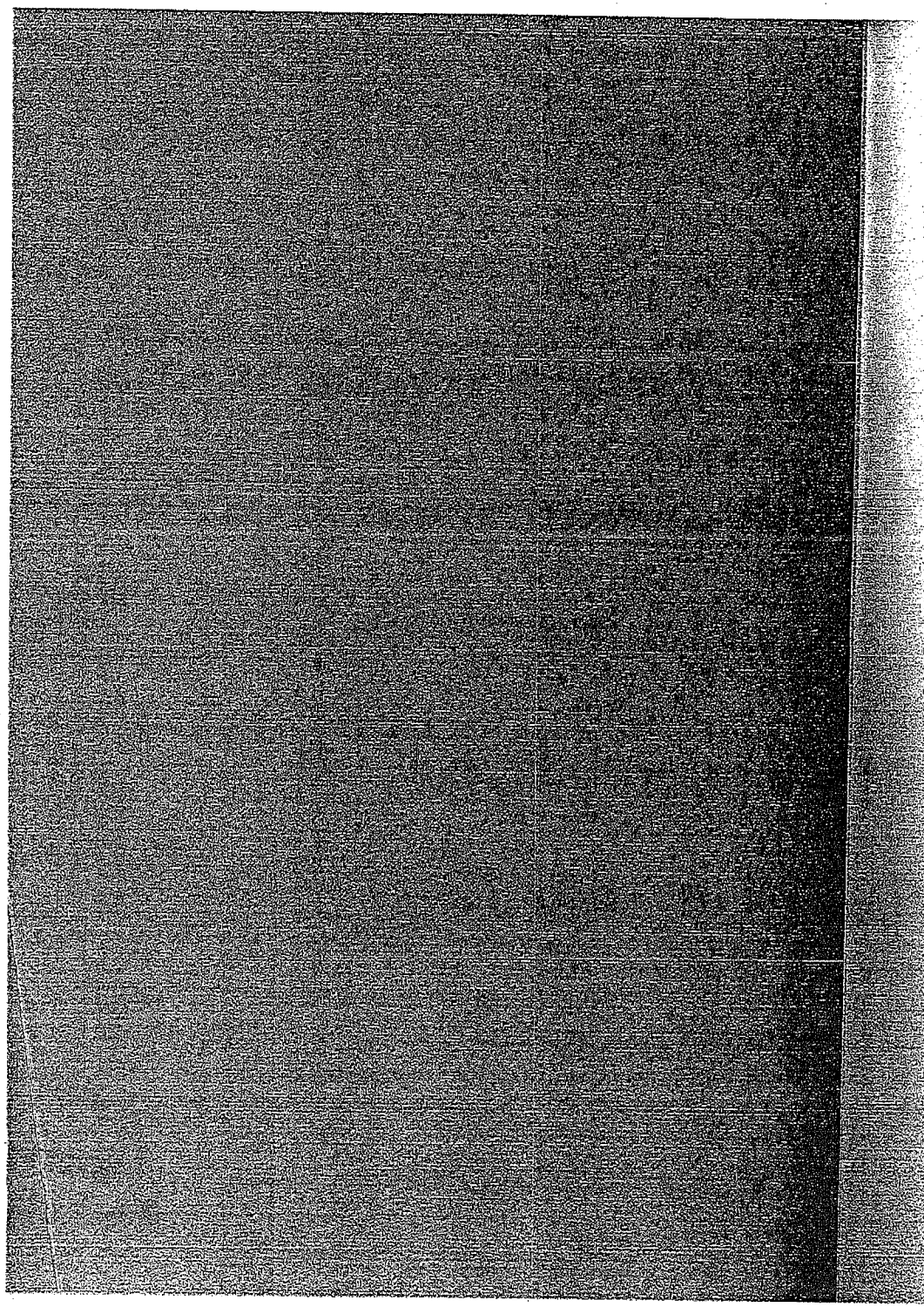

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INVERESK RESEARCH INTERNATIONAL
Report No. 3531

CONFIDENTIAL

[redacted]

ACUTE TOXICITY TESTS

IRI Project No. 234541

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To:

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May 1986

AUTHENTICATION

"I, the undersigned, hereby declare that this work was performed under my direction and in accordance with the principles of Good Laboratory Practice. The study was conducted according to the procedures herein described and this report represents a true and accurate record of the results obtained."

J.A. Cuthbert, B.Sc.
Principal Investigator

Project No. 234541

Report No. 3531

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QUALITY ASSURANCE AUTHENTICATION

The execution of this type of short-term study is not individually inspected. The processes involved are inspected at intervals according to a pre-determined schedule.

This report has been audited by IRI Quality Assurance Personnel according to the appropriate Standard Operating Procedure and is considered to describe the methods and procedures used in the study. The reported results accurately reflect the original data of the study.

IRI Project No. 234541

Report No. 3531

Signed: D Watson
pp(Head of Quality Assurance)

Date: 10th July 1986

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PERSONNEL INVOLVED IN PROJECT 234541

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SUMMARY

The acute toxicity potential of [redacted] was investigated.

ACUTE ORAL TOXICITY (LD50) TEST IN RATS

Mortality was as follows:

Dose Level				
$ml.kg^{-1}$ =	$g.kg^{-1}$	♂	♀	♂ + ♀
1.96	4.0	0/5	0/5	0/10
2.21	4.5	0/5	0/5	0/10
2.45	5.0	1/5	2/5	3/10

Clinical signs included hypokinesia, sedation, piloerection, soiled coat, ataxia and haemodacryorrhoea.

Post mortem observations were limited to gut contents fluid and red stained in premature decedents only.

The Median Oral Lethal Dose (LD50) could not be calculated but may be given as greater than 5 $g.kg^{-1}$.

SKIN IRRITATION TEST IN RABBITS

Moderate to severe erythema and oedema (score 3-4) was noted up to 72 h in all animals following a 4 h occluded exposure to [redacted] (as supplied).

[redacted] may be considered to be severely irritant to rabbit skin.

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BUEHLER SENSITISATION TEST IN GUINEA PIGS

Both the test and control groups reacted at challenge with [redacted] (as supplied). Since there was no difference between the groups, with scores of up to 2-3 being recorded, no clear conclusions can be reached on the sensitisation potential of [redacted]

INTRODUCTION

Montefluos SPA requires information on the acute toxicity potential of [redacted] This report gives details of the following tests performed on [redacted]

1. Acute Oral Toxicity (LD50) test in rats
2. Skin Irritation test in rabbits
3. Buehler Sensitisation test in guinea pigs

The test programme, which was carried out at Elphinstone Research Centre, field station of Inveresk Research International Limited (IRI), was commenced on 28 November 1985 and completed on 27 February 1986.

All data generated and recorded during this study will be stored in the Scientific Archives of Inveresk Research International Limited for 5 years after issue of the final report.

TEST MATERIAL AND ANIMALS

Test Material

Approximately 150 ml of the test material, [redacted], Batch No. L377/1, were received at IRI on 2 October 1985. The test material was a clear colourless liquid and was stored in the dark under ambient conditions.

In addition, a further 100 ml sample of [redacted] batch No. L377/2, was received at IRI on 10 December 1985. The test material was a clear colourless liquid and was stored in the dark under ambient conditions.

The specific gravity of the test material was determined to be 2.04 $g.ml^{-1}$.

Animals

Rats were supplied by Charles River (U.K.) Limited and arrived at Elphinstone Research Centre on 7 November 1985 and 14 January 1986.

Rabbits were supplied by Cheshire Rabbit Farms Limited and arrived at Elphinstone Research Centre on 15 October 1985 and 19 November 1985.

Guinea pigs were supplied by David Hall Limited and arrived at Elphinstone Research Centre on 16 October 1985 and 6 November 1985.

EXPERIMENTAL PROCEDURE

ACUTE ORAL TOXICITY (LD50) TEST IN RATS

Test Material

[redacted]

Animals

Male and female rats of the Sprague-Dawley strain were used. They were housed in suspended plastic cages with mesh floors with a maximum of 5 animals per cage. Mean environmental maximum and minimum temperatures were 21°C and 18°C respectively and mean relative humidity was 31%.

The rats were allowed an acclimatisation period of 41 days before the pre-dose ranging study, 23 days before the dose ranging study and 30 days before the main study.

The animals were fed Special Diet Services Expanded Rat and Mouse Maintenance Diet No. 1 of satisfactory analysis, but were deprived of food for 16-18 h before dosing. Water was available ad libitum throughout the study.

Method

[redacted] was administered orally in a single dose by means of a gavage. The test material was used without dilution, the dose levels under investigation being achieved by varying the dose volumes.

Pre-dose Ranging Study

In the pre-dose ranging study [redacted] was administered to one male and one female at each of 4 dose levels. The dose levels investigated were 1, 2, 3 and 5 $ml.kg^{-1}$ (equivalent to 2.04, 4.08, 6.12 and 10.20 $g.kg^{-1}$).

The rats were observed frequently on the day of dosing and for 5 days following dosing. They were weighed immediately prior to dosing.

Dose Ranging Study

From the results of the Pre-dose Ranging Study [redacted] was administered to one male and one female at each of 7 dose levels. The dose levels investigated were 0.49, 0.98, 1.47, 1.96, 2.45, 2.94 and 3.43 $ml.kg^{-1}$ (equivalent to 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 $g.kg^{-1}$).

The rats were observed frequently on the day of dosing and for 14 days following dosing. They were weighed immediately prior to dosing and at death or sacrifice at the end of the 14 day observation period.

At death or at the end of the observation period and sacrifice by nitrogen asphyxiatiion, each animal was subjected to a gross post-mortem observation.

Main Study

From the results of the dose ranging study, 3 dose levels were selected for the main study in which groups of 5 male and 5 female rats were dosed once with [redacted] by oral intubation at dose levels of 1.96, 2.21 and 2.45 $ml.kg^{-1}$ (equivalent to 4, 4.5 and 5 $g.kg^{-1}$).

The rats were observed frequently on the day of dosing and for 14 days following dosing. They were weighed immediately prior to dosing, 7 days after dosing at death or sacrifice at the end of the 14 day observation period.

At death or at the end of the observation period and sacrifice by nitrogen asphyxiation, each animal was subjected to a gross post mortem examination.

SKIN IRRITATION TEST IN RABBITS

Test Material

[redacted]

Animals

Two male and one female New Zealand White rabbits (order weight 2.5 kg-3.0 kg) were used. They were fed on Special Diet Services Rabbit Diet of satisfactory analysis and allowed food and water ad libitum. They were housed individually in cages with grid floors beneath which were peat moss filled trays. Mean environmental maximum and minimum temperatures were 16°C and 16°C respectively and mean relative humidity was 65%.

The rabbits were allowed an acclimatisation period of 62 days before test commencement.

Method

The hair was clipped from the back of each rabbit approximately 24 h before treatment.

The test material (0.5 ml) was applied to each rabbit under a 2.5 cm x 2.5 cm patch of gauze.

The patch was covered with Micropore tape and the entire trunk was bound loosely with Elastoplast Elastic Bandage which remained in position for 4 h. At the end of this period the patches were removed and the skin wiped with damp tissues to remove surplus test material.

Skin reactions were assessed 1 h after patch removal and 24, 48 and 72 h after patch application using the scoring system as detailed in Appendix 1.

BUEHLER SENSITISATION TEST IN GUINEA PIGS

Test Material

[redacted]

Since no suitable vehicle was available, all procedures were carried out with [redacted] as supplied.

Animals

Young adult (less than one year old), female, nulliparous and non-pregnant albino guinea pigs of the Dunkin-Hartley strain were used.

Twenty test group and 20 control group guinea pigs were housed 5 to a cage. The cages had grid floors beneath which were peat moss filled trays. The animals were fed Special Diet Services Limited FD1 diet supplemented with hay. They were allowed food and water ad libitum. The mean maximum and minimum temperatures were 20°C and 19°C and the mean relative humidity was 44%. They were allowed an acclimatisation period of 12 days before the test commencement.

Method

The Buehler test comprises 2 procedures:

The induction procedure, which consists of a 6 h closed patch topical application of the test material on 3 consecutive days each week for 3 consecutive weeks.

The challenge procedure, which consists of a closed patch topical application, is carried out 4 weeks (in Week 5) after commencement of the induction procedure (i.e. 2 weeks after application of the final patch of the induction phase).

i) Induction

The hair was shaved from a 4 cm x 6 cm area of mid back region of 40 guinea pigs. The animals were allocated as follows:

Test group	20 guinea pigs
Control group	20 guinea pigs

A Webril patch (2.5 cm x 2.5 cm) was coated with approximately 0.4 ml of the test material (as supplied), placed on the shaved back and the whole area covered with Blenderm occlusive tape wrapped around the entire trunk of the animal. This closed patch was left in position for 6 h and then removed. This procedure was carried out on 3 successive days of 3 successive weeks.

The control guinea pigs were similarly treated but with the test material being replaced by distilled water.

Twenty four hours after patch application the test sites were assessed for irritancy.

ii) Challenge

The test and control group animals were challenged 4 weeks (in Week 5) after commencement of the induction procedure.

The hair was removed from 5 cm x 5 cm area on the left flank using electric clippers.

Twenty four hours later, 0.4 ml of the test material, [redacted] (as supplied), and distilled water were applied to the prepared sites of each animal in the test and control groups and covered in the same method employed for induction. The patches were left in place for 6 h after which time they were removed.

