Huntingdon

[redacted]

ACUTE DERMAL TOXICITY TO THE RAT

CONFIDENTIAL

DPT 433/984485/AC

[redacted]

ACUTE DERMAL TOXICITY TO THE RAT

Sponsor

DuPont Speciality Chemicals
Jackson Laboratory
Chambers Works
Deepwater
NJ 08023
USA

Research Laboratory

Huntingdon Life Sciences Ltd
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ENGLAND

Report issued 5 May 1999

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COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

The UK Good Laboratory Practice Regulations 1997 (Statutory Instrument No 654).

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

EC Council Directive 87/18/EEC of 18 December 1986 (Official Journal No L 15/29).

05 May 1999

Date

Lewis A. McRae, M.I.Sc.T., C.Biol., M.I.Biol.,
Study Director,
Huntingdon Life Sciences Ltd.

QUALITY ASSURANCE STATEMENT

The following have been inspected or audited in relation to this study

Study Phases Inspected	Date of Inspection	Date of Reporting
Standard Protocol Audit	24 November 1997	24 November 1997
Process Based Inspections		
Housing/Environment	14 September 1998	17 September 1998
Husbandry	14 September 1998	17 September 1998
Weighing of Animals	14 September 1998	17 September 1998
Treatment Procedure	14 September 1998	17 September 1998
Scoring	14 September 1998	17 September 1998
Records Audit	14 September 1998	17 September 1998
Training Records	14 September 1998	17 September 1998
Report Audit	12 February 1999	16 February 1999

Standard Protocol Audit: An audit of the standard protocol generated for this type of study design was conducted and reported to Company Management as indicated above.

Process based inspections: At or about the time this study was in progress inspections and audits of other routine and repetitive procedures employed on this type of study were carried out. These were promptly reported to appropriate Company Management

Report Audit: This report has been audited by the Quality Assurance Department. This audit was conducted and reported to the Study Director and Company Management as indicated above.

The methods, procedures and observations were found to be accurately described and the reported results to reflect the raw data.

Margaret Blows
Margaret Blows,
Quality Assurance Group Leader,
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.

29 April 1999
Date

RESPONSIBLE PERSONNEL

Lewis A. McRae, M.I.Sc.T., C.Biol., M.I.Biol.,
Study Director,
Department of Acute Toxicology.

SUMMARY

A study was performed to assess the acute dermal toxicity of [redacted] to the rat. The method followed was based on that described in:

EEC Methods for the determination of toxicity, Annex to Directive 92/69/EEC (Official Journal No. L383A, 29.12.92), Part B, Method B.3. Acute toxicity (dermal).

OECD Guideline for Testing of Chemicals No. 402 "Acute Dermal Toxicity". Adopted: 24 February 1987.

A group of ten rats (five males and five females) received a single topical application of the test substance administered at a dose level of 2000 mg/kg bodyweight with dose volume increased to compensate for water content (75%). All dosages referenced hereafter are expressed as dose concentrations after adjustment for the 75% water content of [redacted] The main study dose level selection was supported by preliminary study findings and in compliance with the test guidelines.

All animals were killed as scheduled at study termination (Day 15) and subjected to a macroscopic examination.

There was no clinical signs of reaction to treatment observed in any animal throughout the study.

Primarily transient slight to well-defined dermal irritation (erythema/oedema up to Grade 2) was notable in the majority of rats with a similar level of response more persistent in one female. These reactions were accompanied in one rat by desquamation over the treatment site. In the majority of rats, all reactions had resolved by Day 7 and in one rat by Day 12.

Minor bodyweight fluctuations were noted in the occasion animal. However, the majority of animals were considered to have achieved satisfactory bodyweight gains throughout the study.

No macroscopic abnormalities were observed for animals killed at study termination on Day 15.

The acute lethal dermal dose to rats of [redacted] was demonstrated to be greater than 2000 mg/kg bodyweight.

On the basis of findings in this study and in accord with EU hazard classification [redacted] will not require labelling with the risk phrase R21 "Harmful in contact with skin", in accordance with Commission Directive 93/21/EEC.

INTRODUCTION

The study was designed to assess the toxicity o[redacted]following a single dermal administration to the rat. The rats were dosed by topical application as the test substance may come in contact with the skin during handling or use.

