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ACUTE DERMAL TOXICITY STUDY IN RATS

RBM EXP. No. 970593

EEC Guidelines (B.3)
OECD Guidelines (402)

Issued on March 24, 1998

SPONSOR

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

PERFORMING LABORATORY

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

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RBM Exp. No. 970593

TITLE OF THE STUDY

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

PURPOSE OF THE STUDY

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

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This report consists of 48 pages.

Ivrea, March 24, 1998

Dr. Ping Yu
RBM Study Director

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BIOSCIENCE

RBM Exp. No. 970593

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 dermal route in Sprague Dawley Crl: CD(SD) BR rat (RBM-Experiment No. 970593), 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 24, 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. 970593

QUALITY ASSURANCE STATEMENT

RBM Experiment number: 970593

Study title:

"Acute dermal 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.

Dates of inspection/audit	Dates of report to Study Director and Management
January 13, 1998 March 20 - 23, 1998	January 13, 1998 March 23, 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 23, 1998

Date : March 23, 1998

Enrico Invernizzi
Head of Quality Assurance Unit

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RBM MANAGEMENT DECLARATION OF GLP COMPLIANCE

Study No. 970593 entitled :

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

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

Dr. Ping Yu
RBM Study Director

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

Ivrea, March 27, 1998

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SCIENTISTS INVOLVED IN THE STUDY

STUDY No. 970593

"Acute dermal 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

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MATERIALS AND METHODS

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EXPERIMENTAL DESIGN

RBM Experiment No.:	970593
Test article:	[redacted]
Administration route:	epidermal
Exposure period:	about 24 hours
Duration of treatment period:	single administration
Duration of post-treatment observation period:	14 days after the 24-hour exposure period

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.3) and with Organization for Economic Cooperation and Development Guidelines (section 4, subpart 402, 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
Dosages administered	2000 mg/kg in 5 males and 5 females 1000, 500 and 200 mg/kg in 5 males/dose

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Body weight (at randomization):	Males: 230 - 304 g Females: 221 - 261 g
Age (at randomization):	no more than three months
Supplier:	Charles River Italia S.p.A. Via Indipendenza, 11 22050 CALCO (Lecco) Shipping slips No.s 8504 (December 12, 1997), 8353 (December 5, 1997), 597 (January 23,1998) and 793 (January 30,1998)
Acclimatation:	more than 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 declare 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 diet or drinking water.

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TEST ARTICLE IDENTIFICATION, CHARACTERIZATION AND FORMULATE

The test article was supplied by the Sponsor as follows:

Identification:	[redacted]
Batch:	19387/20
Characteristics:	white solid
Purity:	>99%
Manufacturing date:	December, 1997
Expiry date:	December, 2000
Storage conditions:	at room temperature

TEST DESCRIPTION

Administration route: epidermal

Reason for selection of administration route: possible accidental exposure in humans

Experimental design:

Dose mg/kg		Treatment date	Final killing
2000	males:	January 15, 1998	found dead
2000	females*:	January 23, 1998	found dead
1000	males:	February 6, 1998	February 28, 1998
500	males	February 27,1998	March 14, 1998
200	males	February 27, 1998	March 14, 1998

* 5 females were treated at the dose of 2000 mg/kg since there were no clinical signs observed in the males given the same dose during the first days of treatment.

Preparation of animals skin: approximately 24 hours before the test, fur was clipped from the dorsal and ventral area of the trunk of the test animals. Care was taken to avoid abrading the skin which could alter its permeability.
An area of about 6x5 cm of the body dorsal surface was cleared for the application of the test article.
This area corresponded to about 10% of the total body surface.

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Administration of the test article:	the test article was applied uniformly onto a porous gauze which was moistened with 0.9% NaCl. The treated area was covered with the porous gauze dressing fixed to the skin with hypoallergenic non-irritating tape. The test site was further covered in a suitable manner in order to ensure that the animals could not ingest the test substance. At the end of the exposure period the residual test article was wiped off with water.
Observation period:	14 days (for the 500 and 200 mg/kg groups) or 22 days (for the 1000 mg/kg group) after the 24-hour exposure period. All animals of the 2000 mg/kg group died within 15 days of dosing.
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 8, 15 and/or 22. Volume of administration was based on day 1 body weight.
Gross pathology:	on animals which died before the end of the study and on animals (fasted overnight) killed by excision of the femoral arteries, after i.p. overdosage anesthesia with 5% sodium pentobarbital, at the end of the observation period
Histology:	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.

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RECORD FILING

The protocol, a reserve sample of the test article used, the raw data bound in a register numbered 970593/1, 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 accomodation 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

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CLINICAL OBSERVATIONS

MORTALITY *(TABLE 1)*

The deaths which occurred in the various dose groups are shown below:

Dose (mg/kg)	200	500	1000	2000
Treated animals	5 M	5M	5M	5M + 5F
Mortality	0	2M	4M	5M+5F
Total (%)	0%	40%	80%	100%

The deaths occurred within 18 days of treatment, with the first case observed on 7 days after dosing in one male of the 2000 mg/kg group.

The LD_{50} was calculated to be 600 mg/kg with 95% confidence limits of 414 - 871 mg/kg.

