LABORATORIES & CLINICS GROUP
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"ACUTE ORAL TOXICITY STUDY IN RATS"

RBM EXP. No. 980430

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

Issued on October 14, 1998

SPONSOR

AUSIMONT
Viale S.Pietro, 50/A
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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. 980430

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. 980430

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. 980430), 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 14, 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. 980430

QUALITY ASSURANCE STATEMENT

RBM Experiment number: 980430

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	May 29, 1998
October 1, 1998	October 1, 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 15, 1998

Enrico Invernizzi
Head of Quality Assurance Unit

RBM Exp. No. 980430

CERTIFICATION OF GLP COMPLIANCE

Study No. 980430 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 42 pages.

Study Director

Dr. Ping Yu

Ivrea, October 21, 1998

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SCIENTISTS INVOLVED IN THE STUDY

Study No. 980430

"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.: 980430

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: 250-312 g Females: 200-232 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.

TEST ARTICLE, CHARACTERIZATION

Identification:	[redacted]
Batch:	3/SPINETTA
Characteristics:	white gummy substance
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 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
145	5 males	July 9, 1998	Found dead
81	5 males	August 4, 1998	August 18
63	5 males	August 20, 1998	September 3, 1998
45	5 males	July 22, 1998	August 5, 1998
45	5 females	August 4, 1998	August 18, 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 days after administration
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.
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 980430 /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.

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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.

RBM Exp. No. 980430

RESULTS

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 5-14 days after dosing, with the first case observed on day 5 after administration in the 145 mg/kg group.

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

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

CLINICAL SIGNS *(TABLE 2 AND APPENDIX 1)*

Piloerection and hunched posture were observed in the animals of the various dose groups, starting 3-8 days after dosing. These changes were accompanied by hypoactivity in rats of the higher dose groups (81 and 145 mg/kg).

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.

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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, intestine congestion and catarrhal and/or hemorrhagic content of the intestine. These changes were mainly confined to animals of the higher dose groups (81 and 145 mg/kg). Moreover, kidney medulla congestion, decreased size of spleen 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. They were clinically observed for 14 days following the treatment. Macroscopic examinations were performed in the 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)	45	63	81	145
Treated animals	5M+5F	5M	5M	5M
Mortality	0	2M	4M	5M
Total (%)	0%	40%	80%	100%

The deaths occurred 5-14 days after dosing, with the first case observed on day 5 after administration in the 145 mg/kg group.
The LD_{50} was calculated to be 67.7 mg/kg with 95% confidence limits of 58.5 – 78.3 mg/kg.
No deaths occurred in the animals of either sex in the lowest dose group (45 mg/kg).

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Piloerection and hunched posture were observed in the animals of the various dose groups, starting 3-8 days after dosing. These changes were accompanied by hypoactivity in rats of the higher dose groups (81 and 145 mg/kg). 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, intestine congestion and catarrhal and/or hemorrhagic content of the intestine.These changes were mainly confined to animals of the higher dose groups (81 and 145 mg/kg). Moreover, kidney medulla congestion, decreased size of spleen 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.

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 intestine were mainly involved) in animals given the higher doses.

Dr. Ping Yu
Study Director
October 14, 1998

Dr. Sergio Peano
Senior Scientist for General Toxicology
Oct. 14, 1998

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RBM Exp. No. 980430

GROUP DATA

Test article: [redacted]
Title : Acute toxicity study in rats
RBM exp. : 980430

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

Males - Females

Dose (mg/kg)		45	63	81	145
Treated animals		10	5	5	5
Day	5	0	0	0	3
	6	0	0	0	1
	7	0	1	0	0
	8	0	1	0	1
	10	0	0	1	0
	12	0	0	2	0
	14	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

Test article: [redacted]
Title : Acute toxicity study in rats
RBM exp. : 980430

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 7d- 8d	4 10d-14d	5 5d- 8d
Hypoactivity	-	-	4 4d-13d	2 5d- 7d
Piloerection	4 7d-10d	5 5d-14d	5 3d-13d	2 5d- 7d
Hunched posture	4 8d-10d	5 6d- 7d	5 4d-13d	2 5d- 7d
Recovery	5 11d	-	1 14d	-

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

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Test article: [redacted]
Title : Acute toxicity study in rats
RBM exp. : 980430

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	3 4d-11d
Recovery	5 12d

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

Test article: [redacted]
Title : Acute toxicity study in rats
RBM exp. : 980430

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	0	0	0
General observation				
cannibalized	-	1 50.00%	1 25.00%	0
Intestine				
congestion	-	0	0	1(2.0) 20.00%
catarrhal hemorrhagic content	-	0	0	2(3.0) 40.00%
catarrhal content	-	1(2.0) 50.00%	0	0
Kidneys				
medulla, congestion	-	0	0	3(2.0) 60.00%

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

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Test article: [redacted]
Title : Acute toxicity study in rats
RBM exp. : 980430

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	0	0	0
Liver				
pale	-	1(3.0) 50.00%	3(2.3) 75.00%	5(2.4) 100.00%
Lungs				
congestion	-	1(3.0) 50.00%	0	0
Spleen				
decreased size	-	0	0	1(2.0) 20.00%
Thymus				
congestion	-	1(2.0) 50.00%	0	1(3.0) 20.00%

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

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Test article: [redacted]
Title : Acute toxicity study in rats
RBM exp. : 980430

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 toxicity study in rats
RBM exp. : 980430

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

APPENDICES

Test article: [redacted]
□
Title : Acute toxicity study in rats
RBM exp. : 980430

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	1	1	1	1	1	1	1	1	5	5	5	5	5	5	5	5
Piloerection																4	4	4	4	4	4	4	4								
Hunched posture																		2	2	4	4	4	4								

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	3	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2	5	5	5	5	5	5
Piloerection										2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3						

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

Test article: [redacted]
Title : Acute toxicity study in rats
RBM exp. : 980430

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	
	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																1		1													
No clinical signs		5	5	5	5	5	5	5	5	5	5	5	5	5	5	4	4	3	3	3	3	3	3	3	3	3	3	1	1	1	1
Piloerection														5	5	4	4											2	2	2	2
Hunched posture																4	4														

