macroscopically appreciable lesions
47F	15	General observation	no macroscopically appreciable lesions
48F	15	General observation	no macroscopically appreciable lesions
49F	15	General observation	no macroscopically appreciable lesions
50F	15	General observation	no macroscopically appreciable lesions

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Test article: [redacted]
Title : Acuate oral toxicity study in rats
RBM exp. : 970592

APPENDIX 3. - Gross pathology examination (p. 4)
(individual)

Final killing

Dose (mg/kg) 82

An#	Death day	T I S S U E	Gross observations
32M	15	General observation	no macroscopically appreciable lesions
35M	15	General observation	no macroscopically appreciable lesions

REDACTED AS TO TRADE NAMES

RESEARCH TOXICOLOGY CENTRE - ROMA

[redacted]

ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

FINAL REPORT

RTC Study Number: 9563-003

RTC Report Number: 9563-003/T/391/2002

Sponsor:
AUSIMONT S.p.A.
Via Lombardia, 20
20021 Bollate (MI)
Italy

Commercial Office

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Cod. Fisc.: 00653120584
Partita IVA 00920611001

RTC Report Number: 9563-003/T/391/2002

COMPLIANCE STATEMENT

We, the undersigned, hereby declare that the following report constitutes a true and faithful account of the procedures adopted, and the results obtained in the performance of this study. The aspects of the study conducted by Research Toxicology Centre S.p.A. were performed in accordance with:

A. Commission Directive 1999/11/EC of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances (adoption of the *"OECD principles on Good Laboratory Practice – as revised in 1997"*) and subsequent revisions.

B. Decreto Legislativo 27 Gennaio 1992, n. 120 published in the Gazzetta Ufficiale della Repubblica Italiana 18 Febbraio 1992 (adoption of the Commission Directive of 18 December 1989 adapting to technical progress the Annex to Council Directive 88/320/EEC on the inspection and verification of Good Laboratory Practice (90/18/EEC)) and subsequent revisions.

C. Longobardi, Biol.D.
(Study Director): Date : 18-03-03

J. Brightwell, Ph.D.
(Scientific Director): Date : 18.03.03

RTC Report Number: 9563-003/T/391/2002

QUALITY ASSURANCE STATEMENT

(Relevant to those aspects of the study conducted by Research Toxicology Centre S.p.A.)

Study phases monitored by RTC's QAU according to current relevant Standard Operating Procedures	Quality Assurance Inspections (Day Month Year)		
	Inspection	Report to Study Director	Report to Company Management
PROTOCOL CHECK	17.04.2002	17.04.2002	17.04.2002
PROCESS-BASED INSPECTIONS			
Allocation	22.05.2002	-	19.06.2002
Dose preparation	21.05.2002	-	23.05.2002
Body weight	06.06.2002	-	19.06.2002
Dosing (oral)	03.05.2002	-	23.05.2002
Clinical observations	11.06.2002	-	19.06.2002
Despatch to necropsy	19.06.2002	-	22.07.2002
Necropsy	31.05.2002	-	14.06.2002
Other routine inspections of a procedural nature were carried out on activities not directly related to this type of study. The relevant documentation is kept on file although specific inspection dates are not reported here.			
FINAL REPORT Review of this report by RTC's QAU found the reported methods and procedures to describe those used and the results to constitute an accurate representation of the recorded raw data.			Review completed 17 March 2003

M.M. Brunetti, Biol.D.
(Head of Quality Assurance)

17/03/03
Date

Contents

1. SUMMARY

The acute toxicity of [redacted] was investigated following administration of a single oral dose to the rat.

A single group of 3 male animals was then dosed at a level of 200 mg/kg and observed for a period of 14 days.
No mortality occurred and no clinical signs were noted.

Three female animals were then dosed at the same level (200 mg/kg) and observed for a period of 14 days. No mortality occurred and no clinical signs were noted.

A single group of 3 male animals was dosed at a level of 2000 mg/kg and observed for a period of 14 days.
No mortality occurred. Clinical signs were limited to reduced activity and piloerection. Recovery had occurred by day 5.

Three female animals were finally dosed at the same level (2000 mg/kg) and observed for a period of 14 days.
Two of the 3 animals had died by day 9. Clinical signs included piloerection, reduced activity, ataxia, semi-closed eyes and hunched posture.
No complete recovery occurred in the surviving animal.