CLINICAL SIGNS *(TABLE 2 AND APPENDIX 1)*

Hypoactivity, piloerection, hunched posture, skin and mucosae pallor and hypothermia were observed in animals of the higher dose groups (500 - 2000 mg/kg), starting on days 6-7 after dosing at 2000 mg/kg and on days 8-15 after dosing at the lower doses. Some animals of the highest dose group (2000 mg/kg) also showed sedation and perineum stained with urine.

In addition, changes at the treatment site including skin edema and erythema were found in animals of the 2000 mg/kg group.

Recovery of the clinical changes in the surviving animals was achieved by day 13 (500 mg/kg group) or by day 21 (1000 mg/kg group) of the observation period.

No changes of note were seen in animals given the test article at the lowest dose (200 mg/kg).

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BODY WEIGHT *(APPENDIX 2)*

Decrease in body weight was found in animals of the higher dose groups (2000 and 1000 mg/kg) during the study period. Body weights of animals in the lower dose groups were found to be unaffected by the test article administration.

POST-MORTEM EXAMINATION

GROSS PATHOLOGY *(TABLE 3 AND APPENDIX 3)*

At the autopsy of animals which died before the end of the observation period the macroscopic findings were liver paleness (2000 mg/kg group) or liver increased size (1000 and 500 mg/kg groups), congestion of stomach, decreased size and/or paleness of spleen and kidney medulla congestion. Moreover, skin edema (treatment site) was found in animals of the 2000 mg/kg group.

At the final killing increased size of liver was seen in animals of the 500 mg/kg group. No appreciable modifications were found in animals of the 200 mg/kg group.

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SUMMARY AND CONCLUSIONS

Experimental data from an acute toxicity study in which Sprague Dawley Crl:CD(SD) BR rats were treated by dermal 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.3) and with Organization for Economic Cooperation and Development Guideline (section 4, subpart 402, Paris 1981 and subsequent revisions).

The test article was applied uniformly onto a porous gauze which was moistened with 0.9% NaCl and then, this porous gauze was fixed to the dorsal and ventral area of trunk of the rats (fur was clipped 24 hours previously). The individual dosages were based on body weight taken just before treatment.

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 8, 15 and/or 22. They were clinically observed for 14 days (for the 200 and 500 mg/kg groups) or 22 days (for the 1000 mg/kg group; all 2000 mg/kg rats died within 15 days) after the 24-hour exposure period. Necropsy examination was performed on all animals which died before the end of the study. On day 16 or day 23 the surviving rats were killed (fasted overnight) by excision of the femoral arteries after i.p. overdosage anesthesia with 5% sodium pentobarbital and were submitted to a thorough autopsy.

The deaths which occurred in the various dose groups are showen below:

Dose (mg/kg)	200	500	1000	2000
Treated animals	5 M	5M	5M	5M + 5F
Mortality	0	2M	4M	5M+5F
Total (%)	0%	40%	80%	100%

The deaths occurred within 18 days of treatment, with the first case observed on 7 days after dosing in one male of the 2000 mg/kg group.

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The LD_{50} was calculated to be 600 mg/kg with 95% confidence limits of 414 - 871 mg/kg.

Hypoactivity, piloerection, hunched posture, skin and mucosae pallor and hypothermia were observed in animals of the higher dose groups (500 - 2000 mg/kg), starting on days 6-7 after dosing at 2000 mg/kg and on days 8-15 after dosing at the lower doses. Some animals of the highest dose group (2000mg/kg) also showed sedation and perineum stained with urine. In addition, local changes including skin edema and erythema (treatment site) were found in animals of the 2000 mg/kg group.

Recovery of the clinical changes in the surviving animals was achieved by day 13 (500 mg/kg group) or by day 21 (1000 mg/kg group).

No changes of note were seen in animals given the test article at the lowest dose (200 mg/kg).

Decrease in body weight was found in animals of the higher dose groups (2000 and 1000 mg/kg) during the study period. Body weights of animals in the lower dose groups were found to be unaffected by the test article administration.

At the necropsy of animals which died before the end of the observation period, the main macroscopic findings were liver paleness (2000 mg/kg group) or liver increased size (1000 and 500 mg/kg groups). Moreover, skin edema (treatment site) was found in animals of the 2000 mg/kg group.

At the final killing, increased size of liver was seen in animals of the 500 mg/kg group. No appreciable modifications were found in animals of the 200 mg/kg group.

In conclusion, the **LD_{50}** of the test article [redacted], when administered by dermal route to the rats, was **600 mg/kg with 95% confidence limits of 414 - 871 mg/kg.**

The compound induced delayed toxicity (liver was mainly involved) and local changes (treatment site) which were confined to the animals treated at the higher doses.