No further shaving was required before assessment which was carried out by trained assessors 24 h and 48 h after patch application. Any animal showing erythema at the site of challenge was considered to have shown a positive response.

iii) Assessment of Response

Reactions are scored according to the following scale:

No visible change.....0
Slight or discrete erythema.....1
Moderate and confluent erythema.....2
Intense erythema and swelling.....3

RESULTS

ACUTE ORAL TOXICITY (LD50) TEST IN RATS

Predose Ranging Study

Mortality was as follows:

Dose Level				
$ml.kg^{-1}$	= $g.kg^{-1}$	♂	♀	♂ + ♀
1.0	2.04	0/1	0/1	0/2
2.0	4.08	0/1	1/1	1/2
3.0	6.12	1/1	1/1	2/2
5.0	10.20	1/1	1/1	2/2

Clinical signs included hypokinesia, piloerection, soiled coat and ataxia.

Dose Ranging Study

Mortality was as follows:

Dose Level				
$ml.kg^{-1}$	= $g.kg^{-1}$	♂	♀	♂ + ♀
0.49	1.0	0/1	0/1	0/2
0.98	2.0	0/1	0/1	0/2
1.47	3.0	0/1	0/1	0/2
1.96	4.0	0/1	0/1	0/2
2.45	5.0	0/1	0/1	0/2
2.94	6.0	1/1	1/1	2/2
3.43	7.0	1/1	1/1	2/2

Clinical signs included hypokinesia, sedation, piloerection, soiled coat, and ataxia.

Post mortem observations were limited to gut contents fluid and red stained in premature decedents only.

Main Study

Mortality was as follows:

Dose Level				
$ml.kg^{-1}$	≈ $g.kg^{-1}$	♂	♀	♂ + ♀
1.96	4.0	0/5	0/5	0/10
2.21	4.5	0/5	0/5	0/10
2.45	5.0	1/5	2/5	3/10

Clinical signs are detailed in Table 1 for males and in Table 2 for females.

Post mortem observations are detailed in Table 3 for males and in Table 4 for females.

The Median Lethal Oral Dose (LD50) could not be calculated but may be given as greater than 5 $g.kg^{-1}$.

Body weight gains were considered to be normal in surviving animals.

Details of body weights are given in Tables 5-7.

Skin Irritation Test in Rabbits

Details are given in Table 8.

Moderate to severe erythema and oedema (score 3-4) were noted in all animals from 1 h to 72 h following a 4 h occluded exposure to [redacted] (as supplied).

BUEHLER SENSITISATION TEST IN GUINEA PIGS

Details of irritation scores noted during the induction phase are given in Table 9.

Details of challenge scores are given in Table 10.

All test and control group animals produced positive responses after challenge with [redacted] (as supplied). These responses are indicative of irritation and not sensitisation. However, it was not possible to dilute the test material to enable a rechallenge with a non-irritant concentration.

Body weights are given in Table 11.

Body weight gains were considered to be normal.

No clinical signs, other than those induced by treatment, were noted at anytime during the study.

DISCUSSION AND CONCLUSIONS

ACUTE ORAL TOXICITY (LD50) TEST IN RATS

One male and two females died following a single oral administration of [redacted] at a dose level of 5 $g.kg^{-1}$ (2.45 $ml.kg^{-1}$).

The Median Oral Lethal Dose (LD50) could not be calculated but may be given as greater than 5 $g.kg^{-1}$.

SKIN IRRITATION TEST IN RATS

Severe irritant responses were noted following a 4 h occluded exposure to [redacted] (as supplied).

[redacted] may be considered to be severely irritant to rabbit skin.

BUEHLER SENSITISATION TEST IN GUINEA PIGS

Both the test and control groups reacted at challenge to [redacted] (as supplied). It is clear from the control group that the test material was causing marked irritation, with scores of 2-3. Since it was not possible to eliminate this irritation the reactions in the test group may be irritation only or irritation with sensitisation. It is not possible to determine a difference in responses between groups because of the high scores.

TABLE 1

[redacted]

Acute Oral Toxicity Test in Male Rats
Main Study: Clinical Signs - Animal Affected and Duration

Dose Level (g.kg^{-1})	Observation	No. Affected/Time																				
		1 min	½ h	1 h	2 h	4 h	1 d	2 d	3 d	4 d	5 d	6 d	7 d	8 d	9 d	10 d	11 d	12 d	13 d	14 d
4.0	NAD	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4.5	NAD	5	5	5	5	5					5	5	5	5	5	5	5	5	5	5	5	5
	Hypokinesia								5													
	Sedation						5	5														
	Piloerection						5	5	5	5												
	Soiled coat						5	5	5													
	Ataxia							5	5													
5.0	FDC							1														
	NAD	5	5	5	5	5	5					4	4	4	4	4	4	4	4	4	4	4
	Hypokinesia								4	4												
	Sedation							4	4													
	Piloerection							4	4	4	4											
	Soiled coat							4	4	4												
	Ataxia							4	4	4												
	Haemodacryorrhoea							4														

min = Minutes after dosing
FDC = Found dead in cage
h = Hours after dosing
NAD = No abnormality detected
d = Days after dosing

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TABLE 2

[redacted]

Acute Oral Toxicity Test in Female Rats
Main Study: Clinical Signs - Animal Affected and Duration

Dose Level (g.kg^{-1})	Observation	No. Affected/Time																				
		1 min	½ h	1 h	2 h	4 h	1 d	2 d	3 d	4 d	5 d	6 d	7 d	8 d	9 d	10 d	11 d	12 d	13 d	14 d		
4.0	NAD	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5		
4.5	NAD	5	5	5	5	5					5	5	5	5	5	5	5	5	5	5		
	Hypokinesia								5													
	Sedation						5	5														
	Piloerection						5	5	5	5												
	Soiled coat						5	5	5													
	Ataxia							5	5													
5.0	FDC							2														
	NAD	5	5	5	5	5	5					3	3	3	3	3	3	3	3	3		
	Hypokinesia									3												
	Sedation							5	3													
	Piloerection							3	3	3	3											
	Soiled coat							3	3	3												
	Ataxia							3	3	3												
	Haemodacryorrhoea							3														

min = Minutes after dosing
FDC = Found dead in cage
h = Hours after dosing
NAD = No abnormality detected
d = Days after dosing

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TABLE 3

[redacted]

Acute Oral Toxicity Test in Male Rats
Main Study: Post Mortem Observations

Dose Level ($g \cdot kg^{-1}$)	Animal No.	Post Mortem Observation	Time of Death or Sacrifice
4.0	55	NAD	S
	56	NAD	S
	57	NAD	S
	58	NAD	S
	59	NAD	S
4.5	65	NAD	S
	66	NAD	S
	67	NAD	S
	68	NAD	S
	69	NAD	S
5.0	75	NAD	S
	76	NAD	S
	77	NAD	S
	78	NAD	S
	79	Gut contents fluid, red stained	2 d

d = Days after dosing
S = Sacrificed 14 days after dosing (day 15)
NAD = No abnormality detected

TABLE 4

[redacted]

Acute Oral Toxicity Test in Female Rats
Main Study: Post Mortem Observations

Dose Level ($g \cdot kg^{-1}$)	Animal No.	Post Mortem Observation	Time of Death or Sacrifice
4.0	60	NAD	S
	61	NAD	S
	62	NAD	S
	63	NAD	S
	64	NAD	S
4.5	70	NAD	S
	71	NAD	S
	72	NAD	S
	73	NAD	S
	74	NAD	S
5.0	80	NAD	S
	81	NAD	S
	82	NAD	S
	83	Gut contents fluid, red stained	2 d
	84	Gut contents fluid, red stained	2 d

d = Days after dosing
S = Sacrificed 14 days after dosing (day 15)
NAD = No abnormality detected

TABLE 5

[redacted]

Acute Oral Toxicity Test in Rats
Main Study: 4 g.kg^{-1}
Body Weights

Animal No.	Body Weight (g) At Dosing	After 7 Days	After 14 Days	Overall Weight Gain (g)
55♂	189	242	285	96
56	200	248	301	101
57	201	256	305	104
58	196	249	300	104
59	188	238	292	104
Mean	195	247	297	102
± S.D.	6	7	8	3
60♀	162	190	245	83
61	170	195	251	81
62	155	191	250	85
63	156	191	248	82
64	171	194	255	84
Mean	157	192	250	83
± S.D.	4	2	4	2

TABLE 6

[redacted]

Acute Oral Toxicity Test in Rats
Main Study: 4.5 g.kg^{-1}
Body Weights

Animal No.	Body Weight (g) At Dosing	After 7 Days	After 14 Days	Overall Weight Gain (g)
65♂	190	246	302	112
66	192	244	300	108
67	198	251	305	107
68	200	256	311	111
69	186	249	296	110
Mean	193	249	303	110
± S.D.	6	5	6	2
70♀	160	194	245	85
71	161	194	242	81
72	170	195	251	81
73	158	183	221	63
74	168	192	246	78
Mean	163	192	241	78
± S.D.	5	5	12	9

TABLE 7

[redacted]

Acute Oral Toxicity Test in Rats
Main Study: 5 $g \cdot kg^{-1}$
Body Weights

Animal No.	Body Weight (g) At Dosing	After 7 Days	After 14 Days	Overall Weight Gain (g)
75♂	191	240	298	107
76	195	241	294	99
77	200	248	301	101
78	192	236	286	94
79	184	+	+	-
Mean	192	241	295	100
± S.D.	6	5	7	5
80♀	161	184	225	64
81	162	185	230	68
82	158	180	230	72
83	171	+	+	-
84	165	+	+	-
Mean	163	183	228	68
± S.D.	5	3	3	4

+ = Animal dead

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TABLE 8*

[redacted]

Skin Irritation in Rabbits

Rabbit No./Sex	Reaction Score: Erythema 1 h	Erythema 24 h	Erythema 48 h	Erythema 72 h	Oedema 1 h	Oedema 24 h	Oedema 48 h	Oedema 72 h	Mean Erythema	Mean Oedema	Total* Irritation Score
25♂	3	3	4	4	3	3	4	4	3.5	3.5	7.0
26♀	3	3	4	4	3	3	4	4	3.5	3.5	7.0
27♂	4	4	4	4	4	4	4	4	4.0	4.0	8.0
Mean Score†	3.3	3.3	4.0	4.0	3.3	3.3	4.0	4.0	3.7	3.7	7.3ϕ

* = For each rabbit
† = For each time point
ϕ = Total irritation score for the test

TABLE 9

[redacted]

Buehler Sensitisation Test in Guinea Pigs
Induction Results

Group	Cage	Animal No.	Week/Application/Score								
			1			2			3		
			1	2	3	1	2	3	1	2	3
Test	28	1	1	1	1	0	1	1	0	0	0
		2	1	1	2	0	1	1	0	0	1
		3	1	1	2	0	0	0	0	1	1
		4	1	0	1	0	1	1	0	0	0
		5	1	1	1	0	0	0	0	1	0
	29	1	1	1	2	2	2	2	2	2	2
		2	1	1	2	1	1	1	1	2	1
		3	1	2	2	1	1	2	1	1	0
		4	1	2	2	2	2	2	2	2	1
		5	1	2	2	2	2	2	2	2	2
	30	1	1	2	2	1	1	2	1	2	2
		2	1	2	2	1	1	2	1	1	2
		3	1	1	1	1	1	1	2	2	1
		4	1	2	2	1	2	1	1	1	1
		5	1	2	1	1	1	1	1	0	0
	31	1	1	1	2	1	1	2	1	1	0
		2	1	1	1	1	1	1	0	1	0
		3	1	1	2	0	0	1	0	1	2
		4	1	1	1	1	1	1	0	1	1
		5	1	1	1	1	1	1	1	2	2
Control	32	1	0	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0	0
	33	1	0	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0	0
	34	1	0	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0	0
	35	1	0	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0	0

TABLE 10*

[redacted]

Buehler Sensitisation Test in Guinea Pigs
Challenge Results

Group	Cage	Animal No.	Score			
			24 h		48 h	
			Test	Control	Test	Control
Test	28	1	3	0	3	0
		2	3	0	3	0
		3	2	0	3	0
		4	3	0	3	0
		5	3	0	3	0
	29	1	3	0	3	0
		2	3	0	3	0
		3	3	0	3	0
		4	3	0	3	0
		5	3	0	3	0
	30	1	3	0	3	0
		2	3	0	3	0
		3	2	0	3	0
		4	2	0	3	0
		5	3	0	3	0
	31	1	2	0	3	0
		2	3	0	3	0
		3	3	0	3	0
		4	2	0	3	0
		5	2	0	3	0
Control	32	1	3	0	2	0
		2	3	0	2	0
		3	2	0	2	0
		4	3	0	3	0
		5	3	0	3	0
	33	1	2	0	2	0
		2	2	0	2	0
		3	3	0	3	0
		4	2	0	2	0
		5	3	0	3	0
	34	1	3	0	3	0
		2	3	0	3	0
		3	3	0	3	0
		4	2	0	2	0
		5	3	0	3	0
	35	3	3	0	3	0
		3	3	0	3	0
		3	3	0	3	0
		2	2	0	2	0
		3	3	0	3	0