The study was conducted in compliance with the following guidelines:

EEC Methods for the determination of toxicity, Annex to Directive 92/69/EEC (Official Journal No. L383A, 29.12.92), Part B, Method B.3. Acute toxicity (dermal).

OECD Guideline for Testing of Chemicals No. 402 "Acute Dermal Toxicity". Adopted: 24 February 1987.

The rat was chosen as the test species as it has been shown to be a suitable model for this type of study and is the animal recommended by the test guidelines.

The dose level (2000 mg/kg) for the study was chosen after review of preliminary study findings and in compliance with the test guidelines.

The protocol was approved by Huntingdon Life Sciences Management on 7 July 1998, by the Sponsor on 17 July 1998 and by the Study Director on 27 August 1998.

The experimental phase of the study was conducted between the 7 September and 17 December 1998.

TEST SUBSTANCE

Identity:	[redacted]
Chemical name:	[redacted]
Intended use:	[redacted]
Appearance:	[redacted]
Storage conditions:	Room temperature
Lot number:	[redacted]
Expiry:	Two years from date of receipt
Purity:	[redacted]
Sample received:	23 June 1998

EXPERIMENTAL PROCEDURE

ANIMAL MANAGEMENT

The animals chosen for this study were selected from a stock supply of healthy male and female CD rats of Sprague-Dawley origin (Hsd:Sprague-Dawley (CD)) obtained from Harlan U.K. Ltd., Bicester, Oxon, England.

Animals in the main study were in the weight range of 221 to 308 g and approximately eight to eleven weeks of age prior to dosing (Day 1). All the rats in the main study were acclimatised to the experimental environment for a period of seven days prior to the start of the study.

The rats were allocated without conscious bias to cages within the treatment groups. They were housed in groups of up to five rats of the same sex in metal cages during the acclimatisation period and on commencement of study individually housed in metal cages (RS Biotech Sub-Dividable Rodent Cages - polished stainless steel (20cm high x 39cm wide x 39cm long)). The cages were fitted with grid floors to ensure rapid removal of waste material to undertrays. The cages were suspended in mobile stainless steel racks in Room 6 of Building R14. All animals were returned to group housing on Day 12 of the study (by which time all dermal reactions had resolved).

A standard laboratory rodent diet (Special Diet Services RM1(E) SQC expanded pellet) and drinking water were provided *ad libitum*. The batch(es) of diet used for the study was analysed for certain nutrients, possible contaminants and micro-organisms. Results of routine physical and chemical examination of drinking water, as conducted by the supplier, are made available to Huntingdon Life Sciences Ltd. as quarterly summaries.

Thermostatic controls were set to maintain a temperature of 22 ± 3°C. Relative humidity was not fully controlled but was anticipated to be in the range 30 - 70%. Temperature and humidity were recorded continuously using a seven day recorder. Actual measurement of these parameters revealed that animal room temperature was in the range 20 to 24°C and relative humidity was in the range 30 - 60%. Permanent daily recordings of these parameters were made and these are archived with other Departmental raw data. Lighting was controlled by means of a time switch to provide 12 hours of artificial light (0700 - 1900 hours) in each 24-hour period.

Each animal was identified by cage number and ear punching. Each cage was identified by a coloured label displaying the dose level, study schedule number, animal mark and the initials of the Study Director and Home Office licensee.

TEST SUBSTANCE PREPARATION

[redacted] was administered as supplied [redacted] at a dose volume increased sufficiently to compensate for the water content [redacted] of the test material in order to achieve a dose level of 2000 mg/kg bodyweight in the main study.

The absorption and characterisation of the homogeneity, stability in vehicle and purity of the test substance was not undertaken in this study and remains the responsibility of the Sponsor.

TREATMENT PROCEDURE

Preliminary study

In the absence of precise dermal irritation information a preliminary study comprising of two rats (one male and one female) was dosed at 500 mg/kg bodyweight to assist in defining potential irritation/toxicity of the test substance and aid in selection of a suitable dosage for the main study.

Main study

A group of ten rats (five males and five females) received a single dermal application of the test substance at a dose level of 2000 mg/kg bodyweight. The dose level was chosen after review of preliminary study results and in compliance with the test guidelines.