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

Test article: [redacted]
Title : Acute toxicity study in rats
RBM exp. : 980430

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	
	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
Death																				1				2				1	
No clinical signs		5	5	5	5	5	5	4	4																	1	1	1	1
Hypoactivity										1	1	1	1	1	1	2	2	3	3	4	4	3	3	3	3	1	1	1	1
Piloerection								1	1	5	5	5	5	5	5	5	5	5	5	5	5	4	4	4	4	2	2	1	1
Hunched posture										4	4	4	4	3	3	3	3	4	4	5	5	4	4	4	4	2	2	1	1

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

Test article: [redacted]
Title : Acute toxicity study in rats
RBM exp. : 980430

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

Dose (mg/kg) 145

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

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

Test article: [redacted]
Title : Acute toxicity study in rats
RBM exp. : 980430

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	294	290	299	312	308	232	225	221	220	200
1	1	268	264	271	290	284	217	207	208	206	186
1	3	274	280	284	313	290	223	222	209	219	200
2	8	259	278	259	316	268	205	226	208	220	200
2	14	278	289	264	349	268	229	240	215	233	218

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Test article: [redacted]
Title : Acute toxicity study in rats
RBM exp. : 980430

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

Dose (mg/kg) 63

Animal #		51M	52M	53M	54M	55M
Week	day					
	0	269	281	272	283	264
1	1	282	294	278	295	272
1	3	277	308	269	288	272
2	8	294	319		299	
2	14	277	356		254	

Test article: [redacted]
Title : Acute toxicity study in rats
RBM exp. : 980430

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

Dose (mg/kg) 81

Week	day	Animal # 41M	42M	43M	44M	45M
	0	280	250	273	283	263
1	1	256	230	251	260	238
1	3	264	228	258	283	245
2	8	201	168	202	283	188
2	14				324	

Test article: [redacted]
Title : Acute toxicity study in rats
RBM exp. : 980430

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

Dose (mg/kg) 145

Animal #		21M	22M	23M	24M	25M
Week	day					
	0	301	268	305	303	312
1	1	279	249	284	280	291
1	3	281	242	300	283	289

Test article: [redacted]
Title : Acute toxicity study in rats
RBM exp. : 980430

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	7. M2	Intestine	catarrhal content, diffuse, moderate
		Liver	pale, diffuse, severe
		Lungs	congestion, diffuse, severe
		Thymus	congestion, diffuse, moderate
55M	8 M2	General observation	cannibalized

Death code : M2(Natural death)

Test article: [redacted]
Title : Acute toxicity study in rats
RBM exp. : 980430

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

Dead or agonal sacrificed an.

Dose (mg/kg) 81

An#	Death day/code#	TISSUE	Gross observations
41M	12 M2	Liver	pale, diffuse, moderate
42M	10 M2	General observation	cannibalized
43M	14 M2	Liver	pale, diffuse, severe
45M	12 M2	Liver	pale, diffuse, moderate

Death code : M2(Natural death)

Test article: [redacted]
Title : Acute toxicity study in rats
RBM exp. : 980430

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	Intestine	congestion, diffuse, moderate catarrhal hemorrhagic content, diffuse, severe
		Liver	pale, diffuse, severe
22M	5 M2	Intestine	catarrhal hemorrhagic content, diffuse, severe
		Kidneys	medulla, congestion, diffuse, moderate
		Liver	pale, diffuse, severe
23M	8 M2	Kidneys	medulla; congestion, diffuse, moderate
		Liver	pale, diffuse, moderate
		Spleen	decreased size, diffuse, moderate
24M	5 M2	Liver	pale, diffuse, moderate
		Thymus	congestion, diffuse, severe
25M	6 M2	Kidneys	medulla, congestion, diffuse, moderate
		Liver	pale, diffuse, moderate

Death code : M2(Natural death)

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Test article: [redacted]
Title : Acute toxicity study in rats
RBM exp. : 980430

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

Final killing

Dose (mg/kg) 45

An#	Death day	TISSUE	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	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 toxicity study in rats
RBM exp. : 980430

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	15	General observation	no macroscopically appreciable lesions
52M	15	General observation	no macroscopically appreciable lesions
54M	15	General observation	no macroscopically appreciable lesions

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Test article: [redacted]
Title : Acute toxicity study in rats
RBM exp. : 980430

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
44M	15	General observation	no macroscopically appreciable lesions

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[redacted]

[redacted] ce $(C_3F_6O)_2CF_2COO^-Na^+$

"ACUTE ORAL TOXICITY STUDY IN RATS"

RBM EXP. No. 980428

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

Issued on October 14, 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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Istituto di Ricerche Biomediche "Antoine Marxer" - RBM S.p.A.
Capit. Soc. 10.092.000.000 C.C.I.A.A. Torino 421838. C. Fiscale e P.IVA 00688380013. Reg. Trib. IVREA N. 1287

RBM Exp. No. 980428

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. 980428

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RBM Exp. No. 980428

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. 980428), 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 14, 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. 980428

QUALITY ASSURANCE STATEMENT

RBM Experiment number: 980428

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	May 29, 1998
October 12 – 13, 1998	October 13, 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 15, 1998

Enrico Invernizzi
Head of Quality Assurance Unit

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RBM Exp. No. 980428

CERTIFICATION OF GLP COMPLIANCE

Study No. 980428 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 39 pages.

Study Director

Dr. Ping Yu

Ivrea, October 21, 1998

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RBM Exp. No. 980428

SCIENTISTS INVOLVED IN THE STUDY

Study No. 980428

"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. 980428

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

RBM Experiment No.: 980428

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. 04120 (June 5, 1998), 04317 (June 12, 1998), 04635 (June 26, 1998) and 04980 (July 10, 1998)
Age (at randomization):	no more than three months
Body weight (at randomization):	Males: 273-350 g Females: 211-269 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%

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.

TEST ARTICLE, CHARACTERIZATION

Identification:	[redacted]
Batch:	1/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.

When the formulates were sospension they were kept magnetically stirred until the end of administration and 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 15, 1998	Found dead
126	5 males	August 14, 1998	September 4, 1998
90	5 males	July 28, 1998	August 18, 1998
90	5 females	August 20, 1998	September 3, 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 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 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 980428 /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. 980428

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 5-14 days after dosing, with the first case observed on day 5 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 3-4 days (162 mg/kg group) or 4-11 days (the lower doses) after dosing. One male of the 126 mg/kg group showed also abdominal dilatation during the latter stage of the observation period.