Test = [redacted] (as supplied)
Control = Distilled water

TABLE 11

[redacted]

Buehler Sensitisation Test in Guinea Pigs
Body Weights

Group	Cage	Animal No.	Body Weight (g) Start of Study	End of Study	Gain
Test	28	1	453	582	129
		2	409	585	176
		3	417	620	203
		4	423	599	176
		5	414	596	182
	29	1	430	618	188
		2	420	596	176
		3	409	647	238
		4	442	705	263
		5	414	651	237
	30	1	424	608	184
		2	425	603	178
		3	425	614	189
		4	349	493	144
		5	404	587	183
	31	1	362	582	220
		2	418	608	190
		3	385	619	234
		4	400	607	207
		5	430	655	225
Control	32	1	423	616	193
		2	404	597	193
		3	446	665	219
		4	393	580	187
		5	442	707	265
	33	1	473	668	195
		2	374	615	241
		3	383	583	200
		4	379	491	112
		5	422	622	200
	34	1	419	611	192
		2	430	604	174
		3	455	573	118
		4	415	606	191
		5	397	528	131
	35	1	402	589	187
		2	390	536	146
		3	396	621	225
		4	420	667	247
		5	414	584	170

APPENDIX 1

OECD: Acute Dermal Irritation Test in Rabbits
Grades for Skin Lesions

Erythema and Eschar Formation	Grade
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

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

APPENDIX 2

OECD: Acute Eye Irritation/Corrosion Test in Rabbits
Grades for Ocular Lesions

	Grade
Cornea	
No ulceration or opacity	0
Scattered or diffuse areas of opacity, details of iris clearly visible	1*
Easily discernible translucent areas of opacity, details of iris slightly obscured	2*
Nacreous areas of opacity, no details of iris visible, size of pupil barely discernible	3*
Complete corneal opacity, iris not discernible	4*
Ulceration, absence of a gross patch of corneal epithelium	4*
Iris	
Normal	0
Markedly deepened folds, congestion, swelling, moderate circumcorneal injection (any of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)	1*
No reaction to light, haemorrhage, gross destruction (any or all of these)	2*

APPENDIX 2 (continued)

	Grade
Conjunctivae	
Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris):	
Vessels normal	0
Some vessels definitely injected	1
Diffuse crimson red, individual vessels not easily discernible	2*
Diffuse beefy red	3*
Chemosis	
No swelling	0
Any swelling above normal (including nictitating membrane)	1
Obvious swelling with partial eversion of lids	2*
Swelling with lids about half closed	3*
Swelling with lids more than half closed	4*
Ulceration or necrosis of palpebral and bulbar conjunctivae or nictitating membrane	4*
Discharge	
Mild	1
Moderate	2
Severe	3

* Figures indicate the grades considered positive.

REDACTED AS TO TRADE NAMES

RTC

RESEARCH TOXICOLOGY CENTRE - ROMA

[redacted]

ACUTE ORAL TOXICITY STUDY IN THE RAT

FINAL REPORT

RTC Study Number: 8832-001

RTC Report Number: 8832-001/T/051/2002

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

RTC Report Number: 8832-001/T/051/2002

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 : 21-03-2002

J. Brightwell, Ph.D.
(Scientific Director): Date : 21.03.2002

RTC Report Number: 8832-001/T/051/2002

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	16.07.2001	16.07.2001	16.07.2001
PROTOCOL AMENDMENT CHECK	20.11.2001	20.11.2001	20.11.2001
PROCESS-BASED INSPECTIONS			
Allocation	05.09.2001	-	08.11.2001
Dose preparation	26.10.2001	-	20.11.2001
Body weight	06.09.2001	-	14.11.2001
Dosing (oral)	03.08.2001	-	09.11.2001
Clinical observations	12.11.2001	-	30.11.2001
Despatch to necropsy	13.07.2001	-	19.09.2001
Necropsy	14.08.2001	-	21.08.2001
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 19 March 2002		

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

19/03/02
Date

Contents

1. SUMMARY

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

An initial limit test was performed in which a single group of 5 male and 5 female animals was dosed at 2000 mg/kg. Mortality occurred and, as a result, this limit test was followed by a dose-ranging screen to allow selection of dose levels for a subsequent main study. In the dose-ranging screen 5 groups, each of 1 male and 1 female animal, were dosed at levels of 100, 200, 400, 800 and 1600 mg/kg. In the main study 3 groups, each of 5 male and 5 female animals, were dosed at levels of 1000, 1800 and 3240 mg/kg in order to span the expected median lethal dose.

In the limit test at 2000 mg/kg, piloerection, hunched posture, liquid/soft faeces, lethargy, dirty appearance around the urogenital region, yellow staining in the litter tray and brown staining around the muzzle/eye were seen following dosing. Individual animals also showed salivation and rales. Four of the 5 males and all females died between Day 2 and Day 11 of the observation period. Necropsy examination revealed an abnormal coloration of a number of organs in the abdominal cavity. Cannibalisation by cages mates was also observed.

In the main study mortality occurred at the two higher dose levels investigated. The mortality pattern observed allowed the calculation of the median lethal dose (LD50) for males, females and combined sexes. Mortality data relative to the limit test were included in the calculation.
Results are summarised as follows:

Sex	LD50	95% Confidence interval
Males	1610 mg/kg	1359 – 1908 mg/kg
Females	1781 mg/kg	Not calculable
Combined	1676 mg/kg	1533 – 1832 mg/kg

In the main study hunched posture, piloerection, liquid/soft faeces and/or mucoid material in the litter tray and a dirty appearance around the urogenital region were commonly observed in animals at all dose levels investigated.
Individual animals also showed lethargy and rales.
Abnormal contents (orange/yellow, fluid/mucoid material) were found in some organs of the abdominal cavity of a number of early decedent animals, dosed at 1800 mg/kg.
Brown staining of the muzzle and urogenital region was also noted at the external examination,
Necropsy examination of animals at the end of the observation period revealed no abnormalities.
Body weight gain appeared slightly reduced in the surviving animals, for animals of this stain and age.
These results indicate that the test item, [redacted] has some toxic effect in the rat following oral administration of single dose. The data obtained indicate the median lethal dose (LD50) to be in the range of 1600-1700 mg/kg.

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

Classification : Required
Symbol : Xn
R phrase : R 22 – Harmful if swallowed

2. INTRODUCTION

The purpose of this study was to assess the acute toxicity of the substance following oral 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 oral toxicity described in OECD guideline Number 401, adopted on 24th February 1987. Methods were in agreement with those described in 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 July 2001 with allocation of animals to the limit test and ended on 2nd October 2001 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	: 90199/66
Expiry date	: 24th April 2005
Received from	: AUSIMONT S.p.A.
Date received	: 14th May 2001
Amount received	: 120 grams
Description	: Opaque liquid
Container	: Opaque plastic bottle
Storage at RTC	: Ambient conditions
RTC reference number	: 5504

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 for the test item, 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 formulated for dosing by mixing with corn oil.

During handling of the substance, precautions were taken to reduce possible operator exposure. This included, but was 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 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. They appeared to be in an acceptable condition following arrival in 3 separate batches for the different phases of the study on 29 June 2001, 17 August and 31 August 2001. A pre-dose acclimatisation period of at least 5 days was allowed during which time the health status of the animals was assessed.

4.1.2 Animal husbandry

Animals were housed, in groups of up to 5 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. This colour coding matched the corresponding colour coded formulation container.

Animal room controls were set to maintain temperature within the range of 20 to 24°C and relative humidity within the range of 40 to 70%. Actual conditions were recorded.

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

4.1.3 Water and diet

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

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

4.2 Experimental design

An initial limit test was undertaken in a single group of 5 male and 5 female animals, at a level of 2000 mg/kg. Mortality occurred and this was followed by a dose-ranging screen which was undertaken in 5 groups, each of 1 male and 1 female animals, at levels of 100, 200, 400, 800 and 1600 mg/kg. A main study was then undertaken, in which 3 groups each of 5 male and 5 female animals were dosed at levels of 1000, 1800 and 3240 mg/kg, in order to allow the median effect point to be established.

4.2.1 Selection and allocation

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

A single group of 5 males and 5 females, was allocated to the limit test as follows:-

Dose level (mg/kg)	Animal number	
	Males	Females
2000	132,134,136,138,140	131,133,135,137,139

A total of 3 groups, each of 5 males and 5 females, were allocated to the main phase of the study as follows:-

Dose level (mg/kg)	Animal number	
	Males	Females
1000	62,64,66,68,70	61,63,65,67,69
1800	72,74,76,78,80	71,73,75,77,79
3240	82,84,86,88,90	81,83,85,87,89

Food was removed from cages overnight prior to dosing.

4.2.2 Dosing

On Day 1 of the study, the appropriate dose volume of the test item, calculated for each fasted animal according to body weight, 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.
Animals used as part of the dose-ranging screen were observed for 7 days only.

4.2.5 Body weight

All animals were weighed on allocation to the study (Day -1), immediately prior to dosing (Day 1) and on Days 8 and 15. Animals found dead were weighed when found.

4.2.6 Termination

All surviving animals from the initial limit test, the subsequent dose-ranging screen and the main study were killed by carbon dioxide narcosis.

Surviving animals used as part of the dose-ranging screen were killed 7 days after dosing and no necropsy examination was undertaken.

Those from the limit test and subsequent main study were killed 14 days after dosing. Animals killed in this way and those dying during the study were subjected to a gross necropsy examination for external 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.

Tissue abnormalities were preserved in 10% buffered formol saline and will not be processed unless requested by the Sponsor.

4.3 Statistical analysis

The observed mortality data from all groups forming the main phase of the study, combined with data from the limit test, were analysed using the methods of Finney. (Finney, D., (1971), Probit analysis, 3rd. Ed., Cambridge University Press).

4.4 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).

4.5 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 (Table 1, Appendix 1)

Mortality occurred at all but the lowest dose level investigated (1000 mg/kg). It generally occurred within 11 days of dosing. The mortality pattern observed allowed the calculation of the median lethal dose (LD50) for males, females and combined sexes. Mortality data relative to the limit test were included in the calculation. Results are summarised as follows:

Sex	LD50	95% Confidence interval
Males	1610 mg/kg	1359 – 1908 mg/kg
Females	1781 mg/kg	Not calculable
Combined	1676 mg/kg	1533 – 1832 mg/kg

5.2 Main phase limit test

5.2.1 Clinical signs (Table 2)

Piloerection, hunched posture, liquid faeces and a dirty appearance around the urogenital region were commonly observed following dosing. Female animals also showed salivation, rales, lethargy and brown staining around the muzzle. Recovery from these signs was generally observed in surviving animals during the second week of the observation period. Lethargy/decreased activity, brown staining of muzzle/eyes, soft faeces, semi-closed eyes were commonly observed from day 2 up to the end of the study.

Four out of the 5 males and all females died between Day 2 and Day 11 of the observation period.

5.2.2 Body weight (Table 3)

Body weight gain in the single surviving animal appeared slightly reduced.

5.2.3 Necropsy (Table 4)

Necropsy examination of the early decedent animals revealed an abnormal coloration (dark or pale) of the spleen, thymus, liver, lungs, adrenals, stomach and cervical lymph nodes. An abnormal content (white or yellow, mucoid material) was found in the stomach and duodenum of 2 early decedent animals and 1 surviving animal. Cannibalisation was also observed.

5.3 Preliminary screen

In the preliminary screen, piloerection was commonly observed in animals following dosing. Animals dosed at 800 and 1600 mg/kg showed hunched posture, liquid faeces and/or mucoid material in the litter tray approximately 4 hours after dosing. Decreased activity was also noted in animals dosed at 1600 mg/kg following dosing.
No mortality occurred at any dose level tested.

5.4 Main study

5.4.1 Clinical signs (Table 5)

Piloerection, liquid/soft faeces and/or mucoid material in the litter tray and a dirty appearance around the urogenital region were commonly observed in animals following dosing at all dose levels investigated. In addition, individual animals showed lethargy, hunched posture, and rales. The majority of these signs were present for the duration of the observation period.

5.4.2 Body weight (Table 6)

Changes in body weight showed a slight reduction in body weight gain in surviving animals. A single animal also showed a body weight loss at the end of the study.

5.4.3 Necropsy (Table 7)

Necropsy examination of the early decedent animals showed the presence of abnormal contents (orange or yellow in colour, fluid or mucoid material) in the stomach, duodenum and jejunum in a number of animals dosed at 1800 mg/kg. Some organs in the abdominal cavity (spleen, prostate, seminal vesicles, stomach and mesentery) appeared abnormal in size and colour in a number of early decedent animals dosed at 3240 mg/kg.
Abnormalities observed at necropsy performed at the end of the observation period were limited to the presence of an abnormal content in the jejunum (orange and fluid/oily material) and in the urinary bladder /white and firm material), seen in a single female animal. External abnormalities included skin/fur staining of different regions of the body surfaces. Cannibalisation by cage mates was also observed.

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.