The treatment regime and constitution of the group(s) are shown below:

Phase of Study	Dates dosed	Dose (mg/kg)	Dose volume (ml/kg)	No. of rats	
				M	F
Preliminary	07.09.98	500	1.75	1	1
Main	26.11.98*	2000	7	1	1
	03.12.98	2000	7	4	4

In the above and following Tables, M: denotes male rats and F: denotes female rats.

*: To ensure at the volume dosed the test substance would not prove to irritant initially only one male and one female were dosed.

ADMINISTRATION OF TEST SUBSTANCE

One day prior to treatment, hair was removed from the dorso-lumbar region of each rat with electric clippers taking care to avoid damaging the skin, exposing an area equivalent to approximately 10% of the total body surface area.

The test substance was applied by spreading it evenly over the prepared skin. The treatment area (approximately 50 mm x 50 mm) was covered with porous gauze held in place with a non irritating dressing, and further covered by a waterproof dressing encircled firmly around the trunk of the animal.

Treatment in this manner was performed on Day 1 (day of dosing) of the study only.

At the end of the 24 hours exposure period the dressings were carefully removed and the treated area of skin was washed with warm water (37°C) to remove any residual test substance. The treated area was blotted dry with absorbent paper.

No control animals were included in this study.

OBSERVATIONS

Mortality

Cages of rats were checked at least twice daily for mortalities.

Clinical signs

Animals were observed soon after dosing and at frequent intervals for the remainder of Day 1. On subsequent days animals were observed on at least two occasions during the day (once in the morning and again at the end of the experimental day, with the exception of the day of study termination - morning only). The nature and severity of clinical signs and the time these were observed were recorded at each observation.

Animals in the preliminary and main study were observed for 7 or 14 days respectively after dosing.

Dermal responses

Local dermal irritation at the treatment site was assessed daily using the following numerical scoring system:

Erythema and eschar formation:

No erythema	0
Slight erythema	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Oedema formation:

No oedema	0
Slight oedema	1
Well-defined oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond the area of exposure)	4

Any other lesion not covered by this scoring system was as described.

Bodyweight

The bodyweight of each rat in the preliminary study was recorded on Days 1 (prior to dosing) and 8 and in the main study on Days 1 (prior to dosing) 8 and 15.

TERMINAL STUDIES

Termination

The animals in the preliminary study were killed on Day 8 and all animals in the main study were killed on Day 15 by cervical dislocation.

Macroscopic pathology

All animals were subjected to a macroscopic examination which consisted of opening the thoracic and abdominal cavities, the cranial cavity was not examined as observations did not indicate any neurotoxicity. The macroscopic appearance of all examined organs was recorded.

ARCHIVES

All raw data and study related documents generated during the course of the study at Huntingdon Life Sciences Ltd, together with a copy of the final report are lodged in the Huntingdon Life Sciences Ltd Archive, Huntingdon.

Such records will be retained for a minimum period of five years from the date of issue of the final report. At the end of the five year retention period the Sponsor will be contacted and advice sought on the future requirements. Under no circumstances will any item be discarded without the Sponsor's knowledge.

DEVIATIONS FROM THE PROTOCOL

There were no deviations from the protocol.

RESULTS

PRELIMINARY STUDY

A group of two rats (one male and one female) was dosed at 500 mg/kg bodyweight. There were no deaths.

No dermal response was seen in either animal throughout the study.

Clinical signs were confined to increased sensitivity to touch seen in the female rat and notable for approximately 3 hours after dosing. There were no other signs of reaction and recovery was complete by Day 2.

Bodyweight changes were considered satisfactory for a study of this nature and duration.

There were no macroscopic abnormalities noted in either animal at the terminal necropsy on Day 8.

The results from the preliminary study indicated the acute lethal dermal dose to rats of [redacted] was greater than 500 mg/kg bodyweight and on this basis no further preliminary study animals were used, as this result was considered adequate justification for progression to the main study at a dosage of 2000 mg/kg.

MAIN STUDY

There were no deaths and no evidence of a systemic response in any animal throughout the study following a single dermal application of [redacted] to a group of ten rats (five males and five females) at a dose level of 2000 mg/kg bodyweight.