Dr. Ping Yu

RBM Study Director

March 24, 1998

Dr. Roberto Maraschin

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

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GROUP DATA

[redacted] 160

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Males - Females

Dose (mg/kg)	200	500	1000	2000
Treated animals	5	5	5	10
Day 7	0	0	0	1
8	0	0	0	1
9	0	0	0	1
10	0	0	0	1
12	0	0	0	1
13	0	2	0	1
14	0	0	0	2
15	0	0	1	2
18	0	0	3	0
Total no. (day 22)	0	2	4	10
Total (%)	.0%	40.0%	80.0%	100.0%

Median lethal dose (LD50) = 600.30

95% confidence limits = 413.92 - 870.61

Slope (SE) = 1.91 .51

Heterogeneity P = .959 NS

Linear regression y =-7.1954+1.9063x

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Males

Dose (mg/kg)	200	500	1000	2000
no. of treated animals	5	5	5	5
Death	-	2 13d	4 15d-18d	5 7d-14d
Sedation	-	-	-	2 7d- 8d
Hypoactivity	-	2 11d-12d	5 8d-17d	3 6d-13d
Piloerection	-	2 11d-12d	5 8d-20d	3 6d-13d
Hunched posture	-	2 11d-12d	5 8d-20d	3 6d-13d
Skin and app. mucosae, pallor	-	2 12d-12d	4 15d-18d	2 6d-13d
Hypothermia	-	2 12d-12d	4 15d-17d	2 6d-13d
Skin treatment site: edema	-	-	-	5 2d-13d

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

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Males

Dose (mg/kg)	200	500	1000	2000
no. of treated animals	5	5	5	5
Skin treatment site: erythema	-	-	-	3 6d- 9d
Perineum stained with urine	-	-	-	1 6d- 6d
Recovery	-	3 13d	1 21d	-

[redacted]

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

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Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

TABLE 2. - Clinical signs (maximum daily frequency) (p. 3)
(no. of animals affected, from-to)

Females

Dose (mg/kg)	2000
no. of treated animals	5
Death	5 8d-15d
Sedation	1 12d-12d
Hypoactivity	5 6d-14d
Piloerection	5 6d-14d
Hunched posture	5 6d-14d
Skin and app. mucosae, pallor	1 7d- 9d
Hypothermia	1 7d- 9d
Skin treatment site: edema	5 2d-13d

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

[redacted]

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Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

TABLE 2. - Clinical signs (maximum daily frequency) (p. 4)
(no. of animals affected, from-to)

Females

Dose (mg/kg)	2000
no. of treated animals	5
Skin treatment site: erythema	1 7d-10d
Perineum stained with urine	2 6d-14d

[redacted]

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

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Dead or agonal sacrificed an. Males

Dose (mg/kg)	200	500	1000	2000
no. of animals	0	2	4	5
no. of animals without appreciable lesions	0	0	0	0
General observation				
cannibalized	-	0	1 25.00%	0
Kidneys				
medulla, congestion	-	0	3(2.3) 75.00%	3(2.0) 60.00%
Liver				
increased size	-	2(2.5) 100.00%	3(2.3) 75.00%	0
pale	-	0	0	5(2.8) 100.00%
Skin treatment area				
edema	-	0	0	4(2.0) 80.00%

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

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Dead or agonal sacrificed an. Males

Dose (mg/kg)	200	500	1000	2000
no. of animals	0	2	4	5
no. of animals without appreciable lesions	0	0	0	0
Spleen				
decreased size	-	0	3(2.0) 75.00%	5(2.0) 100.00%
Stomach				
congestion	-	0	2(2.0) 50.00%	0

[redacted]

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

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Final killing	Males			
Dose (mg/kg)	200	500	1000	2000
no. of animals	5	3	1	0
no. of animals without appreciable lesions	5	0	0	0
Liver				
increased size	0	3(2.0) 100.00%	1(2.0) 100.00%	-

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

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Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Dead or agonal sacrificed an.	Females
Dose (mg/kg)	2000
no. of animals	5
no. of animals without appreciable lesions	0
Kidneys	
medulla, congestion	3(2.0) 60.00%
Liver	
pale	5(2.6) 100.00%
Skin treatment area	
edema	2(2.0) 40.00%
Spleen	
decreased size	3(2.7) 60.00%

[redacted]

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Severity : 0(very slight) 1(slight) 2(moderate) 3(severe)

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Test article: [redacted]
Title : Acute dermal toxicity study in rats
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TABLE 3. - Gross pathology examination (p. 5)
(no. of cases, mean severity, %)

Dead or agonal sacrificed an.	Females
Dose (mg/kg)	2000
no. of animals	5
no. of animals without appreciable lesions	0
Stomach	
congestion	1(2.0) 20.00%

[redacted]

Severity : 0(very slight) 1(slight) 2(moderate) 3(severe)

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APPENDICES

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Dose (mg/kg) 200

Cage # 7M	Day	1				2		3		4		5		6		7		8		9		10		11		12		13		14		15	
	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
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	5	5

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

[redacted]

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Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Dose (mg/kg) 500

Cage # 5M	Day	1				2		3		4		5		6		7		8		9		10		11		12		13		14		15	
	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
Death																												2					
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	3	3	3	3	3	3	3	3	3	3
Hypoactivity																								2	2	2	2						
Piloerection																								2	2	2	2						
Hunched posture																								2	2	2	2						
Skin and app. mucosae, pallor																										2	2						
Hypothermia																										2	2						

[redacted]

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

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Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Dose (mg/kg) 1000

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

Cage # 3M (follows)	Day	18		19		20		21		22	
	Time	M	A	M	A	M	A	M	A	M	A
Death		3									
No clinical signs								1	1	1	1
Piloerection		1	1	1	1	1	1				
Hunched posture		1	1	1	1	1	1				
Skin and app. mucosae, pallor		1	1								