Surviving animals were killed at the end of the observation period. All animals were subjected to necropsy examination.

Changes in body weight in animals dosed at 200 mg/kg were not remarkable.
Body weight losses or a reduced body weight gain were observed in animals dosed at 2000 mg/kg.

One surviving female dosed at 2000 mg/kg showed abnormal contents in the abdominal cavity, an abnormal colour of lungs, mesenteric lymph nodes, pancreas, spleen, liver, an abnormal size of thymus, an abnormal consistency of pancreas and an abnormal shape of the spleen. Cannibalisation by cage mates was also observed.
No abnormalities were found on necropsy of the other animals.

These results indicate that the test item, [redacted], has a toxic effect in the rat following oral administration of a single dose at a level of 2000 mg/kg. The mortality pattern observed demonstrates the LD50 to be less than 2000 mg/kg but greater than 200 mg/kg body weight.

European Directives concerning the classification, packaging and labelling of dangerous substances (67/548/EEC and subsequent revisions) would indicate the following:-

Classification : Required
Symbol : Xn
R phrase : R22 – Harmful if swallowed

2. INTRODUCTION

The purpose of this study was to assess the acute toxicity of the test item, [redacted], following oral administration of a single dose to the rat.

The procedures used were designed to meet the requirements of the test for acute oral toxicity described in OECD guideline Number 423, adopted on 22nd March 1996. Methods were in agreement with those of B.1 *tris* detailed in Directive 96/54/EEC. The rat was used, being a species indicated in the guidelines for this test. The route of administration is a potential route of exposure during manufacturing, handling or use of the substance.

The study was carried out at: Research Toxicology Centre S.p.A.
Via Tito Speri, 12
00040 Pomezia (Roma)
Italy

On behalf of: AUSIMONT S.p.A.
Via Lombardia, 20
20021 Bollate (MI)
Italy

The study started on 22nd March 2002 with signing of the protocol by the Study Director. The experimental work described in this report started on 9th April 2002 with allocation of the first 3 male animals to the study and ended on 9th July 2002 when termination of the study. The study was completed on the date shown against the Study Director signature at the front of this report.

3. TEST ITEM

Details of the test item received at RTC are as follows:

Name	:	[redacted]
Lot or Batch Number	:	90215/91
Cas Number	:	330809-92-2
Expiry date	:	February 2004
Purity	:	>90% referred to dry salt
Concentration of active ingredient	:	5% in water
pH	:	6.5
Received from	:	AUSIMONT S.p.A.
Date received	:	11th February 2002
Amount received	:	2000 grams
Description	:	Colourless liquid
Container	:	Opaque plastic tank
Storage at RTC	:	Ambient conditions
RTC reference number	:	6535

Detailed characterisation of the substance was not undertaken at the testing facility. The determination of the identity, strength, purity, composition, stability and method of synthesis and/or derivation of the substance was the responsibility of the Sponsor. A certificate of analysis, supplied by the Sponsor, can be found in Addendum 1 of this report. An aliquot of the supplied substance was taken and will be retained within the RTC archives for a period of 10 years prior to disposal.

The test item was formulated for dosing by dissolution/suspension in distilled water to give concentrations of 200 and 20 mg/ml.

During handling of the substance, precautions were taken to reduce possible operator exposure. These included, but were not limited to, use of a face mask, eye protection and the wearing of gloves.

4. METHODS

Any deviations from the protocol are detailed within the text of the report. No deviations occurred which were considered to have compromised the purpose or conduct of the study.

Dated and signed records were made of all activities relating to the day by day conduct and maintenance of the study.

4.1 Animal management

4.1.1 Animal supply

Healthy rats of the Hsd: Sprague Dawley SD strain were ordered and obtained from Harlan Italy S.r.l., 33049 San Pietro al Natisone (UD), Italy. Animals were ordered weighing 126 to 150 grams and aged approximately 5 to 6 weeks with female animals nulliparous and non-pregnant. Animals appeared to be in an acceptable condition following arrival in batches for the different phases of the study, on 29th March and 31st May 2002. A pre-dose acclimatisation period of at least 5 days was allowed.