Piloerection was the only clinical change observed in the females received the test article at the lowest dose (6-11 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, erosion and congestion of stomach, intestine congestion and decreased size of spleen. The two latter changes were mainly confined to animals of the highest dose group (162 mg/kg). Moreover, kidney medulla congestion or pale kidney was 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 or solution (depending on the concention of the test article in the vehicle) 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 5-14 days after dosing, with the first case observed on day 5 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 3-4 days (162 mg/kg group) or 4-11 days (the lower doses) after dosing. One male of the 126 mg/kg group showed also abdominal dilatation during the latter stage of the observation period. Piloerection was the only clinical change observed in the females that received the test article at the lowest dose (6-11 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, erosion and congestion of stomach, intestine congestion and decreased size of spleen. The two latter changes were mainly confined to animals of the highest dose group (162 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 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 stomach were involved) mainly in animals given the higher doses.

Dr. Ping Yu
Study Director
October 14, 1998

Dr. Sergio Peano
Senior Scientist for General Toxicology
Oct. 14, 1998

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RBM Exp. No. 980428

GROUP DATA

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980428

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

Males - Females

Dose (mg/kg)		90	126	162
Treated animals		10	5	5
Day	5	0	0	1
	7	0	0	1
	8	0	0	1
	9	0	0	1
	10	0	0	1
	14	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. : 980428

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 14d	5 5d-10d
Hypoactivity	2 8d-10d	5 11d-14d	3 4d- 9d
Piloerection	5 4d-16d	5 4d-21d	5 3d- 9d
Hunched posture	3 4d-10d	5 5d-13d	4 3d- 9d
Abdominal dilatation	-	1 16d-21d	-
Recovery	5 17d	-	-

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- (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. : 980428

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 6d-11d
Recovery	5 12d

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

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980428

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	0
General observation			
cannibalized	-	1 33.33%	1 20.00%
Kidneys			
pale	-	1(2.0) 33.33%	0
medulla, congestion	-	0	2(2.0) 40.00%
Liver			
pale	-	2(2.5) 66.67%	4(2.0) 80.00%
Spleen			
decreased size	-	0	4(2.8) 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. : 980428

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	0
Stomach			
congestion	-	0	3(2.0) 60.00%
erosion	-	0	1(2.0) 20.00%

- (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. : 980428

TABLE 3. - Gross pathology examination (p. 3)
(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. : 980428

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

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

APPENDICES

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980428

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																					1	1	3	3	5	5
Hypoactivity																		2	2	2	2	2	2													
Piloerection										5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	4	4	2	2		
Hunched posture										2	2	2	2	2	2	2	2	3	3	3	3	3	3														

Cage # 7M (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

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							3	3	3	3	3	3	5	5	5	5	5	5
Piloerection														5	5	5	5	5	5	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. : 980428

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	5	5	2	2						
Hypoactivity																																					
Piloerection										5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	2	2	2	2	2	2	2	2
Hunched posture												5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5								
Abdominal dilatation																																		1	1	1	1

Cage # 9M (follows)	Day	18		19		20		21	
	Time	M	A	M	A	M	A	M	A
Piloerection		2	2	2	2	2	2	2	2
Abdominal dilatation		1	1	1	1	1	1	1	1

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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. : 980428

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		10
	Time	30m	2h	4h	6h	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M
Death												1				1		1		1		1
No clinical signs		5	5	5	5	5	5			1	1											
Hypoactivity										1	1					3	3	2	2	1	1	
Piloerection								5	5	4	4	4	4	4	4	3	3	2	2	1	1	
Hunched posture								1	1	1	1	1	1	4	4	3	3	2	2	1	1	

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

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980428

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	300	305	301	324	350	261	269	248	211	238
1	1	274	286	280	306	319	270	275	250	218	248
1	3	260	275	260	295	317	262	276	245	214	244
2	8	222	251	211	269	260	250	269	240	217	240
2	14	215	283	237	212	275	293	297	263	233	261
3	21	258	343	279	252	298					

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Test article: [redacted]
Title : Acute oral tcxicity study in rats
RBM exp. : 980428

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

Dose (mg/kg) 126

Animal #		41M	42M	43M	44M	45M
Week	day					
	0	289	287	312	280	306
1	1	267	254	282	253	274
1	3	252	251	282	268	279
2	8	233	248	269	280	251
2	14		254		293	
3	21		314		348	

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980428

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

Dose (mg/kg) 162

Animal #		21M	22M	23M	24M	25M
Week	day					
	0	277	333	320	320	273
1	1	252	311	294	292	294
1	3	234	299	280	289	232
2	8	164			216	

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980428

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	14 M2	General observation	cannibalized
43M	14 M2	Liver	pale, diffuse, moderate
45M	14 M2	Kidneys	pale, diffuse, moderate
		Liver	pale, diffuse, severe

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Death code : M2(Natural death)

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980428

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	10	M2	Kidneys	medulla, congestion, diffuse, moderate
			Liver	pale, diffuse, moderate
			Spleen	decreased size, diffuse, severe
22M	8	M2	Liver	pale, diffuse, moderate
			Spleen	decreased size, diffuse, severe
			Stomach	congestion, diffuse, moderate erosion, multifocal, moderate
23M	7	M2	Liver	pale, diffuse, moderate
			Spleen	decreased size, diffuse, moderate
			Stomach	congestion, diffuse, moderate
24M	9	M2	Kidneys	medulla, congestion, diffuse, moderate
			Liver	pale, diffuse, moderate
			Spleen	decreased size, diffuse, severe
			Stomach	congestion, diffuse, moderate

Death code : M2(Natural death)

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980428

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

Dead or agonal sacrificed an.

Dose (mg/kg) 162

An#	Death day/code#	TISSUE	Gross observations
25M	5 M2	General observation	cannibalized

Death code : M2(Natural death)

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980428

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

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 980428

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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[redacted]

[redacted] $ce(C_3F_6O)_{2,5}CF_2-COO^-Na^+$

"ACUTE ORAL TOXICITY STUDY IN RATS"

RBM EXP. No. 970594

Issued on March 26, 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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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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This report consists of 36 pages.