Statistical analysis of mortality data showed the LD50 to be lower than 2000 mg/kg. Therefere, European Directives concerning the classification, packaging and labelling of dangerous substances would indicate the following:-

Classification : Required
Symbol : Xn
R phrase : R 22 – Harmful if swallowed

SINGLE DOSE ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

TABLE 1 - MORTALITY

Dose level (mg/kg)	Mortality (Number dead/Number in group)		
	Males	Females	Combined
1000	0/5	0/5	0/10
1800	4/5	3/5	7/10
2000*	4/5	5/5	9/10
3240	5/5	5/5	10/10

Sex	LD50	95% Confidence Interval
Males	1610 mg/kg	1359 - 1908 mg/kg
Females	1781 mg/kg	Not calculable
Combined	1676 mg/kg	1533 - 1832 mg/kg

*Limit test

ACUTE ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

TABLE 2 - LIMIT TEST - 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	0	0	0	0	0	0	0	0	0
Piloerection	0	5	5	5	5	5	5	3	2	2
Hunched posture	0	5	5	5	5	5	5	3	2	2
Liquid faeces	0	0	0	5	5	0	0	0	0	0
Dirty appearance - urogenital region	0	0	0	5	5	5	5	3	2	2
Brown staining, eye	0	0	0	0	1	1	1	0	0	0
Soft faeces	0	0	0	0	0	5	5	3	2	0
Lethargy	0	0	0	0	0	0	2	0	0	0
Brown staining, muzzle	0	0	0	0	0	0	0	0	1	1
MORTALITY	0	0	0	0	0	0	0	2	1	0

Sign observed	Day 8	9	10	11	12	13	14	15
No abnormalities detected	0	0	0	0	0	0	1	1
Piloerection	2	2	2	1	1	1	0	0
Hunched posture	2	2	1	0	0	0	0	0
Dirty appearance - urogenital region	2	2	0	0	0	0	0	0
Decreased activity	2	2	1	0	0	0	0	0
Semi-closed eyes	0	0	1	0	0	0	0	0
Lethargy	0	0	1	0	0	0	0	0
Yellow staining in litter tray	0	0	0	1	1	1	0	0
MORTALITY	0	0	0	1	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 ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

TABLE 2 - 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	0	0	0	0	0	0	0	0	0
Salivation	0	2	0	3	2	0	0	0	0	0
Piloerection	0	5	5	5	3	3	3	2	0	0
Hunched posture	0	5	5	5	3	3	3	2	2	2
Liquid faeces	0	0	0	5	3	0	0	0	0	0
Dirty appearance - urogenital region	0	0	0	4	3	3	3	0	0	0
Rales	0	0	0	1	0	0	0	0	0	0
Brown staining, muzzle	0	0	0	3	3	3	3	2	2	0
Lethargy	0	0	0	2	0	0	1	0	2	2
Soft faeces	0	0	0	0	0	3	3	0	0	0
MORTALITY	0	0	0	0	2	0	0	1	0	0

Sign observed	Day 8	9	10	11	12	13	14	15
No abnormalities detected	0	-	-	-	-	-	-	-
Hunched posture	1	-	-	-	-	-	-	-
Dirty appearance - urogenital region	1	-	-	-	-	-	-	-
Lethargy	1	-	-	-	-	-	-	-
MORTALITY	1	1	-	-	-	-	-	-

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
- : Decedent

ACUTE ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

TABLE 3 - LIMIT TEST - BODY WEIGHT

Dose level (mg/kg)	Sex	Animal identity number	Body weight (g) on Day -1	1	8	15	Change in body weight (g) Days 1 - 15
2000	M A L E S	132	275	249	226	280	31
		134	264	238	-	-	-
		136	270	247	222	-	-
		138	260	235	-	-	-
		140	275	250	-	-	-
		Mean	268.8	243.8	N/V	N/C	N/C
		S.Dev.	6.7	6.8	N/V	N/C	N/C
	F E M A L E S	131	193	176	-	-	-
		133	200	185	166	-	-
		135	201	180	-	-	-
		137	187	171	-	-	-
		139	191	174	-	-	-
		Mean	194.4	177.2	N/C	N/C	N/C
		S.Dev.	6.0	5.4	N/C	N/C	N/C

KEY: - = Decedent
N/V = Not valid due to low sample size
N/C = Not calculable

ACUTE ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

TABLE 4 - LIMIT TEST - NECROPSY

DOSE LEVEL: 2000 mg/kg

MALES

Animal number	Tissue/organ	Finding
132		Terminal kill
	Duodenum	Contained a yellow, mucoid material
134		Early decedent
	Spleen	Small (28x5x2mm)
	Thymus	Dark in colour
136		Early decedent
	External surfaces	Yellow staining, urogenital region
	Liver	Pale in colour
	Lungs	Dark in colour
	Stomach	Dark, glandular region
138		Early decedent
	Cervical Lymph nodes	Dark in colour
	Spleen	Small 30x5x1mm
	Thymus	Dark in colour
	Adrenals	Dark in colour
	Jejunum	Contained a yellow, mucoid material
140		Early decedent
		No abnormalities detected

ACUTE ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

TABLE 4 - Continued

DOSE LEVEL: 2000 mg/kg

FEMALES

Animal number	Tissue/ organ	Finding
131		Early decedent No abnormalities detected
133		Early decedent No abnormalities detected
135		Early decedent
	External surfaces	Brown staining, muzzle Yellow staining, urogenital region
	Spleen	Small 26x9x2mm
	Stomach	Contained white mucoid material
137		Early decedent
	Abdominal cavity	All organs cannibalised
	Thoracic cavity	All organs cannibalised
139		Early decedent
	External surfaces	Brown staining, muzzle Yellow staining, urogenital region
	Spleen	Small (30x10x2mm)
	Adrenals	Dark in colour

ACUTE ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

TABLE 5 - LD50 ASSESSMENT - CLINICAL SIGNS

DOSE LEVEL: 1000 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	0	0	0	0	2	2	2	5	5
Piloerection	0	5	5	5	0	0	0	0	0	0
Dirty appearance - urogenital region	0	0	0	3	5	3	3	3	0	0
Liquid faeces	0	0	0	5	0	0	0	0	0	0
Soft faeces	0	0	0	0	5	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 ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

TABLE 5 - Continued

DOSE LEVEL:1000 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	0	0	0	0	0	5	5	5	5
Piloerection	0	5	5	5	0	0	0	0	0	0
Liquid faeces	0	0	0	5	0	0	0	0	0	0
Dirty appearance - urogenital region	0	0	0	1	1	1	0	0	0	0
Soft faeces	0	0	0	0	5	5	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 ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

TABLE 5 - Continued

DOSE LEVEL: 1800 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	5	0	0	0	0	0	0	0	0
Piloerection	0	0	5	5	5	4	3	2	2	1
Hunched posture	0	0	5	5	5	0	0	0	0	0
Liquid faeces	0	0	5	5	0	0	0	0	0	0
Dirty appearance - urogenital region	0	0	5	5	5	4	3	2	2	1
Brown staining, muzzle	0	0	0	0	5	4	3	2	2	1
Soft faeces	0	0	0	0	5	4	3	0	0	0
Mucous in litter tray	0	0	0	0	0	4	0	0	0	0
MORTALITY	0	0	0	0	0	1	1	1	0	1

Sign observed	Day 8	9	10	11	12	13	14	15
No abnormalities detected	0	0	0	0	0	0	0	1
Piloerection	1	1	1	1	1	1	1	0
Dirty - urogenital region	1	1	1	1	1	1	1	0
Brown staining - muzzle	1	1	0	0	0	0	0	0
neck	0	1	1	1	1	1	1	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 ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

TABLE 5 - Continued

DOSE LEVEL: 1800 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	0	0	0	0	0	0	0	3
Piloerection	0	0	5	5	5	5	3	3	3	0
Hunched posture	0	0	5	5	5	0	0	0	0	0
Liquid faeces	0	0	5	5	0	0	0	0	0	0
Dirty appearance - urogenital region	0	0	5	3	5	5	3	3	3	0
Brown staining, muzzle	0	0	0	0	5	3	3	3	3	0
Soft faeces	0	0	0	0	5	0	0	0	0	0
MORTALITY	0	0	0	0	0	0	2	0	0	0

Sign observed	Day 8	9	10	11	12	13	14	15
No abnormalities detected	2	2	0	0	0	0	0	1
Piloerection	0	0	2	2	2	2	2	0
Emaciated	0	0	0	0	0	0	0	1
MORTALITY	1	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 ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

TABLE 5 - Continued

DOSE LEVEL: 3240 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	5	0	0	0	0	0	0	0	0
Hunched posture	0	0	5	5	4	0	0	0	0	1
Piloerection	0	0	5	5	4	1	1	1	1	1
Dirty appearance - urogenital region	0	0	5	5	4	1	1	1	1	1
Liquid faeces	0	0	5	5	0	0	0	0	0	0
Rales	0	0	0	1	0	0	0	0	0	0
Lethargy	0	0	0	0	4	0	0	0	0	0
Brown staining, muzzle	0	0	0	0	0	0	0	0	0	1
Soft faeces	0	0	0	0	0	0	0	0	0	1
Decreased activity	0	0	0	0	0	0	0	0	0	1
MORTALITY	0	0	0	0	1	3	0	0	0	0

Sign observed	Day 8	9	10	11	12	13	14	15
No abnormalities detected	-	-	-	-	-	-	-	-
MORTALITY	1	-	-	-	-	-	-	-

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
- = Decedent

ACUTE ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

TABLE 5 - Continued

DOSE LEVEL: 3240 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	0	0	0	-	-	-	-	-
Hunched posture	0	0	5	5	1	-	-	-	-	-
Piloerection	0	0	5	5	1	-	-	-	-	-
Dirty appearance - urogenital region	0	0	5	5	1	-	-	-	-	-
Liquid faeces	0	0	5	5	0	-	-	-	-	-
Soft faeces	0	0	0	0	1	-	-	-	-	-
Brown staining, muzzle	0	0	0	0	1	-	-	-	-	-
Lethargy	0	0	0	0	1	-	-	-	-	-
MORTALITY	0	0	0	0	4	1	-	-	-	-

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
- = Decedent

ACUTE ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

TABLE 6 - MAIN STUDY - BODY WEIGHT

Dose level (mg/kg)	Sex	Animal identity number	Body weight (g) on Day -1	Body weight (g) on Day 1	Body weight (g) on Day 8	Body weight (g) on Day 15	Change in body weight (g) Days 1 - 15
1000	MALES	62	264	240	284	311	71
		64	243	225	257	288	63
		66	233	214	256	283	69
		68	253	233	271	318	85
		70	250	235	263	305	70
		Mean	248.6	229.4	266.2	301.0	71.6
		S.Dev.	11.5	10.2	11.6	15.0	8.1
	FEMALES	61	183	170	198	196	26
		63	184	174	188	197	23
		65	199	186	195	204	18
		67	185	171	202	209	38
		69	183	166	201	207	41
		Mean	186.8	173.4	196.8	202.6	29.2
		S.Dev.	6.9	7.6	5.6	5.9	9.9
1800	MALES	72	268	247	235	264	17
		74	241	220	-	-	-
		76	259	235	-	-	-
		78	266	247	-	-	-
		80	258	240	-	-	-
		Mean	258.4	237.8	N/C	N/C	N/C
		S.Dev.	10.6	11.2	N/C	N/C	N/C
	FEMALES	71	188	170	-	-	-
		73	200	180	-	-	-
		75	185	170	162	197	27
		77	196	180	-	-	-
		79	189	170	136	114	-56
		Mean	191.6	174.0	N/V	N/V	N/V
		S.Dev.	6.2	5.5	N/V	N/V	N/V

Key: N/C = Not calculable
N/V = Not valid due to low sample size
- = Decedent

ACUTE ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

TABLE 6 - Continued

Dose level (mg/kg)	Sex	Animal identity number	Body weight (g) on Day -1	Body weight (g) on Day 1	Body weight (g) on Day 8	Body weight (g) on Day 15	Change in body weight (g) Days 1 - 15
		82	265	242	-	-	-
	M	84	248	228	-	-	-
	A	86	249	226	-	-	-
	L	88	236	218	-	-	-
	E	90	257	236	-	-	-
	S						
		Mean	251.0	230.0	-	-	-
		S.Dev.	10.8	9.3	-	-	-
3240							
		81	193	177	-	-	-
	F	83	197	181	-	-	-
	E	85	188	172	-	-	-
	M	87	196	176	-	-	-
	A	89	188	169	-	-	-
	L						
	E	Mean	192.4	175.0	-	-	-
	S	S.Dev.	4.3	4.6	-	-	-

Key: - = Decedent

ACUTE ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

TABLE 7 - MAIN STUDY - NECROPSY

DOSE LEVEL: 1000 mg/kg

MALES

Animal number	Tissue/ organ	Finding
62		Terminal kill No abnormalities found
64		Terminal kill No abnormalities found
66		Terminal kill No abnormalities found
68		Terminal kill No abnormalities found
70		Terminal kill No abnormalities found

FEMALES

Animal number	Tissue/ organ	Finding
61		Terminal kill No abnormalities found
63		Terminal kill No abnormalities found
65	Jejunum	Terminal kill Contained orange, fluid oily material
67	Jejunum Urinary bladder	Terminal kill Contained orange, fluid oily material Thickened Contained white firm material
69		Terminal kill No abnormalities found