4.1.2 Animal husbandry

Animals included in the study were housed, in groups of 3 animals of the same sex, in polycarbonate cages measuring 59x20x39 cm and equipped with a stainless steel mesh lid and floor. Cages were suspended over trays holding an absorbent material which was inspected daily and changed as necessary. Throughout the study each cage was identified by a colour coded label recording the study number, animal number and the details of treatment.

Animal room controls were set to maintain temperature within the range of 22°C ± 2°C and relative humidity within the range of 55% ± 15%. Actual conditions were recorded.

The room was lit by fluorescent tubes controlled to give an artificial cycle of 12 hours light and 12 hours dark each day.

4.1.3 Water and diet

Animals were offered drinking water supplied to each cage via a water bottle and a commercially available laboratory rodent diet (Altromin MT, Altromin, D-32770 Lage, Postfach 1120, Germany) *ad libitum* throughout the study except for an overnight fast prior to dosing and a period of approximately 4 hours after dosing.

There was no information to indicate that any component present in the drinking water or diet was at a level likely to interfere with the purpose or conduct of the study.

4.2 Experimental design

A single group of 3 males was dosed at a level of 200 mg/kg. Three females were subsequently dosed at the same dose level.

A single group of 3 males was then dosed at a level of 2000 mg/kg. This was a deviation from protocol in which was indicated to dose the animals initially at 2000 mg/kg. This deviation was due to the information about the toxicity of the test item supplied by the Sponsor, which indicated 2000 mg/kg to be toxic.

4.2.1 Selection and allocation

The required number of animals for the study was allocated to treatment groups. Individuals were permanently identified on arrival by a combination of ear notch (units) and tattoo on the feet. Males were identified by even numbers and females by odd numbers.

Groups of 3 males and 3 females were allocated to the study as follows:-

Dose level (mg/kg)	Animal number	
	Males	Females
200	64, 66, 68	63, 65, 67
2000	58, 60, 62	21, 23, 25

Food was removed from cages overnight prior to dosing.

4.2.2 Dosing

On Day 1 of the study, the amount of the formulated test item to be administered was calculated for each fasted animal according to body weight. This was administered, by gavage at a dose volume of 10 ml/kg, using a rubber catheter attached to a syringe of suitable capacity.

Food was made available approximately 4 hours after dosing.

4.2.3 Mortality and morbidity

Throughout the study all animals were checked twice daily.

4.2.4 Clinical signs

Animals were observed for clinical signs immediately upon dosing, approximately 1, 2 and 4 hours after dosing and daily thereafter for a total of 14 days.

4.2.5 Body weight

All animals were weighed at allocation to the study (Day -1), immediately prior to dosing (Day 1) and on Days 8 and 15 where appropriate. Early decedent animals were weighted when found.

4.2.6 Termination

Surviving animals were killed on Day 15 by carbon dioxide narcosis.

All animals were subjected to a gross necropsy examination for both external and internal abnormalities. The cranial, thoracic and abdominal cavities were opened to allow examination of their contents. Larger organs were sectioned. Both the stomach and representative sections of the gastro-intestinal tract were opened for examination of the mucosal surfaces.

4.3 Classification

The results obtained were used to indicate if classification of the test item is necessary according to the requirements of European Directives concerning the classification, packaging and labelling of dangerous substances (67/548/EEC and subsequent revisions).

4.4 Archives

The raw data and documentation generated during the course of this study will be retained at RTC for a period of 5 years after which the Sponsor will be contacted for instructions regarding despatch or disposal of the material.

5. RESULTS

5.1 **Mortality and clinical signs** (Appendix 1; Tables 1 and 2)

No mortality occurred following dosing in male or female animals at a level of 200 mg/kg and no clinical signs were observed.

Piloerection and reduced activity were observed in the 3 males dosed at 2000 mg/kg from the day of dosing up to Day 4. Recovery had occurred by Day 5.
Two of the females dosed at 2000 mg/kg died on Days 9 and 11. Observed clinical signs included piloerection, reduced activity, ataxia, semi-closed eyes and hunched posture, noted from Day 10 for the duration of the observation period.