Ivrea, March 26, 1998

Dr. Ping Yu
RBM Study Director

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LABORATORIES & CLINICS GROUP
BIOSCIENCE

RBM Exp. No. 970594

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. 970594), 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 26, 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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LABORATORIES & CLINICS GROUP BIOSCIENCE

RBM Exp. No. 970594

QUALITY ASSURANCE STATEMENT

RBM Experiment number: 970594

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 26, 1998	March 26, 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 31, 1998

Enrico Invernizzi
Head of Quality Assurance Unit

Date : March 31, 1998

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LABORATORIES & CLINICS GROUP
BIOSCIENCE

RBM Exp. No. 970594

RBM MANAGEMENT DECLARATION OF GLP COMPLIANCE

Study No. 970594 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 31, 98

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SCIENTISTS INVOLVED IN THE STUDY

STUDY No. 970594

"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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MATERIALS AND METHODS

EXPERIMENTAL DESIGN

RBM Experiment No.:	970594
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 82,102 and 128 mg/kg 5 females at the dose of 82 mg/kg

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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: 230 - 348 g Females: 197 - 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:	18732/40
Characteristics:	white solid
Manufacturing date:	October 14, 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
102	5 males	February 27, 1998	March 13, 1998
82	5 males	February 17, 1998	March 3, 1998
82	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 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.

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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 970594/1, 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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RESULTS

CLINICAL OBSERVATIONS

MORTALITY *(TABLE 1)*

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

Dose (mg/kg)	82	102	128
Treated animals	5M+5F	5M	5M
Mortality Total (%)	0 0%	2M 40%	5M 100%

The deaths occurred within 13 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 99.5 mg/kg with 95% confidence limits of 92.1 - 108 mg/kg.

CLINICAL SIGNS *(TABLE 2 AND APPENDIX 1)*

The main clinical changes induced by administration of the test article were piloerection and hunched posture, starting days 6-9 after dosing in males of all dose groups. In the highest dose dose group (128 mg/kg), these changes were accompanied by sedation or hypoavtivity.

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

No changes of note were seen in females given the lowest dose (82 mg/kg).

BODY WEIGHT *(APPENDIX 2)*

Decrease in body weight or retarted growth was found in animals of the various dose groups mainly during the first week of the observation period.

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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 decreased size of spleen. Moreover, kidney medulla congestion was seen in one rat.

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 82, 102 and 128 mg/kg to groups of 5 males/dose and at the dose of 82 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)	82	102	128
Treated animals	5M+5F	5M	5M
Mortality	0	2M	5M
Total (%)	0%	40%	100%

The deaths occurred within 13 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 99.5 mg/kg with 95% confidence limits of 92.1 - 108 mg/kg.

The main clinical changes induced by administration of the test article were piloerection and hunched posture, starting days 6-9 after dosing in males of all dose groups. In the highest dose dose group (128 mg/kg), these changes were accompanied by sedation or hypoavtivity.

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

No changes of note were seen in females given the lowest dose (82 mg/kg).

Depression in body weight growth was found in animals of the various dose groups mainly during the first week of 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 decreased size of spleen.

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

In conclusion, the **LD_{50}** of the test article [redacted], when administered to rats as a single dose by oral route, was **99.5 mg/kg (95% confidence limits: 92.1-108 mg/kg)**. The compound induced delayed toxicity (liver was mainly involved) in animals given the higher doses.

Dr. Ping Yu

RBM Study Director

March 26, 1998

Dr. Roberto Maraschin

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

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LABORATORIES & CLINICS GROUP BIOSCIENCE

RBM Exp. No. 970594

GROUP DATA

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 970594

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

Males - Females

Dose (mg/kg)	82	102	128
Treated animals	10	5	5
Day 6	0	0	1
7	0	0	2
8	0	0	2
11	0	1	0
13	0	1	0
Total no. (day 14)	0	2	5
Total (%)	.0%	40.0%	100.0%

Median lethal dose (LD50) = 99.54

95% confidence limits = 92.08 - 107.59

Slope (SE) = 7.67 1.68

Heterogeneity P = .348 NS

Linear regression y =-30.2867+7.6702x

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 970594

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

	Males		
Dose (mg/kg)	82	102	128
no. of treated animals	5	5	5
Death	-	2 11d-13d	5 6d- 8d
Sedation	-	-	1 7d- 7d
Hypoactivity	-	-	1 7d- 7d
Piloerection	3 9d-12d	3 9d-12d	4 6d- 7d
Hunched posture	3 9d- 9d	3 8d-12d	3 6d- 7d
Recovery	5 13d	3 13d	-

- (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. : 970594

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

	Females
Dose (mg/kg)	82
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)

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 970594

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

Dead or agonal sacrificed an. Males

Dose (mg/kg)	82	102	128
no. of animals	0	2	5
no. of animals without appreciable lesions	0	0	0
General observation			
cannibalized	-	1 50.00%	0
Kidneys			
medulla, congestion	-	0	1(2.0) 20.00%
Liver			
pale	-	1(2.0) 50.00%	5(3.0) 100.00%
Spleen			
decreased size	-	1(2.0) 50.00%	4(2.0) 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. : 970594

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

Final killing	Males		
Dose (mg/kg)	82	102	128
no. of animals	5	3	0
no. of animals without appreciable lesions	5	3	0

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 970594

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

Final killing	Females
Dose (mg/kg)	82
no. of animals	5
no. of animals without appreciable lesions	5

APPENDICES

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Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 970594

APPENDIX 1. - Clinical signs incidence (p. 1)
(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
No clinical signs		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	2	2	2	2	2	2	4	4	5	5	5	5
Piloerection																				2	2	3	3	3	3	1	1				
Hunched posture																				3	3										

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	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)

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 970594

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

Dose (mg/kg) 102

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
Death																								1				1			
No clinical signs		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	2	2	2	2	2	2	3	3	3	3	3	3	3	3
Piloerection																				3	3	3	3	1	1	1	1				
Hunched posture																		3	3	3	3	3	3	1	1	1	1				

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

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REDACTED AS TO TRADE NAMES

BIOSCIENCE LABORATORIES & CLINICS GROUP

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 970594

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

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

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 970594

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

Dose (mg/kg) 82

Animal #		31M	32M	33M	34M	35M	36F	37F	38F	39F	40F
Week	day										
	0	329	330	335	348	345	204	197	214	210	206
1	1	306	311	315	329	327	187	185	186	188	189
1	3	310	316	313	337	325	209	200	221	223	216
2	8	287	256	300	323	320	218	209	230	231	222
2	14	368	375	354	388	388	229	220	244	246	248

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 970594

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

Dose (mg/kg) 100

Animal #		41M	42M	43M	44M	45M
Week	day					
	0	245	247	230	230	257
1	1	218	228	193	192	234
1	3	226	229	193	183	229
2	8	224	241	150	190	171
2	14	238	289		246	

207

REDACTED AS TO TRADE NAMES

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 970594

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

Dose (mg/kg) 128

Animal #		21M	22M	23M	24M	25M
Week	day					
	0	296	287	275	281	295
1	1	266	260	257	261	264
1	3	275	255	246	253	266

208

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 970594

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

Dead or agonal sacrificed an.