ACUTE ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

TABLE 7 - Continued

DOSE LEVEL: 1800 mg/kg

MALES

Animal number	Tissue/ organ	Finding
72		Terminal kill No abnormalities found
74		Early decedent
	External surfaces	Brown staining, muzzle and urogenital region
	Jejunum	Contained orange fluid
76		Early decedent
	Stomach	Contained yellow mucoid material
	Duodenum	Contained yellow mucoid material
	Jejunum	Contained yellow mucoid material
78		Early decedent
	External surfaces	Brown staining, muzzle and urogenital region
	Stomach	Contained white mucoid and clear oily material
80		Early decedent
	External surfaces	Red staining, muzzle Brown staining, urogenital region

ACUTE ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

TABLE 7 - Continued

DOSE LEVEL: 1800 mg/kg

FEMALES

Animal number	Tissue/ organ	Finding
71		Early decedent No abnormalities found
73		Early decedent
	External surfaces	Ventral region, cannibalised
		Liver, cannibalised
		Spleen, cannibalised
		Intestines, cannibalised
		Kidneys, cannibalised
		Adrenals, cannibalised
		Pancreas, cannibalised
75		Terminal kill No abnormalities found
77		Early decedent Slightly autolysed
	External surfaces	Brown staining, muzzle and urogenital region
79		Terminal kill No abnormalities found

ACUTE ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

TABLE 7 - Continued

DOSE LEVEL: 3240 mg/kg

MALES

Animal number	Tissue/ organ	Finding
82		Early decedent
	Stomach	Contained white mucoid material
84		Early decedent
	External surfaces	Brown staining, muzzle and urogenital region
	Stomach	Contained white mucoid and clear oily material
	Jejunum	Contained yellow mucoid material
86		Early decedent
	External surfaces	Brown staining, muzzle and urogenital region
	Spleen	Small (27x5x2mm)
	Mesenteric L/N	Enlarged (up to 8x5x4mm)
	Prostate	Small in size
	Seminal vescicles	Small in size
	Stomach	Multiple dark pin point areas and glandular region
88		Early decedent
	External surfaces	Brown staining, muzzle and urogenital region
	Stomach	Contained white mucoid material
90		Early decedent
	External surfaces	Brown staining, muzzle and urogenital region
	Stomach	Contained white mucoid material and clear oily material
	Jejunum	Contained yellow mucoid material

ACUTE ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

TABLE 7 - Continued

DOSE LEVEL: 3240 mg/kg

FEMALES

Animal number	Tissue/ organ	Finding
81		Early decedent
	Stomach	Contained white mucoid material
83		Early decedent
	Stomach	Contained white mucoid material
	Jejunum	Contained yellow oily material
85		Early decedent
	Stomach	Contained white mucoid material
	Duodenum	Contained yellow oily material
87		Early decedent
	External surfaces	Brown staining, muzzle and urogenital region
	Stomach	Contained white mucoid material
	Jejunum	Contained yellow mucoid material
89		Early decedent
	Stomach	Contained white mucoid material
	Duodenum	Contained yellow oily material

ACUTE ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

APPENDIX 1 - STATISTICAL ANALYSIS OF MORTALITY DATA

MALES

Dose (mg/kg)	No Dead/No Tested	% Mortality	Expected % Dead
1000	0/5	0.00	0.76
1800	4/5	80.00	71.48
2000	4/5	80.00	86.53
3240	5/5	100.00	99.98

Number of animals	20
Chi square degrees of freedom	2
.05 Chi square level at above degrees of freedom	5.99
Dose effect curve has Chi square value	0.40
Calculated value for LD16	1324.85
Calculated value for LD50	1610.31
Calculated value for LD84	1938.36
Calculated slope function	11.74
95 % confidence interval for calculated LD50	1359.17 - 1907.73
95 % confidence interval for calculated slope ...	5.27 - 18.20

ACUTE ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

APPENDIX 1 - STATISTICAL ANALYSIS OF MORTALITY DATA

FEMALES

Dose (mg/kg)	No Dead/No Tested	% Mortality	Expected % Dead
1000	0/5	0.00	0.00
1800	3/5	60.00	60.00
2000	5/5	100.00	99.70
3240	5/5	100.00	100.00

Number of animals	20
Chi square degrees of freedom	2
.05 Chi square level at above degrees of freedom	5.99
Dose effect curve has Chi square value	200.02
Calculated value for LD16	1707.50
Calculated value for LD50	1780.82
Calculated value for LD84	1853.41
Calculated slope function	54.46
95 % confidence interval for calculated LD50	Not calculable
95 % confidence interval for calculated slope	Not calculable

ACUTE ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

APPENDIX 1 - STATISTICAL ANALYSIS OF MORTALITY DATA

COMBINED SEXES

Dose (mg/kg)	No Dead/No Tested	% Mortality	Expected % Dead
1000	0/10	0.00	0.01
1800	7/10	70.00	69.86
2000	9/10	90.00	90.10
3240	10/10	100.00	100.00

Number of animals	40	
Chi square degrees of freedom	2	
.05 Chi square level at above degrees of freedom	5.99	
Dose effect curve has Chi square value	0.00	
Calculated value for LD16	1461.73	
Calculated value for LD50	1675.77	
Calculated value for LD84	1908.13	
Calculated slope function	16.76	
95 % confidence interval for calculated LD50	1533.23 -	1831.53
95 % confidence interval for calculated slope ...	3.28 -	30.23

ACUTE ORAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8832-001

ADDENDUM 1 - CERTIFICATE OF ANALYSIS FOR THE TEST ITEM

REDACTED AS TO TRADE NAMES

AUSIMONT SpA

Montedison Centro Ricerche e Sviluppo

pag

Richiesta analisi N. [redacted]

Data 08/03/01

Risposta Numero 208/01

Data 12/3/2001

CDC	Commessa	Richiedente	Destinatario
367	D234	S. Fontana	

Firma [redacted]

Prodotto e suoi riferimenti [redacted] 90199/66

[redacted] 90215/20

Precauzioni antinfortunistiche

guanti ed occhiali

Firma S. Fontana

Analisi effettuate o in corso sul campione

ACIDIMETRIC TITRATION

[redacted]

Lavoro richiesto

PESO EQUIVALENTE

Risultati

P.E. = 590

Distribuzione

Riferimenti

Analitica e Strutturistica

Firma

105

REDACTED AS TO TRADE NAMES

RTC

RESEARCH TOXICOLOGY CENTRE - ROMA

[redacted]

ACUTE DERMAL IRRITATION STUDY IN THE RABBIT

FINAL REPORT

RTC Study Number: 8835-001

RTC Report Number: 8835-001/T/308/2001

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

RTC Report Number: 8835-001/T/308/2001

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 : 14-11-2001

J. Brightwell, Ph.D.
(Scientific Director): Date : 14.11.2001

RTC Report Number: 8835-001/T/308/2001

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			
Selection	30.07.2001	-	21.09.2001
Dose preparation	17.09.2001	-	26.09.2001
Body weight	17.08.2001	-	21.08.2001
Dosing	07.08.2001	-	09.08.2001
Clinical observations	17.08.2001	-	26.09.2001
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 12.11.2001

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

12.11.2001
Date

Contents

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, 7 and 14 days after the end of the exposure period.

A well defined erythema and a very slight oedema were observed in all the 3 animals approximately 1 hour after the end of the exposure.
A well defined erythema and slight oedema were still present at the 24 hour examination in the area surrounding the treated site. Discolouration was seen at the treated site at this observation.
A severe (eschar formation) and slight to severe oedema were observed in all animals at 48 and 72 hour examination. This reaction was maintained in 1 of the three animals up to the end of the observation period, while it appeared slightly reduced in the 2 remaining animals at 7 and 14 day examinations.

There was no indication of a systemic effect of treatment.

Body weight changes were not remarkable.

These results indicate that [redacted] has a severe irritant effect on the skin of the rabbit and European Directives concerning the classification, packaging and labelling of dangerous substances would indicate the following:-

Classification : Required
Symbol : Xi
R Phrase : R 38 - Irritating to skin

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 10th September 2001 with allocation of animals to the study and ended on 25th September 2001 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	: 90199/66
Expiry date	: 24th April 2005
Received from	: AUSIMONT S.p.A.
Date received	: 14th May 2001
Amount received	: 120 grams
Description	: Opaque liquid
Container	: Opaque plastic bottle
Storage at RTC	: Ambient condition
RTC reference number	: 5504

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. This included, but was 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 30th August 2001. 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% (batch 5) at a dose level of 0.4 ml/animal.

4.1.2 Housing

Animals were individually housed in stainless steel cages measuring 48 x 63 x 41 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%. Actual conditions were recorded. This was a deviation from the study protocol, in which a range of 22 ± 2°C was erroneously indicated.

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, 72 hours and 7 and 14 days 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

Any reaction to treatment outside the limits of this numerical scale was fully described.

4.5 Body weight

All animals were weighed on preparation (Day -1) and on termination of the study (Day 15).

4.6 Termination

The study was terminated after 14 days, 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)

A well defined erythema (score of 2) and a very slight oedema (score of 1) were observed in all the 3 animals approximately 1 hour after the end of the exposure period.
A well defined erythema and a slight oedema (score of 2) were still present at the 24 hour examination in the area surrounding the treated site. Discolouration (start of necrosis) was seen at the treated site of the 3 animals at this observation.
A severe erythema (score of 4) and a slight to severe (scores of 2 to 4) oedema were observed in all animals at 48 and 72 hour examination.
A slight improvement of this reaction was seen in 1 of the 3 animals at the 7 day examination where a moderate erythema (score of 3) and a slight oedema (score of 2) were observed. A severe reaction was still present in the other 2 animals (score of 4 with eschar formation). The severe erythema and moderate oedema (scores of 4 and 3, respectively) were observed up to day 15 in 1 animal.
A moderate erythema (score of 3) and a slight oedema (score of 2) were seen in a second animal, while a well defined erythema (score of 2) and a very slight oedema (score of 1) were observed in the third animal at this examination.
Scabs on treated site were noted in all animals at the 14 day examination.

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 a severe irritant effect on the skin of the rabbit, this appearing not completely reversible.

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

Classification : Required
Symbol : Xi
R Phrase : R 38 - Irritating to the skin

ACUTE DERMAL IRRITATION STUDY IN THE RABBIT

RTC STUDY NUMBER: 8835-001

TABLE 1 - IRRITATION - MEAN VALUES

Animal Number	Erythema	Oedema
1045	3.3	2.0
1047	3.3	2.7
1049	3.3	3.3
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: 8835-001

TABLE 2 - IRRITATION - INDIVIDUAL FINDINGS

Animal Number: 1045			
Time of examination	Erythema	Oedema	Additional comments
1 hour	2	1	None
24 hours:	2*	2	*Discolouration of treated site
48 hours:	4#	2	#Reddened area around treated site
72 hours:	4#	2	#Reddened area around treated site
7 days:	4°	3	° Eschar formation
14 days:	4	3	Scab on treated site

Animal Number: 1047			
Time of Examination	Erythema	Oedema	Additional comments
1 hour	2	1	None
24 hours:	2*	2	*Discolouration of treated site
48 hours:	4#	3	#Reddened area around treated site
72 hours:	4#	3	#Reddened area around treated site
7 days:	3°	2	° Eschar formation
14 days:	3	2	Scab on treated site

ACUTE DERMAL IRRITATION STUDY IN THE RABBIT

RTC STUDY NUMBER: 8835-001

ADDENDUM 1 - CERTIFICATE OF ANALYSIS FOR THE TEST ITEM

REDACTED AS TO TRADE NAMES

AUSIMONT SpA

Montedison Centro Ricerche e Sviluppo

[illegible]	pag
Richiesta analisi Numero:	Data 08/03/01
Risposta Numero: 208/01	Data 12/3/2001

CDC	Commessa	Richiedente	Destinatario
367	D 234	S. Fontana Firma [redacted]	[redacted]

Prodotto e suoi riferimenti: 90199/66 90215/68

Precauzioni antinfortunistiche

guanti ed occhiali

Firma S. Fontana

Analisi effettuate o in corso sui campioni

ACIDIMETRIC TITRATION [redacted]

Lavoro richiesto

PESO EQUIVALENTE

Risultati

P.E. = 590

Distribuzione

Riferimenti

Analitica e Strutturistica

Firma

121

REDACTED AS TO TRADE NAMES

RTC

RESEARCH TOXICOLOGY CENTRE - ROMA

[redacted]

ACUTE EYE IRRITATION STUDY IN THE RABBIT

FINAL REPORT

RTC Study Number: 8834-001
RTC Report Number: 8834-001/T/407/2001

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

146

RTC Report Number: 8834-001/T/407/2001

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 : 24-01-2002

J. Brightwell, Ph.D.
(Scientific Director): Date : 23.01.2002

RTC Report Number: 8834-001/T/407/2001

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	16.07.2001	16.07.2001	16.07.2001
PROCESS-BASED INSPECTIONS			
Selection	30.07.2001	-	21.09.2001
Dose preparation	17.09.2001	-	26.09.2001
Body weight	17.08.2001	-	21.08.2001
Dosing	13.08.2001	-	21.08.2001
Clinical observations	11.10.2001	-	30.11.2001
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 22 Jan 2002		

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

23/01/02
Date

Contents

Tables

Addenda

1. SUMMARY

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

A 0.1 ml aliquot of the substance was introduced into the right eye of a total of 3 animals. The resulting reaction to treatment was assessed 1, 24, 48, 72 hours and 7, 14 and 21 days after dosing.