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

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Dose (mg/kg) 2000

Cage # 1M	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
Death																1				1						1				2
No clinical signs		5	5	5	5																									
Sedation																2	2	1	1											
Hypoactivity														1	1			1	1	1	1	3	3	3	3	2	2	2	2	
Piloerection														1	1	2	2	2	2	2	2	3	3	3	3	2	2	2	2	
Hunched posture														2	2	2	2	2	2	2	2	3	3	3	3	2	2	2	2	
Skin and app. mucosae, pallor														1	1									1	1			2	2	
Hypothermia														1	1									1	1			2	2	
Skin treatment site: edema						5	5	5	5	5	5	5	5	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Skin treatment site: erythema														3	3	2	2	2	2	1	1									
Perineum stained with urine														1	1															

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

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

APPENDIX 1. - Clinical signs incidence (p. 5)
(no. of animals affected)

Dose (mg/kg) 2000

Cage # 2F	Day	1				2		3		4		5		6		7		8		9		10		11		12		13		14		15
	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
Death																		1				1						1				2
No clinical signs		5	5	5	5																											
Sedation																										1	1					
Hypoactivity														1	1	5	5	4	4	4	4	3	3	3	3	2	2	2	2	2	2	
Piloerection														2	2	5	5	4	4	4	4	3	3	3	3	3	3	2	2	2	2	
Hunched posture														1	1	5	5	4	4	4	4	3	3	3	3	2	2	2	2	2	2	
Skin and app. mucosae, pallor																1	1			1	1											
Hypothermia																1	1			1	1											
Skin treatment site: edema						5	5	5	5	5	5	5	5	5	5	2	2	2	2	2	2	2	2	2	2	2	2	2	2			
Skin treatment site: erythema																1	1	1	1	1	1	1	1									
Perineum stained with urine														2	2	2	2	1	1	2	2	1	1	1	1	2	2	2	2	2	2	

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

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Dose (mg/kg) 200

Week	day	Animal # 31M	32M	33M	34M	35M
	0	230	230	230	230	230
1	1	235	231	232	230	231
2	8	295	291	298	290	286
3	15	349	329	320	326	315

176 971

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Dose (mg/kg) 500

Week	day	Animal # 21M	22M	23M	24M	25M
	0	236	232	230	232	234
1	1	241	236	234	237	238
2	8	278	253	269	255	254
3	15		278	294	269	

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Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Dose (mg/kg) 1000

Animal #		11M	12M	13M	14M	15M
Week	day					
	0	295	296	294	272	250
1	1	300	308	310	280	286
2	8	228	258	227	215	263
3	15	167	177	167		188
4	22					261

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Dose (mg/kg) 2000

Animal #		1M	2M	3M	4M	5M	6F	7F	8F	9F	10F
Week	day										
	0	296	289	270	286	304	237	261	221	222	222
1	1	302	294	279	285	310	249	258	225	216	229
2	8	233	235	191	239		185		155	201	170

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Dead or agonal sacrificed an.

Dose (mg/kg) 500

An#	Death day/code#	TISSUE	Gross observations
21M	13 M2	Liver	increased size, diffuse, severe
25M	13 M2	Liver	increased size, diffuse, moderate

Death code : M2 (Natural death)

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Dead or agonal sacrificed an.

Dose (mg/kg) 1000

An#	Death day/code#	TISSUE	Gross observations
11M	18 M2	Kidneys	medulla, congestion, diffuse, moderate
		Liver	increased size, diffuse, moderate
		Spleen	decreased size, diffuse, moderate
		Stomach	congestion, diffuse, moderate
12M	18 M2	Kidneys	medulla, congestion, diffuse, moderate
		Liver	increased size, diffuse, moderate
		Spleen	decreased size, diffuse, moderate
13M	18 M2	General observation	cannibalized
14M	15 M2	Kidneys	medulla, congestion, diffuse, severe
		Liver	increased size, diffuse, severe
		Spleen	decreased size, diffuse, moderate
		Stomach	congestion, diffuse, moderate

Death code : M2(Natural death)

REDACTED AS TO TRADE NAMES

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Dead or agonal sacrificed an.

Dose (mg/kg) 2000

An#	Death day/code#	TISSUE	Gross observations
1M	14 M2	Liver	pale, diffuse, severe
		Skin treatment area	edema, diffuse, moderate
		Spleen	decreased size, diffuse, moderate
2M	12 M2	Kidneys	medulla, congestion, diffuse, moderate
		Liver	pale, diffuse, severe
		Skin treatment area	edema, diffuse, moderate
		Spleen	decreased size, diffuse, moderate
3M	9 M2	Kidneys	medulla, congestion, diffuse, moderate
		Liver	pale, diffuse, moderate
		Skin treatment area	edema, diffuse, moderate
		Spleen	decreased size, diffuse, moderate
4M	14 M2	Liver	pale, diffuse, severe
		Spleen	decreased size, diffuse, moderate

Death code : M2 (Natural death)

1/82-183

REDACTED AS TO TRADE NAMES

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Dead or agonal sacrificed an.