Dose (mg/kg) 102

An#	Death day/code#	TISSUE	Gross observations
43M	13 M2	Liver	pale, diffuse, moderate
		Spleen	decreased size, diffuse, moderate
45M	11 M2	General observation	cannibalized

209

Death code : M2 (Natural death)

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 970594

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	8 M2	Liver	pale, diffuse, severe
		Spleen	decreased size, diffuse, moderate
22M	7 M2	Liver	pale, diffuse, severe
23M	6 M2	Liver	pale, diffuse, severe
		Spleen	decreased size, diffuse, moderate
24M	7 M2	Liver	pale, diffuse, severe
		Spleen	decreased size, diffuse, moderate
25M	8 M2	Kidneys	medulla, congestion, diffuse, moderate
		Liver	pale, diffuse, severe
		Spleen	decreased size, diffuse, moderate

Death code : M2(Natural death)

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 970594

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

Final killing

Dose (mg/kg) 82

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	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

8/11

Test article: [redacted]
Title : Acute oral toxicity study in rats
RBM exp. : 970594

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

Final killing

Dose (mg/kg) 102

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
44M	15	General observation	no macroscopically appreciable lesions

2/2

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: 9563-002

RTC Report Number: 9563-002/T/333/2002

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

Commercial Office

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Reg. Soc. Trib. di Roma n. 2828/72
Cod. Fisc. 00653120584
Partita IVA 00920611001

RTC Report Number: 9563-002/T/333/2002

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 : 20.03.03

J. Brightwell, Ph.D.
(Scientific Director): Date : 20.03.03

RTC Report Number: 9563-002/T/333/2002

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	10.06.2002	-	18.07.2002
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 13 March 2003

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

18/03/2003
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 dosed at a level of 200 mg/kg and observed for a period of 14 days.

No mortality occurred and no clinical signs were observed.

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 observed.

A single group of 3 male animals was then dosed at a level of 2000 mg/kg.
All animals had died by day 3. Clinical signs included reduced activity, piloerection, ataxia, difficulty in breathing, ocular discharge and pronation.

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

Changes in body weight observed in treated animals were not remarkable.

No abnormalities were found on necropsy of the surviving animals at termination of the study.
The early decedent animals at necropsy examination showed skin/fur staining of different regions of the body surfaces.

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 observed mortality pattern suggests 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 20th June 2002 when the study was terminated. 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	: 90215/92
CAS number	: 330809-80-8
Expiry date	: February 2004
Purity	: > 90% (referred to dry salt)
pH	: 6.6
Received from	: AUSIMONT S.p.A.
Date received	: 11th February 2002
Amount received	: 500 grams
Description	: Colourless liquid
Container	: Opaque plastic container
Storage at RTC	: Ambient conditions
RTC reference number	: 6533

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 for the test item, 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 20 and 200 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. During the first phase of the study the measurements of temperature and humidity were carried out in the access room and not directly inside the animal room.

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	58, 60, 62	57, 59, 61
2000	52, 54, 56	-

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.

4.2.6 Termination

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

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 **Clinical signs** (Table 1 and Appendix 1)

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

One of the males dosed at 2000 mg/kg died on day 2 and the remaining 2 animals died on day 3. Clinical signs observed were, reduced activity, piloerection, ataxia, difficulty in breathing, ocular discharge and pronation.

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

Changes in body weight observed during the period of the study were within the range expected for this strain and age of animals.

5.3 **Necropsy** (Table 2 and Appendix 4)

No abnormalities were found on necropsy of the surviving animals at termination of the study.
The early decedent animals at necropsy examination showed skin/fur staining around the muzzle or the urogenital region.

6. CONCLUSION

The results of this study 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 observed mortality pattern suggest the LD50 to be less than 2000 mg/kg but in excess of 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

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

TABLE 1.1 - Clinical signs - Incidence Table - Day 1

STUDY NO.: 9563-002

MALES

200 mg/kg

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

TABLE 1.1 - Clinical signs - Incidence Table - Day 1

STUDY NO.: 9563-002

FEMALES

200 mg/kg

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

TABLE 1.1 - Clinical signs - Incidence Table - Day 1

STUDY NO.: 9563-002

MALES

2000 mg/kg

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
Piloerection		0/3	0/3	3/3	3/3
Ataxia		0/3	0/3	0/3	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] ACUTE ORAL TOXICITY STUDY IN RATS (ACUTE TOXIC CLASS METHOD)

TABLE 1.2 - Clinical signs - Incidence Table - Days 2 to 15

STUDY NO.: 9563-002

MALES

200 mg/kg

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 - Days 2 to 15

STUDY NO.: 9563-002

FEMALES

200 mg/kg

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 - Days 2 to 15

STUDY NO.: 9563-002

MALES

2000 mg/kg

Clinical Sign	Day of phase 2	3	4	5	6	7	8	9	10	11	12	13	14	15
No significant signs	0/3	0/2	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Reduced activity	2/3	0/2	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Piloerection	2/3	0/2	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Ataxia	1/3	0/2	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Difficulty in breathing	1/3	0/2	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Ocular discharge	1/3	0/2	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Pronation	1/3	0/2	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Dead	1/3	2/2	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

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 - Macroscopic observations - Group incidence

STUDY NO.: 9563-002

200 mg/kg

	-- Males	-- Females --
Group:	1	1
Number in group:	3	3
Whole animal		
No abnormalities detected	3	3

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

TABLE 2 - Macroscopic observations - Group incidence

STUDY NO.: 9563-002

2000 mg/kg

	-- Males
Group:	1
Number in group:	3
Head	
Staining	1
Skin	
Staining	2

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

APPENDIX 1 - Mortality - Individual data

STUDY NO.: 9563-002

Animal Number	Dosage	Sex	Study Phase	Date of Death	Day	Status	Terminal Body Weight (g)
95630052	2000 mg/kg	M	Dosing phase	20.June.02	3	Found dead	205.8
95630054	2000 mg/kg	M	Dosing phase	19.June.02	2	Found dead	221.8
95630056	2000 mg/kg	M	Dosing phase	20.June.02	3	Found dead	206.2