Well defined to moderate conjunctival irritation, a slight iris inflammation and well defined corneal opacity were observed in the 3 animals at examinations performed approximately 1, 24 and 48 hours after dosing. The severity of this reaction was increased in 2 of the 3 animals at 72 hour and 14 day examinations, when a severe chemosis and marked iris inflammation were observed (the latter in 1 animal only).
A slight decrease in severity of the conjunctival irritation was observed 7, 14 and 21 days after dosing. Iris inflammation and well defined corneal opacity were observed up to day 22.

There was no indication of a systemic effect of treatment.

Changes in body weight were not remarkable.

These results indicate that the test item, [redacted] has a marked irritant effect in the eye. European Directives concerning the classification, packaging and labelling of dangerous substances would indicate that classification would be required.

2. INTRODUCTION

The purpose of this study was to investigate the degree of ocular irritation produced following introduction of the test item into the eye of the rabbit.

The procedures used were designed to meet the requirements of the test for acute eye irritation described by OECD guideline Number 405, adopted on 24th February 1987. These methods are in agreement with those of B5 described 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 17th September 2001 with allocation of a single animal to treatment in a preliminary screen and ended on 8th October 2001 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	: 90199/66
Expiry date	: 24th April 2005
Received from	: AUSIMONT S.p.A.
Date received	: 14th May 2001
Amount received	: 120 grams
Description	: Opaque liquid
Container	: Opaque plastic bottle
Storage at RTC	: Ambient conditions
RTC reference number	: 5504

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 administered in the condition supplied by the Sponsor.

During handling of the substance, precautions were taken to reduce possible operator exposure. This included, but was 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.

As the irritancy of the substance was unknown, there was the potential for the substance to cause severe irritation. A single animal was dosed and the response evaluated. As the response to treatment was considered to be within acceptable limits a further 2 animals were dosed, bringing the group size to 3.

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 30th August 2001. Animals were ordered weighing approximately 2 kg, 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. A treatment with Pyrantel 6% (prep. 5) at a dose volume of 0.4 ml/animal was also performed following arrival.

4.1.2 Housing

Animals were individually housed in stainless steel cages measuring 48 x 63 x 41 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 15 to 21°C and relative humidity within the range of 40 to 70%. 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

Available stock animals were examined and a total of 3 animals, whose eyes were free from any irritation or other defect, were selected for use on the study.

4.3 Dosing

A 0.1 ml aliquot of the test item was introduced into the right eye of each animal by gently pulling away the lower lid from the eyeball to form a cup into which the test item was placed. The lids were then held shut for a few seconds to prevent loss of the test item. The left eye remained untreated.

4.4 Observations

Approximately 1, 24, 48 and 72 hours 7, 14 and 21 days after dosing the animals were observed under standard conditions and the treated eye examined macroscopically for damage or irritation to the cornea, iris and conjunctivae using the untreated eye as a comparator control. The observed irritation was allocated a numerical value based on the table below:-

Conjunctivae

1)	Reddening of the palpebral and bulbar conjunctivae	
	Blood vessels normal	0
	Some blood vessels definitely hyperaemic (injected)	1
	Diffuse, crimson colour, individual vessels not easily discernible	2
	Diffuse, beefy red	3
2)	Chemosis of the lids and/or nictitating membrane	
	No swelling	0
	Any swelling above normal (including nictitating membranes)	1
	Obvious swelling with partial eversion of the lids	2
	Swelling with lids about half closed	3
	Swelling with lids more than half closed	4
3)	Discharge (Lachrymation)	
	No discharge	0
	Any amount different from normal (does not include small amount observed in inner canthus of normal animals)	1
	Discharge with moistening of the lids and hair just adjacent to the lids	2
	Discharge with moistening of the lids and hair for a considerable area around the eye	3

Iris

Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia, or injection, any of these or combination of any thereof, iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, haemorrhage, gross destruction (any or all of these)	2

Cornea

1) Degree of opacity: (Area most dense taken for reading)

No ulceration or opacity	0
Scattered or diffuse area of opacity (other than slight dulling of normal lustre), details of iris clearly visible	1
Easily discernible translucent area, details of iris slightly obscured	2
Nacreous area, no details of iris visible, size of pupil barely discernible	3
Opaque cornea, iris not discernible through the opacity	4

2) Area of cornea involved

One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

4.5 Body weight

All animals were weighed on selection (Day 1) and on termination of the study (Day 21).

4.6 Termination

The study was terminated after 21 days, 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 of the group. The test item would be classified as an irritant to the eye if the value, in 2 or more animals, for corneal opacity equalled or exceeded 2.0, iris inflammation equalled or exceeded 1.0, conjunctival redness equalled or exceeded 2.5 or conjunctival chemosis equalled or exceeded 2.0. If the test item was to be considered irritant, labelling and use of the risk phrase (R 36) "Irritating to eyes" and symbol "Xi" would be required.

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)

A well defined to moderate chemosis, redness and discharge (scores of 2 and 3), a slight iris inflammation (score of 1) and a slight to well defined corneal opacity (scores of 1 and 2) were observed in the 3 animals approximate 1, 24 and 48 hours after dosing.

A moderate redness (score of 3), a moderate to severe chemosis (scores of 3 and 4), a well defined to moderate ocular discharge (scores of 2 and 3) and a well defined corneal opacity (score of 2) were seen at the 72 hour examinations in the 3 animals. A slight iris inflammation (score of 1) was still present in 2 animals, while a score of 2 was seen in the third animal.

A slight to well defined ocular discharge (scores of 1 and 2), a moderate to severe chemosis (scores of 3 and 4), a slight (1 animal, score of 1) to well defined (score of 2) redness and a well defined corneal opacity (score of 2) were seen up to Day 22 of the observation period. A slight iris inflammation (score of 1) was present in 1 animal at 7, 14 and 21 day examinations and in a second animal at the 7 day examination. An iris inflammation of 2 was seen in 2 animals at 14 and 21 day examinations.

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 a marked irritant effect in the eye. European Directives concerning the classification, packaging and labelling of dangerous substances would indicate that classification would be required.

ACUTE EYE IRRITATION STUDY IN THE RABBIT

RTC STUDY NUMBER: 8834-001

TABLE 1 - IRRITATION - MEAN VALUES

PRELIMINARY SCREEN				
Animal Number	Conjunctival Redness	Conjunctival Chemosis	Iris Inflammation	Corneal Opacity
1089	3.0	3.3	1.0	2.0

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

MAIN PHASE				
Animal Number	Conjunctival Redness	Conjunctival Chemosis	Iris Inflammation	Corneal Opacity
1091	2.7	3.3	1.3	2.0
1093	2.7	3.0	1.0	2.0

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

ACUTE EYE IRRITATION STUDY IN THE RABBIT

RTC STUDY NUMBER: 8834-001

TABLE 2 - IRRITATION - INDIVIDUAL FINDINGS

PRELIMINARY SCREEN		Animal Number: 1089						
Region of eye	Parameter	Ocular reaction						
		Hours				Days		
		1	24	48	72	7	14	21
	Discharge:	2	2	3	2	1	1	1
Conjunctivae:	Chemosis:	2	3	3	4	3	3	3
	Redness:	3	3	3	3	2	2	2
Iris:	Inflammation:	0	1	1	1	1	1	1
Cornea:	Opacity:	1	2	2	2	2	2	2
	Area:	4	4	4	4	4	4	4

ACUTE EYE IRRITATION STUDY IN THE RABBIT

RTC STUDY NUMBER: 8834-001

TABLE 2 - IRRITATION - INDIVIDUAL FINDINGS (Continued)

MAIN PHASE		Animal Number: 1091						
Region of eye	Parameter	Ocular reaction						
		Hours				Days		
		1	24	48	72	7	14	21
	Discharge:	2	2	3	3	2	2	1
Conjunctivae:	Chemosis:	2	3	3	4	4	4	3
	Redness:	3	3	2	3	2	1	1
Iris:	Inflammation:	0	1	1	2	2	2	2
Cornea:	Opacity:	1	2	2	2	2	2	2
	Area:	4	4	4	4	4	4	4

MAIN PHASE		Animal Number: 1093						
Region of eye	Parameter	Ocular reaction						
		Hours				Days		
		1	24	48	72	7	14	21
	Discharge:	2	3	3	3	1	1	1
Conjunctivae:	Chemosis:	2	3	3	3	3	3	3
	Redness:	3	3	2	3	2	2	2
Iris:	Inflammation:	0	1	1	1	1	2	2
Cornea:	Opacity:	1	2	2	2	2	2	2
	Area:	4	4	4	4	4	4	4

ACUTE EYE IRRITATION STUDY IN THE RABBIT

RTC STUDY NUMBER: 8834-001

TABLE 3 - BODY WEIGHT - INDIVIDUAL VALUES

PRELIMINARY SCREEN			
Animal Number	Body weight (kg) on Day:-		Change in body weight (kg) Day 1 to 21
	Day 1	Day 21	
1089	2.7	2.9	0.2

MAIN PHASE			
Animal Number	Body weight (kg) on Day:-		Change in body weight (kg) Day 1 to 21
	Day 1	Day 21	
1091	2.8	3.2	0.4
1093	3.0	3.5	0.5

ACUTE EYE IRRITATION STUDY IN THE RABBIT

RTC STUDY NUMBER: 8834-001

ADDENDUM I – Certificate of analysis for the test item

REDACTED AS TO TRADE NAMES

AUSIMONT SpA

Montedison Centro Ricerche e Sviluppo

pag

Richiesta analisi

Data 08/03/01

Risposta

Data 12/3/2001

C.D.C.	Commessa	Richiedente	Destinatario
367	D234	S. Fontana	

Firma [redacted]

Prodotto e suoi riferimenti [redacted]

Lotto n. 90199/66 90215/20

Precauzioni antinfortunistiche

guanti ed occhiali

Firma S. Fontana

Analisi effettuate o in corso sul campione

ACIDIMETRIC TITRATION

[redacted]

Lavoro richiesto

1/50 EQUIVALENTE

Risultati

P.E. = 590

Distribuzione

Riferimenti

Analitica e Strutturistica

Firme

164

RTC

RESEARCH TOXICOLOGY CENTRE - ROMA

[redacted]

DELAYED DERMAL SENSITISATION STUDY IN THE GUINEA PIG (MAGNUSSON AND KLIGMAN TEST)

FINAL REPORT

RTC Study Number: 8836-001
RTC Report Number: 8836-001/T/380/2001

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

RTC Report Number: 8836-001/T/380/2001

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 : 17-12-2001

J. Brightwell, Ph.D.
(Scientific Director): Date : 17.12.2001

RTC Report Number: 8836-001/T/380/2001

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	29.08.2001	29.08.2001	29.08.2001
PROCESS-BASED INSPECTIONS			
Allocation	05.09.2001	-	08.11.2001
Dose preparation	17.09.2001	-	26.09.2001
Body weight	06.09.2001	-	14.11.2001
Dosing (induction dermal)	04.06.2001	-	08.06.2001
Dosing (induction intradermal)	11.09.2001	-	02.10.2001
Dosing (challenge)	14.08.2001	-	21.08.2001
Clinical observations	02.10.2001	-	19.10.2001
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 14.12.2001		

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

14.12.2001
Date

Contents

1. SUMMARY

The potential of the test item, [redacted] to induce and elicit delayed dermal sensitisation was assessed by a guinea pig model using the maximisation test of Magnusson and Kligman.

The concentrations of the test item used in the main study were determined by the results of preliminary screening tests. The main sensitisation test was undertaken using a test group of 20 animals and a control group of 10 animals. In an attempt to induce sensitisation, test animals were intradermally injected with an emulsion of Freund's complete adjuvant and the test item at 0.1% concentration in both the selected vehicle (corn oil) and an emulsion of Freund's complete adjuvant. One week later this was boosted by topical application of the test item at 5% concentration over the injection sites. Control group animals were treated in the same manner but the selected vehicle (corn oil) was used in place of the test item. Two weeks after the second induction stage, all animals were challenged by topical application of both the vehicle (corn oil) and the test item at 0.5% concentration.

At the challenge with the test item at 0.5% concentration no response to the test item was apparent in any animal of the test or control groups. No reaction to the vehicle alone was observed in any animal.

These results indicate that the test item, [redacted] does not elicit a sensitisation response in the guinea pig, there being no reaction observed at challenge following an induction period of exposure.

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 the study was to assess the ability of the test item, [redacted] to cause delayed dermal sensitisation by use of a guinea pig model.

The procedures used were those of the maximisation test for skin sensitisation described by Magnusson and Kligman. These methods meet the requirements of OECD guideline Number 406, adopted on 17th July 1992. Methods were in agreement with those of B.6 detailed in Commission Directive 96/54/EEC. The species and route of administration were those stated in the regulations, giving a valid model for the assessment of sensitisation.

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 29th August 2001 with allocation of animals of the preliminary phase to treatment and ended on 4th October 2001 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	: 90199/66
Expiry date	: 24th April 2005
Received from	: AUSIMONT S.p.A.
Date received	: 14th May 2001
Amount received	: 120 grams
Description	: Opaque liquid
Container	: Opaque plastic bottle
Storage at RTC	: Ambient conditions
RTC reference number	: 5504

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 prepared for dosing by mixing with corn oil for the induction phase and for challenge phase of the study. A range of concentrations was selected for the preliminary tolerance phase of the study. A concentration of 0.1% was selected for the intradermal injection phase of the induction procedure. Suitable concentrations for topical application were investigated in a screening study and the test item at 5% concentration was used in the induction phase, being tolerated by the test system. A lower concentration of 0.5% was selected for the challenge phase.