DERMAL RESPONSES (Table 1)

Primarily transient slight to well-defined dermal irritation (erythema/oedema up to Grade 2) was notable in the majority of rats with a similar level of response more persistent in one female. These reactions were accompanied in one rat by desquamation over the treatment site. In the majority of rats, all reactions had resolved by Day 7 and in one rat by Day 12.

BODYWEIGHT (Tables 2 and 3)

Minor bodyweight fluctuations were noted in the occasion animal. However, the majority of animals were considered to have achieved satisfactory bodyweight gains throughout the study.

MACROSCOPIC EXAMINATION

No macroscopic abnormalities were observed for animals killed at study termination on Day 15.

CONCLUSION

The acute lethal dermal dose to rats of [redacted] was demonstrated to be greater than 2000 mg/kg bodyweight.

TABLE 1

Dermal reactions

Dose (mg/kg)	Animal No.& Sex		Days after dosing										
			2	3	4	5	6	7	8	9	10	11	12 to 15
2000	21 M	E	1	0	0	0	0	0	0	0	0	0	0
		O	1	0	0	0	0	0	0	0	0	0	0
		#	-	-	-	-	-	-	-	-	-	-	-
	23 M	E	0	0	0	0	0	0	0	0	0	0	0
		O	0	0	0	0	0	0	0	0	0	0	0
		#	-	-	-	-	-	-	-	-	-	-	-
	24 M	E	0	0	0	0	0	0	0	0	0	0	0
		O	0	0	0	0	0	0	0	0	0	0	0
		#	-	-	-	-	-	-	-	-	-	-	-
	25 M	E	2	1	0	0	0	0	0	0	0	0	0
		O	1	0	0	0	0	0	0	0	0	0	0
		#	-	-	-	-	-	-	-	-	-	-	-
	26 M	E	0	0	0	0	0	0	0	0	0	0	0
		O	0	0	0	0	0	0	0	0	0	0	0
		#	-	-	-	-	-	-	-	-	-	-	-
	22 F	E	1	0	0	0	0	0	0	0	0	0	0
		O	0	0	0	0	0	0	0	0	0	0	0
		#	-	-	-	-	-	-	-	-	-	-	-
	27 F	E	1	2	2	2	2	2	1	1	1	1	0
		O	1	1	1	1	1	1	1	0	0	0	0
		#	-	-	-	-	-	-	-	-	-	-	-
	28 F	E	2	2	1	0	0	0	0	0	0	0	0
		O	1	2	1	0	0	0	0	0	0	0	0
		#	-	c	c	c	c	-	-	-	-	-	-
	29 F	E	0	0	0	0	0	0	0	0	0	0	0
		O	0	0	0	0	0	0	0	0	0	0	0
		#	-	-	-	-	-	-	-	-	-	-	-
	30 F	E	1	0	0	0	0	0	0	0	0	0	0
		O	0	0	0	0	0	0	0	0	0	0	0
		#	-	-	-	-	-	-	-	-	-	-	-

Key	
E	Erythema
O	Oedema
#	Other reactions
c.	Desquamation characterised by dryness, sloughing and /or scaling on treatment site (reported as desquamation)
-	Not applicable

TABLE 2

Individual and group mean bodyweights (g)

Phase of study	Dose (mg/kg)	Animal No. & Sex	Bodyweight (g) at Day		
			1*	8	15
Preliminary	500	1 M	284	311	-
		2 F	234	234	-
Main	2000	21 M	238	287	334
		23 M	278	302	326
		24 M	294	341	375
		25 M	289	325	361
		26 M	308	319	362
		Mean	281	315	352
		22 F	241	248	255
		27 F	227	235	249
		28 F	221	239	249
		29 F	239	253	254
		30 F	233	235	233
		Mean	232	242	248

*: Prior to dosing

TABLE 3

Individual and group mean bodyweight changes (g)

Phase of study	Dose (mg/kg)	Animal No. & Sex	Bodyweight gains (g)	
			Day 1-8	Day 8-15
Preliminary	500	1 M	27	-
		2 F	0	-
Main	2000	21 M	49	47
		23 M	24	24
		24 M	47	34
		25 M	36	36
		26 M	11	43
		Mean	33	37
		22 F	7	7
		27 F	8	14
		28 F	18	10
		29 F	14	1
		30 F	2	-2
		Mean	10	6