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

APPENDIX 2 - Body weight (g) - Individual data

STUDY NO.: 9563-002

Animal Number		Day of Phase 1!	1"	8	15
MALES - 200 mg/kg					
95630058		218	199	255	300
95630060		229	209	279	323
95630062		218	200	258	303
	(n)	3	3	3	3
	Mean	221.7	202.7	264.0	308.7
	SD	6.4	5.5	13.1	12.5
FEMALES - 200 mg/kg					
95630057		200	187	218	213
95630059		205	186	232	218
95630061		209	193	228	221
	(n)	3	3	3	3
	Mean	204.7	188.7	226.0	217.3
	SD	4.5	3.8	7.2	4.0
MALES - 2000 mg/kg					
95630052		240	221	-	-
95630054		247	224	-	-
95630056		241	218	-	-
	(n)	3	3		
	Mean	242.7	221.0		
	SD	3.8	3.0		

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

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

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

STUDY NO.: 9563-002

200 mg/kg

Animal Number		Day of Phase 8	15
MALES			
95630058		56	101
95630060		70	114
95630062		58	103
	(n)	3	3
	Mean	61.3	106.0
	SD	7.6	7.0
FEMALES			
95630057		31	26
95630059		46	32
95630061		35	28
	(n)	3	3
	Mean	37.3	28.7
	SD	7.8	3.1

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

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

APPENDIX 4 - Macroscopic observations - Individual data

STUDY NO.: 9563-002

MALES

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

APPENDIX 4 - Macroscopic observations - Individual data

STUDY NO.: 9563-002

FEMALES

200 mg/kg

Animal Number	Tissue / Observation(s)
95630057	Whole animal No abnormalities detected
95630059	Whole animal No abnormalities detected
95630061	Whole animal No abnormalities detected

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

APPENDIX 4 - Macroscopic observations - Individual data

STUDY NO.: 9563-002

MALES

2000 mg/kg

Animal Number	Tissue / Observation(s)
95630052	Early decedent Head Staining, red, muzzle
95630054	Early decedent Skin Staining, brown, urogenital region
95630056	Early decedent Skin Staining, brown, urogenital region

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

ADDENDUM 1 - CERTIFICATE OF ANALYSIS FOR THE TEST ITEM

STUDY NO.: 9563-002

AUSIMONT S.p.A.

Bollate, 30 gennaio 2002

Certificato di analisi

Prodotto:	[redacted]
Batch:	90215/92
Concentrazione della soluzione:	20 % peso
PH della soluzione:	6.6

Caratteristiche del precursore acido:

Peso equivalente:	534
Metodo:	titolazione acidimetrica

REDACTED AS TO TRADE NAMES

RESEARCH TOXICOLOGY CENTRE - ROMA

[redacted]

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

FINAL REPORT

RTC Study no.: 15300-002

Sponsor:
AUSIMONT S.p.A.
Via Lombardia, 20
20121 Bollate (MI)
Italy

Commercial Office

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Reg. Soc. Trib. di Roma n. 2828/72
Cod. Fisc.: 00653120584
Partita IVA: 00920611001

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 : 12 - 06 - 2003

J. Brightwell, Ph.D.
(Scientific Director): Date : 12 - 6 - 2003

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	11.12.2002	11.12.2002	11.12.2002
PROCESS-BASED INSPECTIONS			
Allocation	07.11.2002	-	28.11.2002
Dose preparation	06.12.2002	-	12.12.2002
Body weight	03.12.2002	-	02.01.2003
Dosing (oral)	10.01.2003	-	14.01.2003
Clinical observations	06.12.2002	-	13.01.2003
Despatch to necropsy	12.02.2003	-	20.03.2003
Necropsy	12.02.2003	-	10.03.2003
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 11.06.2003

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

11.06.2003
Date

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Tables

Appendices

1. SUMMARY

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

A single group of 3 female animals was dosed at a level of 2000 mg/kg.
One animal was found dead on Day 10 and the remaining 2 animals died on Day 13.
Reduced activity was noted on the day of dosing. No clinical signs were observed from Day 2 to Day 6. Thereafter, clinical signs included hunched posture, a thin and pale appearance, reduced activity, piloerection and reduced faeces.

An additional group of 3 female animals was dosed at the same level (2000 mg/kg).
One animal was found dead on Day 10 and the remaining 2 animals died on Day 11.
All animals were prone on the day of dosing. No clinical signs were noted from Day 2 to Day 6. Thereafter, clinical signs observed were brown staining around the muzzle, hunched posture, reduced activity, a thin appearance, piloerection and red staining in the litter tray.

A group of 3 female animals was then dosed at 300 mg/kg and observed for a period of 14 days. No mortality occurred and no clinical signs were noted.

A group of 3 female animals was subsequently dosed at the same level (300 mg/kg) and observed for a period of 14 days. No mortality occurred and clinical signs were observed.

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

Marked body weight losses were seen in all animals dosed at 2000 mg/kg on Day 8. Changes in body weight observed in animals dosed at 300 mg/kg were not remarkable.

Three of the early decedent animals showed no internal abnormalities at necropsy examination. An abnormal content in the stomach, an abnormal size of the thymus, spleen and the uterus, an abnormal colour of the spleen and staining of the muzzle were observed in the other 3 early decedent animals, dosed at 2000 mg/kg. The remaining animal showed abnormal size of the thymus and the spleen which also had an abnormal colour.
No abnormalities were observed in terminal kill animals, dosed at 300 mg/kg.

These results indicate that the test item, [redacted] has a severe toxic effect in the rat following oral administration of a single dose at a level of 2000 mg/kg. The lack of mortality at 300 mg/kg, demonstrates the LD50 to be greater than 300 mg/kg, but less than 2000 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 17th December 2001. 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
20121 Bollate (MI)
Italy

The study started on 14th November 2002 with signing of the protocol by the Study Director. The experimental work described in this report started on 16th December 2002 with allocation of the first 3 female animals to the study and ended on 6th February 2003 with 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	: 90347/36
CAS number	: 330809-80-8
Purity	: >90%
Expiry date	: 30th November 2004
Received from	: AUSIMONT S.p.A.
Date received	: 21st November 2002
Amount received	: 1000 grams
Description	: Colourless liquid
Container	: Opaque plastic bottle
Storage at RTC	: Ambient temperature
RTC reference number	: 7493

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.
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 in sterile distilled water to give concentrations of 200 and 30 mg/ml. Concentrations were calculated and expressed in terms of test item as supplied.