5.2 **Body weight** (Appendices 2 and 3)

Changes in body weight in animals dosed at 200 mg/kg observed during the period of the study were within the range expected for this strain and age of animal.
Body weight losses were observed during the study in the females dosed at 2000 mg/kg.
In addition, a single male animal dosed at 2000 mg/kg showed a reduced body weight gain, while changes in the remaining males were in the range expected for this strain and age of animal.

5.3 **Necropsy** (Table 3 and Appendix 4)

The surviving female dosed at 2000 mg/kg showed abnormal contents (clear, fluid) in the abdominal cavity, a pale colour of mesenteric lymph nodes, lungs (with dark pinpoints), pancreas, spleen, liver, an abnormally small thymus. In addition, the pancreas was oedematous and the spleen swollen.
No abnormalities were found on necropsy of the other animals. Cannibalisation by cage mates was observed in 1 of the early decedent females.

6. CONCLUSION

These results indicate that the test item, [redacted], has a toxic effect in the rat following oral administration of a single dose at a level of 2000 mg/kg. Mortality was observed at this dose level, as well as a number of clinical signs. No mortality and no signs of toxicity were observed at 200 mg/kg. On the basis of these results, the LD50 is estimated to be less than 2000 mg/kg but greater then 200 mg/kg.

European Directives concerning the classification, packaging and labelling of dangerous substances (67/548/EEC and subsequent revisions) would indicate the following:-

Classification : Required
Symbol : Xn
R phrase : R22 – Harmful if swallowed

[redacted]: ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

TABLE 1.1 - Clinical signs - Incidence Table - 200 mg/kg - Day 1

STUDY NO.: 9563-003

MALES

Clinical Sign	Day ---->	1	1	1	1
	Session ---->	1	2	3	4
No significant signs		3/3	3/3	3/3	3/3

Key: Number of animals with sign at least once during interval /number of animals alive at start of interval
Session: 1: At dosing
2: Approximately 1 hour after dosing
3: Approximately 2 hours after dosing
4: Approximately 4 hours after dosing

REDACTED AS TO TRADE NAMES

[redacted] ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

TABLE 1.1 - Clinical signs - Incidence Table - 200 mg/kg - Day 1

STUDY NO.: 9563-003

FEMALES

Clinical Sign	Day ---->	1	1	1	1
	Session ---->	1	2	3	4
No significant signs		3/3	3/3	3/3	3/3

Key: Number of animals with sign at least once during interval /number of animals alive at start of interval
Session: 1: At dosing
2: Apprcximately 1 hour after dosing
3: Approximately 2 hours after dosing
4: Approximately 4 hours after dosing

[redacted] ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

TABLE 1.2 - Clinical signs - Incidence Table - 200 mg/kg - Days 2 to 15

STUDY NO.: 9563-003

MALES

Clinical Sign	Day of phase 2	3	4	5	6	7	8	9	10	11	12	13	14	15
No significant signs	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3

Key: Number of animals with sign at least once during interval /number of animals alive at start of interval

[redacted] ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

TABLE 1.2 - Clinical signs - Incidence Table - 200 mg/kg - Days 2 to 15

STUDY NO.: 9563-003

FEMALES

Clinical Sign	Day of phase 2	3	4	5	6	7	8	9	10	11	12	13	14	15
No significant signs	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3

Key: Number of animals with sign at least once during interval /number of animals alive at start of interval

[redacted]: ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

TABLE 2.1 - Clinical signs - Incidence Table - 2000 mg/kg - Day 1

STUDY NO.: 9563-003

MALES

Clinical Sign	Day ---->	1	1	1	1
	Session ---->	1	2	3	4
No significant signs		3/3	0/3	0/3	0/3
Reduced activity		0/3	3/3	3/3	3/3

Key: Number of animals with sign at least once during interval /number of animals alive at start of interval
Session: 1: At dosing
2: Approximately 1 hour after dosing
3: Approximately 2 hours after dosing
4: Approximately 4 hours after dosing

REDACTED AS TO TRADE NAMES

[redacted] ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

TABLE 2.1 - Clinical signs - Incidence Table - 2000 mg/kg - Day 1

STUDY NO.: 9563-003

FEMALES

Clinical Sign	Day ---->	1	1	1	1
	Session ---->	1	2	3	4
No significant signs		3/3	3/3	3/3	3/3