Dose (mg/kg) 2000

An#	Death day/code#		TISSUE	Gross observations
5M	7	M2	Kidneys	medulla, congestion, diffuse, moderate
			Liver	pale, diffuse, severe
			Skin treatment area	edema, diffuse, moderate
			Spleen	decreased size, diffuse, moderate
6F	15	M2	Liver	pale, diffuse, moderate
			Spleen	decreased size, diffuse, severe
7F	8	M2	Kidneys	medulla, congestion, diffuse, moderate
			Liver	pale, diffuse, severe
			Skin treatment area	edema, diffuse, moderate
8F	10	M2	Kidneys	medulla, congestion, diffuse, moderate
			Liver	pale, diffuse, severe
			Skin treatment area	edema, diffuse, moderate
			Spleen	decreased size, diffuse, moderate

Death code : M2 (Natural death)

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Dead or agonal sacrificed an.

Dose (mg/kg) 2000

An#	Death day/code#		TISSUE	Gross observations
8F	10	M2	Stomach	congestion, diffuse, moderate
9F	13	M2	Liver	pale, diffuse, moderate
10F	15	M2	Kidneys	medulla, congestion, diffuse, moderate
			Liver	pale, diffuse, severe
			Spleen	decreased size, diffuse, severe

Death code : M2 (Natural death)

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Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

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

Final killing

Dose (mg/kg) 200

An#	Death day	T I S S U E	Gross observations
31M	16	General observation	no macroscopically appreciable lesions
32M	16	General observation	no macroscopically appreciable lesions
33M	16	General observation	no macroscopically appreciable lesions
34M	16	General observation	no macroscopically appreciable lesions
35M	16	General observation	no macroscopically appreciable lesions

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

APPENDIX 3. - Gross pathology examination (p. 7)
(individual)

Final killing

Dose (mg/kg) 500

An#	Death day	T I S S U E	Gross observations
22M	16	Liver	increased size, diffuse, moderate
23M	16	Liver	increased size, diffuse, moderate
24M	16	Liver	increased size, diffuse, moderate

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

APPENDIX 3. - Gross pathology examination (p. 8)
(individual)

Final killing

Dose (mg/kg) 1000

An#	Death day	T I S S U E	Gross observations
15M	23	Liver	increased size, diffuse, moderate

REDACTED AS TO TRADE NAMES

RESEARCH TOXICOLOGY CENTRE - ROMA

[redacted]

ACUTE DERMAL IRRITATION STUDY IN THE RABBIT

FINAL REPORT

RTC Study Number: 8835-006

RTC Report Number: 8835-006/T/183/2002

Sponsor:
AUSIMONT S.p.A.
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Partita IVA 00920611001

RTC Report Number: 8835-006/T/183/2002

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 : 08-08-2002

J. Brightwell, Ph.D.
(Scientific Director): Date : 9.08.2002

RTC Report Number: 8835-006/T/183/2002

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	17.07.2001	17.07.2001	17.07.2001
PROCESS-BASED INSPECTIONS			
Dose preparation	18.04.2002	-	02.05.2002
Body weight	19.03.2002	-	29.04.2002
Dosing	09.04.2002	-	29.04.2002
Clinical observations	15.02.2002	-	19.03.2002
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 09.08.2002

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

09.08.2002
Date

Contents

Tables

1. SUMMARY

The acute dermal irritation of [redacted] was investigated in the rabbit.

A 0.5 ml aliquot of the substance was applied to the prepared dorsal skin of 3 animals for a period of 4 hours. The resulting reaction to treatment was assessed 1, 24, 48 and 72 hours after the end of the exposure period.

No irritation was apparent following a 4 hour period of exposure to the test item.

There was no indication of a systemic effect of treatment.

Body weight changes were not remarkable.

These results indicate that [redacted] has no irritant effect on the skin of the rabbit and European Directives concerning the classification, packaging and labelling of dangerous substances (67/548/EEC and subsequent revisions) would indicate the following:-

Classification : Not required
Symbol : None indicated
R Phrase : None indicated

2. INTRODUCTION

The purpose of this study was to investigate the degree of irritation produced on the intact skin of the rabbit following 4 hours contact with the substance. This allowed hazard assessment as required by European Directives concerning the classification, packaging and labelling of dangerous substances (67/548/EEC and subsequent revisions).

The procedures used were designed to meet the requirements of the test for acute dermal irritation described by OECD guideline Number 404, adopted on 17th July 1992. These methods are in agreement with those of B4 detailed in COM(93)638, a compilation of Council Directive 67/548/EEC. The rabbit 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 1st June 2001 with signing of the protocol by the Study Director. The experimental work described in this report started on 8th April 2002 with allocation of animals to the study and ended on 12th April 2002 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 were as follows:

Name	:	[redacted]
Lot or Batch Number	:	90215/91
Expiry date	:	1st February 2004
Purity	:	90%
Concentration of active ingredient	:	5% in water
Received from	:	AUSIMONT S.p.A.
Date received	:	11th February 2002
Amount received	:	2000 grams
Description	:	Colourless liquid
Container	:	Opaque plastic bottle
Storage at RTC	:	Ambient condition
RTC reference number	:	6535

Detailed characterisation of the test item 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 test item was the responsibility of the Sponsor. An aliquot of the test item was taken and will be retained within the RTC archives for a period of 10 years prior to disposal.

The test item was used in the condition 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 of all activities relating to the day by day conduct and maintenance of the study were made.