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 female 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 176 to 200 grams and aged approximately 6 to 8 weeks, nulliparous and non-pregnant. Animals appeared to be in an acceptable condition following arrival in different batches on 6th December 2002 and 10th January 2003. 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 in polycarbonate cages measuring 42x26x18 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 (4 RF 18, Mucedola S.r.l, Via G. Galilei, 4, 20019, Settimo Milanese (MI) Italy) *ad libitum* throughout 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 female animals was dosed at a level of 2000 mg/kg. All animals died.
A single group of 3 females was then dosed at the same dose level (2000 mg/kg). All animals died. This was a deviation from protocol, which indicated that the animals be dosed at a lower dose level. This deviation was due to the delayed mortality of the animals of the first step, which occurred towards the end of the observation period, when the second step was already planned.
Two groups of 3 females were subsequently dosed at 300 mg/kg.

4.2.1 Selection and allocation

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

Single groups of 3 females were allocated to the study as follows:

Dose level (mg/kg)	Step	Animal number Females
2000	1	13, 15, 17
2000	2	75, 77, 79
300	3	133, 135, 137
300	4	159, 161, 163

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 30 minutes, 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 2, 8 and 15. Animals found dead were weighed when found.

4.2.6 Termination and necropsy

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 (Tables 1, 2, 3, 4 and Appendix 1)

Following dosing of the first 3 female animals at a 2000 mg/kg, one animal was found dead on Day 10 and the remaining 2 animals died on Day 13 of the observation period. Reduced activity was noted on the day of dosing. No clinical signs were observed from Day 2 to Day 6. Thereafter, clinical signs included hunched posture, a thin and pale appearance, reduced activity, piloerection and reduced faeces.

Of the second 3 female animals dosed at the same level (2000 mg/kg), one animal was found dead on Day 10 and the remaining 2 animals died on Day 11.
All animals were prone on the day of dosing. No clinical signs were noted from Day 2 to Day 6. Thereafter, clinical signs observed were brown staining around the muzzle, hunched posture, reduced activity, a thin appearance, piloerection and red staining in the litter tray.

No mortality occurred and no clinical signs were noted in the first group of 3 female animals dosed at 300 mg/kg and observed for a period of 14 days.

No mortality occurred and no clinical signs were observed following dosing of the subsequent 3 female animals dosed at the same level (300 mg/kg) and observed for a period of 14 days

5.2 Body weight (Appendices 2 and 3)

Marked body weight losses were observed in all animals dosed at 2000 mg/kg during the first week of the observation period. Changes in body weight observed during the period of the study in the other animals were within the range expected for this strain and age of animal.

5.3 Necropsy (Table 5 and Appendix 4)

Three of the early decedent animals showed no internal abnormalities at necropsy examination. Of the three remaining decedents, one had abnormal contents in the stomach (brown fluid), one showed abnormal size of the thymus, spleen and the uterus, which also had abnormal contents (clear fluid) and staining of the muzzle. The remaining animal showed abnormal size of the thymus and the spleen which also had an abnormal colour (red). No abnormalities were observed in terminal kill animals.

6. CONCLUSION

These results indicate that the test item, [redacted] has a severe toxic effect in the rat following oral administration of a single dose at a level of 2000 mg/kg resulting in the mortality of all animals. The lack of mortality or other significant clinical signs, at 300 mg/kg, demonstrates the LD50 to be greater than 300 mg/kg, but less than 2000 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

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

TABLE 1.1 - Clinical signs - Step 1 - Incidence Table - Day 1

STUDY NO.: 15300-002

2000 mg/kg

Clinical Sign	Day ---->	1	1	1	1
	Session ---->	1	2	3	4
No significant signs		3/3	3/3	0/3	0/3
Reduced activity		0/3	0/3	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 30 minutes 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 - Step 1 - Incidence Table - Days 2 to 15

STUDY NO.: 15300-002

2000 mg/kg

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	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/0	0/0
Hunched posture	0/3	0/3	0/3	0/3	0/3	3/3	3/3	3/3	2/3	2/2	2/2	0/2	0/0	0/0
Thin	0/3	0/3	0/3	0/3	0/3	0/3	3/3	3/3	2/3	2/2	2/2	0/2	0/0	0/0
Pale	0/3	0/3	0/3	0/3	0/3	0/3	3/3	3/3	2/3	2/2	2/2	0/2	0/0	0/0
Reduced activity	0/3	0/3	0/3	0/3	0/3	0/3	0/3	3/3	2/3	2/2	2/2	0/2	0/0	0/0
Piloerection	0/3	0/3	0/3	0/3	0/3	0/3	0/3	3/3	2/3	2/2	2/2	0/2	0/0	0/0
Reduced faeces	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/2	2/2	0/2	0/0	0/0
Dead	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/2	0/2	2/2	0/0	0/0

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 - Step 2 - Incidence Table - Day 1

STUDY NO.: 15300-002

2000 mg/kg

Clinical Sign	Day ----> / Session ---->	1 / 1	1 / 2	1 / 3	1 / 4
No significant signs		3/3	0/3	0/3	0/3
Pronation		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 30 minutes 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 2.2 - Clinical signs - Step 2 - Incidence Table - Days 2 to 15

STUDY NO.: 15300-002

2000 mg/kg

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	1/3	0/3	0/3	0/3	0/2	0/0	0/0	0/0	0/0
Brown staining - muzzle	0/3	0/3	0/3	0/3	0/3	1/3	2/3	2/3	2/3	0/2	0/0	0/0	0/0	0/0
Hunched posture	0/3	0/3	0/3	0/3	0/3	2/3	3/3	3/3	2/3	0/2	0/0	0/0	0/0	0/0
Reduced activity	0/3	0/3	0/3	0/3	0/3	0/3	3/3	3/3	2/3	0/2	0/0	0/0	0/0	0/0
Thin	0/3	0/3	0/3	0/3	0/3	0/3	3/3	3/3	2/3	0/2	0/0	0/0	0/0	0/0
Piloerection	0/3	0/3	0/3	0/3	0/3	0/3	3/3	3/3	2/3	0/2	0/0	0/0	0/0	0/0
Red staining - litter tray	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	2/3	0/2	0/0	0/0	0/0	0/0
Dead	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	2/2	0/0	0/0	0/0	0/0