Key: Number of animals with sign at least once during interval /number of animals alive at start of interval
Session: 1: At dosing
2: Approximately 1 hour after dosing
3: Approximately 2 hours after dosing
4: Approximately 4 hours after dosing

REDACTED AS TO TRADE NAMES

[redacted] ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

TABLE 2.2 - Clinical signs - Incidence Table - 2000 mg/kg - Days 2 to 15

STUDY NO.: 9563-003

MALES

Clinical Sign	Day of phase 2	3	4	5	6	7	8	9	10	11	12	13	14	15
No significant signs	0/3	0/3	0/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Reduced activity	3/3	3/3	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Piloerection	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

Key: Number of animals with sign at least once during interval /number of animals alive at start of interval

[redacted] ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

TABLE 2.2 - Clinical signs - Incidence Table - 2000 mg/kg - Days 2 to 15

STUDY NO.: 9563-003

FEMALES

Clinical Sign	Day of phase 2	3	4	5	6	7	8	9	10	11	12	13	14	15
No significant signs	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/3	0/2	0/2	0/1	0/1	0/1	0/1
Piloerection	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	2/2	1/2	1/1	1/1	0/1	0/1
Reduced activity	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	2/2	1/2	1/1	1/1	1/1	0/1
Ataxia	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	2/2	1/2	0/1	0/1	0/1	0/1
Semi-closed eyes	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/2	1/2	1/1	1/1	0/1	0/1
Hunched posture	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/2	1/2	1/1	1/1	1/1	1/1
Dead	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/2	1/2	0/1	0/1	0/1	0/1

Key: Number of animals with sign at least once during interval /number of animals alive at start of interval

REDACTED AS TO TRADE NAMES

[redacted] ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

TABLE 3 - Macroscopic observations - Group incidence

STUDY NO.: 9563-003

	-- Males		-- Females --	
Dosage (mg/kg):	200	2000	200	2000
Number in group:	3	3	3	3#
Whole animal				
No abnormalities detected	3	3	3	1
Abdominal region				
Cannibalised	0	0	0	1
Abdominal cavity				
Abnormal contents	0	0	0	1
Mesenteric lymph nodes				
Abnormal colour	0	0	0	1
Pancreas				
Abnormal colour	0	0	0	1
Abnormal consistency	0	0	0	1
Spleen				
Abnormal colour	0	0	0	1
Abnormal shape	0	0	0	1
Liver				
Abnormal colour	0	0	0	1
Thymus				
Abnormal size	0	0	0	1
Abnormal colour	0	0	0	1
Lungs				
Abnormal areas	0	0	0	1

= Includes the 2 early decedent animals

REDACTED AS TO TRADE NAMES

[redacted]: ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

APPENDIX 1 - Mortality - Individual data

STUDY NO.: 9563-003

Animal Number	Dosage	Sex	Study Phase	Date of Death	Day	Status	Terminal Body Weight (g)
95630021	2000 mg/kg	F	Dosing phase	03.Jul.02	9	Found dead	163.8
95630023	2000 mg/kg	F	Dosing phase	05.Jul.02	11	Found dead	178.5

REDACTED AS TO TRADE NAMES

[redacted] ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

APPENDIX 2 - Body weight (g) - Individual data

STUDY NO.: 9563-003

200 mg/kg

Animal Number		Day of Phase 1!	1"	8	15
MALES					
95630064		235	211	292	333
95630066		211	194	273	321
95630068		212	193	255	291
	(n)	3	3	3	3
	Mean	219.3	199.3	273.3	315.0
	SD	13.6	10.1	18.5	21.6
FEMALES					
95630063		202	184	224	230
95630065		201	183	201	211
95630067		211	194	236	233
	(n)	3	3	3	3
	Mean	204.7	187.0	220.3	224.7
	SD	5.5	6.1	17.8	11.9

Note: ! = Pretest phase (Day -1); " = Dosing phase

REDACTED AS TO TRADE NAMES

[redacted] ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

APPENDIX 2 - Body weight (g) - Individual data

STUDY NO.: 9563-003

2000 mg/kg

Animal Number		Day of Phase 1!	1"	8	15
MALES					
95630058		260	235	223	194
95630060		263	238	260	295
95630062		260	236	250	273
	(n)	3	3	3	3
	Mean	261.0	236.3	244.3	254.0
	SD	1.7	1.5	19.1	53.1
FEMALES					
95630021		206	188	174	-
95630023		220	197	189	-
95630025		216	197	185	195
	(n)	3	3	3	1
	Mean	214.0	194.0	182.7	N/C
	SD	7.2	5.2	7.8	N/C