4.1 Animals, husbandry and diet

4.1.1 Animals

Female rabbits of the New Zealand White strain were ordered from, and supplied by, Charles River Italia S.p.A., (Como) and bred by P.O.A.D.A., Mandello Lario, (CO), Italy and were delivered to the testing facility on 28th March 2002. Animals were ordered weighing approximately 2 kg and 9 to 11 weeks of age, nulliparous and non-pregnant.

Animals were examined following arrival and identified in the ear by tattoo with an individual number. An acclimatisation period of at least 10 days was allowed before dosing. The health status of animals was assessed during this time. Following arrival the animals were treated with Pyrantel 6% at a dose level of 0.4 ml/animal.

4.1.2 Housing

Animals were individually housed in stainless steel cages measuring 69 x 45 x 51 cm and equipped with grid floors. Cages were suspended over trays and each tray held 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. This colour coding matched the corresponding colour coded formulation container.

Animal room controls were set to maintain temperature within the range of 17 to 21°C and relative humidity within the range of 40 to 70%. This was a deviation from the study protocol, in which a range of 22 ± 2°C was erroneously indicated. Actual conditions were recorded.

Artificial lighting by fluorescent tubes was set to a 24 hour cycle of 12 hours light/12 hours dark.

4.1.3 Water and diet

Animals were offered drinking water supplied to each cage via water bottles and a commercially available anti-biotic free pelleted laboratory diet (Altromin MSK, Altromin, D-32770 Lage, Postfach 1120, Germany) *ad libitum* throughout the study.

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

4.2 Animal selection and preparation

Animals were selected for treatment from available stock. The day before dosing commenced the dorsal surfaces of the trunk of each animal, on both sides of the mid-line, were clipped free of hair using an electric clipper equipped with a suitable blade. Care was taken to avoid damage to the skin.

4.3 Dosing

Each selected animal was removed from its cage and gently restrained. A 0.5 ml aliquot of the test item was spread evenly over a gauze square measuring 2.5x2.5 cm. The gauze square was then placed onto the animal's skin with the test item in direct contact with the skin. A strip of aluminium foil was placed over the treated site and the whole assembly held in place by encircling the trunk of the animal with a length of elastic adhesive bandage, this forming a semi-occlusive barrier.

After a period of 4 hours, the adhesive bandage and gauze patch were removed from the treated site of each animal which was cleaned by gentle swabbing of the skin with cotton wool soaked in water at approximate body temperature.

4.4 Observations

The treated skin site on each animal was examined approximately 1 hour after the end of the exposure period. Additional examinations were performed 24, 48 and 72 hours after dosing.

Animals were examined under standard conditions and any observed irritation, in comparison with adjacent untreated skin, was allocated a numerical value based on the table below.

Erythema and eschar formation	**Value**
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to eschar formation preventing grading of erythema	4

Oedema formation	**Value**
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

4.5 Body weight

All animals were weighed on preparation (Day -1) and on termination of the study (Day 4).

4.6 Termination

The study was terminated after 72 hours, the objectives having been achieved.

After termination animals were killed by the intravenous injection of a suitable anaesthetic agent. No necropsy examination was undertaken.

4.7 Classification

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

The numerical scores obtained on assessing irritation at the 24, 48 and 72 hour examinations were summed and a mean calculated for each animal. The values for erythema and eschar formation were calculated separately from those obtained on assessing oedema. When the mean value for either erythema or oedema equalled or exceeded 2.0, in two or more animals, the test item would be considered irritant to the skin. Labelling would then be required with the risk phrase (R 38) "Irritating to the skin" and symbol "Xi".

4.8 Archives

All 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 regarding despatch or disposal of the material.

5. RESULTS

5.1 Irritation (Tables 1 and 2)

No irritation or other reaction was apparent on the treated skin of any animal.

5.2 Systemic effects

There was no indication of a systemic effect of treatment.

5.3 Body weight (Table 3)

Changes in body weight during the course of the study were not remarkable.

6. CONCLUSION

The results of this study indicate that the test item, [redacted], has no irritant effect on the skin of the rabbit.

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

Classification : Not required
Symbol : None indicated
R Phrase : None indicated

ACUTE DERMAL IRRITATION STUDY IN THE RABBIT

RTC STUDY NUMBER: 8835-006

TABLE 1 - IRRITATION - MEAN VALUES

Animal Number	Erythema	Oedema
321	0.0	0.0
323	0.0	0.0
325	0.0	0.0

The mean score recorded for each animal is the average of the individual scores observed at the 24, 48 and 72 hours examinations

ACUTE DERMAL IRRITATION STUDY IN THE RABBIT

RTC STUDY NUMBER: 8835-006

TABLE 2 - IRRITATION - INDIVIDUAL FINDINGS

Animal Number: 321			
Time of examination	Erythema	Oedema	Additional comments
1 hour	0	0	-
24 hours:	0	0	-
48 hours:	0	0	-
72 hours:	0	0	-

Animal Number: 323			
Time of examination	Erythema	Oedema	Additional comments
1 hour	0	0	-
24 hours:	0	0	Scab on ears
48 hours:	0	0	Scab on ears
72 hours:	0	0	Scab on ears