Freund's complete adjuvant (FCA), a mixture of paraffin oil, an emulsifier and killed mycobacteria, was used to enhance the potential of the substance to cause a delayed contact hypersensitivity reaction. It was used as a 50% v/v emulsion of FCA in sterile water.

During handling of the substance and its formulations, precautions were taken to reduce possible operator exposure. This included, but was 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

Young adult female guinea pigs of Dunkin-Hartley strain were ordered from Harlan Italy S.r.l., San Pietro al Natisone (UD), Italy. Animals were ordered nulliparous and non-pregnant, within the weight range of 300 to 350 grams and 4 to 5 weeks of age. They were supplied by Harlan Netherlands, Kreuzelweg 53, 5961 NM Horst, P.O. Box 6174 NL, 5960 AD Horst, Netherlands and appeared to be in an acceptable condition following arrival on 24th August 2001. Animals were identified by tattoo on the ear following arrival and an acclimatisation period of at least 5 days was permitted before undertaking any dosing procedure.

4.1.2 Animal husbandry

Animals were housed during the study, in groups of up to 5 animals in stainless steel cages, with a grid floor. Cages were suspended over metal trays which held an absorbent material. This was inspected daily and changed as necessary. Throughout the study, each cage was identified by a label, colour-coded according to group, recording the study number, animal numbers and details of treatment.

Controls for the animal room were set to maintain temperature within the range of 20 to 24°C, and relative humidity within the range of 40 to 70%. This was a deviation from the study protocol in which the range of temperature was erroneously indicated as 18-22°C. Actual conditions achieved were recorded daily. The room was lit by fluorescent light to give an artificial cycle of 12 hours light/12 hours dark.

4.1.3 Water and diet

Animals were offered drinking water supplied to each cage via a water bottle and a commercially available 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 was present in either diet or drinking water at a level likely to interfere with the purpose or conduct of the study.

4.2 Experimental design

The study was divided into 2 distinct phases. The first of these was a dose-ranging screen which was used to determine suitable dose levels for use in the second phase. This second phase formed the main study, a determination of the sensitisation potential of the test item.

4.2.1 Allocation to groups

Animals were selected and allocated to treatment groups prior to each phase of the study.

4.2.2 Intradermal injection tolerance test

Two animals were selected from those available and the hair over the scapulae was removed using an electric clipper with suitable blade. Six sites were selected on each animal and these injected intradermally with 0.1 ml of the test item. Each site was injected with a single concentration of the test item. The 2 animals were treated with the substance at concentrations of 100%, 50%, 20%, 10%, 5% and 1% in corn oil. The treated sites of the animals were not examined 7 days later for any signs of reaction to treatment as they were found dead on days 2 and 4, respectively.
The preliminary phase of the tolerance test was repeated since the animals were found dead on the second and the fourth days after injection of the test item. Two additional animals were treated with the test item at concentrations of 5%, 1%, 0.5%, 0.1%, 0.05% and 0.01% in corn oil and any sign of reaction was recorded 6 days after treatment according to the Draize scoring scale.

Erythema and eschar formation	Value
No response	0
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema to slight eschar formation	4

4.2.3 Topical application tolerance test

Five animals were selected from those available and the hair over the scapulae was removed using an electric clipper with suitable blade. Each animal was then injected intradermally at the prepared site with two injections, each of 0.1 ml, of emulsified Freund's complete adjuvant.

Seven days later, the flanks of each animal were clipped free of hair. Each animal was dosed with 2 concentrations of the test item, 1 on either flank. A gauze patch measuring at least 20 x 20 mm was soaked with 0.2 ml of the selected concentration of the test item. This was then placed onto the selected treatment site. When both sites of the animal had been treated, they were covered with a strip of aluminium foil to act as an occlusive barrier and the trunk of the animal was wrapped with an elastic adhesive bandage to maintain the test item in contact with the skin.

All animals were treated in this manner such that a total of 5 concentrations (100%, 50%, 20%, 10% and 5% in corn oil) of the test item were each dosed in duplicate. The adhesive dressings and gauze patches were removed after 24 hours contact with the skin.

Twenty four and 48 hours after removal of the dressings, the treated sites were examined for signs of reaction to treatment. Each site was assessed and scored on the following scale:-

Reaction observed	Value
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

4.2.4 Main study - Induction - Intradermal injection

Animals were allocated to treatment to give a test group of 20 animals and a control group of 10 animals. On the day of dosing (Day 1) the hair was clipped from the scapular region of each animal over an area approximately 20 x 40 mm. Three pairs of intradermal injections were made at the prepared skin site of each animal. All injections were made at the edge of the prepared site and the anterior and median injections were positioned close together and distant from the posterior injections. A volume of 0.1 ml was injected at each point.

Animals of the test group were treated as follows:-

Injection site	Treatment
Anterior	Emulsified Freund's complete adjuvant
Median	0.1% test item in corn oil
Posterior	0.1% test item in emulsified Freund's complete adjuvant

Animals of the control group were treated in the same manner except that the test item was replaced by the vehicle alone. The treatment plan was:-

Injection site	Treatment
Anterior	Emulsified Freund's complete adjuvant
Median	Vehicle (corn oil)
Posterior	Vehicle mixed with emulsified Freund's complete adjuvant

Skin reaction at the injection sites was assessed approximately 24 hours after injection.

4.2.5 Main study - Induction - Topical application

Seven days after injection (Day 8 of the study) the area surrounding the injection sites on each animal was clipped free of hair. On Day 8 animals of the test group were treated with the test item at 5% concentration. A gauze patch was covered with 0.4 ml of the substance and this placed over the injection sites, with the substance in contact with the skin, and covered with a strip of aluminium foil to serve as an occlusive barrier. The animal was then wrapped with a length of elastic adhesive bandage to maintain the gauze patch in contact with the skin. All animals of the test group were treated in this manner. Animals of the control group were similarly treated with the vehicle alone (corn oil). After a contact period of 48 hours the dressings were removed and the treated sites gently cleaned by washing with warm water.

Reaction to treatment was assessed approximately 24 hours after removal of the dressings using the following scoring system:-

Reaction observed	Value
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

4.2.6 Main study - Challenge

On Day 22, 3 weeks after preparing the animals for the first induction phase of the main study, all animals were prepared for challenge by clipping the flanks free of hair to expose areas approximately 50 mm x 50 mm on each flank.

Patches of gauze measuring 20 mm x 20 mm were coated with 0.2 ml aliquots of the test item at 0.5% concentration. These were placed on the right flank of each animal, of both test and control groups, in the centre of the prepared skin site. The left flank of each animal was similarly treated with patches coated with 0.2 ml of the vehicle alone (corn oil). The treated sites were covered with a strip of aluminium foil to act as an occlusive barrier and each animal then wrapped with a length of elastic adhesive bandage to keep the test item and vehicle in contact with the skin. After a contact period of 24 hours the dressings and patches were removed.

Approximately 22 hours after removal of the dressings and patches, the treated sites were closely clipped to remove any hair that may have grown. This was a deviation from protocol in which this procedure was indicated to be performed approximately 21 hours after patches removal. Approximately 2 hours later, 24 hours after removal of the dressings, the treated sites were examined for any signs of reaction to treatment.

The following scoring scale was used to describe any observed reaction:-

Reaction observed	Value
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

This examination was repeated 24 hours later, 48 hours after removal of the dressings.

4.2.7 Body weight

Animals used in the main sensitisation assessment were weighed at the start of treatment (Day 1) and on completion of the challenge (Day 25).

4.2.8 Termination and necropsy

All animals were killed by carbon dioxide narcosis following the end of the experimental procedure. Necropsy examination was performed on the 2 early decedent animals.

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).

The test would be considered positive if 30% or more of animals in the test group exhibited erythema or dermal swelling following challenge with a non-irritant concentration of the test item. The non-irritant nature of the test item at the concentration used at challenge would be demonstrated by the lack of dermal responses in the control group.

Should the test have been considered positive, the test item would require labelling with the risk phrase (R 43) "May cause sensitisation by skin contact" and symbol "Xi".

4.4 Archives

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

5. RESULTS

5.1 Preliminary tolerance tests (Table 1)

The preliminary tolerance test to establish a suitable concentration for injection in the main sensitisation test indicated that the test item at 0.1% concentration should be easily injected.
The topical application test indicated that the test item at 5% concentration should be tolerated by the test system. A lower concentration of 0.5% was judged to be non-irritant and was selected for use at challenge.

5.2 Induction (Tables 2 and 3)

Well defined erythema (score of 2) was apparent at the sites of intradermal injection following administration of Freund's complete adjuvant (test and control groups), the vehicle mixed with Freund's complete adjuvant (control group) and the test item in the vehicle (test group). Moderate erythema with a beginning of necrosis was found following administration of the test item mixed with Freund's complete adjuvant in the test group. No response was observed at sites treated with the vehicle alone (control group).

Discrete erythema was found in 3 out of 20 test animals (score of 1). No reaction was observed around the injection sites of the remaining test animals or in the control group following 24 hours topical exposure.

5.3 Challenge (Table 4)

At challenge no response was observed in any animal of either the test or control groups following 24 hours topical exposure to the test item at 0.5%.
No reaction to the vehicle alone was observed in any animal of test or control groups.

Group	Treatment	Incidence of response (%) at challenge:-	
		24 Hours	48 Hours
Control	Test item	0%	0%
	Vehicle	0%	0%
Test	Test item	0%	0%
	Vehicle	0%	0%

5.4 Body weight (Table 5)

Changes in body weight of animals during the period of the study were generally similar in animals from both test and control groups during the study.

5.5 Necropsy

Two animals of the preliminary phase (intradermal injection) were found dead 2 and 4 days after dosing. Necropsy examination of these animals revealed no abnormalities which could be clearly attributed to the treatment with the test item.

6. CONCLUSION

The results obtained in this study indicate that the test item, [redacted] does not elicit a sensitisation response in the guinea pig, there being no reaction observed at challenge following an induction period of exposure to the substance.

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

DELAYED DERMAL SENSITISATION STUDY IN THE GUINEA PIG
(MAGNUSSON AND KLIGMAN TEST)

RTC STUDY NUMBER: 8836-001

TABLE 1 - PRELIMINARY SCREENS - INDIVIDUAL RESULTS

These tables detail the results of examination of injection sites 6 days after injection of a range of concentrations of the test item ([redacted] in the selected vehicle (corn oil).

Animal number	Test item Concentration	Erythema	Additional comments
1	100%	-	Found dead
	50%	-	
	20%	-	
	10%	-	
	5%	-	
	1%	-	
3	100%	-	Found dead
	50%	-	
	20%	-	
	10%	-	
	5%	-	
	1%	-	

Animal number	Test item Concentration	Erythema	Additional comments
351	5%	N	-
	1%	N	-
	0.5%	N	-
	0.1%	1	-
	0.05%	1	-
	0.01%	1	-
353	5%	N	-
	1%	N	-
	0.5%	N	-
	0.1%	1	-
	0.05%	1	-
	0.01%	1	-

KEY: 0 = No response
1 = Very slight erythema
2 = Well defined erythema
3 = Moderate to severe erythema
4 = Severe erythema to slight eschar formation
N = Necrosis

DELAYED DERMAL SENSITISATION STUDY IN THE GUINEA PIG
(MAGNUSSON AND KLIGMAN TEST)

RTC STUDY NUMBER: 8836-001

TABLE 1 - PRELIMINARY SCREENS - INDIVIDUAL RESULTS

This table details the results of examination of treated sites following topical application of a range of concentrations of the test item ([redacted] in the selected vehicle (corn oil).

Animal Number	Observation Time	Test item concentration				
		100%	50%	20%	10%	5%
5	24 hours	N	N			
	48 hours	N	N			
7	24 hours		N	N		
	48 hours		N	N		
9	24 hours			3	1	
	48 hours			N	1	
11	24 hours				0	0
	48 hours				0	0
13	24 hours	N				0
	48 hours	N				0

KEY: 0 = No visible change
1 = Discrete or patchy erythema
2 = Moderate and confluent erythema
3 = Intense erythema and swelling
N = Necrosis

DELAYED DERMAL SENSITISATION STUDY IN THE GUINEA PIG
(MAGNUSSON AND KLIGMAN TEST)

RTC STUDY NUMBER: 8836-001

TABLE 2 - MAIN STUDY - INDUCTION INJECTION - INDIVIDUAL RESULTS

This table details the responses observed 24 hours after injection of animals with the test item ([redacted]) at 0.1% concentration or the vehicle alone (corn oil) in the initial phase of induction.