Note: ! = Pretest phase (Day -1); " = Dosing phase; - = Decedent; N/C = Not calculable

REDACTED AS TO TRADE NAMES

[redacted]: ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

APPENDIX 3 - Body weight change°(g) - Individual data

STUDY NO.: 9563-003

200 mg/kg

Animal Number		Day of Phase 8	15
MALES			
95630064		81	122
95630066		79	127
95630068		62	98
	(n)	3	3
	Mean	74.0	115.7
	SD	10.4	15.5
FEMALES			
95630063		40	46
95630065		18	28
95630067		42	39
	(n)	3	3
	Mean	33.3	37.7
	SD	13.3	9.1

Note: Data for Dosing phase
° = Body weight change relevant to Day 1 of study

REDACTED AS TO TRADE NAMES

[redacted] ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

APPENDIX 3 - Body weight change°(g) - Individual data

STUDY NO.: 9563-003

2000 mg/kg

Animal Number		Day of Phase 8	15
MALES			
95630058		-12	-41
95630060		22	57
95630062		14	37
	(n)	3	3
	Mean	8.0	17.7
	SD	17.8	51.8
FEMALES			
95630021		-14	-
95630023		-8	-
95630025		-12	-2
	(n)	3	1
	Mean	-11.3	N/C
	SD	3.1	N/C

Note: Data for Dosing phase; - = Decedent; N/C = Not calculable
° = Body weight change relevant to Day 1 of study

REDACTED AS TO TRADE NAMES

[redacted] ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

APPENDIX 4 - Macroscopic observations - Individual data

STUDY NO.: 9563-003

MALES

200 mg/kg

Animal Number	Tissue / Observation(s)
95630064	Whole animal No abnormalities detected
95630066	Whole animal No abnormalities detected
95630068	Whole animal No abnormalities detected

REDACTED AS TO TRADE NAMES

[redacted] ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

APPENDIX 4 - Macroscopic observations - Individual data

STUDY NO.: 9563-003

FEMALES

200 mg/kg

Animal Number	Tissue / Observation(s)
95630063	Whole animal No abnormalities detected
95630065	Whole animal No abnormalities detected
95630067	Whole animal No abnormalities detected

[redacted] ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

APPENDIX 4 - Macroscopic observations - Individual data

STUDY NO.: 9563-003

MALES

2000 mg/kg

Animal Number	Tissue / Observation(s)
95630058	Whole animal No abnormalities detected
95630060	Whole animal No abnormalities detected
95630062	Whole animal No abnormalities detected

REDACTED AS TO TRADE NAMES

[redacted] ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

APPENDIX 4 - Macroscopic observations - Individual data

STUDY NO.: 9563-003

FEMALES

2000 mg/kg

Animal Number	Tissue / Observation(s)
95630021	Early decedent
	No abnormalities detected
95630023	Early decedent
	Abdominal region
	Cannibalised
95630025	Abdominal cavity
	Abnormal contents, clear fluid
	Mesenteric lymph nodes
	Abnormal colour, pale
	Pancreas
	Abnormal colour, pale
	Abnormal consistency, oedematous
	Spleen
	Abnormal colour, pale
	Abnormal shape, swollen
	Liver
	Abnormal colour, pale
	Thymus
	Abnormal size, small
	Abnormal colour, pale
	Lungs
	Abnormal areas, multiple, pale, dark pinpoint

REDACTED AS TO TRADE NAMES

[redacted] ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

ADDENDUM 1 - CERTIFICATE OF ANALYSIS FOR THE TEST ITEM

STUDY NO.: 9563-003

AUSIMONT SpA

Bollate, 30 gennaio 2002

Certificato di analisi

Prodotto:	[redacted]
Batch:	90215/91
Concentrazione della soluzione:	5 % peso
PH della soluzione:	6.5

Caratteristiche del precursore acido:

Peso equivalente:	560
Metodo:	titolazione acidimetrica