Animal Number: 325			
Time of Examination	Erythema	Oedema	Additional comments
1 hour	0	0	-
24 hours:	0	0	-
48 hours:	0	0	-
72 hours:	0	0	-

ACUTE DERMAL IRRITATION STUDY IN THE RABBIT

RTC STUDY NUMBER: 8835-006

TABLE 3 - BODY WEIGHT - INDIVIDUAL VALUES

Animal Number	Body weight (kg) on Day:-		Change in body weight (kg)
	Day –1	Day 4	Day –1 to 4
321	2.4	2.5	0.1
323	2.6	2.7	0.1
325	2.6	2.6	0.0

REDACTED AS TO TRADE NAMES

RESEARCH TOXICOLOGY CENTRE - ROMA

[redacted] ACUTE DERMAL TOXICITY STUDY IN THE RAT

FINAL REPORT

RTC Study Number: 8833-006

RTC Report Number: 8833-006/T/217/2002

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RTC S.p.A.
Capitale sociale 10.000.000.000
C.C.I.A.A. n. 375376
Reg. Soc. Trib. di Roma n. 2828/72
Cod. Fisc. 00653120584
Partita IVA 00920611001

RTC Report Number: 8833-006/T/217/2002

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 : 09-08-2002

J. Brightwell, Ph.D.
(Scientific Director): Date : 9.08.2002

RTC Report Number: 8833-006/T/217/2002

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 **PROTOCOL AMENDMENT (1) CHECK**	30.07.2001 08.08.2002	30.07.2001 08.08.2002	30.07.2001 08.08.2002
PROCESS-BASED INSPECTIONS			
Allocation	22.05.2002	-	19.06.2002
Dose preparation	18.04.2002	-	02.05.2002
Body weight	15.03.2002	-	23.04.2002
Dosing (dermal)	18.04.2002	-	29.04.2002
Clinical observations	17.05.2002	-	18.07.2002
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 09.08.2002

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

09.08.2002
Date

Contents

1. SUMMARY

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

A single dose of 2000 mg/kg was administered to a group of 5 male and 5 female animals for a 24 hour period. A 14 day period followed after which all animals were killed and subjected to a necropsy examination.

No mortality occurred following dosing and no signs of systemic toxicity were noted.

Changes in body weight were generally within the expected range.

Necropsy examination revealed no abnormalities.

These results indicate that the test item, [redacted] has no systemic toxic effect in the rat following dermal exposure over a 24 hour period at a level of 2000 mg/kg. European Directives concerning the classification, packaging and labelling of dangerous substances would indicate the following:-

Classification : Not required
Symbol : None indicated
R Phrase : None indicated

2. INTRODUCTION

The purpose of this study was to assess the acute toxicity of the substance following dermal administration of a single dose to the rat. This allowed hazard assessment as required by European Directives concerning the classification, packaging and labelling of dangerous substances (67/548/EEC and subsequent revisions).

The procedures used were designed to meet the requirements of the test for acute dermal toxicity described in OECD guideline Number 402, adopted on 24th February 1987. Methods were in agreement with European Directives described by COM(93)638, a compilation of Council Directive 67/548/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 1st June 2001 with signing of the protocol by the Study Director. The experimental work described in this report started on 17th April 2002 with allocation of animals to treatment and ended on 2nd May 2002 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 were as follows:

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

Detailed characterisation of the test item 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 test item was the responsibility of the Sponsor. A certificate of analysis, supplied by the Sponsor, can be found in Addendum 1 of this report. An aliquot of the test item was taken and will be retained within the RTC archives for a period of 10 years prior to disposal.

The test item was used in the condition 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 rats of the Hsd: Sprague Dawley SD strain were ordered from and supplied by 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 with female animals nulliparous and non-pregnant. They appeared to be in an acceptable condition following arrival on 5th April 2002. A pre-dose acclimatisation period of at least 5 days was allowed during which time the health status of the animals was assessed. Following arrival animals were identified by a combination of ear notch and tattoo on the feet.

4.1.2 Animal husbandry

Animals were individually housed in polycarbonate cages measuring 42 x 26 x 18 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. This colour coding matched the corresponding colour coded formulation container.

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

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

4.1.3 Water and diet

Animals were offered drinking water supplied to each cage via a water bottle and a commercially available laboratory rodent diet (Altromin MT, Altromin, D-32770 Lage, Postfach 1120, Germany) *ad libitum* throughout the study.

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 5 male and 5 female animals were dosed at a level of 2000 mg/kg.

4.2.1 Selection and animal preparation

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

A single group of 5 males and 5 females were allocated to the study as follows:-

Dose level (mg/kg)	Animal number	
	Males	Females
2000	22,24,26,28,30	21,23,25,27,29

All animals were within a body weight range of 209 to 284 grams when prepared for dosing. The fur was removed from the dorsal surfaces of the trunk over an area estimated to be at least 10% of the total body surface of each animal. An electric clipper with suitable blade was used and care was taken to avoid any irritation or damage to the skin.

4.2.2 Dosing

On Day 1 of the study, the amount of supplied test item to be administered, at a dose level of 2000 mg/kg body weight, was calculated for each animal according to body weight. This was spread evenly over a gauze patch the size of the treatment site. The gauze patch was then placed onto the animal's skin, with the test substance in direct contact with the skin. A strip of aluminium foil was placed over the treated site and the whole assembly held in place by encircling the trunk of the animal with a length of elastic adhesive bandage. All animals were treated in the same manner.