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 3.1 - Clinical signs - Step 3 - Incidence Table - Day 1

STUDY NO.: 15300-002

300 mg/kg

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 30 minutes 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 3.2 - Clinical signs - Step 3 - Incidence Table - Days 2 to 15

STUDY NO.: 15300-002

300 mg/kg

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 4.1 - Clinical signs - Step 4 - Incidence Table - Day 1

STUDY NO.: 15300-002

300 mg/kg

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 30 minutes 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 4.2 - Clinical signs - Step 4 - Incidence Table - Days 2 to 15

STUDY NO.: 15300-002

300 mg/kg

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 5 - Macroscopic observations - Group incidence

STUDY NO.: 15300-002

Dosage (mg/kg)	2000	2000	300	300
Step:	1	2	3	4
Number in group:	3	3	3	3
Whole animal				
No abnormalities detected	3	0	3	3
Head				
Staining	0	1	0	0
Spleen				
Abnormal size	0	2	0	0
Abnormal colour	0	1	0	0
Uterus				
Abnormal size	0	1	0	0
Abnormal contents	0	1	0	0
Thymus				
Abnormal size	0	2	0	0
Stomach				
Abnormal contents	0	1	0	0

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

APPENDIX 1 - Mortality - Individual data

STUDY NO.: 15300-002

Animal Number	Dosage	Sex	Study Phase	Date of Death	Day	Status	Terminal Body Weight (g)
15300017	2000 mg/kg	F	Dosing phase	26.Dec.02	10	Found dead	126.3
15300013	2000 mg/kg	F	Dosing phase	29.Dec.02	13	Found dead	113.2
15300015	2000 mg/kg	F	Dosing phase	29.Dec.02	13	Found dead	112.3
15300079	2000 mg/kg	F	Dosing phase	09.Jan.03	10	Found dead	136.6
15300075	2000 mg/kg	F	Dosing phase	10.Jan.03	11	Found dead	137.4
15300077	2000 mg/kg	F	Dosing phase	10.Jan.03	11	Found dead	148.5

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

APPENDIX 2 - Body weight (g) - Individual data

STUDY NO.: 15300-002

Animal Number		Day of Phase 1!	1"	2	8	15
STEP 1 - 2000 mg/kg						
15300013		192	186	189	136	-
15300015		201	177	185	135	-
15300017		199	185	192	137	-
	(n)	3	3	3	3	
	Mean	197.3	182.7	188.7	136.0	
	SD	4.7	4.9	3.5	1.0	
STEP 2 - 2000 mg/kg						
15300075		238	216	218	158	-
15300077		243	223	228	168	-
15300079		226	205	210	153	-
	(n)	3	3	3	2	
	Mean	235.7	214.7	218.7	159.7	
	SD	8.7	9.1	9.0	7.6	

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

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

APPENDIX 2 - Body weight (g) - Individual data

STUDY NO.: 15300-002

Animal Number		Day of Phase 1!	1"	2	8	15
STEP 3 -300 mg/kg						
15300133		212	191	202	209	218
15300135		196	176	195	213	224
15300137		212	190	205	213	222
	(n)	3	3	3	3	3
	Mean	206.7	185.7	200.7	211.7	221.3
	SD	9.2	8.4	5.1	2.3	3.1
STEP 4 - 300 mg/kg						
15300159		206	188	212	222	240
15300161		213	193	215	220	240
15300163		203	181	200	216	226
	(n)	3	3	3	3	3
	Mean	207.3	187.3	209.0	219.3	235.3
	SD	5.1	6.0	7.9	3.1	8.1

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

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

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

STUDY NO.: 15300-002

Animal Number		Day of Phase 2	Day of Phase 8	Day of Phase 15
STEP 1 - 2000 mg/kg				
15300013		3	-50	-
15300015		8	-42	-
15300017		7	-48	-
	(n)	3	3	
	Mean	6.0	-46.7	
	SD	2.6	4.2	
STEP 2 - 2000 mg/kg				
15300075		2	-58	-
15300077		5	-55	-
15300079		5	-52	-
	(n)	3	2	
	Mean	4.0	-55.0	
	SD	1.7	3.0	

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

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

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

STUDY NO.: 15300-002

Animal Number		Day of Phase 2	8	15
STEP 3 - 300 mg/kg				
15300133		11	18	27
15300135		19	37	48
15300137		15	23	32
	(n)	3	3	3
	Mean	15.0	26.0	35.7
	SD	4.0	9.8	11.0
STEP 4 - 300 mg/kg				
15300159		24	34	52
15300161		22	27	47
15300163		19	35	45
	(n)	3	3	3
	Mean	21.7	32.0	48.0
	SD	2.5	4.4	3.6

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

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

APPENDIX 4 - Macroscopic observations - Individual data

STUDY NO.: 15300-002

Animal number	Tissue / Observation(s)
STEP 1 - 2000 mg/kg	
15300013	Early decedent Whole animal No abnormalities detected
15300015	Early decedent No abnormalities detected
15300017	Ealry decedent Whole animal No abnormalities detected
STEP 2 - 2000 mg/kg	
15300075	Early decedent Whole animal Head Staining, red, muzzle Spleen, Abnormal size, small (25x5x2 mm) Uterus, Abnormal size distended (6 mm diam.) abnormal content, clear, fluid Thymus, Abnormal size, small

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

APPENDIX 4 - Macroscopic observations - Individual data

STUDY NO.: 15300-002

Animal number	Tissue / Observation(s)
15300077	Early decedent Spleen, Abnormal size, small (26x5x2 mm) Abnormal colour, dark Thymus, Abnormal size, small
15300079	Early decedent Stomach Abnormal content, brown, fluid

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

APPENDIX 4 - Macroscopic observations - Individual data

STUDY NO.: 15300-002

Animal number	Tissue / Observation(s)
STEP 3 - 300 mg/kg	
15300133	Whole animal No abnormalities detected
15300135	Whole animal No abnormalities detected
15300137	Whole animal No abnormalities detected
STEP 4 - 300 mg/kg	
15300159	Whole animal No abnormalities detected
15300161	Whole animal No abnormalities detected
15300163	Whole animal No abnormalities detected