Group Function	Animal number	Dermal response					
		Anterior site (FCA emulsion)		Median site (Vehicle)		Posterior site (Vehicle/FCA)	
		Left	Right	Left	Right	Left	Right
CONTROL	81	2	2	0	0	2	2
	83	2	2	0	0	2	2
	85	2	2	0	0	2	2
	87	2	2	0	0	2	2
	89	2	2	0	0	2	2
	91	2	2	0	0	2	2
	93	2	2	0	0	2	2
	95	2	2	0	0	2	2
	97	2	2	0	0	2	2
	99	2	2	0	0	2	2

Group Function	Animal Number	Dermal response					
		Anterior site (FCA emulsion)		Median site (Test item)		Posterior site (Test item/FCA)	
		Left	Right	Left	Right	Left	Right
TEST	101	2	2	2	2	3b	3b
	103	2	2	2	2	3b	3b
	105	2	2	2	2	3b	3b
	107	2	2	2	2	3b	3b
	109	2	2	2	2	3b	3b
	111	2	2	2	2	3b	3b
	113	2	2	2	2	3b	3b
	115	2	2	2	2	3b	3b
	117	2	2	2	2	3b	3b
	119	2	2	2	2	3b	3b
	121	2	2	2	2	3b	3b
	123	2	2	2	2	3b	3b
	125	2	2	2	2	3b	3b
	127	2	2	2	2	3b	3b
TEST	129	2	2	2	2	3b	3b
	131	2	2	2	2	3b	3b
	133	2	2	2	2	3b	3b
	135	2	2	2	2	3b	3b
	137	2	2	2	2	3b	3b
	139	2	2	2	2	3b	3b

KEY: 0 = No response
1 = Very slight erythema
2 = Well defined erythema
3 = Moderate to severe erythema
4 = Severe erythema to slight eschar formation
b = Beginning of necrosis

DELAYED DERMAL SENSITISATION STUDY IN THE GUINEA PIG
(MAGNUSSON AND KLIGMAN TEST)

RTC STUDY NUMBER: 8836-001

TABLE 3 - MAIN STUDY - TOPICAL INDUCTION - INDIVIDUAL RESULTS

This table details the findings at the injection sites of each animal 24 hours following 48 hours topical exposure to either the test item [redacted] at 5% concentration or the vehicle alone (corn oil) during the second stage of the induction procedure.

Group function	Animal number	Dermal response
CONTROL	81	0
	83	0
	85	0
	87	0
	89	0
	91	0
	93	0
	95	0
	97	0
	99	0
TEST	101	0
	103	0
	105	0
	107	0
	109	1
	111	0
	113	0
	115	0
	117	0
	119	0
	121	0
	123	1
	125	0
	127	1
	129	0
	131	0
	133	0
	135	0
	137	0
	139	0

KEY: 0 = No visible change
1 = Discrete or patchy erythema
2 = Moderate and confluent erythema
3 = Intense erythema and swelling

DELAYED DERMAL SENSITISATION STUDY IN THE GUINEA PIG
(MAGNUSSON AND KLIGMAN TEST)

RTC STUDY NUMBER: 8836-001

TABLE 4 - MAIN STUDY - CHALLENGE - INDIVIDUAL RESULTS

This table details the responses observed 24 and 48 hours following the challenge by 24 hours topical exposure to the test item ([redacted]) at 0.5% concentration and the vehicle alone (corn oil).

Group function	Animal number	Dermal response			
		Vehicle		Test item	
		24 hours	48 hours	24 hours	48 hours
CONTROL	81	0	0	0	0
	83	0	0	0	0
	85	0	0	0	0
	87	0	0	0	0
	89	0	0	0	0
	91	0	0	0	0
	93	0	0	0	0
	95	0	0	0	0
	97	0	0	0	0
	99	0	0	0	0
TEST	101	0	0	0	0
	103	0	0	0	0
	105	0	0	0	0
	107	0	0	0	0
	109	0	0	0	0
	111	0	0	0	0
	113	0	0	0	0
	115	0	0	0	0
	117	0	0	0	0
	119	0	0	0	0
	121	0	0	0	0
	123	0	0	0	0
	125	0	0	0	0
	127	0	0	0	0
	129	0	0	0	0
	131	0	0	0	0
	133	0	0	0	0
	135	0	0	0	0
	137	0	0	0	0
	139	0	0	0	0

KEY: 0 = No visible change
1 = Discrete or patchy erythema
2 = Moderate and confluent erythema
3 = Intense erythema and swelling

DELAYED DERMAL SENSITISATION STUDY IN THE GUINEA PIG
(MAGNUSSON AND KLIGMAN TEST)

RTC STUDY NUMBER: 8836-001

TABLE 5 - MAIN STUDY - BODY WEIGHT - INDIVIDUAL VALUES

This table details the body weights of animals used in the study.

Group function	Animal Number	Body weight (g) on Day:-		Change in body weight (g) Day 1 to Day 25
		1	25	
CONTROL	81	544	603	59
	83	578	660	82
	85	556	641	85
	87	543	599	56
	89	531	680	149
	91	536	611	75
	93	540	626	86
	95	536	641	105
	97	537	598	61
	99	490	550	60
	Mean	539.1	620.9	81.8
	S. Dev.	22.0	37.0	28.3
TEST	101	568	629	61
	103	488	591	103
	105	518	623	105
	107	483	587	104
	109	530	617	87
	111	501	589	88
	113	498	570	72
	115	576	680	104
	117	536	622	86
	119	514	607	93
	121	530	605	75
	123	553	634	81
	125	548	625	77
	127	504	593	89
	129	536	615	79
	131	490	572	82
	133	526	603	77
	135	530	608	78
	137	574	621	47
	139	512	590	78
	Mean	525.8	609.1	83.3
	S. Dev.	28.0	24.8	14.6

DELAYED DERMAL SENSITISATION STUDY IN THE GUINEA PIG
(MAGNUSSON AND KLIGMAN TEST)

RTC STUDY NUMBER: 8836-001

TABLE 6 - RELIABILITY CHECK - SUMMARY

This table summarises the results obtained in the most recent reliability check.

RTC STUDY NUMBER:	8442-002INT		
REFERENCE SUBSTANCE:	α-HEXYLCINNAMALALDEHYDE		
CONCENTRATION:	INDUCTION	(INJECTION) -	20% in Corn oil
		(TOPICAL) -	50% in Corn oil
	CHALLENGE	-	10% in acetone
CRITICAL DATES:	INDUCTION	(INJECTION) -	11th September 2001
		(TOPICAL) -	18th September 2001
	CHALLENGE	-	2nd October 2001
RESULTS:	100% response in test group and 0% response in control group at challenge		
INTERPRETATION:	Incidence at challenge acceptable Test system regarded as valid		

DELAYED DERMAL SENSITISATION STUDY IN THE GUINEA PIG (MAGNUSSON AND KLIGMAN TEST)

RTC STUDY NUMBER: 8836-001

ADDENDUM 1 - CERTIFICATE OF ANALYSIS FOR THE TEST ITEM

REDACTED AS TO TRADE NAMES

AUSIMONT SpA

Montedison Centro Ricerche e Sviluppo

pag

Richiesta analisi

Numero

Data 08/03/01

Risposta Numero 208/01

Data 12/3/2001

CDC	Commessa	Richiedente	Destinatario
567	D 234	S. Fontana Firma	

Prodotto e suoi riferimenti

[redacted] 90199/66

[redacted] 90215/28

Precauzioni antinfortunistiche

guanti ed occhiali

Firma S. Fontana

Analisi effettuate o in corso sul campione

ACIDIMETRIC TITRATION

[redacted]

Lavoro richiesto

PESO EQUIVALENTE

Risultati

P.E. = 590

Distribuzione

Riferimenti

Analitica e Strutturistica

Firme

145

REDACTED AS TO TRADE NAMES

RTC

RESEARCH TOXICOLOGY CENTRE - ROMA

[redacted]

ACUTE DERMAL TOXICITY STUDY IN THE RAT

FINAL REPORT

RTC Study Number: 8833-1

RTC Report Number: 8833-1/T/317/2001

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

213

RTC Report Number: 8833-1/T/317/2001

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.

pp Cinzia Longoland

M. A. Antonelli, Biol. D., Spec. Tox.
(Study Director): Date : 14-11-2001

J. Brightwell, Ph.D.
(Scientific Director): Date : 12.11.2001

RTC Report Number: 8833-1/T/317/2001

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	30.07.2001	30.07.2001	30.07.2001
PROCESS-BASED INSPECTIONS			
Allocation	07.05.2001	-	24.07.2001
Dose preparation	13.08.2001	-	21.08.2001
Body weight	08.06.2001	-	26.06.2001
Dosing (dermal)	03.05.2001	-	26.06.2001
Clinical observations	16.08.2001	-	03.10.2001
Despatch to necropsy	13.07.2001	-	19.09.2001
Necropsy	14.08.2001	-	21.08.2001
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 12.11.2001

M. Brunetti

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

12.11.2001
Date

Contents

Tables

Addenda

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. Hardening of the treated sites followed by necrosis and scabs were observed during the 14 day observation period.

Changes in body weight were within the expected range.

Necropsy examination revealed no significant abnormalities. Abrasions and scabs were observed on the treated sites of most animals.

These results indicate that the test item 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 will allow 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 1st August 2001 with allocation of animals to treatment and ended on 16th August 2001 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	: 90199/66
Expiry date	: 24th April 2005
Received from	: AUSIMONT S.p.A.
Date received	: 14th May 2001
Amount received	: 120 grams
Description	: Opaque liquid
Container	: Opaque plastic bottle
Storage at RTC	: Ambient conditions
RTC reference number	: 5504

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. This included, but was 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 20th July 2001. 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 36 x 19 x 24 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 20 to 24°C and relative humidity within the range of 40 to 70%. 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	92, 94, 96, 98, 100	91, 93, 95, 97, 99

All animals were within a body weight range of 204 to 281 grams and animals were 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

The next day (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, 2 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. Hardening of the treated sites followed by necrosis and scabs were observed during the 14 day observation period.

5.2 **Body weight** (Table 2)

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

5.3 **Necropsy** (Table 3)

No abnormalities were found on necropsy of animals on termination of the study. Abrasions and scabs were observed on the treated sites of most animals.

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: 8833-1

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	5	5	5	5	5	5	5	5	5
Hardening - treated site	0	0	0	0	0	0	0	0	5	5
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
Hardening - treated site	5	3	0	0	0	0	0	0
Beginning of necrosis - treated site	0	2	5	4	2	2	1	0
Necrosis - treated site	0	0	0	1	3	3	3	3
Scabs - treated site	0	0	0	0	0	0	0	2
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: 8833-1

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	5	5
Hardening - treated site	0	0	0	0	0	0	0	0	3	0
Beginning of necrosis - treated site	0	0	0	0	0	0	0	0	0	0
Necrosis - treated site	0	0	0	0	0	0	0	0	2	5
Scabs - treated site	0	0	0	0	0	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
Hardening - treated site	0	0	0	0	0	0	0	0
Beginning of necrosis treated site	0	0	0	0	0	0	0	0
Necrosis - treated site	5	5	5	5	5	5	5	3
Scabs - treated site	0	0	0	0	0	0	0	2
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: 8833-1

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
	92	271	276	289	309	33
M	94	275	273	300	321	48
A	96	272	276	298	328	52
L	98	252	254	274	299	45
E	100	281	287	313	340	53
S						
	Mean	270.2	273.2	294.8	319.4	46.2
	S.Dev.	10.9	12.0	14.4	16.0	8.0
	91	204	201	215	230	29
F	93	219	222	225	231	9
E	95	234	230	245	256	26
M	97	208	205	220	238	33
A	99	222	221	230	246	25
L						
E	Mean	217.4	215.8	227.0	240.2	24.4
S	S.Dev.	11.9	12.3	11.5	10.9	9.2

ACUTE DERMAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8833-1

TABLE 3 - NECROPSY

DOSE LEVEL: 2000 mg/kg

Sex	Animal number	Tissue/ organ	Finding
MALES	92		Terminal kill No abnormalities found
	94	Treated site	Terminal kill Abrasion No abnormalities found
	96	Treated site	Terminal kill Abrasion No abnormalities found
	98	Treated site	Terminal kill Multiple scabs up to 2x2 mm No abnormalities found
	100	Treated site	Terminal kill Abrasion No abnormalities found
FEMALES	91	Treated site	Terminal kill Single abrasion up to 25x20 mm No abnormalities found
	93	Treated site	Terminal kill Multiple scabs up to 2x2 mm No abnormalities found
	95	Treated site	Terminal kill Single abrasion up to 20x15 mm No abnormalities found
	97	Treated site	Terminal kill Single abrasion up to 16x10 mm No abnormalities found
	99	Treated site	Terminal kill Multiple scabs up to 2x2 mm No abnormalities found

ACUTE DERMAL TOXICITY STUDY IN THE RAT

RTC STUDY NUMBER: 8833-1

ADDENDUM 1 - CERTIFICATE OF ANALYSIS FOR THE TEST ITEM

REDACTED AS TO TRADE NAMES

RÜSIMONT SpA

Monsindoro Centro Ricerche e Sviluppo

nag

Richiesta analisi
Numero

Data
08/03/01

Risposta
Numero 208/01

Data 12/3/2001

CDC	Commessa	Richiedente	Destinatario
367	D 134	S. Fontana	

Firma

Prodotto e suoi riferimenti

[redacted] batch 90199/66

[redacted] batch 90215/20

Precauzioni antinfortunistiche

guanti ed occhiali

Firma S. Fontana

Analisi effettuate o in corso sul campione

ACIDIMETRIC TITRATION

[redacted]

Lavoro richiesto

RESO EQUIVALENTE

Risultati

P.E. = 590

Distribuzione

Riferimenti

Analitica e Strutturistica

Firme

230

mal