After a period of 24 hours, the adhesive bandage and gauze dressings were removed. The treated skin was washed gently with warm water to remove residual test item.

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 and 4 hours after dosing and daily thereafter for a total of 14 days.

4.2.5 Body weight

All animals were weighed on allocation to the study (Day -1), immediately prior to dosing (Day 1) and at weekly intervals thereafter (Days 8 and 15).

4.2.6 Termination

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

They 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. Particular attention was paid to the treated site.

4.3 Classification

The results obtained on testing were used to classify the test item 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)

No mortality occurred following dosing. Clinical signs were limited to staining around the urogenital region in one male animal on days 1 and 2 of the observation period. Observations of the treated site showed the presence of erythema in one female on days 6 to 9.

5.2 **Body weight** (Table 2)

Changes in body weight were within the expected range for the male animals of this age and strain. A slight reduction in body weight gain and, in a single animal a decrease in body weight, were observed in the females.

5.3 **Necropsy** (Table 3)

No abnormalities were found on necropsy of animals on termination of the study.

6. CONCLUSION

The results of this study indicate that the test item, [redacted], has no systemic toxic effect in the rat following dermal exposure over a 24 hour period at a level of 2000 mg/kg.

European Directives concerning the classification, packaging and labelling of dangerous substances would indicate the following:-

Classification : Not required
Symbol : None indicated
R Phrase : None indicated

ACUTE DERMAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8833-006

TABLE 1 - CLINICAL SIGNS

DOSE LEVEL: 2000 mg/kg

MALES - Number of animals with signs (Number of animals dosed = 5)

Sign observed	Day 1 Time 0	1	2	3	Day 2	3	4	5	6	7
No abnormalities detected	5	4	4	4	4	5	5	5	5	5
Staining - urogenital region	0	1	1	1	1	0	0	0	0	0
MORTALITY	0	0	0	0	0	0	0	0	0	0

Sign observed	Day 8	9	10	11	12	13	14	15
No abnormalities detected	5	5	5	5	5	5	5	5
MORTALITY	0	0	0	0	0	0	0	0

KEY Day 1 : Time 0 : At dosing
Time 1 : Approximately 1 hour after dosing
Time 2 : Approximately 2 hours after dosing
Time 3 : Approximately 4 hours after dosing

ACUTE DERMAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8833-006

TABLE 1 - Continued

DOSE LEVEL: 2000 mg/kg

FEMALES - Number of animals with signs (Number of animals dosed = 5)

Sign observed	Day 1 Time 0	1	2	3	Day 2	3	4	5	6	7
No abnormalities detected	5	5	5	5	5	5	5	5	4	4
Erythema - treated site	0	0	0	0	0	0	0	0	1	1
MORTALITY	0	0	0	0	0	0	0	0	0	0

Sign observed	Day 8	9	10	11	12	13	14	15
No abnormalities detected	4	4	5	5	5	5	5	5
Erythema - treated site	1	1	0	0	0	0	0	0
MORTALITY	0	0	0	0	0	0	0	0

KEY Day 1 : Time 0 : At dosing
Time 1 : Approximately 1 hour after dosing
Time 2 : Approximately 2 hours after dosing
Time 3 : Approximately 4 hours after dosing

ACUTE DERMAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8833-006

TABLE 2 - BODY WEIGHT

DOSE LEVEL: 2000 mg/kg

Sex	Animal identity number	Body weight (g) on day				Change in body weight (g)
		-1	1	8	15	Days 1 - 15
	22	271	280	302	327	47
M	24	254	259	290	312	53
A	26	284	295	296	337	42
L	28	278	288	304	325	37
E	30	271	282	303	324	42
S						
	Mean	271.6	280.8	299.0	325.0	44.2
	S.Dev.	11.2	13.5	5.9	8.9	6.1
	21	223	228	229	249	21
F	23	209	219	219	235	16
E	25	223	230	233	210	-20
M	27	213	217	220	233	16
A	29	223	225	236	243	18
L						
E	Mean	218.2	223.8	227.4	234.0	10.2
S	S.Dev.	6.7	5.6	7.6	14.9	17.0

ACUTE DERMAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8833-006

TABLE 3 - NECROPSY

DOSE LEVEL: 2000 mg/kg

Sex	Animal number	Tissue/ organ	Finding
MALES	22		Terminal kill No abnormalities found
	24		Terminal kill No abnormalities found
	26		Terminal kill No abnormalities found
	28		Terminal kill No abnormalities found
	30		Terminal kill No abnormalities found
FEMALES	21		Terminal kill No abnormalities found
	23		Terminal kill No abnormalities found
	25		Terminal kill No abnormalities found
	27		Terminal kill No abnormalities found
	29		Terminal kill No abnormalities found

ACUTE DERMAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8833-006

ADDENDUM 1 - CERTIFICATE OF ANALYSIS FOR THE TEST ITEM

AUSIMONT SpA

Bollate, 30 gennaio 2002

Certificato di analisi

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

Caratteristiche del precursore acido:

Peso equivalente:	560
Metodo:	titolazione acidimetrica